ADVANCED PHARMACEUTICAL AND HERBAL NANOSCIENCE FOR TARGETED DRUG DELIVERY SYSTEMS

PART I

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Swarnlata Saraf
Ram Kumar Sahu
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Bentham Books

Advanced Pharmaceutical Herbal Nanoscience: Targeted Drug Delivery System

Part I

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Advanced Pharmaceutical Herbal Nanoscience: Targeted Drug Delivery System (Part I)

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ISBN (Online): 978-981-5036-51-0

ISBN (Print): 978-981-5036-52-7

ISBN (Paperback): 978-981-5036-53-4

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FOREWORD

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Nanoscience knowledge has been developed exponentially over the last 20 years. We now have a much clearer understanding of novel drug delivery system technologies. The improvement of herbal nanoscience technology has accepted the creation of a new novel drug delivery system that uses physical enhancers for many of the mentioned therapeutical applications.

The aim of the book entitled "Advanced Pharmaceutical Herbal Nanoscience: Targeted Drug Delivery System" is to offer a regular understanding of various herbal novel drug delivery systems, their preparations, characterisation and exciting range of applications. Herbal nanoscience targeted drug delivery system demands a broad range of awareness in novel drug delivery systems. It is very important to have detailed knowledge and awareness of new technology or new process about the various herbal nanoscience products and their composition. The awareness of knowledge about the properties of various herbal constituents of a nanoformulation is also very important because it decides the determination of the formulation of the delivery systems and broadly ensures its properties. This book covers a broad spectrum of herbal nanoscience topics required for appropriately providing formulation procedures, evaluation and applications of herbal drug delivery systems and correlate it with treating many diseases. The book not only focuses on theoretical knowledge but also considers its practical aspects. The book is quite beneficial for students and researchers across the globe who are indulged in the reading and investigation of advanced pharmaceutical herbal nanoscience and related nanotechnology.

This book offers an overview of the latest trends in advanced pharmaceutical herbal anoscience targeted drug delivery system. The book covers research targets at various hierarchical levels of herbal novel drug delivery systems, from the nano molecular level up to the psychological implications. The richness of the information and the coverage of the topics are the strengths of this book, which offers the informed reader a mixture of the herbal delivery system in vivid disease and new technology in advanced pharmaceutical herbal nanoscience targeted drug delivery system. In this book, the current volume should be considered useful to stimulate innovative interdisciplinary research pathways.

The contributors to this text have been directed to emphasize updates on above mentioned herbal nanoscience technologies involved in therapeutic applications. Authors were selected due to their knowledge and reputation in their subject area and for their ability to address the topics of this book objectively. I strongly feel that this addition to literature will be particularly useful to undergraduate pharmacy/post-graduate pharmacy students/scientists/industry/ academics. The book will serve as a sound source of systematic information for the herbal nanoscience targeted drug delivery system.

PREFACE

The objective of the book entitled "**Advanced Pharmaceutical Herbal Nanoscience: Targeted Drug Delivery System"** is to offer an understanding of various herbal novel drug delivery systems, their preparations, characterisation and exciting range of applications. Herbal nanoscience targeted drug delivery system demands a broad range of awareness in novel drug delivery systems. It is very important to have detailed knowledge and awareness of new technology or new process about the various herbal nanoscience products and their composition. The awareness of knowledge about the properties of various herbal constituents of a nanoformulation is also very important because it decides the determination of the formulation of the delivery systems and broadly ensures its properties. This book covers a broad spectrum of herbal nanoscience topics required to appropriately give formulation procedure, evaluation and applications of herbal drug delivery systems and correlate it with treating many diseases.

It expresses huge awareness and knowledge regarding advanced pharmaceutical, herbal nanoscience targeted drug delivery systems in the aspect of the application in drug delivery and herbal nanomedicine. In addition to this, the book covers all major topics like drug development issues, adaptation to clinical use, market prospects and industrial commercialization too, which come under advanced pharmaceutical nanoscience and nanotechnology application. Apart from the application section, it discusses in detail the safety, herbal nanotechnology, regulatory, targeting aspects and social scenario of pharmaceutical nanoscience. The book not only focuses on theoretical knowledge but also considers practical aspects. The book is quite beneficial for students and researchers across the globe who are indulged in the reading and investigation of advanced pharmaceutical, herbal nanoscience and related nanotechnology, thereby spreading awareness all over the globe and promoting anticipated trends in the field of nanoscience and nanotechnology. The major objective of this initiative is to bring all the fundamental concepts, target delivery, herbal bioactive, and nanomedicine, all in a common platform that will provide knowledge about all the possible advanced pharmaceutical, herbal nanoscience drug delivery systems. Some major chapters to be published in the book include nucleic acid-based therapeutic, electrosomes, aquasomes, phytosomes, guggulosome, niosomes, self nano/micro emulsified drug delivery system, the concept of targeted drug delivery system, application of herbal Bioactive and many more. This valuable resource will make the readers more aware of novel drug delivery systems as well as their promising applications in drug targeting and nanotheronostics, thereby improving the pharmaceutical world's situation.

The book's aim is to provide a single volume covering a detailed description of various herbal drug delivery systems, their principles and how these are put in use for the treatment of vivid diseases. The book has been divided into four sections. The first section deals with the fundamentals of advanced pharmaceutical nanoscience, whereas the second section deals with a detailed overview of the novel and efficient advanced pharmaceutical nanoscience delivery systems in the field of pharmaceutical science in which some major topics will be published in the book, which includes nucleic acid-based therapeutic, electrosomes, aquasomes, phytosomes, guggulosome, niosomes, self nano/micro emulsified drug, *etc*. This section is quite unique as it elaborately describes the major main beliefs and techniques of the preparations of the herbal drug delivery systems. This furnishes a quite unique and updated coverage of the essential areas that guide the underlying science behind these therapeutic delivery systems. The distinguished authors have emphasized on providing a complete insight into the advanced pharmaceutical nanoscience drug delivery systems with their convenient applications in the field of herbal nanoscience technology. The third section mainly involves diseases specific to

advanced pharmaceutical nanoscience targeted drug delivery systems like cancer, infectious diseases, brain diseases, *etc*. Finally, the fourth and the last section of the book provides the application of herbal bioactive in pharmaceutical, herbal nanoscience targeted drug delivery systems.

This advanced book has been designed keeping in mind the young and new researchers and scientists who are working dedicatedly in the field of health/medicine and the pharmaceutical sector. This book promises a detailed, informative description of advanced herbal and absolutely modern pharmaceutical dosage forms and drug delivery systems. The information furnished in the book is sure to serve society.

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CHAPTER 1

Introduction to Nanotechnology and Herbal-Based Nanoparticulate Systems

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Abstract: This introductory chapter reviews the history of nanotechnology and its benefits and challenges in the pharmaceutical field. In general, the chapter summarizes the types of nanoparticles and the techniques used to formulate nanoparticles. In detail, it discusses the principle of nanotechnology in improving solubility and dissolution rate. It discusses and describes different types of nanoparticles, including polymeric, metallic nanoparticles, and other types, such as solid lipid nanoparticles (SLN) and liposomes. Nanosization can be performed by various techniques, including top-down, bottom-up, and combination techniques. The method of these techniques has been discussed in this chapter. One of the disadvantages of nanoparticles is their stability. Nanoparticles suffer from various types of instability problems, including aggregation, sedimentation, and crystal growth. Therefore, in this chapter, the authors discuss the problem of stabilization of nanoparticles and describe the different pathways of physical instability and the mechanism of stabilizers to stabilize the colloidal system. Finally, the importance of herbs and natural products in the medical field and how the use of nanotechnology addresses various drawbacks of herbal products are also discussed.

Keywords: Aggregation, Crystal growth, Dissolution rate, Herbal nanoparticles, Herbal Stabilizer, Liposomes, Marketed nanoparticle products, Metallic nanoparticles, Solid lipid nanoparticles, Solubility, Stability, Stabilization.

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Swarnlata Saraf, Ram Kumar Sahu & Vivek Dave (Eds.)

INTRODUCTION

Nanotechnology is a technology used for the transformation of nanosized materials in the range of 1 to 100 nm. These nanosized materials have different electrical, mechanical, magnetic, and biological properties than conventional materials.

The term nano was introduced in 1959 by Richard Feynman with the hypothesis, "Why can not we write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin?" He described a vision of how machines could be used to develop smaller machines at the molecular level [1]. After that, in 1974, a Japanese scientist named Norio Taniguchi defined the term "nanotechnology" as follows: "Nanotechnology consists mainly of the processing of separation, consolidation, and deformation of materials by an atom or a molecule" [2].

However, the Romans already knew about nanoparticles in the fourth century AD. An ancient cup from the Roman era, the Lycurgus cup, is a good example of nanoparticles being used at that time. This cup can show different colors depending on whether it is illuminated from inside or outside. The cup is made of a special type of glass called dichroic glass, which contains a small amount of 7 nm gold and silver. The presence of metal nanoparticles is responsible for changing the color of the beaker from opaque green to a translucent, bright red when exposed to light [3].

Ancient nanotubes were also used in the 13^{th} century. "Damascus saber blades were made of nanowires and carbon nanotubes to provide strength and sharpness [4].

Later, in the 19^{th} century, Faraday demonstrated that materials containing nanoparticles had unique optical and electronic properties. He studied the properties of a colloidal gold suspension and how it produced different colors under certain lighting conditions [5].

Nowadays, nanotechnology is used in various fields, such as medicine, science, computer science, and engineering.

The Principle of Particle Size Reduction to Improve the Solubility and Dissolution Rate of Poorly Water-soluble Drugs

The solubility of a drug is often significantly related to the particle size. When the particle size becomes smaller, the area-to-volume ratio increases, which consequently allows greater interaction between the large surface area of the particles and the solvent, which increases solubility [6]. The conventional methods

to reduce the particle size rely on mechanical stress, such as crushing and spray drying, to break down the drug. Particle size reduction improves solubility through efficient, reproducible, and economical methods. However, the mechanical forces induced by comminution, such as grinding and crushing, often result in significant physical stress on the active ingredient, which can lead to degradation [6]. Thermosensitive and unstable APIs can be affected by the thermal stress that can occur during comminution and spray drying, which is a concern during processing. Conventional processes for poorly soluble APIs may not be able to increase solubility to the desired level. One of the conventional techniques for reducing particle size is micronization.

Micronization involves increasing the surface area, which increases the dissolution rate of drugs; however, it does not increase equilibrium solubility. These drugs' dissolving rate is increased by reducing particle size and increasing surface area. Micronization of drugs is performed by grinding techniques using rotor-stator colloid mills, jet mills, *etc.* Micronization cannot be used for drugs with high dose numbers because it does not change the saturation solubility of the drug [7]. These techniques were used for griseofulvin, progesterone, spironolactone, diosmin, and fenofibrate. For each drug, micronization improved the absorption of these drugs from the gastrointestinal tract and thus their bioavailability and clinical efficacy. Conventional particle size reduction remains a fundamental size reduction technique, but particle size reduction techniques now include nanotechnology and nanonization, which are being extensively studied for formulation approaches of drugs with poor aqueous solubility [8, 9].

Recently, numerous nanonization strategies have been developed to improve the bioavailability and dissolution rate of many drugs that exhibit poor aqueous solubility. Some of these strategies include changing the crystalline form of the drug, developing new nanomaterials that can serve as carriers for the drug for controlled release, and increasing the surface area to volume ratio of drug powders [10, 11]. Nanonization can lead to improved solubility and pharmacokinetics of the drug and can also reduce systemic side effects [12]. Alternatively, nanotechnology can also be used in drug delivery systems such as polymeric micelles and nanoemulsions [11]. In the last decade, several drugs in nanoformulations have been clinically approved or are currently under clinical investigation [10, 11]. Research has focused on the development of nanoformulation technologies, new pharmaceutical materials, and quality control to improve product properties while minimizing production costs. New technological developments and unmet clinical needs are a major driving force for the research and development of nanonization strategies [12].

Advantages of Nanotechnology in the Pharmaceutical and Medicinal Fields

Nanotechnology offers a solution to many problems. The reduction of particle size to the nanoscale will improve the solubility of poorly water-soluble drugs (class II and IV). It will also improve the permeability and transport of the drug through the epithelial and endothelial barriers, which will solve the problem with drugs of classes III and IV. Nanotechnology will improve drug targeting to cancer cells by passive diffusion using the improved permeability and retention effect, which will increase the target to non-target ratio and reduce the side effects. Moreover, it combines therapeutic and diagnostic modalities in one agent as in theranostics [13].

Challenges

Although nanotechnology offers a solution to many pharmaceutical problems and improves the diagnostic and treatment efficacy of many drugs, these benefits are accompanied by several scientific challenges. One of these challenges is the high risk of toxicity. The smaller the particle size of the materials, the greater their ability to penetrate cells, raising concerns about toxicity. Another issue is related to the regulatory requirement. Nanomaterials have unique properties that are different from molecular substances. Therefore, the FDA treats nanomedicine as a new drug for marketing approval; thus, extensive clinical trials must be conducted [14, 15].

The use of alternative medicine, “herbal medicine,” in the treatment and cure of diseases still needs time to be approved and marketed. The natural source of these medicines is the biggest challenge. Using a natural product carries a high risk of toxicity and immunogenicity. In addition, because it is a bioproduct, instability in the pharmaceutical formulation has been a major concern for most industries. Due to these problems, many herbal medicines have failed during clinical trials [16].

This chapter deals with the different approaches used in nanotechnology. It also deals with the different types of nanoparticles. Plant-based nanoparticles and various plant-based active ingredients and stabilizers are also discussed.

CLASSES OF NANOPARTICLES DRUG DELIVERY SYSTEM

The applications of nanoscience and nanotechnologies are becoming increasingly popular and include almost all scientific fields, such as electronics, physics, chemistry, energy, material science, engineering, agriculture, biology, medicine, and environmental protection (pollution control) [17 - 19]. The pharmaceutical and medical applications of nanoparticles (NPs) will have a great impact on diagnostics and biosensing, bioimaging, new drug development, and targeted drug delivery.

This could be due to the small particle size (less than 150 nm), large surface area, and easy surface functionalization, which improves cell membrane permeation [20, 21]. Several types of nanoparticles are now approved and available for the treatment of certain diseases such as cancer, infectious diseases, diabetes, and immune diseases [22].

Different materials have been used for the preparation of nanoparticles, such as natural and synthetic polymers, organic materials, inorganic metals, lipid materials, and oils. Accordingly, the main drug delivery vehicles based- nanoparticles can be classified into polymeric NPs, metallic NPs, solid lipid nanoparticles (SLN), liposomes and nano-emulsion, quantum dot, carbon nanotubes, and nanocrystals, *etc.*, as shown in Fig. (**1**) [23].

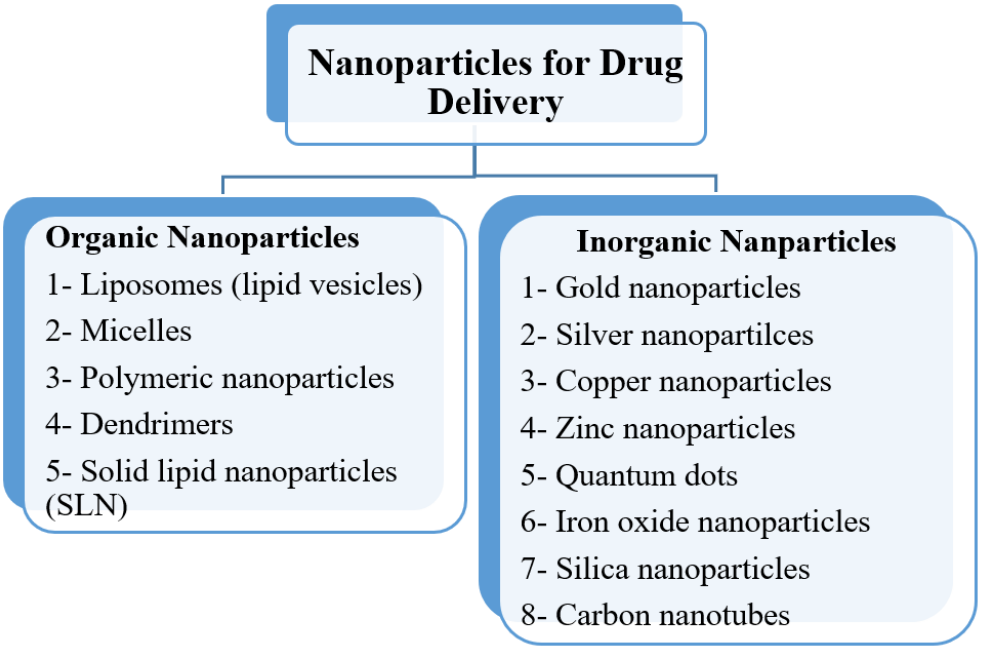

Fig. (1). Different types of nanoparticles used for targeted drug delivery.

Polymeric Nanoparticles

Unlike other nanoparticles, polymeric NPs are gaining interest as vehicles for drug delivery due to their biocompatibility, biodegradability, greater safety, and lower toxicity, ability to be loaded with a large number of active ingredients, and ease of manipulation and targeting [24]. Various natural, semi-synthetic and synthetic polymers have been widely used for the preparation of polymeric nanoparticles.

The most commonly used natural polymers for the preparation of NPs include chitosan, alginate, cellulose, starch, proteins, natural gums, carrageenan, hyaluronic acid, and pectin. The most commonly used synthetic polymers for the production of NPs are PLA PLGA, PLC, PVA, PEG, and methacrylic acid derivatives (Eudragit). Another class of semi-synthetic polymers, such as cellulose derivatives, which combine the advantages of natural and synthetic polymers, have also been used for the preparation of NPs [25, 26].

The possibility of drug intake by all routes of administration, excellent pharmacokinetic properties, and the ability of relative accumulation in specific parts of the body are the characteristics that make polymeric nanoparticles more advantageous than other types of nanoparticles for drug delivery [26]. For example, PLGA nanoparticles loaded with benzydamine HCl incorporated into a thermosensitive hydrogel have been used for the local treatment of oral mucositis. In addition, many authors have described the administration of drug-loaded polymeric nanoparticles by intravenous, intramuscular, subcutaneous, ocular, topical, and oral routes [27]. Cancer treatment is one of the major applications of polymeric nanoparticles because the drug is encapsulated in the nanoparticles, providing a protective shell for the drug and minimizing its interaction with normal cells, which in turn greatly reduces the side effects of chemotherapeutic agents [22, 24].

Metallic Nanoparticles

Recently, metallic (inorganic) NPs have attracted greater attention due to their unique physicochemical properties, which mainly depend on the shape, size, surface charge, and composition of the nanoparticles [28]. Metallic nanoparticles are colloidal dispersions, and the dispersed metallic NPs have the main characteristic properties of both liquids and solids. In other words, metallic NPs are considered as the intermediate state between solids and liquids. This state gives metallic NPs unique characteristics and properties that are not possible for NPs in solid, liquid, or polymer-based states. In particular, properties such as chemical inertness, good stability, and ease of surface functionalization make inorganic NPs charming platforms for many applications [29].

The most commonly used metals for the synthesis of inorganic nanoparticles are gold, silver, copper, zinc, iron, selenium, and cobalt [29 - 33]. Drug-loaded gold nanoparticles were first used for cancer treatment by combining the following two treatment options: chemotherapy and physical therapy by thermal ablation [29]. Silver nanoparticles possess potent antibacterial activity and have been used to treat various bacterial infections, especially infections caused by resistant bacteria [30]. Other metallic nanoparticles have been used for the treatment of microbial infec-

tions, bioimaging and diagnosis, biosensing, and targeted drug delivery [30, 31, 33].

Due to biodegradability and safety properties, metallic nanoparticles are considered less safe than nanoparticles of organic origin. This is because such nanoparticles settle and accumulate in certain organs such as the liver and kidney and are taken up by the reticuloendothelial system. These properties limit the application of metallic nanoparticles in the treatment of diseases inside the body, and so far, their use has been limited to the treatment of topical and local diseases [28].

Solid Lipid Nanoparticles (SLN) and Solid Lipid Carriers (SLC)

Solid lipid nanoparticles are considered the next generation of colloidal dispersion systems, especially in the field of drug delivery. Compared to other nanoparticles such as liposomes, nanoemulsions, and polymeric nanoparticles, SLNs consist of a biodegradable solid lipid matrix that forms a protective shell enclosing a core of various types of drugs and active pharmaceutical ingredients. In addition to their high stability, they possess many advantages, such as increasing the bioavailability of the loaded drug, modifying the release pattern, lowering toxicity, protecting the entrapped sensitive drugs, and improving the permeation and delivery of many drugs through the skin, but they also suffer from certain disadvantages, such as poor drug loading and drug leakage [34, 35].

Solid lipid carriers (SLCs) are the second generation (advanced version) of SLNs, which consist of a mixture of solid and liquid lipids (oils). The incorporation of oils in NLC reduces the degree of crystallization of the solid lipid core of SLN, increasing the drug loading capacity and improving physical and chemical long-term stability [36 - 38].

The main components of SLN and SLC are solid lipids, oils surfactants, and water. Moreover, SLN may be coated with charge modifiers and stealthing agents to increase the blood circulation time in the body and improve the targeting ability. Different types of drug-loaded SLN are available and can be easily obtained depending on several factors such as the composition of formulation, a technique used for preparation, and preparation temperature [37 - 39].

Solid Solution Type of SLN

This type is obtained when the cold homogenization technique is applied. The lipid and drug solid solution is prepared without the use of surfactants.

Core-shell Model With Drug-enriched Shell

This type of SLNs is prepared by using a hot homogenization technique corresponding to the drug-enriched shell model. As a hot o/w nano-emulsion is cooled, precipitation of lipid takes place, thereby resulting in a steady rise in the concentration of drug in remaining molten lipids. This results in the solidification of the outer shell containing a large amount of the drug. The drug enriched shell gives an initial high drug release rate (burst release) due to dose dumping.

Core-shell Model With Drug-enriched Core

In this type of SLNs, the precipitations of the drug occur before the lipid solidifies or crystallizes. The drug concentration in lipids is high and close to its saturation solubility. As nano-emulsion cools down, drug supersaturation in molten lipid occurs and, hence, precipitation before the lipid crystallizes. Subsequent cooling leads to lipid recrystallization around the drug-rich core as a lipid coat.

Liposomes

Liposomes are small artificial vesicles, ranging from nano-sized to micro-sized, are composed of natural or semi-synthetic phospholipids, cholesterol, and water in addition to other additives. According to the structure, liposomes are described as vesicles of an aqueous compartment (where the hydrophilic drug is dissolved) covered with a phospholipid bilayer, where lipophilic drugs can be entrapped (Fig. **2**). Due to their biocompatibility, biodegradability, low toxicity, and ability to trap hydrophilic as well as hydrophobic drugs and site-specific drug delivery, liposomes have attracted the attention of many scientists, especially those who are working in the area of drug delivery [40 - 44].

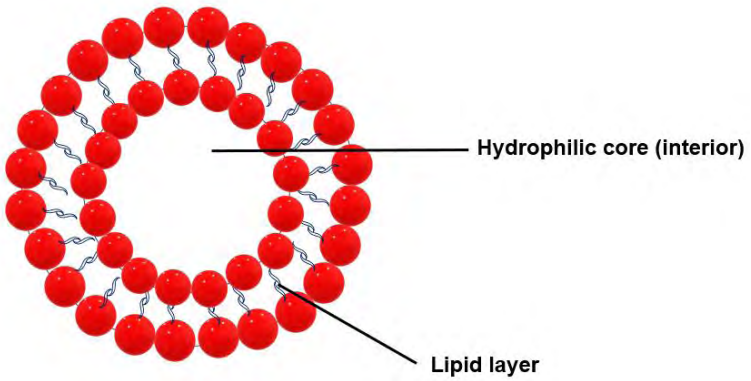

Fig. (2). General structure of liposomes.

Liposomes or vesicular nanoparticles can be classified into different types according to their size and structure [45].

According to the Structure

Liposomes can be structurally divided into several categories (Fig. **3**); i) Unilamellar vesicles (UV), which consist of a single lipid bilayer and are generally 50-250 nm in diameter. They contain a large aqueous core and are preferred for the encapsulation of water-soluble drugs. ii) Multilamellar Vesicles (MLV) consist of multiple concentric bilayers. The high lipid content of these vesicles enable them to efficiently entrap lipid-soluble drugs. iii) Oligo-lamellar vesicles (OLV) contain fewer layers than MLV and are used for diagnostic and therapeutic purposes. iv) Multivesicular liposomes consist of multiple non- concentric vesicles encapsulated in a single bilayer.

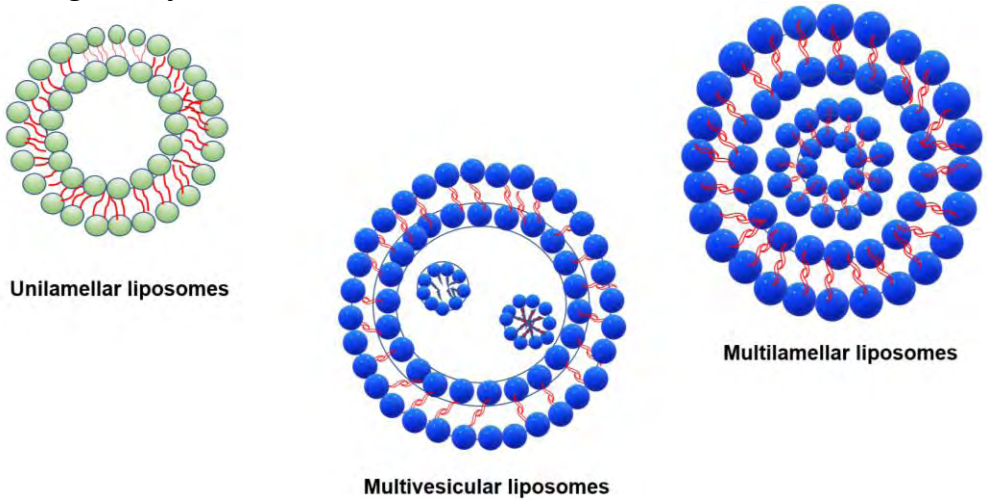

Fig. (3). Types of liposomes according to their structure.

According to Size

Liposomes can also be classified according to their size. Unilamellar liposomes can be divided into small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). Table **1** shows the size difference between the different types of liposomes.

Table 1. Classification of liposomes

Term Used		Approx. Size (µm)
Small unilamellar	SUV	0.025-0.050

(Table 1) cont.....

Term Used		Approx. Size (μm)
Large unilamellar	LUV	0.1-0.5
Large multilamellar	MLV	0.5-10

Carbon Nanotubes (CNTs)

Carbon nanotubes are the latest class of nanoparticles that have a wide range of pharmaceutical and medical applications. Typically, nanotubes have a diameter of 20-150 A^{o} and a length of 1000-2000 A^{o}. The small nanoscale dimensions and high length-to-width ratio make them a promising carrier with a wide range of valuable applications. Carbon nanotubes (CNTs) have proven to be good carriers as they serve as transporters for biomolecules to the target site. These nanostructures can be loaded with various drugs and biomolecules, such as vaccines, small peptides, proteins, nucleic acids, vitamins, and sugars. The applications of CNTs have also been extended to gene therapies, tissue regeneration, biosensor diagnosis, enantiomer separation of chiral drugs, extraction and analysis of drugs and pollutants [46, 47].

CNTs can be classified based on their length into long or short and based on their structure into single-walled, double-walled, or multi-walled (depending on the number of concentric cylindrical layers in their nanostructure). They can also be classified as open (both ends of the tube are open) or closed type. Carbon nanotubes are also classified based on their crystallographic configurations, such as a zigzag, armchair, and chiral, depending on how the graphene layer is coiled [48 - 50].

API NANONIZATION TECHNIQUES

Nanonization of API particles can be performed using either “bottom-up” or “top-down” techniques, depending on the nature of the starting material, as shown in Fig. (**4**).

Bottom-up Technologies

Bottom-up technologies fabricate nanoparticles by building them from molecular states, *e.g*., precipitation with antisolvents, supercritical fluids, droplet evaporation by spray drying, and so on [51].

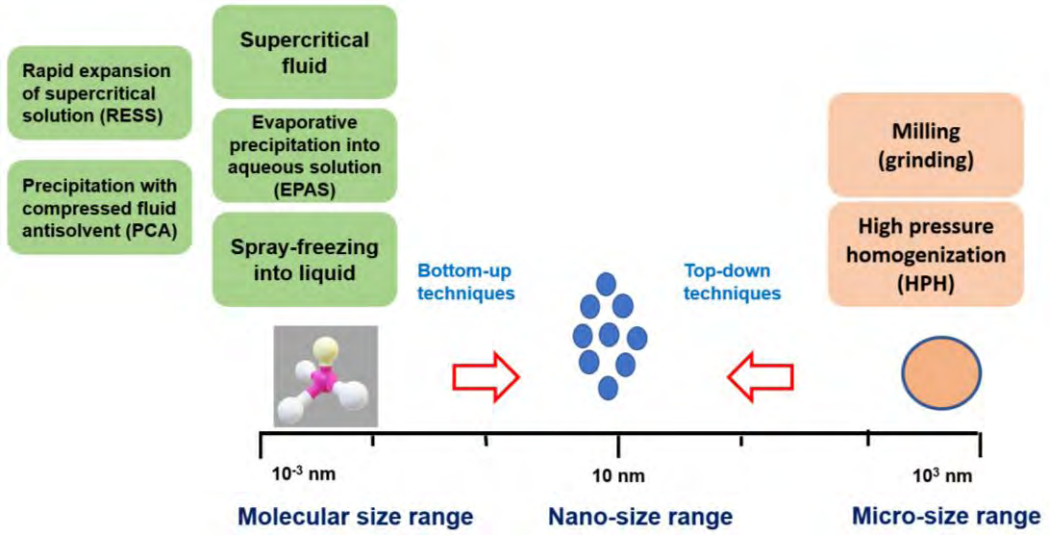

Fig. (4). Bottom-up and Top-down nanonization techniques.

Precipitation with a Compressed Liquid Antisolvent (PCA)

In this nanonization technique, the organic solvent of the drug is added to supercritical carbon dioxide as an antisolvent. The solvent expands into carbon dioxide, creating a supersaturated solution that leads to the precipitation of drug crystals. These particles are then subjected to mass transfer to reduce their size to the nanoscale [52]. This method was applied to improve the dissolution rate of quercetin, a poorly soluble drug, by converting it into nanoparticles [52]. Many parameters were studied, such as the influence of drug concentration, solvent to antisolvent ratio, stirring speed, and flow rate. Using this technique, the prepared nanoparticles showed a higher dissolution rate than the pure drug [51 - 53].

The Rapid Expansion of Supercritical Solution (RESS)

In this method, the drug is dissolved in a supercritical fluid (SCF) containing a surfactant to obtain its nanoscale particles. A surfactant is used to stabilize the medium and reduce its ability to aggregate [54, 55]. By using the RESS, the particle size of raloxifene was reduced from 45 μm to 19 nm, and the dissolution rate of nanoparticles was increased sevenfold compared to the dissolution of the drug from microparticles [56].

Supercritical Fluid (SCF)

Here, the drug is dissolved in SCF such as carbon dioxide, nitrous oxide, ethylene, or water. The particle size produced by SCF can range from micro to nanoparticles. Due to the safety and reproducibility as well as the cost-effective property of the

method, many pharmaceutical manufacturers use this method to improve the dissolution rate and bioavailability of various poorly soluble drugs [15, 57].

Spray Freezing (SFL)

In liquid spray freezing, the drug is brought into a solution, emulsion, or suspension using a solvent (aqueous, organic, or a mixture of both). The solvent is then atomized *via* a nozzle into a cryogenic liquid, such as argon, nitrogen, or halocarbon. The resulting droplets exit the nozzle and are forced into cold halocarbon vapor, which causes gradual solidification of the droplets [15]. The particles prepared at SF exhibit good wettability with an increased surface area, which has a positive effect on the dissolution rate of the drug. However, the particles prepared by this technique have a wide size range due to the agglomeration of the particles in the nozzle during the spraying process. Purvis *et al*. [58] showed that the oral bioavailability of itraconazole (a class II drug) was improved by SFL prepared nanoparticles compared to the commercial product, and the survival time of mice was prolonged after the inhalation of itraconazole nanoparticles.

Evaporative Precipitation Anti Solvent (EPAS)

EPAS is based on dissolving the active ingredient in an organic solvent with a low boiling point. The solution is sprayed into a heated aqueous solution, forming an amorphous suspension. In this process, the addition of a surfactant is very important to obtain a stable nanosuspension and to minimize the aggregation of particles. This method was first developed in 2001 and patented by the College of Texas at Austin [59]. Dharmalingam *et al*. [60] studied the release properties of naproxen after preparing the drug in gel form using the EPAS method. The results showed that the dissolution rate of naproxen was significantly improved compared to pure naproxen.

Top-down Techniques

“Top-down” techniques mean the reduction of large drug crystals or particles to nanosize either by milling (grinding) or high-pressure homogenization (HPH). The starting material in top-down nanotechnology is coarse particles that are reduced to the nanoscale by either wet milling or high-pressure homogenization (HPH).

Media Milling Techniques

Media milling is the most widely used industrial technology for the production of nanonized drug particles due to its low cost and ability for rapid production. Several commercial nanoparticle-based pharmaceutical products are produced by media milling. In this technique, the grinding vessel is filled with a grinding ball, a dispersion medium (*e.g*., water), API powder, and a suitable stabilizer (or more). During the grinding process, the grinding beads or balls are subjected to high- speed rotation to generate strong shear forces that grind the APIs into nanoparticles [61]. The grinding beads can be made of glass, zirconium, steel, or plastic. However, zirconium balls are more suitable for producing very small nanosized particles and minimizing the erosion of the balls. In nanonization by wet milling, stabilizers for the nanoparticles should be added to obtain a stable formulation [62].

High-Pressure Homogenization (HPH)

The nanonization procedures using HPH are carried out by cavitation, which includes the generation of vapor bubbles in the milling medium by applying high pressure [63]. HPH could be performed in either aqueous or non-aqueous milling media. Non-aqueous solvents (*e.g*., PEG 400) are suitable for water-sensitive APIs. A suspension of solid drug and suitable stabilizer is forced through the narrow gap of a homogenizer at high pressure (500–2000 bar). The pressure generates disruptive forces such as collision, cavitation, and shearing, which grind large drug crystals to nanoparticles. HPH gives an exceptional alternative for the production of industrial-scale high-quality drug nanoparticles. Controlling homogenization pressure and speed, as well as the number of homogenization cycles, might result in producing tiny particles in the nanosize range [64].

Drug nanonization by top-down technologies (mechanical milling or high- pressure homogenization) suffers from some drawbacks as it needs long nanonization times, results in impurities, and low flexibility in monitoring particle surface and shapes.

Combination of Top-Down and Bottom-Up Techniques (Second Generation of Drug Nanocrystals; SmartCrystal®)

A combination of a pre-treatment step with the following annealing step by rendering high energy has been introduced to compensate for the drawbacks of the single “bottom-up” or “top-down” strategies [65]. This 'annealing' step is defined as a single or repeated energy input for the transformation of amorphous particles into crystalline materials [64].

SmartCrystal® technology is considered as the second generation of drug nanoparticles that has a combination of different methods owned by Abbott Lab [65]. Combining a pre-treatment step followed by HPH produces nanoparticles less than 100 nm in size and results in improved drug dissolution mimicking the injection of solution [63, 66]. H69 cavitation-precipitation techniques combine precipitation in the cavitation zone with the classic HPH. In H42 and H96, spray-drying and lyophilization procedures, respectively, are followed by HPH to obtain nanocrystals below 100 nm [67]. Nanopure® technique is also a member of the smartCrystal® family [65]. The smartCrystals® could be regarded as a toolbox containing specialized combinative strategies. Besides, these techniques also provide additional physical nanoparticles' stability, which could not be attained by using single-step techniques [51].

PHYSICAL STABILITY OF NANOPARTICLES

Aggregation

The decrease in particle size can lead to an increase in the surface area of the particles and thus an increase in the surface energy, which can lead to agglomeration of the particles as tiny particles tend to agglomerate to minimize the surface energy and rebalance the formula. This agglomeration can trigger crystal growth. To solve this problem, it is recommended that the formulator add a stabilizer that can prevent particle agglomeration [68, 69].

Crystal Growth

Crystal growth (also called Ostwald ripening) occurs mainly in colloidal dispersions and occurs when the solubility of the drug depends on its particle size. The theory of Ostwald ripening states that small particles have relatively high saturation solubility compared to larger particles, resulting in a concentration gradient between the small and large particles [70]. Therefore, the drug molecules are transferred from the high concentration (small particles) to the lower concentration (large particles), and accordingly, the large particles become supersaturated, which increases their tendency to form crystals [70]. Crystal growth could be minimized by using a narrow particle size distribution in the formula, especially if a stabilizer is added [71]. Otherwise, the addition of nanoparticle stabilizers could minimize crystal growth *via* another mechanism (discussed later).

Storage time and environmental conditions (such as humidity and temperature) can also mimic Ostwald remaining. Van Eerdenbrugh *et al*. [69] investigated the effects of storage time and temperature on 9 nanosuspension formulations stabilized with TPGS (D-alpha-tocopherol-polyethylene glycol-1000-succinate).

They found that crystal growth occurred in eight of nine nanosuspensions after 3 months of storage. They found that increasing the storage temperature to 40 °C increased crystal growth.

Sedimentation in Nanosuspensions

The sedimentation rate of nanoparticles in nanosuspension is affected by the size of the particles, viscosity of the medium, and density, as described by Stokes' law [70]. In aqueous nanosuspensions, sedimentation is very low because certain surfactants or polymers are added to act as stabilizers and reduce the size of nanoparticles to minimize sedimentation. In addition, aqueous nanosuspensions can be dried by spray drying or freezing, which reduces the sedimentation of particles in non-aqueous nanosuspensions that have a very high sedimentation rate. The formulator can increase the stability of non-aqueous nanosuspensions by adding surfactants or by changing the particle surface [72].

The Crystalline State

The crystalline state of the nanoparticles can affect the dissolution and stability of the drug as well as the therapeutic efficacy. The amorphous state is less stable than the crystalline state. High-energy amorphous particles could change to a lower-energy crystalline form over time, depending on the temperature, the type of stabilizer used, and the nature of the crystalline form. Lindfors *et al*. [73] observed that amorphous felodipine nanoparticles are unstable due to the existence of trace crystalline drug particles, which is due to the difference in the saturation solubility of the drug between amorphous and crystalline states, by using the antisolvent precipitation method under sonication. Ali *et al*. [74] indicated that the hydrocortisone nanosuspension prepared by the precipitation method showed good stability after storage at 25 °C for 3 months. In addition, the amorphous form of retinoic acid showed good stability after 6 months of storage at 4 °C [75].

STABILIZATION OF NANOPARTICLES

Mechanism of Stabilization

Nanosized particles have a high surface free energy (ΔG) due to their small size, therefore, these particles tend to be closer together to reduce their surface free energy. This leads to an increase in particle size and a decrease in surface area. Therefore, a stabilizer leads to a decrease in ΔG by decreasing the interfacial tension γs /l, as shown in Equation 1 [76, 77].

$$\Delta G = \Delta A \times \gamma s/1 \quad \textbf{(1)}$$

Where ΔG is the change in surface free energy (joules) and ΔA is the change in surface area (m^2).

Polymeric or non-ionic surfactants exert their stabilizing effect on the nanoparticles by covering the surface of the nanocrystals and creating a steric barrier to stabilize them. On the other hand, ionic surfactants act by covering the surface of nanoparticles with the same charges that lead to increased repulsive forces between neighboring nanoparticles, thus reducing their aggregation. Forces between nanosized particles can be the result of dispersion or van der Waals forces.

Stabilizers for Nanoparticles

Nanoparticle stabilizers have a critical function in the formulation and stabilization of nanoparticles. The main roles of a stabilizer are to thoroughly wet the drug particles and inhibit Ostwald ripening [78] and to agglomerate the nanosuspension to formulate a physically stable formulation with steric or ionic barriers. The type and amount of stabilizer have a significant impact on the physical stability and *in vivo* behavior of the nanosuspension [61, 64]. Stabilizers explored to date include poloxamers, polysorbates, lecithins, and povidones [79]. Hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), polyvinylpyrrolidone (PVP K30) are polymers suitable for use as stabilizers. The polymer chains should be long enough to form a steric layer on the surface of the nanoparticles. However, a too-long polymer chain may delay the dissolution of the drug [79]. Choi *et al*. [80] found that the interactions between stabilizers and drugs are complex and depend on many variables, such as the presence of functional groups and surface energy.

Polysorbate 80 (nonionic), sodium lauryl sulfate (SLS), and docusate sodium salt (DOSS) (both anionic) are some examples of suitable surfactants used as stabilizers for the physical stability of nanosuspensions. Surfactants often help in wetting, electrostatic stabilization by charging and dispersion of drug particles, which are usually very hydrophobic. Some of the stabilizers that have been used in nanoparticle formulations of drugs on the market today include HPMC, PVP, DOSS, and SLS [81]. In parenteral formulation, lecithin has always been a necessary stabilizer to achieve a stable nanosuspension. The ratio of active ingredient to the stabilizer in the formulation can vary from 2:1 to 20:1 and should be evaluated on a case-by-case basis [81].

HERBAL BASED NANOPARTICLES

Herbal Active Ingredients

Herbal or natural products have been used since ancient times for the treatment of human diseases. Herbs have been used to treat diseases in various regions of the world, including China, Egypt, India, and the Americas. Some of these treatments were recorded as in the Ebers Papyrus, however, others were not. Due to process difficulties, herbal products were not developed. Nowadays, several medicines with natural herbal sources are available in the market (Table **2**).

Table 2. Marketed drug products from herbal origin.

Drug	Source	Medical uses
Artemotil®	Artemisia annua L.	Malaria treatment
Reminyl®	Galanthus woronowii Losinsk	Alzheimer's disease treatment
Paclitaxel®	Taxus brevifolia plant	Cancer treatment
Vinblastine and vincristine	Catharanthus roseus	Cancer treatment
Silymarin	Silybummarianum	Liver disease treatment

Most herbal products are characterized by poor solubility, resulting in poor absorption and consequently low bioavailability. Formulation of herbal products as one of the nanoproducts improves their solubility and bioavailability and, at the same time, reduces toxicity to a lesser extent [82].

Nowadays, herbal medicinal products are formulated as nanoparticles, liposomes, nanoemulsions, and SLN. In addition, the herbal drug "curcumin" was developed as nanotubes in combination with paclitaxel to enhance the therapeutic effect [83].

By merging herbal medicine and nanotechnology, several disadvantages of conventional herbal medicines are overcome. Herbal products are characterized by poor water solubility, and some might be acid labile and unable to tolerate the acidic media of the stomach. Nanotechnology offers a good solution to these problems. By reducing particle size, solubility is increased, allowing optimal absorption and improving bioavailability. Reducing particle size decreases the incidence of toxicity by reducing the dose and frequency of administration. Particle size reduction can serve as a targeted approach by improving absorption in a specific area, such as cancer cells, where the nanomedicine can be targeted through improved permeability and retention. In addition, the reduction in particle size allows the high molecular weight plant-derived drug to penetrate the lipid membrane [84].

Herbal drugs are characterized by their poor water solubility, and the use of nanotechnology has overcome this barrier. Nanotechnology improves the solubility and delivery of herbal medicines [85].

Several herbal products have been used as active ingredients and possess pharmacological activity. Camptothecin is a natural plant alkaloid extracted from *Camptotheca acuminata Decne*. It has potent anticancer activity and can be targeted against intracellular topoisomerase. However, its poor solubility and instability (unstable lactone ring) pose a major challenge in formulation development. Min and other collaborators prepared hydrophobically modified glycol chitosan nanoparticles loaded with camptothecin. The system showed good stability and significant antitumor activity compared to the free drug [86]. In another study, camptothecin was incorporated into pegylated liposomes. The formulated liposome showed potent antitumor activity in mice with low side effects. The accumulation index was also very high compared to drug solutions [87].

Quercetin is a natural flavonoid with anti-inflammatory and antioxidant activity. Quercetin is prepared in the form of solid lipid nanoparticles and nanostructured lipid carriers. Both systems improve the bioavailability of the active ingredient compared to the free active ingredient. In addition, the anti-inflammatory effect is enhanced [88, 89].

The anxiolytic effect of quercetin was studied by Priprem *et al*. [90]. Quercetin was prepared in the form of liposomes for intranasal administration. The study showed that the anxiolytic effect of the intranasal liposomal formulation of quercetin was greater than that of the oral liposomal formulation.

Curcumin is a yellow polyphenol derived from the R*hizomes of Curcuma longa (Zingiberaceae*). Its poor solubility and low bioavailability limit its clinical use. The formulation of nanoparticles containing curcumin improved its solubility, and the anti-tumor activity was enhanced. Curcumin was prepared as polymeric nanoparticles with PLGA using the emulsion solvent evaporation technique. The prepared nanoparticles showed a 35% reduction in cell *via*bility in the prostate cancer cell line [91].

Breviscapin is a natural flavonoid isolated from *Erigeron breviscapus*. It has a protective effect on the brain against ischemic damage. Breviscapin was prepared in the form of multivesicular liposomes (MVLs) designed for sustained release. The *in vitro* study showed significantly prolonged release, which was 5 times higher than the normal liposomal formulation. The *in vivo* study showed a prolonged release duration of up to 5 days [92].

Triptolide is a herbal drug with potent anti-inflammatory activity. It is a poorly soluble in water and severely toxic when administered. Liu and other collaborators formulated PLA nanoparticles loaded with triptolide. The formulated nanoparticles improve the solubility of the drug. In addition, the *in vivo* study showed significant inhibition of adjuvant-induced arthritis with anti- inflammatory effects up to 30 days [93].

Herbal Stabilizer

A plant product was used to stabilize and formulate metallic nanoparticles using a green synthesis method. Yasmin *et al*. [94] formulated stable gold nanoparticles (16-30 nm) using *Hibiscus rosa-Sinensis* using a microwave heating method.

In another study, *Prunus domestica* gum at a concentration of 0.5% was used as a stabilizer in the formulation of gold and silver nanoparticles. The resulting nanoparticles had a size of 5-30 nm. The anticancer activity of *Prunus domestica* gum loaded with gold and silver nanoparticles was investigated by IC50. The results showed a significantly high potential anticancer effect. The IC50 value of 2.14 ± 0.15 μg/mL for gold nanoparticles was observed, while silver nanoparticles had an IC50 value of 3.45 ± 0.23 μg/mL, compared with the standard IC50 value of 1.89 ± 0.12 μg/mL for cisplatin [95].

Nowadays, an herbal extract has been used for the green synthesis of gold and silver nanoparticles. It is considered an environmentally and economically friendly alternative method for the synthesis of AuNPs and AgNPs. It also provides a large yield of nanoparticles with a defined size.

Marketed Product

The global market for nanoparticles in life sciences was estimated at over $29.6 billion in 2014. This market was forecasted to grow to more than $79.8 billion by 2019 [96].

The nanomedicine market is in the growth phase. The largest area of research is drug delivery, and nano-enhanced drug delivery products are already commercially available. In addition, several advanced nanotechnology-based drugs and medical devices are in clinical trials.

To date, several products containing nanoparticles have been approved by the FDA and are commercially available. Table **3** shows some of these marketed products.

Table 3. Marketed nanoparticles drug products.

Commercial name (Company)	Ingredient Active	Application	Dosage Form
Doxil®/Caelyx™ (Janssen)	Doxorubicin	Karposi's sarcoma; Ovarian cancer; multiple myeloma	Injection
Abelcet® (Sigma-tau)	Amphotericin B lipid complex	Fungal infection	Injection
DaunoXome® (Galen)	Daunorubicin	Karposi's sarcoma	Injection
DepoCyt® (Sigma-Tau)	Cytarabine	Lymphomatous meningitis	Injection
AmBisome® (Gilead Sciences)	Amphotericin B	Fungal and/or protozoal infections	Injection
Curosurf®/Poractant alpha (Chiesei farmaceutici)	Proteins SP-B and SP-C	Lung activator for stress disorder; pulmonary surfactant for respiratory distress syndrome	Injection
DepoDur® (Pacira Pharmaceuticals)	Morphine sulfate	Prolonged release	Injection
Copaxone® (Teva)	Glatopa	Multiple sclerosis	Injection
Renagel® (Sanofi)	Sevelamer hydrochloride or sevelamer carbonate	Chronic renal diseases	Tablets
PegIntron® (Merck)	*Interferon-alpha* (*IFN*-α2b)	Hepatitis C	Injection
Pegasys® (Genentech)	*Interferon-alpha* (*IFN*-α2a)	Hepatitis B and C	Injection
Eligard® (Tolmar)	Leuprolide acetate	Prostate cancer	Injectable suspension
Neulasta® (Amgen)	PEG-filgrastim	Neutropenia induced by chemotherapy	Injection
Somavert® (Pfizer)	PEG-visomant	Acromegaly	Injection
Cimzia® (UCB)	Certolizumab pegol	Crohn's disease; rheumatoid arthritis; psoriatic arthritis, and ankylosing spondylitis	Injection
Plegridy® (Biogen)	*Interferon*-beta (*IFN*-β1a)	Multiple sclerosis	Injection
Rapamune® (Wyeth Pharmaceuticals)	Sirolimus	Immunosuppressant	Oral solution & tablets
Megace ES® (Par Pharmaceuticals)	Megestrol acetate	Anti-anorexic	Oral suspension
Avinza® (Pfizer)	Morphine sulfate	Mental stimulant	Extended-release capsules
Ritalin LA® (Novartis)	Methylphenidate HCl	Mental stimulant	Extended-release capsules

(Table 3) cont.....

Commercial Name (Company)	Ingredient Active	Application	Dosage Form
Zanaflex® (Acorda)	Tizanidine HCl	Muscle relaxant	Tablets & capsules
Emend® (Merck)	Aprepitant	Antiemetic drug	Capsules
Tricor® (Lupin Atlantis)	Fenofibrate	Hyperlipidemia	Tablets
Invega® Sustenna® (Janssen Pharms)	Paliperidone palmitate	Schizophrenia schizoaffective disorder	Extended-release injectable suspension
Ryanodex® (Eagle Pharmaceuticals)	Dantrolene sodium	Malignant hypothermia	Injection
Estrasorb™ (Novavax)	Estradiol	Menopause hormone therapy	Topical emulsion
Abraxane® (Celgene)	Paclitaxel (ABI-007)	Breast cancer; non-small cell lung cancer, and pancreatic cancer	Injectable suspension
Ferrlecit® (Sanofi Avertis)	Sodium ferric	Chronic kidney failure with iron deficiency	Injection
GastroMARK™; umirem® (AMAG pharmaceuticals)	Superparamagnetic iron oxide nanoparticles (SPION)	Imaging material	Oral suspension

CONCLUSION

In this chapter, the importance of nanotechnology as a drug delivery system has been focused on based on the wide range of applications of nanotechnology in pharmacy and medicine. Nanotechnology is considered as a pronouncing mean to enhance the dissolution rate and bioavailability of poorly water-soluble drugs. The relationship between particle size and solubility has been discussed in this chapter. The chapter also pointed out the two major nanonization techniques, namely bottom-up and top-down. The interdisciplinary nature of nanotechnology enables the formation of different types of nanoparticles, including organic and inorganic nanoparticles. In addition, the stability issues of nanoparticulate systems, as nanoparticles represent a great challenge during the formulation, have been detailed. Moreover, the chapter sheds light on the mechanism of nanoparticles' stabilization through several types of stabilizers to deliver the drug more effectively. Several nanoparticle-based products that have been approved by the FDA and marketed have been mentioned for parenteral and oral administration.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 2

Nucleic Acid-Based Therapeutic Drug Delivery System

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Abstract: The targeted drug delivery system aims to deliver medication to the target tissue to reduce side effects and improve the overall efficacy of the medication. The drastic reduction of the dose has been an advantage of this system, coupled with a significant increase in the delivered pharmaceutical agent's therapeutic index. Nucleic acid, as a naturally occurring chemical compound, carries information molecules and monitors protein synthesis in the cells, making it an important therapeutic target for drug delivery to tissues. They are made up of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The purpose of this chapter is to discuss the nucleic acid-based therapeutic drug delivery system with its associated principles and mechanisms, barriers to nucleic acid-based therapeutics transfection and their delivery systems, primary delivery systems used in transferring nucleic acid-based therapy, and nucleic acid-based therapeutic drug delivery system as a tool of advanced pharmaceutical, herbal nanoscience. This is meant to support innovations in the field of pharmaceutical and herbal nanoscience.

Keywords: Barriers, Delivery, Efficacy, Herbal, Innovations, Mechanisms, Medication, Nanoscience, Nucleic Acid.

INTRODUCTION

Herbal medicine involves the use of plants and plant extracts for healing purposes. Herbal medicines could also be described as naturally occurring plant-derived products with little or no pharmaceutical industry processing to treat illnesses within a locality. It is a known fact that most of the processed drugs used today are of plant origin. This is so because the use of herbs in the healing process as a medical practice predates recorded historical facts and modern medicine as well [1 - 3].

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The quest to improve the availability of plant products has transformed the pharmaceutical nanoscience field, which involves applying characteristics and properties that are unique to nanoparticles and the utilization of such materials in pharmaceutical industries to produce products, such as nanomedicines [4].

Therefore, to improve the specificity and delivery of a therapeutic agent to an intended location, a system known as the targeted drug delivery system, which leads to the concentration of such agents only on the targeted tissue, was introduced, thereby reducing indiscriminate exposure of healthy cells to such agents. This method can reduce the toxic effects of such agents and reduce instances of the development of drug-resistant pathological cells. The mechanism of action of the targeted drug delivery system is ligand-based and could identify the physiological and structural differences between healthy and diseased cells. The developed ligand is conjugated with the therapeutic agent to be delivered through covalent or noncovalent methods [5, 6].

The best approach to a drug delivery system remains the pharmaceutical nanoscience approach, which could deliver the drug to a target location and achieve a sufficient concentration over a desired therapeutic period [7]. The nanoscience drug delivery system possesses the capability of delivering high drug concentrates to the target sites. Small particle delivery aids quick dissolution, maintains the concentration for a longer time at the delivery site, does not require ligand moiety, reduces the observed side effects, and reduces the dose administered [8].

Low solubility and bioavailability of herbal constituents in the human body are significant challenges to herbal medicine. These challenges can be overcome by reducing the size of bioactive compounds such that they can be adequately absorbed and made available in the human body [7].

The advancement in fields like genomics, biotechnology, combinatorial chemistry, and nanotechnology has contributed immensely to the advancement of drug delivery systems, which has overcome the constraints associated with herbal pharmaceuticals. Pharmaceutical and herbal nanoscience aims to provide targeted site-specific delivery of phytomedicines, a sustained drug release to prevent repeated administration, and improve patient compliance while enhancing the specificity, solubility, safety, and efficacy of the delivered herbal drugs [8, 9].

According to another study [10], the targeted drug delivery system can be classified into passive and active targeting. Passive targeting involves the accumulation of drugs at a specific site based on the physiochemical nature or pharmacological nature of the disease condition. This is the most widely exploited option for the targeted delivery of the drug to the tumour sites, and it utilizes enhanced permeability and retention, exploiting the leaky tumour vasculature [10, 11]. Active

targeting involves a therapeutic agent's conjugation being delivered to a tissue or a cell-specific ligand. Active targeting refers to a specific receptor- ligand type of interaction for the localization of agents after blood circulation and extravasations. The approach can be classified as first-order targeting, which distributes the therapeutic agents only to the capillary bed of a target site, second- order targeting, which involves the delivery of a therapeutic agent to specific cell types and third-order targeting, which delivers therapeutic agents to a specific intracellular target site [10, 12].

Phytochemicals gained some attention when herbal pharmaceutical researchers answered questions about their pharmacokinetics, mechanisms of action, and action sites. Understanding the mechanism of activity prompted the need for the adaptation of a novel drug delivery system to enhance the delivery, efficiency, and efficacy of phytochemicals as therapeutic agents. New drug delivery systems were developed, including liposomes, nanoparticles, stable lipid nanoparticles, microemulsions, matrix systems, and solid dispersions. Nanocarriers used in pharmaceutics and herbal nanoscience should be made of safe materials like synthetic biodegradable polymers, lipids, and polysaccharides [13, 14]. There are drug vectors called drug delivery vehicles. They are nonimmunogenic, nontoxic, and highly biodegradable [10]. Examples of these drug vectors are polymers, conjugates, polymeric nanoparticles, lipid-based carriers (liposomes, micelles, and phytosomes), dendrimers, carbon nanotubes, and gold nanoparticles [14 - 16]. This chapter discusses the nucleic acid-based therapeutic drug delivery system, the principles, mechanisms, procedures, and limitations of its use in herbal nanoscience, and related innovations.

NUCLEIC ACID-BASED THERAPEUTIC DRUG DELIVERY SYSTEM

Nucleic Acid-Based Therapy And Its Principle

The first authorized human gene therapy was achieved in 1989 when a viral (retroviral) delivery system was used to tag tumour-infiltrating lymphocytes genetically [17]. In 2017, the number of clinical trials being conducted globally had increased to about 2600. Furthermore, it was reported that the majority of these trials (75%) used viral vectors to deliver genes into the host cell [18].

Nucleic acid is a naturally occurring chemical compound that functions as an information-carrying molecule in cells and also aids in directing protein synthesis in the cell [19]. Furthermore, there are two main classes of nucleic acids, which are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [19].

The nucleic acids or closely related compounds themselves can serve as a remedy in treating genetic diseases. Thus, they are termed therapeutic nucleic acids (TNAs). Several examples of TNAs share a common mechanism of action mediated by sequence-specific recognition of endogenous nucleic acid through Watson-Crick base pairing (formation of a hydrogen bond between nucleotides) [20].

As significant advancements in biotechnology and molecular biology have upsurged the number of therapeutic nucleic acids and contributed to a noteworthy increase in the production of these macromolecules in large quantities, there have been marked advancements in the art of nucleic acid-based therapy for varieties of illnesses [21]. The basic principle that has been holding up the hope of exploring nucleic acid-based therapy for treatment is that the over-expression of functional genes (which have been silenced) and the silencing of diseased genes (which are being over-expressed) can be achieved easily by various means of drug delivery technologies. Of the two main nucleic acid classes, RNA has a more potent therapeutic effect due to its significant role in translational regulation, retroviral application, RNA splicing, and RNA stability controls [22]. Furthermore, nucleic acid-based therapeutics are be divided into two main categories (DNA-based and RNA-based therapeutics) and will be discussed briefly.

DNA-Based Therapeutics and Their Mechanism

The DNA-based therapeutics include plasmids, DNA aptamers, DNAzymes, antisense, and antigene DNA.

Plasmids are double-stranded DNA molecules containing transgenes that encode specific proteins. Upon cellular internalization, the transcription and translation of the employed DNA biologically synthesise the target protein, which serves as the therapeutic agent [23]. Its mechanism of action requires that the DNA molecules gain entry into the cell nucleus, where they eventually regulate and express the desired gene. Plasmids are DNA-based therapeutics, but they are also used in DNA vaccines to define the host cell's desired antigen [24].

DNA aptamer is a single-stranded or double-stranded DNA that interacts with proteins outside the cell. Interaction of the aptameters with the target proteins alters the protein's physiological effect, thereby inhibiting its function [25].

DNAzymes: DNAzyme acts as an enzyme and binds to intracellular RNA substrates through Watson-Crick base pairing. After binding to these substrates, it cleaves sequences that include purine-pyrimidine nodes [26]. DNAzymes are similar to ribozymes in the sense that they are both catalytic nucleic-acid-based the-

rapeutics. Although they are similar, DNAzymes are more stable than ribozymes and are easier to synthesise [27].

Antisense and Antigene DNA: Upon internalization of the oligonucleotide (a short-stranded segment of DNA or RNA), the DNA binds to precise complementary areas and forms a duplex with RNA (mRNA or pre-mRNA) through Watson-Crick base pairing, thereby inhibiting the RNA transition and consequently inhibiting the synthesis of the target protein [28].

For the antigene, the short-stranded DNA must gain entry into the nucleus, attach to the double-stranded genomic DNA and consequently inhibit the targeted gene expression, thereby negatively influencing the biosynthesis of protein [27]. For treatment purposes, oligodeoxyribonucleotides can be used to block the expression of proteins associated with diseases. For example, MG98 is an approved antisense drug that inhibits methyltransferase synthesis and c-raf kinase in cancer cells [29].

RNA-Based Therapeutics And Their Mechanism

RNA-based therapeutics include antisense RNA, RNA aptameters, RNA decoys, ribozymes and small regulatory RNAs.

Antisense RNA: This is a specific type of non-coding RNA (ncRNA) similar to antisense DNA; there are limitations to the use of antisense RNA in the sense that their interference with the RNA produced by the cells is competitive, and thus their effect results in gene knockdown instead of gene knockout [22]. Also, the stoichiometry disadvantage of antisense RNA is that there is a need for high expression before it can successfully interact with the target protein [27].

RNA aptameters: These are single-stranded RNAs capable of interacting with their specific target with great affinity. Recently, many RNA aptameters have been identified as being capable of binding with various targets through a process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment); these targets include nucleotides, proteins, organic compounds, and whole-cell [30, 31]. The RNA aptameter is smaller in size than its counterpart (DNA aptameters) of the same length, giving them easier cell entry than their counterpart [31].

RNA decoys: The first endogenous RNA decoy was the miRNA decoy, which was demonstrated in the interaction of miR399 in Arabidopsis. The miR399 decoy mimics the actual miR399 and results in the inactivation of the primary miR399 target transcript [32]. From this, it is known that RNA decoy is aimed at providing

competition for the target protein binding site. Also, the decoy can induce instability and prevent translation [27].

Ribozymes are enzymatic RNA molecules that can form and break covalent bonds with nucleic acid molecules [33]. They have more significant advantages than antisense oligonucleotides due to their binding specificity and ability to cleave mRNA substrates. Also, ribozymes inactivate target RNA without depending on the cellular machinery [34]. Thus, the use of ribozymes as a therapeutic agent against viral and cancer diseases has recently been established. Furthermore, ribonuclease is an example of a ribozyme capable of cleaving RNA targets.

Small regulatory RNAs include miRNA (micro-RNA), siRNA (small interfering RNA) and shRNA (short/small hairpin RNA).

(a) Micro RNA: These are small non-coding RNAs (about 20-24 base pairs in length) that occur naturally in the cells and have the primary function of regulating gene expression *via* sequence complementarity [35]. Recent studies have also shown how plant miRNAs are produced, degraded, and utilised in repressing target gene expression [35].

(b) Small interfering RNA: siRNA are long double-stranded RNA molecules that are synthesized artificially. They are about 19-23 nucleotides in length. Also, they are used in transient slicing of the targeted gene in molecular biology. Upon binding to their target transcript, siRNA elicits an RNAi (RNA interference) response upon sequence complementarity [36]. Currently, siRNA has been a promising therapeutic for viral, cancer, and other genetic diseases, although the medical application of this RNA-based therapy is limited due to the risk of mutations, oncogenic effects, and the fact that siRNA cannot enter the cell itself without the help of a delivery system [37].

(c) Short/small hairpin RNA: The shRNA is a nucleotide with a hairpin-like structure when compared to siRNA molecules; they are slightly larger and are produced in the cell nucleus, unlike siRNA. Like siRNA, tshRNA is used in treating genetic diseases like cancer [38]. Successful studies have been carried out to block galectin-1 expression with shRNA; this results in decreased tumour cells growth and metastasis *in vivo* [39].

Barriers to Nucleic-Acid Based Therapeutics Transfection and Their Delivery Systems

In this section, the overview of naked nucleic-acid transfection and its barriers was discussed. Also, the primary delivery systems used in transferring the nucleic-

acid therapies into the cellular compartments (targets) were examined.

Transfection of Naked Nucleic-Acid Based Therapeutics and its Barriers

The naked nucleic acids are unprotected DNA/RNA-based drugs, which means that they are not protected or coated by proteins, lipids, or any other molecules [40]. Injection of naked nucleic acids has been perceived as a safer alternative to the vast number of gene delivery systems. Although it is safer, the rate at which the naked nucleic acid is cleared from the circulation (first-pass metabolism) and depredated by the serum nucleases has been a significant barrier to the effectiveness of naked nucleic acids [41]. For example, while naked DNA degrades after intravenous administration, it has markedly been a suitable option for intramuscular administration [42]. Also, the systemic administration and transfection of naked nucleic acids are significantly inhibited by extracellular barriers like the shape, size, and polyanionic charge of DNA; these barriers further reduce cell permeability to naked nucleic acid drugs (DNA in particular) uptake, as shown in Fig. (**1**). In addition to the extracellular obstacles, there are intracellular barriers such as lysosomal degradation of nucleic acids, DNA/RNA trafficking, and biological immune barriers (some DNA sequences cause an immune response) [43].

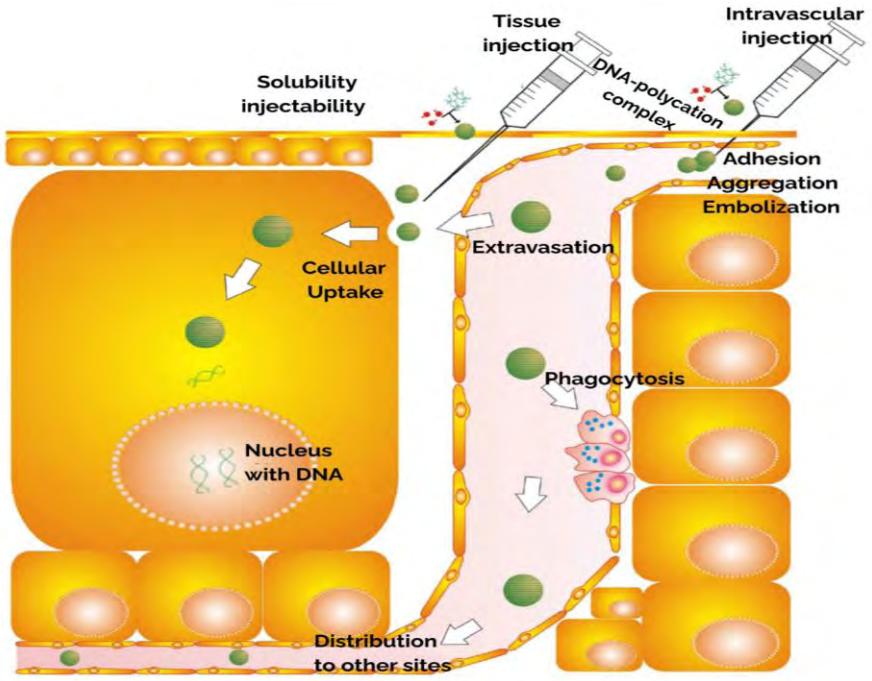

Fig. (1). The barriers in delivering nucleic acids to the cell following an *in vivo* administration [43].

The basic principle of transfection is that negatively charged nucleic acids are transferred across the negatively charged plasma membrane [44]. Therefore, the transfection pathway of an injected naked nucleic acid involves cell internalization, intracellular trafficking, and appropriate expression of the nucleic acids [45]. Recently, various methods for overcoming transfection pathway barriers have been developed, including nucleic acid transfer by chemical methods (chemicals capable of neutralising the charge on DNA/RNA), physical techniques (mechanical and electrical techniques aid in the passage of nucleotides into the cell), and biological methods (viral agents deliver the nucleic acids) [44]. These barriers were partially solved by mixing DNA with polymers, polycationic lipids, or materials capable of forming compounds (ionic bonding) with the DNA [43]. As a result of this, the quest for a novel delivery system suitable for all types of genetic modification is still on [46].

It is important to note that not all delivery systems are suitable for all types of nucleic acids in overcoming this barrier. Thus, the type of genetic modification intended to solve a problem (expression, silencing, repair, *etc.*) determines the delivery system to be used.

Major Delivery Systems Used in Transferring Nucleic-Acids Based Therapy

The successful delivery of nucleic acids (*e.g.*, antisense oligonucleotides, plasma DNA, shRNA, miRNA, and siRNA) depends on the use of efficient and appropriate carriers [47]. In this study, the nucleic-acid-based delivery system is classified into two main groups; physical techniques and vector-based delivery techniques (viral vector and non-viral vector), as shown in Fig. (**2**) below.

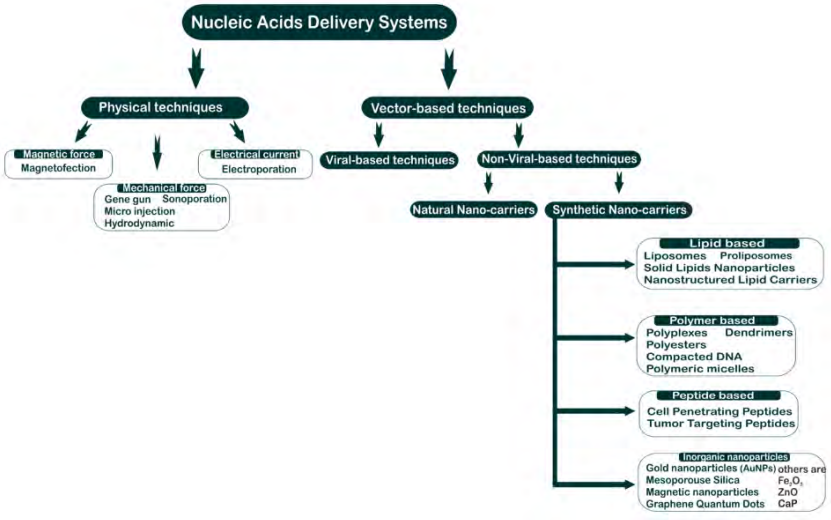

Fig. (2). Nucleic acids delivery systems techniques.

1. Physical techniques: The table below summarises the physical methods used in delivering nucleic acid-based therapeutics.

Table 1. The overview of the method used in physical techniques and their mechanisms.

Physical Techniques	Method	Mechanism	Advantages	Disadvantages	Ref.
Mechanical force	microinjection	The injection is targeted at one cell at a time for the nucleic acid transfer. The needle is about 25-2000 μm long.	Efficient	Time-consuming	[18, 27]
Mechanical Force	Gene gun or Biolistic (particle bombardment)	It involves the ballistic transfer of a bullet containing microparticles (nucleic acid) to a target cell. Thus, gene delivery is achieved.	Efficient	Limited to the target area; Needs evasive procedure for internal organ	[18, 27]
Mechanical Force	Hydrodynamic gene delivery (Hydrodynamic pressure)	Delivery is achieved by injecting a large amount of naked nucleic acid solution into the vein rapidly, creating pressure in the vein and forcing the nucleic acids into the cellular compartment.	Simple Highly efficient Site-specific	Need catheter insertion technique in large animals	[18]
Mechanical Force	Sonoporation (Ultrasound)	Ultrasound is used to increase the cellular uptake of nucleic acids.	Site-specific	Low efficiency High cell mortality and tissue damage	[18]
Magnetic force	Magnetofection	Delivery is achieved by using a magnetic field; this allows the passage of microparticles with nucleic acids into the cell. This method is always accompanied by other ways like lipofection to protect the nucleic acids from degradation.	Site-specific	Low efficiency Limited target area Need surgical procedure for internal organ	[18]
Electric pulse	Electroporation (electric current)	Delivery is achieved by using electrical current to increase the permeability of the cell membrane and facilitate DNA Transfer.	Highly efficient	High cell mortality; Limited target area; Need a surgical procedure for internal organ	[18, 27]

Vector delivery techniques: These are of two types: viral and non-viral delivery techniques.

a. Viral delivery system (biological method): The viral process makes use of non-pathogenic attenuated viruses for delivering nucleic acids, such as plasmids and other nucleic acid molecules, into the intracellular compartment [27]. The viruses used in providing the nucleic acids are of five types: adenoviruses, retroviruses, herpes simplex viruses, lentiviruses, and adeno-associated viruses [46]. The viral vector is more efficient than a non-viral vector, as it results in a high level of gene expression for some time. However, immunogenicity, inflammatory reaction, carcinogenicity, lethal immune reactions, and problems associated with scaling up limit the clinical use of viral vectors [18, 47].

b. Non-viral delivery system (Nano vesicle): An ideal non-viral vector should be safe, deliver the prescribed amount of drugs to the target, and be efficient in transporting the nucleic acids through the extracellular and intracellular environment. Thus, the non-viral delivery system has been a safer method over the viral vectors, but lesser antigenicity, human genome integration, and the period of transgene expression remain hindrances in the race for non-viral vectors [18, 47, 48].

Natural Nano-vesicle (Exosome): Exosomes are nonspherical extracellular vesicles (40-150 nm in diameter) that are naturally secreted by cells. They are made up of lipid bilayers and antigens secreted by the particular cell (disease cell or normal cell) [49, 50]. This is so because the exosomes contain cell tropism and organs [18]. Studies also revealed that exosomes encapsulate and transport various substances, such as plasmids, non-coding RNAs, proteins, miRNAs, mRNAs, *etc.* Studies have shown that the exosome was initially thought to be an organelle used by the cell to discard unwanted cellular debris; however, studies have shown that it is a novel intracellular communication regulator [49, 50]. Recently, since the advent of exosomes as a tool in delivering therapeutic nucleic acids, exosomes have been applied to deliver therapeutic nucleic acids. For example, it is used for conveying CRISPR/Cas9 plasmids into cancer cells to suppress PARP-1 gene expression. The suppression of this gene successfully induced apoptosis in a cancerous cell [18]. Lastly, the exosome-based delivery approach has been perceived as an excellent nucleic acid therapeutic delivery system, although its mechanisms, structures, and preparation need to be properly scrutinized.

Synthetic Nano-vesicles: These include lipid-based nano-vesicles, polymer-based nano-vesicles, peptide-based nano-vesicles, and inorganic-based nanoparticles

that encapsulate or form a reaction with the nucleic acids of interest and aim at delivering them to the target cells.

Lipids-based nano-vesicle: The lipid-based nano-vesicle has been used as a potential carrier due to its specific properties like large surface to mass ratio, shielding less water-soluble drugs, and controlled drug release. There are different types of lipid-based nano-vesicle. Here, we discuss liposomes, NLC (nanostructured lipid carriers), and SLN (solid lipid nanoparticles) [51].

The Liposomes: They are spherical micron/submicron-sized (10-80 nm) nanovesicles that are surrounded by a lipid bilayer. They act as a vehicle for a variety of drugs as they can be used to encapsulate peptides, nucleic acids, genes, plasmids, small proteins, small molecules, hydrophilic and hydrophobic drugs [21, 52]. The liposomes depend on electrostatic bonds between anionic nucleic acids and lipid molecules; these complexes are more compact than applying the naked nucleic acids [21]. Furthermore, because liposomes are plasma membrane- like structures, they easily bind to the cell membrane and transport their contents into the cell for therapeutic purposes [51]. Also, liposomes can contact the ocular surface; therefore, they tend to increase ocular drug absorption [21]. The benefits of using liposomes include low toxicity, fewer side effects, a longer circulatory half-life, biodegradability, and the ability to surface modify [51], while the disadvantages include low encapsulation capacity, poor storage stability, and rapid leakage of water-soluble drugs when present in blood components [52]. Dioleoylphosphatidylethanolamine (DOPE) and dioleylphosphatidylcholine (DOPC) are examples of neutral lipids that increase the efficiency of DNA transfection. Also, lipofection (a liposome) is the mixture of cationic lipid (dioleyloxypropyl trimethyl ammonium chloride (DOTMA) and neutrally charged DOPC. The lipofection combined with antisense oligonucleotides (AS-ODNs) penetrates the cell and releases the ODNs [21].

Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC): The SLN and NLC are the first and second generations of lipid-based nano-vesicles. When compared to regular liposomes, the SLN is easier to make and has a diameter of less than 200 nm. The SLN has been used to deliver nucleic acid-based therapeutics into the tissue to direct the ARPE-19 cell line [21]. Organic solvents are not needed in SLN synthesis, and at the same time, they are biocompatible [51]. The NLC contains solid lipids, unstructured liquid lipids, and surfactants, and this facilitates their preparation. Therefore, they tend to have higher water content, lasting physical stability, and prolonged storage time compared to SLN. The advantages of using SLN and NLC are that they help control the release of drugs and extend the time for hydrophobic medicines (mainly) in circulation [51].

Polymer-Based Nano-vesicles: They are artificially synthesised vesicles in the form of polyplexes, polyesters, compacted DNA nanoparticles, polymeric micelles, and dendrimers [21]. They offer many advantages over liposomes, as they tend to be more stable and circulate in the bloodstream for a more extended period than liposomes and have a better retention effect [52, 53].

Polyplexes are complexes of nucleic acids and cationic polymers that contain several amines, arginines, or lysine groups bound together at a physiological pH. They are 8 nm in diameter and have a large vector capacity. Their stability in a nuclease-rich environment and high efficiency in the transfection of therapeutics for both dividing and nondividing cells have been the advantages of polyplexes [21]. Notably, the long stability and persistent positive charge of polyplexes further lead to cytotoxicity and an acute autoimmune response [18].

Polyesters: This is an example of a synthetic polymer-based nano-vesicle used in delivering nucleic acid-based therapy. They include poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactic-co-glycolic acid (PLGA), and poly-epsilo-caprolactone (PCL). By comparing PCL to other polyesters, its degradation is prolonged, leading to its limited usage in some specific biomedical fields. On the contrary, PGA exhibits a high degradation rate even though it is similar to PLA. Therefore, the PGA cannot be used in advanced delivery systems [54]. Due to their desirable mechanical and processing features, PLA and PLGA have been used in delivering nucleic acid-based drugs into cells [55]. Also, the PLA and PLGA are produced from the fermentation of lactic and glycolic acids sourced from sugarcane; this makes them eco-friendly, safe, and non-toxic, unlike the other polyesters, which are produced from petrochemical sources [54].

Compacted DNA: These are nanoparticles that rely on the principle of DNA condensation. They contain a circular or linear segment of nucleotides that are negatively charged. These segments are compacted with a condensing agent that can vary from organic polyamines to polypeptides and inorganic polycations. Their size varies from 10 – 100 nm. Therefore, they yield medium to high transfection efficacy, which is greater than the observed expression level in naked plasmids [21].

Polymeric micelles: They are amphiphilic block copolymers (covered by the hydrophilic shell and have a core of hydrophobic block). They encapsulate therapeutic compounds such as nucleic acids and formulations like Genexol, which have been reported to be approved in South Korea and some countries as medicinal drugs for cancer [56]. As a vesicle, the polymeric micelles can also protect and entrap poorly soluble molecules in their hydrophobic core. Thus, they improve the pharmacokinetic profile.

Dendrimers are made up of amine-containing cationic polymers like polyamidoamine, polyethyleneimine, and poly-L-lysine dendrimers. They are synthetic macromolecules with a 3D branched-chain structure; they have multifunctional capping properties with a nanometer-size diameter. In addition, they possess an interior cavity in which therapeutic molecules are encapsulated, and thus they protect, increase stability, and deliver drugs to the target site. Also, it has been shown that drug molecules can attach to the dendrimer surface for drug delivery [57]. They function by condensing DNA molecules while interacting with the negatively charged cell membrane to ease cell entry [21]. Polyamidoamine (PAMAM) dendrimers are the most studied class of dendrimers and are considered suitable nanocarriers for drug delivery due to their useful properties such as monodispersity, modifiable surface groups, and extraordinary uniformity [51]. Lastly, 1,2-epoxyhexane-modified G4 PAMAM dendrimers are important dendrimers that can be used as a stabiliser or a reducing agent to form gold nanoparticles (AuNPs). Therefore, nanoparticles stabilised by dendrimers have been said to possess magnetic, sensing, catalytic and optical properties. This makes them a promising molecule in nanotechnologies [57].

In addition to all the listed polymers, hyaluronan (HY), chitosan (CH), and chitosan's oligomer (CHO) are the natural polymeric nanoparticles that are also being studied for the production of nano-vehicles.

Peptide-Based Nano-vesicles: Cell-penetrating peptides (CPPs) are also known as protein transduction domains (PTDs), with sizes ranging from 6 to 30 amino acids in length. They are capable of transporting molecules across the cellular barriers in intact and functional form. The first example of a protein that achieved cell penetration was the Trans-Activator of Transcription (TAT) protein of HIV. This protein aids transduction and leads to intracellular delivery of the viral genome and further leads to viral gene expression in cultured cells [58]. Likewise, the Antennapedia (Antp) transcription factor of Drosophila has also been shown to be capable of entering nerve cells and regulating neural morphogenesis without attaching to receptors. Studies have also shown that gelatin-silica can be modified with peptides like fusogenic peptides and TAT to mobilise and deliver plasmid DNA to cell nuclei *in vivo* [59]. CPPs are divided into two parts, which are cell-specific CPPs and non-cell-specific CPPs. TATs are examples of non-cell-specific CPPs, whereas phage displays are examples of cell-specific CPPs.

Inorganic Nano-particles: Some inorganic materials are unique nanocarriers because they possess functional chemical bonding surfaces that can react and bind with molecules like nucleic acids. The fascinating materials are gold nanoparticles (AuNPs), among the most studied inorganic nanoparticles. This is because they possess a surface that can easily interact with nucleic acids in multiple ways.

AuNPs can be coated with polycations like PAMAMs and PBAEs [60]. Several inorganic nanoparticles can act as nucleic acids carriers; these are calcium phosphate (CaP), lipid-coated CaP (LCPs), mesoporous silica nanoparticles (MSNs), graphene quantum dots, magnetic nanoparticles, *etc.* Many of the above mentioned are mainly used in amalgamation with PEG (PEGylation), PEI, and some polymers (see dendrimers above); therefore, they function best when combined with other carriers.

NUCLEIC ACID-BASED THERAPEUTIC DRUG DELIVERY SYSTEM AS A TOOL OF ADVANCED PHARMACEUTICAL HERBAL NANOSCIENCE

The demand for herbal extracts in treating a variety of diseases in the modern world has been established recently. This is due to an unlimited number of research showing or attempting to develop the therapeutic/preventive effects of an enormous number of herbal extracts. In this section, limitations to herbal extracts and innovations will be discussed. The correlation between nucleic acid and herbal extract delivery systems will also be examined.

Limitations of Herbal Extracts and Innovations

Herbal extracts' properties, such as their high chemical diversity, low toxicity, improved therapeutic effects, and macromolecular specificity, have made them important substances in drug discovery and computational studies [61]. Despite this, a few challenges are limiting the efficiency of herbal extracts in humans. The major challenge impeding the therapeutic efficiency of herbal extracts is that the acidic pH of the stomach ruins their activities, and even if they are absorbed, they tend to be metabolised by liver enzymes (first-pass metabolism), thereby affecting their bioavailability and distribution [13, 62].

Researchers are currently using nucleic acid delivery systems to bypass metabolism, stabilise and minimise herbal extract degradation, reduce side effects, and deliver drugs to their target sites.

Correlation between Nucleic Acid and Herbal Extract Delivery Systems

Since the advent of using nanocarriers in delivering nucleic acids into cells, nanocarriers have been a potential vehicle for shuttling herbal extract into their target sites. This shows that the use of nanocarriers in herbal extracts is one of the novel delivery methods to deliver nucleic acid therapeutics and herbal drugs/extracts, thus making them indispensable substances in nanoformulation for herbal extracts. Furthermore, nanocarriers' use comes with benefits like enhanced bioavailability, permeability, solubility, stability, distribution, and targeted

delivery. These benefits have been a possible way out of the rising dilemma of delivering herbal extract to its target sites. Thus, these dilemmas are overcome by loading herbal extracts into different nanocarriers.

Knowing that nano-carriers are the most common vehicles shared by both nucleic acid and herbal extract delivery systems, the subsection below highlights the established nano vehicle common for the delivery systems and their herbal loading mechanisms.

Nano-Carriers Common for the Nucleic Acid and Herbal Extract Delivery System with their Herbal Loading Mechanisms

This section is focused majorly on the nanocarriers common for the nucleic acid and herbal extract delivery systems. These are broken into four major categories; lipid-based, polymer-based, peptide-based, and inorganic nanoparticle-based delivery systems.

Lipid-Based Nano-vesicle (in herbal extract delivery system): There are different methods of loading herbal extracts into lipid nanocarriers. The most common method is hot and cold emulsification. Nanoparticles are formed when the herbal drugs are stocked with lipids in the presence of a hot surfactant solution (NB: hot emulsification takes place at a temperature higher than the lipid melting point). Simultaneously, cold emulsion methods utilise a process where the herbal extract is dissolved in lipid melt and immediately cooled with cryogenic systems, such as liquid nitrogen [63]. Other forms of herbal loading specific to each lipid- based nanocarrier are discussed below.

Liposomes: They enhance the stability, biodistribution, safety, and targeted drug delivery of herbal extracts. The use of stealth liposomes (having the ability to evade the immune system) to increase drug half-life has been established as an herbal extract delivery method. This is seen in a recent experiment, where essential oil (source; Atractylodes macrocephala Koidz) was entrapped/loaded into liposomes using the rapid expansion of supercritical solutions (RESS) method [61]. In this method, both the liposome and the essential oil were dissolved in a mixture of supercritical carbon dioxide (SC-CO_2) and ethanol. This solution was further sprayed into aqueous media (coaxial nozzle) to form liposome suspension; this process can be altered to attain maximum performance by modifying the temperature of SC-CO_2 and changing the concentration of ethanol [64]. In addition to these extracts, carvacrol, thymol, breviscapine, and berberine have also been loaded into liposomes for delivery [51, 63, 64].

Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs):

Like the liposomes, the SLNs also improve the stability of herbal extracts. The SLNs include solid lipids, water, and emulsifiers, while the NLC contains solid lipids, unstructured liquid lipids, and surfactants, as mentioned above. Like the liposomes, the SLNs and NLCs are prepared to expand through supercritical solutions (RESS) and homogenization techniques rapidly. They can be designed using high-pressure homogenization and supercritical fluid extraction of emulsion (SFEE). The high-pressure homogenizers tend to push high-pressured liquid across a narrow opening of a few microns in diameter; this causes the fluid to accelerate to about 1000 km/h through the narrow gap. Therefore, the high pressure, cavitation, and shear stress forces result in the dissociation of the drug particles down to submicron levels [64]. The extract particle sizes are reduced to submicron due to collision and cavitation when using a microfluidiser [62]. As part of the SFEE techniques, the SLNs are prepared using supercritical carbon dioxide (SC-CO_2), as seen in liposomes [63]. Lastly, examples of herbal extracts that have been loaded into SLNs or NLCs include berberine, doxorubicin, paclitaxel (used in treating varieties of cancers), frankincense, cryptotanshinone, curcuminoids, and myrrh oil [51, 61].

Polymer-Based Nano-vesicle (in herbal extract delivery system): There are different techniques for loading herbal extracts into polymer-based nanocarriers. Solvent displaced or nanoprecipitation methods are most suitable for poorly soluble herbal extracts. In this method, two miscibles are needed: the organic phase (polymer solution) and the aqueous phase (semipolar solvent); the interfacial deposition of polymer results in tension between the two steps. Furthermore, there is an increase in the surface area available to form droplets of organic solvent. The nanoprecipitation methods have also been used in the encapsulation of essential oils [64]. Other methods, like the salting-out method, have also been used in loading extracts into nanospheres; in this method, the nanosphere is formed by the combination of solvent, drug, polymer, and electrolytes (the salting agent). Also, the focus has been on the coacervation or ionic method; this is because hydrophilic polymers like chitosan (from insect chitin), gelatin, and sodium alginate are being used in the preparation of biodegradable nanoparticles [63]. Below is a list of polymers that have been used to encapsulate herbal extracts.

Polymeric nanoparticles: are colloidal structured particles with several advantages, such as increased bioavailability, dose count, enhanced solubility, and enhanced absorption of herbal drugs compared to the standard one. Polymeric nanoparticles have been used as carriers for many herbal drugs; for example, triptolide (*Tripterygium wilfordii* roots extract) has been successfully loaded into poly (DL-lactic acid) nanoparticles to overcome low solubility and toxicity problems. Also, curcumin (from turmeric) has been loaded into polymeric nanoparticles (methoxy polyethene glycol) to deliver cancerous cells. Chitosan

nano-particles have been developed for *Ziziphus mauritiana* extracts, whereby the extract is loaded into the polymer and administered to check its immunomodulatory pastime [13, 61].

Apart from polymeric nanoparticles, herbal extracts like berberine (extracted from several plants, *e.g*., Oregon grape) have been loaded into PAMAM dendrimer nanocarriers to check the effect of its anticancer properties against human breast cancer [51]. Lastly, it has been reported that polymeric micelles have been used in encapsulating 3-acetyl-11-keto-boswellic acid (from frankincense tree) to drastically increase the anti-inflammatory, anti-arthritic, and skin permeability activities of the herbal drug when likened to a standard transdermal drug delivery system [62].

Peptide-Based Nano-vesicle (in herbal extract delivery system): The basic principle of drug loading in cell-penetrating peptides (CPPs) and tumour targeting peptides (TTPs) is their ability to form covalent and non-covalent bonds with their cargo (herbal extract) and deliver it intracellularly or extracellularly [65, 66]. The TTPs are capable of binding to tumour cells selectively but lack the capability to penetrate the plasma membrane themselves. On the contrary, the CPPs can penetrate the plasma membrane, but they lack specificity. As a result of this, TTPs and CPPs are mostly used together to increase cargo delivery efficiency. Also, linear tumour-homing peptides (GHHNGR), as well as nona-arginine peptides, are examples of TTPs that have been reported to be conjugated with curcumin- loaded liposomes in increasing the cytotoxicity of curcumin suspension in the cancer cell line MCF7 (IC_{50} 3.8 μM). Another example is the fusion of a human- derived CPPs (HBD sequence "GPGLWERQAREHSERKKRRRESECKAA") with C terminus of recombinant Tcs (rTcs) in order to improve the translocation efficiency of Trichosanthin (Tcs) (a protein extracted *Trichosanthes kirilowii* root) into tumour cells. Other examples of plant extract conjugated with peptides are paclitaxel, 10-hydroxycamptothecin, camptothecin, docetaxel, *etc.* [66].

Inorganic Nano-particles (in herbal extract delivery system): These encompass metallic or metallic oxide materials employed in both nucleic acid and herbal drug delivery, *e.g*., gold, zinc oxide, iron oxide, or silver. Owing to their sizeable modifiable surface, they can be modified to form hydrophilic and electrostatic charges on their bodies, thus facilitating herbal drug loading. As the metallic nanoparticles enable drug loading and delivery, they also have the advantage of photothermal excitation, which leads to the tracking of the nano vehicle inside the body [67]. Recent studies have reported that gold nanoparticles conjugated with folic acids have been used to increase berberine effects against HeLa cancer cells. Also, berberine has been loaded onto fabricated inorganic ZnO (zinc oxide), where it was used in delivering the herbal extract into lung cancer

cells [51]. Likewise, iron (III) oxide (Fe_2O_3) is an inorganic material used in targeted drug and nucleic acid delivery systems. The advantage of using this material as a carrier is that when it eventually delivers the extract, such as berberine, the iron oxide is stored in the body's natural iron depository as heme and haemoglobin [51, 68]. Lastly, quantum dots (containing semiconductor materials with fluorescent properties) are nanoparticles covered by materials that allow light dispersion and prevent heavy metal leakage that contributes to cell/tissue toxicity. The coated dots glow when illuminated with UV light. Therefore, when the particle binds with the cancer cells' unique protein, it helps bring tumours to light and thus serves as a nano-tracker [13].

Toxicity of Nano-Carrier after Delivering the Cargo

The use of nanotechnology in delivering drugs to a target site has been growing recently. Therefore, the human race is likely to be exposed to deliberately generated nanotoxicity. This is because any intrinsic toxicity caused by nanoparticles will be enhanced by their small size (nm), which will contribute to their uncontrolled chemical reaction in the body. Typically, an urban atmosphere contains about 107 nanoparticles per 1 cm^3 (less than 300 nm in diameter), mostly made up of carbon elements. Due to their smaller size, they can easily access the lungs by inhalation. These have been shown to result in cardiovascular dysfunction [13]. Also, the respiratory, cardiovascular, and nervous systems are the targets of nanoparticles. Thus, the agglutination of nanoparticles in those sites can result in unwanted toxicity.

The human body is complex, and several reactions occur every second. Therefore, when the nanoparticle gains entry into the systemic circulation alongside the herbal extract, they will probably interact with immune cells, and this will result in the production of free radicals. Also, metallic nanoparticles such as gold particles, silver, and titanium with some polymers and peptides have been reported to have a potent cytotoxic effect on the human body. Also, apart from cytotoxicity, the toxicity effects can range from skin toxicity to whole organ dysfunction [69].

As nanoparticles have been shown to exert a toxicological effect, a branch of toxicology called nanotoxicology has emerged. Therefore, the major branch challenge is that different types of engineered nanoparticles have several possible side effects that might not manifest instantly or probably the current technology cannot detect and link them to nanotoxicity.

RECOMMENDATIONS

When evaluating herbal nanoformulations, it is recommended to vigorously consider issues like the toxicity of the nanomaterial and the herbal drug itself. This will help identify the cause of the toxicity itself, as some nanomaterials may be responsible for the toxicity caused by the herbal therapeutics themselves and *vice versa*.

There is a need to regulate the release of nanomaterials as it has been reported earlier that our environment can be polluted by these materials (*e.g*., titanium dioxide). This is because some plants or aquatic species might serve as a reservoir for the released nanoparticles, thereby exposing humans to toxic nanomaterials.

As protein-based nanocarriers have been a promising herbal drug and gene delivery system, it is recommended that homogenous peptides should not be used as carriers as they might contribute to autoimmunity.

Exosomes are naturally occurring nanovesicles, but their mechanisms, structures, and preparation have not been fully explored. Thus, it is recommended that natural nanovesicles such as exosomes should be investigated and preferred as a means of delivering herbal extracts to their target sites. This is due to the fact that exosomes are naturally absorbed by the targeted cells and are a traditional vehicle for transporting genetic materials within cells; thus, herbal therapeutics will be delivered as naturally as possible. Lastly, exosomes also possess pleiotropic effects as they contain antioxidants.

CONCLUSION

This chapter has shown that nanocarriers have been a potential delivery system for both nucleic acid and herbal therapeutic delivery systems. Also, the therapeutic limitations of traditional herbal extracts and drugs have been greatly enhanced through the use of nanocarriers. Given that not all delivery nanosystems are suitable for all herbal extracts, it is concluded that naturally occurring nanovehicles are a possible way out of these hurdles as they will be friendly to the internal environment and will not invoke an immune response.

CONSENT OF PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 3

Herbal Bioactive: A Booster Dose for Advanced Pharmaceutical Nanoscience

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Abstract: The development of bioactive components as delivery systems with the use of advanced nanoscience is opening new therapeutic avenues for the management of various diseases. Among recent novel applications, plant phytopharmaceuticals and nutraceuticals are the fastest growing areas of nanotechnology-based research for effective public healthcare. Bioactive compounds, either encapsulated or in entrapped form within novel drug delivery systems, are reported as a booster treatment for various chronic infections and life-threatening diseases, including cancer, cardiovascular disorders, hypertension, diabetes, asthma, malaria, microbial infections, immune disorders, and gastrointestinal disorders. Recently, considerable progress has surged in understanding the factors associated with these diseases. A variety of nanoscience-based formulations such as polymeric matrix nanoparticles, aerosol inhalers/nebulizers nanoemulsions, and vesicular carrier systems, including liposome, phytosome, transfersome, herbosome, ethosome, niosome, have proven valuable in the delivery of bioactive materials. Moreover, it is reported that herbs and herbal bioactive compounds exhibit notable efficacy compared to phytopharmaceuticals and plant extracts fortified within the conventional method of delivery, with enhanced solubility, bioavailability, stability, tissue distribution, abridged toxicity, improved pharmacological efficacy, and protection from physicochemical degradation. The current chapter focuses on the carrier-based delivery of bioactive as a booster with advanced nanosciences, such as nanoemulsion and vesicular drug delivery systems. In addition, the chapter also elaborates patented technologies along with potential bioactive products available in the market.

Keywords: Herbs and bioactive compounds, Liposome, Nanoemulsion, Nanoscience, Phytosome, Transfersome.

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INTRODUCTION

Medicines with herbs and bioactives have been widely utilized throughout the world for the management of various infectious diseases since the beginning of civilization. Bioactive incorporated pharmaceutical formulations are more popular than allopathic systems due to their effectiveness, relatively fewer side effects, and low cost of production [1]. In addition, physicians and patients have accepted herbal medicines due to their safety and proven therapeutic efficacy. Delivery of bioactive constituents as therapeutic agents demands a strategic approach to enhance patients' compliance and avoid frequent dosing. These can be achieved by incorporating herbal bioactive constituents into advanced drug delivery systems with the use of nanoscience. Formulations of phytoconstituent into dosage form with the use of nanoscience not only reduce repetitive dosing to overcome non-compliance but also help to boost the therapeutic value with reduced toxicity. Moreover, the incorporation of herbal bioactive constituents in novel drug delivery systems boost the therapeutic efficacy by reducing the herbal bulk dosing and enhancing bioavailability [2]. Bioactive constituents are a revolutionary alternative approach for the delivery of poorly soluble bioactive and herbal extract for enhanced efficacy with a reduced dose of administration [3].

Advanced pharmaceutical nanoscience is still the most appropriate way to deliver active pharmaceutical ingredients or herbal bioactive constituents in the body. Advanced pharmaceutical nanoscience amalgamates pharmaceutical technology, polymer science, bio-conjugate chemistry, and molecular biology to offer the required physicochemical properties, including ultra-small and controlled size, large surface area to mass ratio, high reactivity, with functionalizable structure [4]. In the past years, researchers have employed nanotechnology for the deliverance of bioactive constituents from herbal extracts. However, limited solubility and bioavailability remain to be lingering challenges. These limitations could be overcome using several approaches, including size reduction, co-crystallization, with the inclusion of advanced drug delivery systems such as vesicular systems, nanoemulsions, aerosol inhalers, and solid lipid nanoparticles. These carrier-based drug delivery systems (Fig. **1**) are promising due to the potential capability to entrap both hydrophilic and hydrophobic active pharmaceuticals with maximized biodegradability and biocompatibility. Moreover, the nano-sized droplets within nanoemulsion confer superior solubility and bioavailability by channeling phytoconstituent to the targeted site with required blood plasma concentration for a prolonged time, making it the pioneer for encapsulating the herbal bioactive components [5].

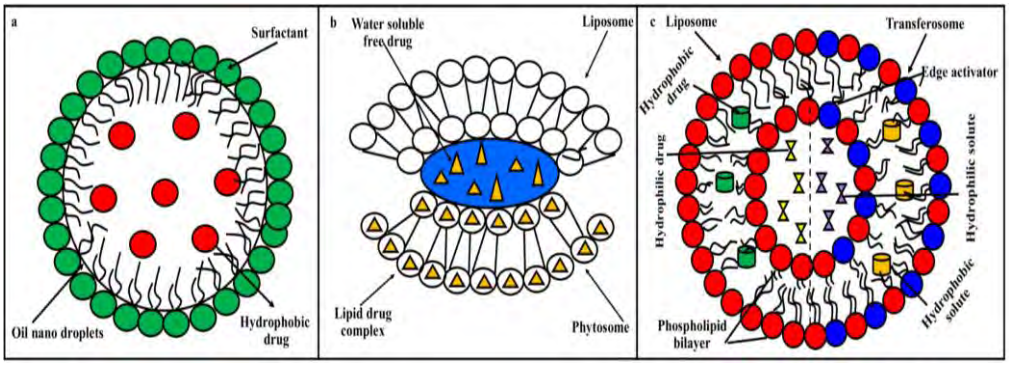

Fig. (1). Herbal bioactive incorporated within the carrier system as a booster dose for advanced pharmaceutical nanoscience. Nanoemulsion **(a)**, phytosome **(b)**, and transferosome **(c)**.

Advantages of Concentric Bilayer Vesicles

- Efficient encapsulations of both hydrophobic and lipophilic drugs with enhanced bioavailability.
- Prolong the existence of the active pharmaceutical ingredient in the systemic circulation with boosted stability in body fluid.
- Targeted delivery of drug with the resolved issue of toxicity.
- Resistance to rapid elimination of the encapsulated bioactive.

Advantages of Nanoemulsion as Advanced Nano-Carrier Drug Delivery System

- It improves the physical stability of encapsulated bioactive constituents.
- The used oil/lipid phase is biocompatible and biodegradable.
- It offers site-specific delivery with the prolonged release of encapsulated bioactive constituents.
- It avoids first-pass hepatic metabolism when applied topically
- It offers value-added delivery for dietary supplements and nutraceuticals.
- Lipid and oil used for the fabrication of nanoemulsion fortified with bioactive constituents are generally recognized as safe (GRAS). Hence cell damage and then associated issues do not arise.
- Nanoemulsion protects entrapped bioactive from hydrolysis and oxidation.

Formulation Facet of Nanoemulsion

Nanoemulsion fabrication requires admixture of two immiscible phases with a stabilizer known as an emulsifier; one of them is oleaginous, and the other is aqueous. The oil phase comprises monoacylglycerols, diacylglycerols, triacylglycerols, and free fatty acids. However, triacylglycerols are the most preferred oleaginous phase, which may be attributed to their low cost, availability, and functional and nutritional properties. Nanoemulsion aqueous phase consists of polar solvents such as water and carbohydrate, protein, and alcohol as co-solvent. The destabilization between the aqueous and oil phase of nanoemulsion occur due to Ostwald ripening, flocculation, coalescence, and gravitational separations. These defects can be prevented by the addition of an optimized quantity of stabilizers such as emulsifiers, ripening retarders, and texture modifier, which can form monolayer, multilayer, and solid particulate within nanoemulsion [6].

Nanoemulsions are comprised of several droplets with the requirement of a huge quantity of shear energy to form a high surface area. The energy required in the formation of nanoemulsion (ΔG) is estimated by the following expression: $\Delta G = \Delta A\gamma - T\Delta S$, whereas ΔA is the increase in the interfacial area, γ represents the surface tension and $T\Delta S$ the entropy of dispersion. The emulsion globule size depends on the constituent, operating conditions, and fabrication process. Generally, spontaneous nano-emulsification with high or low energy techniques is used to produce nanodroplets with in nanoemulsion.

Spontaneous Nanoemulsion Using Low Energy

The chemical potentials are the source of low energy to form nanoemulsion, which utilizes condensation process, occurred due to phase transitions within spontaneous oil and water phase interface. Spontaneous emulsification can be achieved either by the change in temperature without altering the compositions or by varying the composition and interfacial properties by intriguing a constant temperature. Low energy methods involved in nanoemulsion fabrication are solvent diffusions, and phase inversion temperature [7].

Phase Inversion Temperature (PIT)

The phase inversion temperature is based on the alteration of oil-in-water to a water-in-oil emulsion. The PIT method utilizes the phase inversion property of the molecules, wherein the emulsifiers change the lipophilicity at a fixed composition with alteration in the curvature of non-ionic surfactants. The curvature due to the surfactant becomes zero and, the solubility of the emulsifier becomes approximately equal in both water and oil phase at a definite temperature with a lamellar liquid crystalline system formation. With an increase in temperature, the

surfactant layer becomes concave with negative curvature due to the dehydration of the surfactant. Although, due to the reduction in surface tension with the increase in temperature, small globules within nanoemulsion can be formed at the PIT. However, these small globules are not stable, as they coalesce and form macroemulsion. Therefore, it is possible to form emulsions near PIT, although they are unstable. Thus, to produce stable nanoemulsion, a cooling process with storage below PIT is required. Further, if the emulsion is formed near PIT with rapid cooling or heating, kinetically stable emulsions with small droplets and narrow size distribution can be produced. The PIT can be unwavering by quantifying transformation in different properties of nanoemulsion, including conductivity, viscosity, and turbidity [7].

Phase Inversion Composition (PIC)

The phase inversion composition process, also known as a self nano-emulsification, produces nanostructured globules without the incorporation of heat and solvent. The PIC method involves the variation in constituent composition that changes the hydrophilic-lipophilic behavior of the emulsifier. These phenomena involve phase transition throughout the formation of a bi-continuous microemulsion or lamellar liquid crystalline phase [7].

Spontaneous Emulsification

Spontaneous emulsification techniques involve the formation of emulsion by mixing aqueous and oil phases together in the presence of an emulsifier under moderate mixing at a specified temperature. When both phases are mixed together, the emulsifier can penetrate into the aqueous phase, increasing interfacial tension and forming nano oil globules, with the excess oil phase being eliminated under reduced pressure. The scale-up of nanoemulsion following spontaneous emulsification process endure with the drawback of high synthetic surfactants use, which limits the use due to regulatory and sensory issues of food and pharmaceutical industries [8].

Spontaneous Nanoemulsion by a High-Energy Method

Spontaneous nano emulsification using high-energy methods involves mechanical devices such as high-pressure valve homogenizers, microfluidizers, and ultrasonicator, used for the application of disruptive force to disperse phase into petite globules on nanoemulsions.

The most commonly used techniques for nanoemulsion fabrication are ultrasonication and homogenization. The ultrasonication method employs ultrasonic waves *via* probe sonicator to reduce the micron globules into tiny emul-

sion globules. Ultrasonication employs cavitation and turbulence for size reduction and nano-emulsification, while homogenization exploits 500-5000 psi of high pressure for exerting high energy through hydraulic force with shear, a requisite for forming nanoemulsion. The process of homogenization converts microemulsion into nano-size globules, whereas micro-fluidization applies extremely high pressure, approximately to 20000 psi, with the use of a displacement pump to convert micron size emulsion into a nanoemulsion [9].

Characterization of Nanoemulsion

The physicochemical characterization of nanoemulsion includes globules size, size distribution, polydispersity, pH, refractive index, osmolarity, dye solubility, zeta potential, surface interfacial tension, lipid crystallinity, stabilities, rheology, percentage entrapment, and *in-vitro* skin permeability with drug release. The globule's size of nanoemulsion is quantified using dynamic scattering of light, neutron scattering with a small angle, and atomic force microscopy. Nanoemulsion demonstrates low and high viscosity in the presence of high content of water-soluble surfactant. Although the nature of the prepared nanoemulsion can be determined during fabrication, a conductivity test can also be used to determine it. Moreover, the dynamic and structural features of nanoemulsion were assessed by dielectric measurement. The physical stability of the nanoemulsion is determined by performing stability tests, including freeze- thaw cycle, heating cooling, and centrifugation. Briefly, all these tests analyze the time for which the bioactive encapsulated nanoemulsion systems can remain stable without losing integrity throughout the shelf life [9].

Nanoemulsion as a Booster for Delivering Herbal Bioactive

Recently, the nanoemulsion of numerous herbs and herbal bioactive constituents has been investigated to achieve enhanced solubility and bioavailability with the required therapeutic efficacy. Nanoemulsions have received scientific attention towards the oral delivery of herbal drugs due to their protective effect and reduction in gastric degradation of active components encapsulated within globules of nanoemulsion. Moreover, nano-sized globule offers a favorable environment for the uptake of highly lipophilic bioactive constituents. Nanoemulsion regulates the release of active components based on partition coefficient in gastrointestinal fluids. Furthermore, nanoemulsions have been widely investigated for food preservation and protection. The application of nanoemulsion as a booster for delivering herbal bioactive as food preservation is presented in Table **1**.

Table 1. Encapsulated bioactive constituents within the nanoemulsion with potential application.

Bioactive constituents	Oil/excipients	Therapeutic use	Salient features	References
α-tocopherol	Pomegranate seed oil	Food preservation, pharmaceutical	Mean droplet size (36.65 –43.17) nm Zeta potential (–8.51 to – 8.80) mV	[10]
Cinnamon oil	Medium-chain triglyceride oil	Food preservation	Mean droplet 50.71 nm Polydispersity index 0.23	[11]
Anethole, naringenin, taxifolin from anise seed	Tween 80	Food preservation, pharmaceutical	Average droplet size (440 nm) Polydispersity index (0.23)	[12]
β-carotene, peppermint oil	Tripolyglycerol monostearate, casein	Pharmaceutical	Average droplet size (230 –250) nm Zeta potential (–12 to – 16) mV	[13]
β-carotene	Rice bran oil	Preservation and bioavailability enhancement	Mean droplet size (200) nm Polydispersity index (0.30) Zeta potential (–60 mV)	[14]
Thyme oil	Chitosan hydrochloride	Antibacterial and food preservative	Average droplet size (225 nm) Zeta potential (17.50 to 20.50) mV	[15]
Procyanidins	Lecithin	Anti-inflammatory, antioxidant, anti-cancer	Average droplet size (163 nm) Zeta potential (–51.80 mV)	[16]
Carotenoid reach extract from *Paprika oleoresin*	Gum arbic, whey protein, lecithin	Food, beverage, pharmaceuticals	Average droplet size (539–150) nm Polydispersity index (0.27)	[17]
d-Limonene from grapes fruit peel oil	Tween 80	Antimicrobial	Mean droplet size (204 nm) Surface tension 40.21	[18]
Litsea cubeba essential oil	Medium-chain triacylglycerol	Food preservation, antioxidant, and antimicrobial	Average droplet size (101.1 nm)	[19]

(Table 1) cont.....

Bioactive constituents	Oil/excipients	Therapeutic use	Salient features	References
Lapachol	Soybean oil, polysorbate	Anti-tumor	Mean droplet diameter ~175 nm Polydispersity ≤ 0.2 Zeta potential (–20 mV)	[20]
Ginsenoside	Caproyl, tween 80, transcutol	Antiobesity	Mean droplet diameter < 100 nm Zeta potential (–7.02 to –9.92) mV	[21]
Artemisia cina extract	Tween 80	Antiproliferative and anti-cancer	Average droplet size (15 – 16) nm Zeta potential 1 mV	[22]
Cannabinoids	Tween 80 and span 80	Management of chronic pain	Average droplet size (<100 nm)	[23]
Protium heptaphyllum resin essential oil	Sorbitan monooleate and polysorbate 80	Larvicidal	Mean droplet diameter (109 – 115) nm Zeta potential (–21.7 to –34.66) mV	[24]
Curcumin	Acconon Mc8-2 and transcutol HP	Psoriasis	Mean droplet diameter (10.5 – 68.8) nm Zeta potential (–3.9 to –18.17) mV	[25]
Boswellia serrta extract	Glyceryl monooleate, *helianthus annus* seed oil, and propylene glycerol	Dressings for skin disease	Mean droplet diameter (115 – 150) nm Polydispersity index (<0.16) Zeta potential (–80 to –72) mV	[26]
Okra fruit extract	Glyceryl caprylate, propylene glycol, glycerin	Antidiabetic	Mean droplet size 134.7 nm Polydispersity index (<0.512) Zeta potential (–26.72 mV)	[27]
Naringenin (flavonoid)	Capryol 90, transcutol, polyethylene glycol, chitosan	Topical wound healer	Mean droplet size (15.69–156) nm Polydispersity index (0.22 – 0.41) Zeta potential (–8.3 to +44.4) mV	[28]

(Table 1) cont.....

Bioactive constituents	Oil/excipients	Therapeutic use	Salient features	References
Pequi oil from *Caryocar brasilense* Cambess	Lecithin	Anti-tumor	Mean droplet size (120 – 306) nm Zeta potential (–15.03– 68.30) mV	[29]
Opuntia oligacantha C	Lecithin and orange oil	Food preservation	Mean droplet size (118.80 nm ± 5.5) Zeta potential (–69.9 mV)	[30]
Luteolin	Transcutol-HP, polyethylene glycol 400	Enhance bioavailability	Mean droplet size (25.58 nm) Zeta potential (–10.2 mV)	[31]
Ocimum sanctum Linn.	Tea seed oil, avocado oil, almond oil, tween 20, brij S10®, triton X-114, propylene glycol,	Enhanced anti-aging dermal delivery	Mean droplet size (~200 nm) Zeta potential (~ –25 mV)	[32]

Formulation Facet of Vesicular Drug Delivery Systems

Nanoscale vesicles have provided a versatile platform for the transportation of various bioactive therapeutic agents. Vesicular systems are highly structured assemblies of several concentric lipid bilayers formed as a result of amphiphilic building blocks in the occurrence of the aqueous phase. Recently herbal bioactive entrapped vesicular drug delivery systems have gained importance for targeted drug delivery systems due to localized efficacy with lowered dosage and high bioavailability. Vesicular bioactive carriers comprising liposome, phytosome/ herbosome, transfersome, and peptide-based vesicles have exhibited potential characteristics for the development of nanomedicine with enhanced therapeutic efficacy and stability [33].

Liposomes are spherical vesicles made up of lipid bilayers, where an aqueous compartment is entirely enclosed by a bilayer membrane, composed of either synthetic or natural lipids. The essential components of the liposomal drug delivery system include phospholipids (phosphatidylcholine) and cholesterol, with the cholesterol acting as a flexibility buffer. The effectiveness of the herbal bioactive products depends upon their effectual delivery to achieve the required therapeutic concentration. The phytosome technology was first developed by Indena that distinctly enhances the bioavailability of bioactive phytoconstituent. The term “Phyto” means plant while “some” means cell-like; it was a breakthrough technology for striking the enhancement of bioavailability and assuring delivery

to the tissues without compromising nutrients safety. Previous studies demonstrated that water-soluble phytomedicine such as flavonoids and other phenolic compounds can be successfully converted into lipid-friendly complex by encapsulating bio-active compounds within phospholipids. Phytosomes are made by reacting two moles of natural or synthetic phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine, with one mole of bioactive, either alone or in a mixture of aprotic solvents such as dioxane or acetone.Skin permeability is tolerated by vesicular drug delivery systems such as liposome and niosome; however, a different type of vesicular system, the transferosome, can overcome permeability issues. Transferosome is derived from the Latin words "Transfree" meaning to carry across, and "some" meaning body, which was developed by Gregor Cevc in 1991. Thus, transferosomes are ultra deformable, stress-responsive, complex vesicles surrounded by a bilayer of lipid. Transferosomes are composed of one natural amphiphilic lipid, such as phosphatidylcholine and dipalmitoylphosphatidylcholine, supplemented by a bilayer surfactant such as sodium cholate, span 80, and tween 80.

Characterization of Bioactive Encapsulated Vesicular Drug Delivery System

The vesicular drug delivery systems are characterized to ensure their predictable biological performance. The characterization of the vesicular system is broadly classified into three categories, namely physical, chemical, and biological. The physical parameter includes size, polydispersity index, shape, surface tension, phase behavior, entrapped volume, and drug release profile. Chemical and biological characterization includes phospholipid hydrolysis, phospholipid oxidation, encapsulation efficiency, purity, the potency of lipophilic constituents, and therapeutic application [34].

Vesicular Drug Delivery System as a Booster for the Delivery of Herbal Bioactive

Several phytoconstituent molecules fabricated as vesicular drug delivery have been investigated to achieve enhanced solubility, bioavailability, and skin permeability with therapeutic efficacy on the target site of action. The summarized applications of phytoconstituent incorporated in vesicular are presented in Table **2**.

Table 2. Encapsulated bioactive constituents in the vesicular drug delivery system with potential applications.

Bioactive constituents	Lipid/excipients	Therapeutic use	Salient features	References
Ocimum sanctum Linn.	Lecithin, span 20, cholesterol	Enhanced anti-aging	Average vesicle size (460) nm Zeta potential (–46.4) mV	[32]
Citrullus colocynthis (L.) Momordica balsamina and Momordica dioica	Lipoid® S45, Lipoid Co	Antidiabetic	Average vesicle size 450 nm Zeta potential –22.7 mV Entrapment efficiency > 90%	[35]
Curcumin	Lecithin, Tween 80, Span 80	Enhanced skin permeability	Average vesicle size 339.3 nm Zeta potential –26.0 mV Entrapment efficiency (68.2–89.6) %	[34]
Pollen phenolic extract	Lecithin	Enhanced solubility and bio-accessibility	Average vesicle size (86.89–402) nm Zeta potential (–31.2 to 52) mV Entrapment efficiency (81 - 85)%	[36]
Balaocarpol	Cholesterol, Span 80	Breast and ovarian cancer	Average vesicle size (160.6–75.6) nm Entrapment efficiency 39.32%	[37]
Imperialine from *Fritillaria crirrhosa* D.	Lipod S100, cholesterol	Anti-tumor	Average vesicle size 110 nm Zeta potential (–20) mV	[38]
Resveratol	Dioctadecyldimethylammonium bromide, monoolein	Improved bioavailability	Average vesicle size (100–200) µm Zeta potential (+40) mV	[39]

(Table 2) cont.....

Bioactive constituents	Lipid/excipients	Therapeutic use	Salient features	References
Resveratol	Magnetite, lipid	Treatment of cerebral disease	Average vesicle size (124–163) µm Entrapment efficiency (86.94) %	[40]
Camellia sinensis L. Kuntze	Lipoid P30, Span 80	Enhanced skin permeability	Average vesicle size (80.6–95.67) nm Zeta potential (–41.1 to –48.2) mV	[41]
Acronychiabaueri secondary metabolite	Hydrogenated soy phosphatidylcholine	Anti-cancer	Average vesicle size (93.64–112.34) nm Zeta potential (0 to –25) mV	[42]
Berberine	Dipalmitoylphosphatidylcholine, distearoylphosphoethanolamine	Improved bioavailability	Average vesicle size (0.11) µm Polydispersity index (0.043)	[43]
Zataria multiflora Boiss. essential oil	Lecithin	Food preservation	Average vesicle size (272) nm Entrapment efficiency (54.5)%	[44]
Hesperetin	Hydrogenated phosphatidylcholine	Anti-cancer	Average vesicle size (65) nm Zeta potential (–10) mV	[45]
Curcumin	Dipalmitoylphosphatidylcholine, dis-tearoyl phosphoethanolamine	Enhanced aqueous solubility	Average vesicle size (0.18) µm Polydispersity index (0.105)	[46]
Eruca sativa seed extract	Distearoylphosphatidylcholine, dis-tearoyl phosphoethanolamine -polyethylene glycol	Anti-cancer	–	[47]
Chitosan coated curcumin	Phosphatidylcholine	Nutraceutical	Average vesicle size (93.2 – 332.7) nm Zeta potential (–24.37 and +67.09) mV	[48]

(Table 2) cont.....

Bioactive constituents	Lipid/excipients	Therapeutic use	Salient features	References
Epiisopiloturine from *Pilocarpus microphyllus*	Dipalmitoylphosphatidylcholine, cholesterol	Schistosomicidal efficacy	Average vesicle size (188.2 – 222.1) nm Polydispersity index (0.088 – 0.122)	[49]
Quercetin	Phosphatidylcholine	Hepatoprotective	–	[50]
Rose essential oil	Cholesterol	Natural fragrance	Average vesicle size (~132) nm Entrapment efficiency (~88) %	[51]
Chitosan coated tamarind fruit pulp extract	Phosphatidylcholine, Cholesterol	Improved stability and cell proliferation	Average vesicle size (~158) nm Zeta potential (–6.0) mV Entrapment efficiency (~68) %	[52]
Hesperidin	Lecithin, phosphatidylcholine	Enhanced oral bioavailability with antioxidant potential	Entrapment efficiency (80.2 – 92.54) %	[53]
Punicalagins from pomegranate extract	Soya lecithin	Hepatoprotective with antioxidant potential	–	[54]
Mangiferin	Soya phosphatidylcholine	Hepatoprotective with antioxidant potential	Average vesicle size (0.2 –2) µm	[55]
Resveratol	Soya phosphatidylcholine	Improved bioavailability	Average vesicle size (1–5) µm	[56]
Purple glutinous rice extract	Tween 61, cholesterol	Anti-aging	Average vesicle size (135.9) nm Zeta potential (–28.8) mV	[57]
Resveratol	Span 60, cholesterol	Enhanced bioavailability with milk products	Average vesicle size (168.73) nm Zeta potential (–29.6) mV Entrapment efficiency (90.11) %	[58]

(Table 2) cont.....

Bioactive constituents	Lipid/excipients	Therapeutic use	Salient features	References
Curcumin	Cholesterol, tween 20, span 20	Antinociceptive and anti-inflammatory	Average vesicle size (383.67) nm Zeta potential (–9.3) mV Entrapment efficiency (87.42 - 95.18) %	[59]
Berberine	Leciva-S70, cholesterol, span 60	Management of skin cancer	Average vesicle size (74.05) nm Entrapment efficiency (82.54) %	[60]
Turmeric oil	Span 20, 60, 80, cholesterol	Mosquito vector management	Average vesicle size (74.05) nm Zeta potential (–41.3 to 64.4) mV	[61]
Blackberry fruit extract	Span 80, cholesterol	Antioxidant potential	Average vesicle size (267.2) nm Zeta potential (–21.7) mV	[62]
Centella asiatica extract	Span 60, tween 60, cholesterol	Enhanced dermal absorption	Average vesicle size (155) nm Zeta potential (–15) mV	[63]
Rhodomyrtone	Phosphatidylcholine, cholesterol	Anti-acne	Average vesicle size (209.56) nm Zeta potential (–41.19) mV Entrapment efficiency (65.47) %	[64]

Market Potential of Bioactive Encapsulated Advanced Nanoscience

The Swiss-based Cosmetochem International AG Company pioneered the production of high-quality customized herbs and bioactives. Moreover, an Italy-based Indena is the second leading phytopharmaceuticals producer of herbal nanoscience. The list of market available products is presented in Table **3**. Furthermore, the list of patented technology of herbal bioactive as a booster dose for advanced nanoscience is presented in Table **4**.

Table 3. Marketed herbal bioactive as a booster dose for advanced nanoscience.

Brand Name	Bioactive Constituents	Types of Advanced Nanoscience	Company Name	References
Liposome with white tea Herbasec®	*Camellia sinensis*	Liposome	Cosmetochem	[65]
Liposome with green tea Herbasec®	*Camellia sinensis*	Liposome	Cosmetochem	[65]
White hibiscus liposome Herbasec®	*Hibiscus moscheutos*	Liposome	Cosmetochem	[65]
Liposome with aloe vera Herbasec®	*Aloe barbadensis miller*	Liposome	Cosmetochem	[65]
Liposome with guarana Herbasec®	*Paullinia cupana*	Liposome	Cosmetochem	[65]
Centella Phytosome®	Triterpenes from *Centella asiatica leaf*	Phytosome	Indena	[66]
Crataegus Phytosome®	Vitexin-2”-O-rhamnoside from *Crataegus monogyna*	Phytosome	Indena	[66]
Ginkgoselect Phytosome®	Flavoglucoside from *Ginkgo biloba*	Phytosome	Indena	[66]
Ginselect Phytosome®	Ginsenosides from *Panax ginseng*	Phytosome	Indena	[66]
Meriva®	Curcuminoids from turmeric rhizome	Phytosome	Indena	[66]
Sericoside Phytosome®	*Terminalia sericea*	Phytosome	Indena	[66]
Siliphos®	Silybin from *Silybum marianum*	Phytosome	Indena	[66]
Silymarin Phytosome®	Silymarin from *Silybum marianum*	Phytosome	Indena	[66]
Visnadex®	*Ammi visnaga*	Phytosome	Indena	[66]
Grape seed Phytosome®	Procyanidolic oligomers from grape seeds	Phytosome	VitaMedics	[67]

Table 4. Patented technology for herbal bioactive as a booster dose for advanced nanoscience.

Patented technology	Innovation	Patent No.	Reference
Improved bioavailability of olive fruit or leaves complexed with phospholipids	Improved bioavailability	EP/1844785	[68]
A composition comprising *Ginko biloba* derivatives for the management of asthma	Treatment of respiratory disorders	EP/1813280	[69]

(Table 4) cont.....

Patented technology	Innovation	Patent No.	Reference
Dermatological use of fatty acid monoesters and compositions	Cosmetics	EP/1690862	[70]
Cosmetic and dermatological composition for the treatment of aging	Dermatological composition for topical use	EP/1640041	[71]
Treatment of skin and wound repair with thymosin β-4	Skin treatment	US/2007/0015698	[72]
Soluble isoflavone compositions	Improved solubility	WO/2004/045541	[73]
Plant extracts based antioxidants for the treatment of adiposity problems	Treatment of phlebitis varicose veins, arteriosclerosis, hemorrhoids, and high blood pressure	EP/1214084	[74]
Saponins with phospholipids and pharmaceuticals and cosmetic compositions containing them	Improved bioavailability	EP/0283713	[75]
Nanoemulsion based on oxyethylenated or non-oxyethylenated sorbitan fatty esters, and its uses in the cosmetics	Dermal and ophthalmic use	US6335022B1	[76]
Flavor nanoemulsions and methods of preparing the same	Food preservation	WO2016064883A1	[77]
Encapsulated natural antioxidants for preserving fresh and minimally processed foods	Preservation of food product	WO2019039947A1	[78]
Resveratrol-phospholipid complex nanoemulsion	Improved stability and bioavailability for cosmetic use	US20100297199	[79]

Potential and Challenges

The utilization of herbs and herbal bioactive products has gained tremendous popularity over the past few decades, with not less than 80% of people worldwide relying on them for primary health care. Although therapies involving nanoscience-developed bioactive constituent delivery systems have shown promise, with the efficacy of several bioactive products being established, many of them remain untested and their use being insufficiently monitored. Medicinal bioactives as a potential source of therapeutic aid play a noteworthy role in the health care system throughout the world for not only diseased conditions but also as a potential material for proper health maintenance. There is a good possibility that the herbal industry will make significant progress. However, before such a gain, the quality aspects need to be addressed. Moreover, the major drawbacks to the development of bioactives as a booster for advanced nanoscience in herbal pharmaceuticals

lack information on high-throughput screening bioassay with social, industrial, and economic development. At the same time, there are several opportunities for countries rich in herbal resources and traditional knowledge, which are key components for bioprocessing and value-addition.

CONCLUSION

The expansion of advanced nanoscience with the use of bioactives is highlighted and utilized in the field of biomedical, cosmetics, and food science. Herbs and herbal bioactive products have gained the attention of researchers as well as pharmaceutical manufacturers due to their traditional use and efficacy. These herbal products are considered safe and efficacious but constrained by several limitations regarding the choice of carrier biomaterials for the fabrication and clinical trials with maximum efficacy. However, there is still a possibility for the development of bioactive, including flavonoids, terpenoids, tannins, xanthones as booster doses with the use of advanced nanosciences such as aerosol inhalers, nanoemulsion, and vesicle-based drug delivery systems. This pharmaceutical assures the delivery of phytoconstituent to targeted sites such as the liver, brain, heart, kidney, lungs, *etc*., with a low dose compared to conventional herbal extract or phyto-molecules. Thus, utilizing pharmaceutical advanced nanoscience technology as a booster for delivering the bioactive enhances solubility, stability, and therapeutic efficacy. Hence, advanced nanoscience with natural phytoconstituent can be a promising booster for the treatment of various infectious and life-threatening diseases.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 4

Pharmaceutical Nanoscience: Pulmonary Drug Delivery System

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Abstract: Pulmonary diseases impose an immense burden on global health. Asthma, cystic and idiopathic pulmonary fibrosis, pulmonary hypertension, lung cancer, and chronic obstructive pulmonary diseases (COPD) are among the diseases that require efficient pulmonary drug delivery. Targeted-drug delivery refers to the delivery of a drug or a therapeutic agent to a certain tissue or organ; this can be achieved through pulmonary drug delivery (PDD) with nanomaterials. Nanomaterials are made from polymers, ceramics, metals, and biological materials into different sizes and structures. Nanomaterials for PDD entail nanoparticles of therapeutic ingredients, including herbal-based drugs and nanocarriers of the therapeutic bioactive substance. These types of materials used in managing pulmonary diseases are discussed in this chapter.

Keywords: Nanocarrier, Nanomaterial, Nanoparticle, Pulmonary diseases, Pulmonary drug delivery.

INTRODUCTION

Pulmonary diseases impose an immense burden on global health [1]. It is reported that about 4 billion people are exposed to air pollution, which includes indoor toxic smoke, outdoor pollutant air, and tobacco smoke [2]. Exposure to these types of air pollution would lead to various diseases, which include asthma, cystic and idiopathic pulmonary fibrosis, pulmonary hypertension, lung cancer, and chronic obstructive pulmonary diseases (COPD). Every year, it is estimated that about 65 million people suffer from chronic obstructive pulmonary disease (COPD), out of which 3 million die from it each year, contributing as the third leading cause of

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Swarnlata Saraf, Ram Kumar Sahu & Vivek Dave (Eds.)

death globally [3]. Apart from the environmental factors, allergens and genetic factors are also some of the contributing factors that are linked to pulmonary diseases [4, 5]. Exposure to environmental or non- environmental factors usually lead to common clinical symptoms, such as airway inflammation, airflow obstruction, and excessive mucus secretion, which can be managed through medicaments, such as bronchodilators, steroid- and non-steroid- based anti-inflammatory drugs, and antibiotics [6, 7].

In this chapter, a thorough discussion on targeted drug delivery will be done. Targeted drug delivery refers to the delivery of a drug or a therapeutic agent to a certain tissue or organ. Using drug targeted delivery system, there are two strategies that can be adopted; active target delivery, which requires the conjugation of the therapeutic agent or carrier system to a specific tissue or cell- specific ligand [8], whereas passive target delivery involves the incorporation of the therapeutic agent into a carrier that can be a macromolecule or nanoparticle which passively reaches the targeted organ or tissue [9, 10]. In this chapter, we will discuss in detail pulmonary drug delivery (PDD) using nanoparticles.

Nano-sized materials are made from polymers, ceramics, metals, and biological materials [11] of different sizes, shapes, and structures. Nanoparticles (NPs) exhibit better physical, chemical, mechanical, thermal, and biological properties than their bulk materials [12]. These are particles that are less than 1000 nm in at least one dimension and range in size from 1–1000 nm [13 - 15]. Nanoparticles are developed with a bigger surface area compared to volume ratios; thus, they allow high loading capacity and maximise the drug delivery to the target [16]. In addition, due to their small nano-size, they may escape from mucociliary and macrophage clearance, which would eliminate particles larger than 1 μm. Nanoparticles in sizes of more than 20 nm are able to cross mucosal membranes *via* endocytosis, carrier, or receptor-mediated transports [17]. In general, nanoparticles are better internalized by cells [20], able to cross permeability barriers [16], increase cellular uptake, allow long lung retention [21] and mucus penetration in the airways compared to micro or larger particles.

MECHANISMS OF PULMONARY DRUG TRANSPORT

Particles' deposition from inhaled aerosol involves a series of spontaneous mechano-chemical processes, which is under a multi-factorial influence. Inhaled particle deposition involves impaction, sedimentation, and Brownian motion in bronchi, bronchioles, and alveolar regions, respectively. At the same time, the sedimentation of particles involves gravitational forces [18].

As most of the pulmonary administrations are in the form of aerosols, aerosols for PDD undergo two mechanisms; deposition and absorption.

Deposition of Particles

The delivery of the drug to the airways and the amount of drug reaching the target site would determine the clinical efficacy of the drug [19]. Several mechanisms are involved during the deposition process of aerosol particles, which include inertial impaction, sedimentation or gravitational forces, interception, and diffusion (Fig. **1**). Some particle parameters, such as size, shape, and type of particle, which are either prepared in solution, powder, or suspension [20], would usually influence the location, degree, and particle deposition efficiency in PDD [21]. All mechanisms described in this section will act simultaneously, and the relative contribution of each depends on multiple parameters.

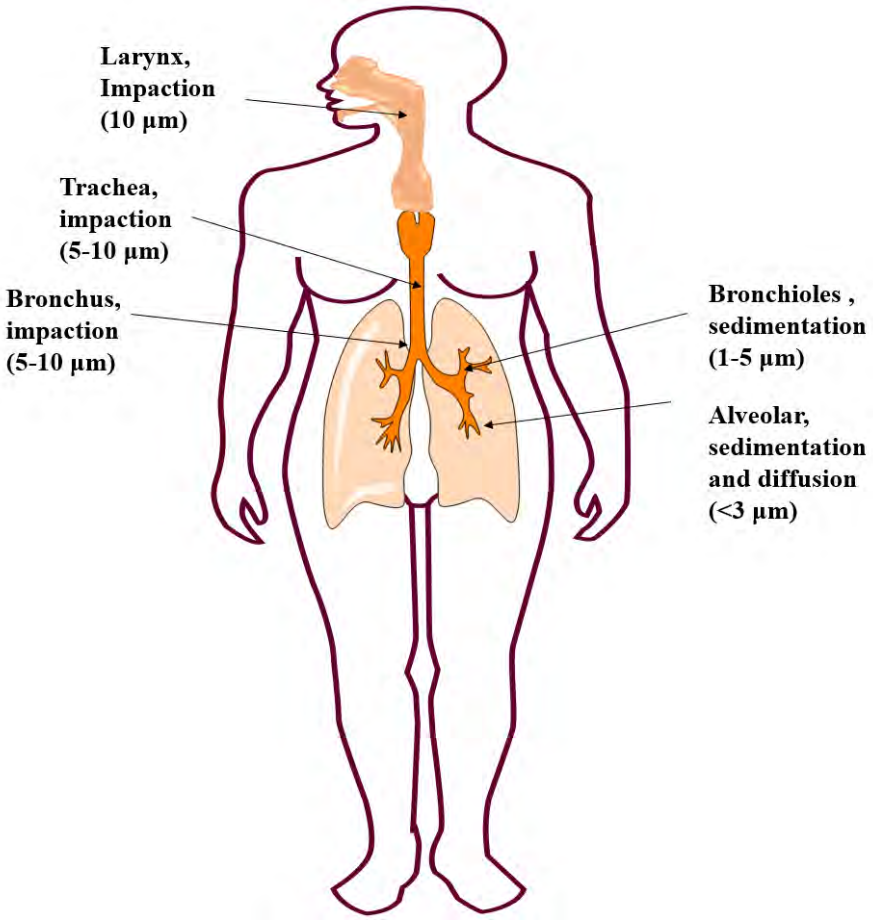

Fig. (1). Deposition of the aerosol particle in the airway entails mechanisms of inertial impaction, sedimentation/gravitational settling, diffusion, and sedimentation depending on the particle size.

a). Inertial Impaction

Inertial impaction is a process of deposition of particles that pass through the conducting airways with directional changes along the branching airways; suspended particles have an impact against airway surfaces, and they are deposited by inertial impaction [10, 21]. This happens for large particles above 3 μm [10, 21] where particles collide with the respiratory wall due to the centrifugal force; therefore, they are deposited [18]. In addition, the deposition by inertial impaction can be improved by increasing the air velocity, breathing frequency, as well as particle size [22]. The mechanism of inertial impaction is the main deposition mechanism that occurs in the tracheobronchial bifurcation [10, 22, 23].

b). Sedimentation

Particles below 3 μm are deposited into the terminal and respiratory bronchioles, where airflow is at a low rate, depending on particles' terminal settling velocity particles, which are deposited through sedimentation by gravitational force [10, 21, 23]. Sedimentation or gravitational settling takes place when the gravitational force, which acts on particles, incapacitates the total force of air resistance [22]. This mechanism usually occurs in the bronchi and the bronchioles. In the alveoli, this mechanism has significant importance, and it is usually pronounced for particles with a range diameter of 0.5-2 μm. Since most therapeutic aerosols are hygroscopic, the relatively increasing airway humidity towards the alveolar lumen allows hypertonic particles to change their size through hygroscopic growth during transport along the airways [10, 21]. Therefore, when particles agglomerate due tocoulombic attraction with opposite electrostatic charge or undergo hygroscopic growth, this particle size is also demonstrated through gravitational settling [23]. Nanoparticle deposition *via* sedimentation is the most enticing method because the particles will be aggregated into micrometer size to produce an adequate mass to sediment in the bronchiolar region [18].

c). Brownian Diffusion

Brownian diffusion is a mechanism of particle deposition in size range of 0.5-1 μm [10, 20, 23]. As particles travel in the terminal and respiratory bronchioles [23], there will be a collision between the particles and surrounding molecules which will exert non-uniform pressures on the particles' surfaces to produce a random Brownian motion [10, 18, 22]. The particles move from a high concentration region to a lower concentration region through Brownian motion [10], and the diffusion process is influenced by the concentration gradient [18]. In contrast to impaction

and sedimentation, Brownian motion increases as particle size decreases, especially for particles with a diameter of less than 0.5 m.

d). Interception

In small airways, the interception depends on the ratio of the particle size to airway diameter, which suggests interception as the most significant for elongated particles such as fibres, which are long in one dimension yet have small enough diameters to enter small airways [23].

e). Electrostatic Effects

Electrostatic attraction is important for electrically charged particles. The deposition of electrically charged particles is affected by electrostatic forces through image charge force, space charge force, and coulombic attraction forces. The cytotoxicity of nanoparticles is primarily determined by their entrance pathway and intracellular site [24], and positively charged particles are usually associated with potential cytotoxicity [25]. For instance, stearylamine-PEG-PLA nanoparticles with positively charged surfaces were reported to enhance pulmonary side effects, which caused systemic toxicity [26]. Even though the positively charged surfaces were more likely to adhere to the negatively charged cell mucosa [27], positively charged silica nanoparticles uptake by the alveolar epithelial cells was inhibited by pulmonary surfactant [28].

Absorption of Drugs in PDD

The human lungs contain over 300 million alveoli, whereby each is lined with pulmonary capillaries, creating a large surface area [18], which account for more than 95% of the lungs' surface area [29], and they are directly linked to the systemic circulation through the pulmonary circulation [21]. For a drug to be absorbed in the pulmonary system, it has to cross the air-blood barrier and enter into the pulmonary blood circulation. This barrier consists of thin alveolar cells (0.6-1nm) and thicker endothelial cells (4-5.8 nm) sides [30]. For absorption to occur, the drug also needs to cross the lung surfactant, surface lining fluid, epithelium, interstitium, basement membrane, and the endothelium [31], as it is believed to be more permeable compared to lung epithelium [32]. Some molecules on the lining, such as proteins, lipids, and tight junctions, will act as primary barriers for the transportation of drug molecules [18].

The lung surfactant may also affect drug absorption by either acting as a hydrophobic barrier that hinders the diffusion of hydrophilic molecules [33] or changing the particles' surface properties when they are adsorbed by inhaled particles [34]. All of these factors would determine the efficiency of drug deposition

and absorption or the elimination of the drug from the system.

Different mechanisms for the transportation of drugs in the lung have been proposed, which include transportation through pores in the membrane, transportation through intracellular tight junctions, vesicular transportation, active transportation (transporter-mediated), and drainage into the lymphatics [35, 36]. Nanoparticles are usually internalized *via* uptake pathway by the cells [24]; for instance, the lipid-based nanoparticles would utilize the clathrin-mediated endocytosis pathway for the cellular uptake [37].

a). Transcellular Transport

Transcellular transport mechanisms involve passive diffusion, transport mediated by caveolae (non-coated vesicles) and receptor-mediated transport molecules. Passive diffusion is the transport of drugs through membrane lipid bilayers *via* concentration gradient [33], especially for hydrophobic drugs [38]. Absorption by transporter proteins *via* active or passive diffusion depends on the nature and chemical structure of the therapeutic compound [18].

The lung consists of more than 40 cells [39], among which are airway ciliated epithelial cells, type I (flattened), and type II (cuboidal alveolar) pneumocytes [25] that are keys to pulmonary drug transport and absorption [21]. The type I pneumocytes are the most abundant, which consist of about 95% of the alveolar surface [40], with carriers endocytotic vesicles, while type II produces surfactant proteins and can be differentiated with type I cells on the basis of the damage caused to epithelial cells [21]. Human alveolar type I epithelial cells internalize nano-size particles [25].

Many lung efflux transporters have been reported with their respective substrates [33]. The trans-membrane ABC family of transporter proteins are considered to be responsible for the efflux of many substrates in an energy-dependent manner [41]; therefore, it is important to determine the presence or absence of drug efflux transport interaction to adjust its dose accordingly [33].

b). Paracellular Transport

The tight junctions are transmembrane and cytoskeleton proteins [42]. These tight barriers decrease from the trachea to distal airways as measured by apical-to-basal electrical resistance across the lung epithelium. The mechanism of drug uptake by the paracellular absorption through the minimum tight junction in distal airways had been described previously [43]. Nanoparticles are able to cross the epithelial monolayer but not the tight junctions between cells [25] in the alveolar region.

c). Transcytosis

Drugs are transported through the cytoplasm of the endothelial and epithelial cell in the membrane vesicles of caveolae (50–100 nm invaginations of the plasmalemma) and clathrin (about 200 nm in diameter) [33, 44]. The clathrin-mediated pathway involves the binding of certain extracellular ligands to cell membrane receptors to form a ligand-receptor complex, in which the complexes are engulfed into the cell inside clathrin-coated vesicles. The vesicles will fuse with early endosomes to reach the lysosomes for the hydrolysis of the content [45, 46]. The caveolae-mediated endocytosis involves detached flask-shaped membrane invaginations (caveolae) that fuse to a cell compartment called caveosomes. Caveosomes will evade lysosomes and protect the contents from hydrolytic enzyme and lysosomal degradation, thus making it an important pathway employed in nanomedicine [47, 48].

Particle size is a key determining factor of transport vesicles clathrin- or caveolae-mediated [33], as suggested particles sizes of 150–200 nm undergo clathrin mediated uptake, while smaller sizes (<120 nm) undergo caveolae-mediated transport. Clathrin-mediated mechanism is the main mechanism of nanoparticles uptake [49]. For example, 50 nm nanoparticles are able to cross the alveolar epithelium *via* non-endocytic pathways, while 100 nm nanoparticles can cross *via* clathrin- and caveolin-mediated endocytosis [25]. It is important to prevent nanoparticles elimination by reticuloendothelial system and prolong its circulation in the blood, thereby increasing drug bioavailability at the target, which is also influenced by the NPs size [24].

d). Nanoparticles Trafficking

Following the uptake, nanoparticles would either encounter the membrane-bound intracellular vesicles endosomes that transport content to lysosomes [24] or be surrounded by autophagosomes and delivered to the lysosomes [50]. However, some nanoparticles can evade these processes and be released into the cytoplasm [51, 52], while others can enter the nucleus or interact with other cellular organelles [25].

Therefore, nanoparticles should be studied individually on a particle-by-particle basis, as small changes in size, charge, particle composition [25], shape, and surface chemistry [24] can alter the cellular uptake and the endpoints of PDD.

METHODS OF PULMONARY DRUG DELIVERY

Particles that are developed for PDD are delivered in the form of aerosols

(aerosolization). Aerosols are solid particles or liquid droplets that have been prepared in gas suspension. The particulate portion of an aerosol is referred to as particulate matter, which can be ultrafine (less than 0.1 micron), fine particles (0.1-2 microns), or coarse particles (larger than 2 microns) [10]. Aerosols are commonly administered by oral inhalation or intranasal inhalation. Presently, there are three types of devices used for pulmonary drug delivery; nebulisers, metered-dose inhalers (MDIs), and dry powder inhalers (DPIs). These inhalers are based on diverse delivery mechanisms and they entail different types of drug formulations.

a). Nebuliser

Nebulisers work by converting solutions or drugs into fine mists for inhalation. Nebulisers produce liquid aerosols by using an external power supply, and they do not use any propellant [53]. High-pressure air is passed through nozzles for air accelerated liquid droplets [54], and high doses of drugs can be aerosolized, which is not possible to achieve through MDIs and DPIs. Nebulisers do not require synchronization between inhalation and actuation by the user, so they are suitable for elderly, paediatric, ventilated, non-conscious patients, or those who are unable to use MDIs or DPIs. This device has been used for many years by asthmatic and COPD patients as an alternative to inhalers.

There are three types of nebulisers; jet nebuliser, ultrasonic nebuliser, and mesh nebuliser. The jet nebulizer functions according to the Bernoulli principle with compressed gas (air or oxygen) passing through a narrow orifice and creating a low-pressure area at the outlet of the adjacent liquid feed tube. This results in the drug solution being pulled up from the solvent reservoir and shattered into droplets in the gas stream. Meanwhile, the ultrasonic nebulizer uses a piezoelectric crystal that vibrates at a high frequency (usually 1–3 MHz) to produce a fountain of liquid in the nebulizer chamber; the higher the frequency, the smaller the droplets produced. Mesh nebulizer, on the other hand, uses micropump for aerosol production. Mesh nebulizer contains apertures or aperture plates which produce aerosol when forces are applied [55]. Mesh nebulizers have higher efficiency than jet nebulizers, and they are able to deliver higher drug doses to patients. The advantages of mesh nebulizers include short treatment duration and high output but low residual volume [56]. Constant output jet nebulizers can aerosolize most drug solutions in large doses. However, treatments using nebulizers can be time-consuming and inefficient as some of the aerosolised drugs are unable to reach the lungs.

b). Metered Dose Inhaler

The pressurized metered-dose inhaler (MDI) is the most widely used inhaler

because it is a simple, economical, multi-dose, portable, and easy method of delivery system to be applied for pulmonary drugs [54, 57]. The MDI is commonly used for asthma medications as well as other respiratory diseases, such as bronchitis, emphysema and chronic lung disease. Through this method, the drug is dissolved or suspended in the propellant under pressure, and it is protected from oxidation, light, and moisture [54]. When the device is activated, a valve system releases a metered volume of drug and propellant. To administer the drug, the patient needs to squeeze the canister of the inhaler and directly inhale the aerosols. With MDI, it takes only 5–15 minutes to have an effect for short-acting bronchodilators (quick-relief medicine), compared to oral asthma medicines, which can take up to 1–3 hours.

However, there are some limitations to the device. The MDIs require a specific breathing technique that involves coordination between inspiration and actuation, slow and steady inspiration, and a breath-hold. Therefore, elderly patients with poor dexterity or weak grip strength might face some difficulties in actuating an MDI device [58]. Furthermore, poor coordination between actuation and inhalation would reduce the drug deposition in the lung [59]. To overcome this problem, spacer attachments and breath-actuated pressurised MDIs are developed, which help trigger the patient's inspiratory force.

c). Dry Powder Inhaler

A dry powder inhaler (DPI) is a breath-activated device that aerosolises solid drug particles on an airstream. The device was developed to overcome the coordination difficulties with the MDI. DPI drugs are commonly prepared by spray drying or spray freeze drying (especially in the case of labile compounds). Most DPIs contain a micronised drug conjugated with carrier particles, such as lactose that prevent aggregation and provide sufficient flowability [60, 61]. DPIs are more durable than aqueous drug solutions, and they do not require cold chain handling or powder reconstitution into nebulization solutions. By using the device, the required powder dose is delivered into the patient's inhaled air [54]; therefore, the patient's inhalation effort determines the level of particle deposition, as insufficient effort deposits powder particles in the upper respiratory airways [18].

d). Soft-mist Inhalers (SMI)

A soft mist inhaler (SMI) is a new generation of propellant-free inhaler that generates a fine aerosol mist. A soft mist inhaler uses spring mechanical power to generate a slow and long-lasting aerosol. SMI is easier than pressurised MDI as it facilitates patient coordination of actuation and inhalation [62]. By using SMI, the mist that comes out from the device is much slower than the mist from an MDI, which would allow for more medication to reach the air passages and deposit in

the lung; thus less medication would be left in the mouth [63].

NANOCARRIERS FOR PULMONARY DRUG DELIVERY

Nanoparticles act as drug carriers that dissolve, entrap, encapsulate, adsorb, or attach to the drug for tissue delivery [64]. Carrier nanoparticles help address the challenges faced by the drug inhomogeneity in aerosols [65], modifying the drug dissolution rate [66] and drug clearance by mucociliary movement and macrophage phagocytosis [67], drug concentration at the disease site, minimizing the drug degradation and creating ease in inhalable formulations [18, 68], compared to the direct drug nanoparticle delivery.

Nanocarriers protect encapsulated drugs from premature degradation and increase drug bioavailability [11]. The pulmonary drug delivery carriers are made up of a lipid base (liposomes, microemulsions, niosomes, lipidic micelles, solid lipid nanoparticles) or polymer-base (dendrimers, polymer micelles, polymeric nanoparticles, nanogels, nanocapsules) [13] or a non-polymer basematerials. Encapsulated drugs are kept in the lungs for a longer duration than in solution form at the same concentration, thereby prolonging their therapeutic effect [13]. Examples of such carriers for PDD are discussed below.

Polymers and Polysaccharide-based Nanocarriers

Therapeutic polymeric nanoparticles are classified into biodegradable or biocompatible materials [11]. Nanomaterial polymers for PDD can be of natural sources such as chitosan, cellulose, alginate, gelatin or synthetic polymers such as polyethylene glycol (PEG), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL) and polyglycolide (PGA) (Fig. **2**). Polysaccharides and proteins are natural biopolymers from plants, animals, microorganisms, and marine sources [69]. The biopolymeric nanoparticles are decomposable, metabolisable, and can be functionalized for specific drugs and targeting ligands [70].

In a drug delivery system, the polymeric nanoparticles would serve as nano- carriers of drug molecules. They provide high drug encapsulation [57], encapsulated drug protection [18], sustained drug release [18, 57], long shelf life [18], and possible surface modification [18, 57]. Some of the polymers and polysaccharide-based nanomaterials for PDD include:

a). Cellulose

Cellulose derivatives, methylcellulose, hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose and cationic hydroxyethyl cellulose, were used as acyclovir carriers for infusion into the nasal mucosa. A study observed an increased acyclovir delivery with cationic hydroxyethyl cellulose. Through the ciliary beat frequency (CBF) assessment, all cellulose derivatives were found safe on tissues and cells of the nasal mucosa [71].

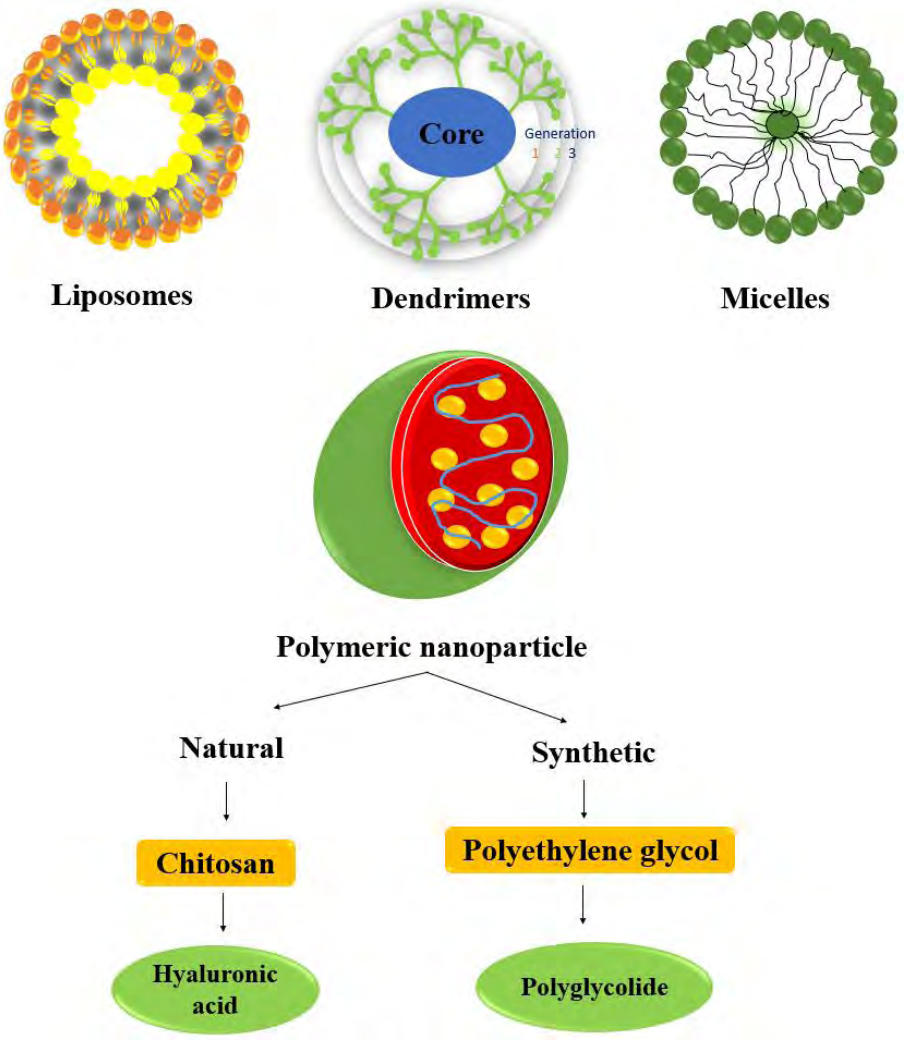

Fig. (2). Different types of nanocarriers commonly used for drug delivery.

b). Chitosan

Chitosan is a cationic polysaccharide that is mucoadhesive, biocompatible, biodegradable, and possesses antimicrobial activity. The chitosan used for medical applications is usually derived from crustacean shell chitin through the deacetylation process [4].

Several studies have reported the use of chitosan as a nanocarrier. Intranasal chitosan IFN-γ-pDNA nanoparticles were reported to be efficient in delivering gene therapy for asthma, as they had efficiently ameliorated inflammation and airway hyperresponsiveness [72]. Glycerosomes were phospholipid or glycerol vesicles that had been combined with trimethyl chitosan or hyaluronic acid [73], and when loaded with rifampicin, they were found to be more stable than conventional liposomes [74]; they had expressed more rifampicin accumulation in the lungs and systemic distribution [75]. Moreover, oleic acid-based nano- emulsions, which had been loaded with rifampicin, were functionalized with chitosan and chitosan-folate to provide increased cell internalization [76].

Spray-dried inhalable powders containing isoniazid-loaded chitosan for tuberculosis had been reported previously [77]. After inhalation, the studies demonstrated greater carrier drug delivery in the lungs compared to the drugs delivered without a carrier. An increased antifungal efficacy was reported with chitosan-stearic acid conjugated nanomicellar carriers of amphotericin B delivered by a jet nebulizer [78]. The budesonide microparticles prepared with porous chitosan (in drug to chitosan ratio of 1:2) were found comparable to conventional budesonide DPI formulations in efficacy [79].

c). Hyaluronic Acid

HA is a natural mucopolysaccharide that consists of repeated disaccharide units, d-glucuronic acid, and N-acetyl-d-glucosamine, which are linked by glycosidic bonds [80]. The polyanionic non-sulfated HA has biocompatibility and mucoadhesive properties. It has a low molecular weight (202 kD) derivative , which allows better penetration properties than chitosan hydrochloride [54]. Rifampicin-loaded glycerosomes of hyaluronic formulation were more likely to demonstrate rifampicin accumulation in the lung, with lower systemic distribution compared to chitosan formulation [76].

d). Cyclodextrin

Cyclodextrins (CD) are cyclic polymers of alpha-D-glucopyranose (cyclic oligosaccharides) [54]. CDs may be used to overcome mucosal and enzymatic barriers to enhance drug permeation. CDs were reported to reduce and minimise the enzymatic activity of nasal mucosa [81]. The methylated b-CDs carriers for nasal drug delivery had a similar effect of the physiological saline and benzalkonium chloride (a preservative for nasal drug formulations) [82]. CDs had improved the permeation of loaded lipophilic, polypeptides, and protein drugs through nasal mucosa [83]; its complexes with formoterol had developed a nebulized solution with improved solubility [84]. Following drug inhalation, it was shown that voriconazole solubilization with sulfobutyl ether-beta-cyclodextrin had offered

rapid and high drug concentrations in plasma [85].

e). Alginates

Alginate, 1,4 linked-D-mannuronic acid-L-guluronic acid, is a natural mucoadhesive, non-immunogenic, and biodegradable polymer [54]. Inhalable anti-tubercular (Rifampin, Isoniazid, Pyrazinamide) drugs loaded in alginate nanoparticles as carriers were reported to exhibit more efficacy compared to the carrier-free drugs [86]. Alginate nanoparticles, which had been loaded with anti-tuberculosis drugs and administered in 3 doses of nebulization in 15 days, had demonstrated better efficacy compared to oral doses of the free drugs for 45 days [87].

f). Synthetic Polymers

Several synthetic polymers have been investigated for PDD, which include PEG, PLA, PLGA, PCL, and PGA. PEGylation of particles improves the encapsulation efficacy and drug uptake sustained release [88] and evades macrophages phagocytosis [34]. Meanwhile, the process involved in PLGA preparations has been proven to preserve sustained release, biocompatibility, biodegradability, and non-toxicity of the drug [54].

A study by Sung, Padilla [89], which encapsulated rifampicin into PLGA nanoparticles by a solvent evaporation process, spray-dried into porous nanoparticle-aggregate and administered through DPI, had shown efficient drug deposition in the lungs, achieved systemic levels of rifampicin, which had been detected for up to six to eight hours, in contrast to rifampicin aerosols alone. In addition, PLGA-rifampicin inhaled nanoparticles were identified by alveolar macrophages and displayed increased uptake at alveolar regions [90]. Successful rifampicin-loaded poly (butyl cyanoacrylate) [91] and PCL [92] nanoparticles had also been demonstrated.

There are studies done on the anti-TB drugs encapsulated in PLGA. PLGA nanoparticles encapsulated with rifampicin [93] isoniazid and pyrazinamide [94] for PDD had improved the bioavailability of the drug. PLG nanoparticles prepared in combination with rifampicin [89], isoniazid and pyrazinamide [94] were also prepared in nebuliser form. Similarly, Johnson *et al*. had reported 5 doses of rifampicin, isoniazid, and pyrazinamide-loaded PLG nanoparticlesthat demonstrated equivalent effectiveness to 46 dosages of the free drugs in guinea pigs infected with TB [95].

An anti-inflammatory compound that consisted of hydroxybenzyl alcohol (HBA) in combination with polyoxalate (HPOX) was prepared using PLGA-based polymeric nanoparticles and later intratracheally administered in ovalbumin-induced mice, which showed a decrease in the level of the pro-inflammatory cytokine [96]. Salbutamol encapsulated with PLGA and Poly (vinysulfonate–co vinyl alcohol) had also demonstrated a significant increase in mean particle size and polydispersibility [97]. Atropine-loaded PLGA nanoparticles aerosolised in ovalbumin-induced Wistar rats model of chronic asthma attenuated airway surfaces, inflammation, hyperresponsiveness, and obstruction [98].

Lipid-based Nanocarriers

a. Liposomes

Liposomes are hydrophobic lipid bilayer vesicles with an aqueous core for drug carriage, which allows hydrophobic molecules absorption; therefore, they are referred to as amphiphilic carriers [99]. Liposomes offer several advantages as they can be prepared in different size ranges; they may effectively encapsulate a broad range of drugs [53], allow surface modification with polymer to improve its circulating properties [100], and they can be made from lungs endogenous compounds (surfactants, phospholipids, cholesterol), thus making them suitable for pulmonary drug delivery [18, 101]. Nano-liposomes with surface phospholipids can escape opsonin attack, have improved fine particle fraction [102], and are able to sustain release properties [18]. Due to their similar compositions with a pulmonary surfactant, liposomes exhibit adsorption, endocytosis, exchange of lipids, or fusion interactions with macrophages [13].

Liposomes have been studied as PDD carriers of different therapeutic drugs and compounds in different disease models, including dapsone [103], ciprofloxacin [104], n-acetylcysteine, vitamin E, glutathione [105], budesonide [106], rifampicin [107], amikacin [108], amphotericin- B [109], glucocorticoid [110], with reported improvement of drug delivery.

b. Micelles

Micelles absorb and transport drugs in quantities far above their inherent water solubility as they possess additional advantages, which include modifiable size and shape with a surface chemistry that could prevent reticuloendothelial system recognition [13]. Beclomethasone dipropionate-loaded polymeric micelles nanoparticles inhalation had shown mucus penetration ability [111]; therefore, it is suggested to overcome the mucosal barrier challenge by reducing the bioavailability of the corticosteroids in the treatment of asthma and COPDs [13]. Similarly, beclomethasone dipropionate loaded micelles using poly-(ethylene

oxide)-block-distearoyl phosphatidylethanolamine (mPEG-DSPE) polymer had demonstrated entrapment efficiency and sustained release properties [112].

c. Solid-lipid Nano

Solid-lipid nano (SLN) are lipid-based nanocarriers with phospholipid [54] or with physiological triglycerides coating like surfactants [113] for emulsification [57]. SLN are usually solid at room temperature and made up of fatty acids, steroids, triglycerides, partial glycerides, and waxes [57]. They have various advantages, including easy cellular uptake, biocompatiblility [113], control and sustained drug release, and fast degradation in the lungs [57]. Beclomethasone [114] and curcumin (from plant *Curcum longa* Spp) [115] loaded SLN had resulted in sustained drug release in the treatment of asthma.

Moreover, liquid-lipid modification of SLN to nanostructured lipid carriers (NLC) could improve their stability, storage, encapsulation percentage of drugs, and release profiles [116]. NLC particles loaded with levofloxacin against *Pseudomonas aeruginosa* infection, particularly in cystic fibrosis (CF) disease, had shown a better-controlled release profile than SLN formulation [117]. For PDD and the treatment of lung diseases, amikacin [118], rifampicin-isoniazid-pyrazinamideb [119], rifabutin [120], rifampicin [121], and quorum sensing inhibitor [122] are among the drugs that have been encapsulated with SLN.

Other PDD Nanocarriers

a. Nano-in-micro Particles

Some nanoparticles exhibit challenges in exhalation instability [123], particle-particle interaction, thermal stability, shape preservation, as well as handling and assembly difficulties due to their small sizes [53, 54]. Therefore, to overcome these challenges, larger particles are developed on the sub-micrometer scale, while the inner materials are developed in nanoscale, these include porous nanoparticle-aggregate particles (PNAPs) [54], nanoparticles-in-microparticles system (NiMS) [124], micron-sized powder carriers [125], modified microparticle carriers [53] for nanoparticles delivery to the lungs. The microcarrier matrix can be consisted of nanoparticles or additional excipients such as amino acids, sugars, or phospholipids as the microparticles dissolve on lung lining fluid to release the nanoparticles [64].

Curcumin nanocrystals (NC) loaded microparticles administered in a rat model of pulmonary arterial hypertension had shown an increase in curcumin bioavailability and therapeutic efficacy [126]. Hea *et al*. [67] had fabricated three sizes of curcumin particles for pulmonary delivery and revealed an improved dissolution rate of small-

sized nanoparticles, as larger-sized NCs remained longer in the lung airway lumen. Leucine microparticles loaded with poly(glycerol adipate-co-ώ- pentadecalactone) (PGA-co-PDL) nanoparticles were also able toreach the broncho-alveolar zone and potentiate dendritic cells uptake experimentally [127]. A wheat germ agglutinin (WGA)-anchored salmeterol xinafoate (SalX)-loaded chitosan nanoparticles-in-microparticles system (NiMS) had shown triggering-effects on drug release from lectin-anchored carriers through sugar lectin interactions, suggesting the possibility of controlling drug release by using specific sugars [128].

b. Liquid Crystalline Nanoparticles (LCNs)

Liquid crystals are intermediate carriers between a crystalline solid and an isotropic liquid state [129] that have been produced from vegetable oils or mineral oils [130]. They enhance active molecules to cell interaction, cellular entry, and pharmacological action [131]. Surface modification with hydrophilic polymers like chitosan can enhance LCN residence time, mucoadhesion, and cellular uptake [132]. Fruits and vegetable bioflavonoid rutin-loaded liquid crystalline nanoparticles (LCNs) had demonstrated a significant reduction in NO and ROS levels and prevented apoptosis in lipopolysaccharide (LPS) induced-human bronchial epithelial cell line (BEAS-2-B) [133].

c. Metal and Metal Oxide Nanoparticles

Metallic nanoparticles of cobalt, nickel, iron, gold, and their respective oxides can be synthesised and modified with therapeutic peptides, proteins, and the DNA attached to their functional chemical groups [134]. Ag NPs were found to reduce mucus glycoproteins (mucins) and perivascular and peribronchial inflammation in lung tissues [135]. Ag NPs had also suppressed pro-inflammatory cytokines, *i.e.*, IL-12 and TNF-α production, and downregulated COX-2 gene expression [136].

HERBAL NANOPARTICLES FOR PDD

Herbal remedies are becoming increasingly common globally to treat a range of diseases. However, herbal extracts have some limitations, which can restrict their therapeutic effects. To improve the drug properties, the integration of the nanocarriers to the herbal-based drugs is essential to maximise drug delivery to the targeted area. There are significant advantages in developing nanoformulations for herbal medicines, which include enhanced solubility and bioavailability, pharmacological activity enhancement, stability enhancement, improved tissue macrophage distribution, sustained delivery, protection from physical and chemical degradation, and so on. Thus, there is a potential for the herbal-based drugs prepared in nano-sized drug delivery systems to improve efficiency and overcome

issues related to herbal medicines.

Some herbal nanoparticles for pulmonary drug delivery have been reported previously. A Phytolacca decandra root extract, Phytolaccaceae, encapsulated in PLGA, had shown enhanced bioavailability and chemo-preventive effect compared to the free Phytolaccaceae in mice when tested on A549 lung cancer cells [137]. Polygalaceae ethanolic extract of Polygala senega encapsulated into PLGA had demonstrated superior anti-cancer effect against A549 lung cancer cells than Polygalaceae alone [138]. Another herbal formulation prepared in PLGA, naringin, had reported favorable nebulization properties with high drug deposition for lung cancer therapy [139].

A lactoferrin-chondroitin sulfate nanocomplex for the co-delivery of doxorubicin and ellagic acid nanocrystals was developed for lung cancer therapy. The nanocomplex, which was transformed into inhalable nanocomposites *via* spray drying, had exhibited deep lung deposition with promising antitumor efficacy against the lung cancer cells [140]. A chitosan polymer, which was used to formulate inhalable magnolia extract lignin honokiol microparticles by spray-drying technique, had shown about 90% drug release after 96 hours [141]. Another study by Papay *et al*. [142] had formulated inhalable nanoparticles loaded with plant flavone apigenin in bovine serum albumin. The spray-dried particles had demonstrated good aerodynamic properties with preserved apigenin anti-oxidant activity. Resveratrol, a natural phenol from grapes, berries, and peanuts, was co-spray dried (co-SD) with budesonide loaded into microparticle DPI aerosols, which had shown a significant antioxidant activity than vitamin C in reducing TNF-α, TGF-β1, and LPS levels for the treatment of extrapulmonary hypertension, lung inflammation and COPD [143].

The cognitive and anxiolytic effects of natural flavonoid quercetin loaded in liposomes and administered intranasally in male Wistar rats had displayed rapid and lower dose efficacy over orally administered free quercetin and quercetin liposomes [138]. Another study had described quercetin in liquid crystalline nanoparticles (LCN), and surface-modified liquid crystalline nanoparticles (sm-LCN), which were prepared by ultrasonication had exhibited anti-inflammatory activities by reducing the production of pro-inflammatory cytokines, *i.e.*, IL-1β, IL-6, and IL-8 in a human primary bronchial epithelial cell line, BCi-NS1.1 [132]. A diphenyl propane flavone fisetin, with anti-asthmatic, anti-cancer, anti-inflammatory, anti-viral and anti-oxidant effects, was reported to exhibit poor aqueous solubility and high lipophilicity [144]. Its low bioavailability was enhanced with different formulations of nanoemulsions, liposomes, nanocochleates, CD, cyclosophoroase dimer, and sulfobutylether-β-cyclodextrin (SBE-β- CD) complexes [145]. Andrographolide that was isolated from *Andrographis paniculata*

was converted into andrographolide-β-CD inclusion complexes, which were prepared by freeze-drying and had enhanced the andrographolide dissolution in the lung and exhibited better anti-pneumonia effect compared to andrographolide and penicillin alone [146]. In addition, curcumin- loaded stearic acid- and lecithin-based SLN, which was prepared by solvent injection method, had demonstrated a significant decrease in cytokine levels, suppressed airway hyperresponsiveness, and inflammatory cell infiltration in the lungs of ovalbumin-induced asthma model [115].

CONCLUSION

Nanoparticle drug formulations offer numerous benefits over traditional formulations. The use of nanoparticles for PDD has a lot of potential in the treatment of lungs diseases. Even though many pieces of research with promising results have been done on the PDD of nanoparticles, more *in vivo* and possible human studies are needed to determine the clinical success of this approach. Since particle size, surface morphology and aerodynamics, and PDD kinetics are well studied, it is essential to study the toxicity of various nanocarriers, phytoconstituents, and their clinical manifestations in *in vivo* settings.

CONSENT OF PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENT

This study was supported by the Research University Grant (1001/PPSK/ 8012344) from Universiti Sains Malaysia.

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CHAPTER 5

Application of Nano-drug Delivery Systems in improving the Therapeutic Efficacy of Bioactive Natural Products

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Abstract: The pharmaceutical industry has witnessed a huge revolution in the past few decades due to the discovery of various novel drugs for the treatment of several life-threatening ailments. However, there is a continued interest in exploring nature for inventing therapeutically active novel compounds. Among the various natural resources, plants constitute an invaluable source of bioactive molecules. Numerous studies have illustrated the exceptional therapeutic efficacy of phytochemicals against various diseases. Some of the well-known drugs, such as paclitaxel, vincristine, vinblastine, *etc.*, which can potentially be used in cancer therapy, were first isolated from plants. Later, several other drug-active molecules, which have demonstrated promising therapeutic effects, were discovered. Despite their outstanding antimicrobial, antidiabetic, and anticancer effects *in vitro*, most of these molecules fail to achieve a similar effect *in vivo*, mainly due to their poor aqueous solubility and bioavailability. The advent of nanotechnology and the application of nano-drug delivery carriers have significantly revolutionized the biomedical industry. Numerous studies indicate the successful utilization of nanoparticles for theragnostic applications. Nanoparticles having a size of approximately 50–250 nm can efficiently interact with cellular structures, eliciting desirable therapeutic effects. Apart from improving the aqueous dispersibility and stability of drugs, nanoparticles enable cell-specific as well as receptor-specific drug targeting, thereby reducing the off-target effects. Moreover, the stealthy nature of surface-modified nanoparticles, in combination with the controlled release of encapsulated drugs, enables prolonged therapeutic activity *in vivo*. This chapter presents an updated summary of the applications and challenges of various nano-drug carrier systems for the delivery of natural bioactive principles.

Keywords: Nanoparticles, Natural compounds, Pharmacokinetics, Phytochemicals, Plants.

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INTRODUCTION

Drug discovery is a complex, laborious, and expensive process. Nonetheless, the past few decades have witnessed an unbelievable revolution in drug discovery. The tremendous costs associated with the discovery of new leads and a reduction in the number of approvals of new drugs pose challenges to the drug development process [1, 2]. Despite the progress made to date, there is still continued interest in finding new drugs with improved efficacy and better pharmacokinetic profiles. Plants have been regarded as invaluable sources of therapeutically beneficial compounds since time immemorial [3, 4]. Traditional medicine practitioners use different parts of plants for preparing crude therapeutic formulations against various diseases [5]. Recently, there has been a renewed interest in developing novel therapeutics from natural products. The bioactive principles isolated from plants have also played significant roles in designing novel lead compounds and scaffolds [6, 7]. The therapeutically beneficial bioactive principles exhibit astonishing chemical diversity, which, in turn, contributes to the diversity in their actions [8]. Several studies illustrate the exceptional therapeutic effects of phytochemicals against various chronic diseases [9 - 12]. Table **1** presents the list of phytochemicals currently in clinical trials.

Table 1. Phytochemicals currently in clinical trials (Courtesy: Choudhari *et al.*, 2020, Ref 9).

Phytochemical	Type of Cancer	Clinical Trial Reference (from www.clinicaltrials.gov.in)
Berberine	Colorectal cancer	NCT03281096
Curcumin	Advanced and metastatic breast cancer	NCT03072992
Epigallocatechin	Colorectal cancer	NCT02891538
Lycopene	Metastatic colorectal cancer	NCT03167268
Quercetin	Prostate cancer	NCT01912820
Resveratrol	Low-grade GI neuroendocrine tumors	NCT01476592
Sulforaphane	Former smokers with a high risk of developing lung cancer	NCT03232138

Some of the well-known drugs currently used in modern medicine, such as paclitaxel, vincristine, vinblastine, *etc.*, were initially isolated from natural sources [13, 14]. Later, several other drug-active molecules, which demonstrated promising therapeutic effects, were discovered from natural sources, mostly plants [15]. Further advancements in medicinal chemistry have enabled scientists to synthesize these bioactive compounds in the laboratory [16]. Despite their outstanding antimicrobial, antidiabetic, and anticancer effects *in vitro*, most of the phyto-

chemicals fail to achieve a similar effect *in vivo*, mainly due to their poor solubility and bioavailability in aqueous biofluids [17, 18]. The majority of phytochemicals have low absorption profiles either due to their large molecular size or hydrophobic nature, which is also responsible for their fast clearance from the body [19]. This ultimately causes reduced bioavailability, which, in turn, leads to poor therapeutic outcomes. Moreover, rapid systemic clearance also necessitates either repeated applications or the administration of high doses to elicit a desirable therapeutic response [20]. High therapeutic dosages may sometimes cause toxic side effects [21]. Most of the phytochemicals present these limitations, which erect hurdles on their path to becoming potential drug candidates.

The advent of nanotechnology has revolutionized almost all fields of science [22]. The discovery and development of novel nanocarriers for drug delivery have caused an incomparable impact on modern pharmaceutical sciences and technology [23]. The encapsulation of drugs in rationally designed nanoparticles enables better dispersion of poorly aqueous-soluble drugs in biofluids, consequently improving their bioavailability and therapeutic efficacy [24]. Moreover, the application of conjugation chemistry principles enables surface conjugation of nanocarriers to cell-targeting ligands as well as the modification of nanoparticles for prolonged systemic circulation of drugs [25]. Further, carefully designed core-shell nanoparticles can deliver multiple drugs, thereby combining molecules with different hydrophilicity/lipophilicity profiles [26]. Interestingly, evidence indicates that the slow and sustained release of drugs from nanocarriers may often slow down the development of drug resistance [27, 28]. Thus, nano- drug delivery systems provide new and alternative options for the treatment of diseases, which otherwise have a slow response to conventional therapeutic strategies. In the present chapter, we will explore the opportunities and challenges of using nanoparticles for the delivery of phytoconstituents.

Bioactive Principles from Phytochemicals

Plants have been a major source of nutrition since time immemorial. However, they are also vital sources of phytochemicals that are crucial in the treatment and prevention of several diseases. Epidemiological studies have shown that intake of some dietary components prevents the occurrence of various diseases, such as cancer, diabetes mellitus, cardiovascular diseases, hypertension, *etc.* [29]. The selection of the right plant species for achieving the desired therapeutic effect is one of the major challenges in phytochemical-based drug discovery. However, one can depend on ancient literature or traditional knowledge for this purpose. The application of *in silico* modelling tools and virtual screening platforms also enable the selection of the right phytochemical for further research.

Phytochemicals are secondary metabolites produced by plants through several chemical pathways that are non-nutritive in nature and provide protection against microbial infections. They can be classified as alkaloids, flavonoids, phenolics, tannins, glycosides, gums, resins, and oils [30]. According to reports, among more than 250,000 species of plants, only 10% have been subjected to pharmacological research [31].

Although several modern-age technology platforms have accelerated the discovery of therapeutic lead molecules, drug discovery remains a lengthy and cost-intensive process that involves target determination and evaluation, compound screening, lead identification, compound optimization, *in vitro* and *in vivo* testing/preclinical evaluation, and clinical trials. Reports have shown that, on average, it takes about 12 to 15 years for a lead molecule to become a market- ready drug, with costs of approximately €1 billion. Furthermore, only half of the drug candidates become unsuccessful in the later stages of clinical trials [32]. These factors have prompted pharmaceutical companies to focus on the development of drugs derived from natural bioactive principles. Most often, these natural therapeutics are isolated and characterized from phytochemical crude formulations. The majority of these phytopharmaceuticals are better tolerated in an *in vivo* environment as compared to several synthetic drugs conventionally used in clinics [33]. Notably, most of them possess considerable antioxidant potential [34]. They have been reported to have multiple molecular targets, and hence, their molecular activities are complex and have a wide scope. These properties can allow them to be the most suitable candidates for the development of novel drugs [35]. Phytochemicals are found to have a different mechanism of action in diseased cells as compared to normal cells. They can selectively kill fast-dividing cells, as in the case of cancer, reduce oxidative stress, regulate the expression of signal transduction molecules and transcription factors [36].

However, the quality and quantity of bioactive compounds vary with latitude, altitude, climate, and season [37]. Sometimes, bioactive principles isolated from natural sources such as plants need to be converted into druggable forms [38]. Bioassay-guided fractionation is one of the strategies used for the isolation of bioactive components from a combination of compounds by employing various analytic techniques based on their activity in biological testing [39, 40]. The development of advanced chromatography systems has aided the isolation of a substantial number of compounds from crude extracts. The therapeutic efficacy of isolated fractions can be tested *in vitro* and *in vivo*. Silica, Sephadex, and Superdex are usually used as matrices for fractionation [40]. Solvents are used in the increasing order of their polarity. A natural extract with confirmed bioactivity is used for fractionation. The eluted fractions are further analyzed for biological efficacy, and active fractions are subjected to various analytical tests, such as

thin-layer chromatography (TLC), HPLC, FTIR, mass spectroscopy (MS), *etc*. Bioactivity-guided fractionation is often regarded as a low-cost procedure with a higher success rate. Some of the most important phytopharmaceuticals with proven therapeutic benefits include curcumin, quercetin, thymol, rutin, rosmarinic acid, β-carotene, allicin, gingerol, epigallocatechin gallate, coumarin, *etc.* [39]. Although antimicrobial, anti-fungal, anti-diabetic, and anti-inflammatory effects of phytochemicals are well documented, most research on phytochemicals is intended for the development of novel therapeutic drugs against cancer, considering that it is the leading cause of death worldwide, followed by cardiovascular diseases [41]. Extensive studies are being performed for evaluating the chemopreventive and chemosensitizing effects of phytochemicals and compounds isolated from natural sources, in addition to chemotherapeutic applications [39, 42]. Chemoprevention refers to the use of any molecule, either natural or synthetic, to prevent cancer or its development at any stage, *i.e.*, initiation, promotion, or progression. Chemopreventives can be classified as primary, secondary, or tertiary based on the stage at which they intervene [43]. Chemosensitization refers to the use of one drug or a compound, either natural or synthetic, to enhance the activity of another drug and, consequently, reducing the side effects [44]. To date, numerous studies have illustrated the mechanism through which phytochemicals exert their therapeutic effects both *in vitro* and *in vivo*. Phytochemicals are non-toxic to normal and healthy cells and are better tolerated *in vivo* since most of them are dietary components [37].

Most phytochemicals exert their therapeutic actions through a multitude of mechanisms [45]. In fact, most of them perform antioxidant activities by directly absorbing reactive oxygen species (ROS) or by promoting the action of antioxidant enzymes such as superoxide dismutase, glutathione, and catalase [46]. Curcumin, a polyphenolic compound isolated from *Curcuma longa*, is one of the widely studied phytochemicals to date. It exhibits exceptional anti-microbial, anti-diabetic, and most importantly, anti-cancer effects [47]. This compound has been shown to upregulate the expressions of p21, p16, p53, Bax and Caspase- 3, 8, 9 proteins and downregulate the levels of Bcl-2, mTOR, p65, Bcl-xL, Akt, EGFR, NF-κB, c-myc, and cyclin D1 proteins, thereby exhibiting anti-cancer activity in a wide range of cancers [48, 49]. Curcumin has been shown to exhibit profound chemosensitizing, chemotherapeutic, as well as chemopreventive effects [50]. Curcumin has been shown to inhibit pro-carcinogenic signals and mutagenesis induced by B[a]PDE in lung cancer cells [51]. Furthermore, it has been found to sensitize cervical cancer cells to paclitaxel chemotherapy and breast cancer cells to 5-FU chemotherapy [52, 53].

Epigallocatechin gallate (EGCG), a catechin polyphenol isolated from green tea, exerts anticancer effects through a multitude of mechanisms. It can successfully

obstruct the proliferation of cancer cells by inhibiting the activity of ornithine decarboxylase, which plays a prominent role in the proliferation of cells. It has also been found to exhibit significant inhibition potential against NF- κB, Bcl-2, COX-2, matrix metallopeptidase-9 (MMP-9), VEGF, and Erk, JNK, and MMP-9 expressions [54, 55]. Ellagic acid, a phenol antioxidant found in several fruits and vegetables, is effective in inducing apoptosis in prostate and breast cancer cells and inhibiting metastasis in a variety of cancers [56]. Luteolin, a type of flavonoid, has been found to obstruct epithelial-mesenchymal transition in the case of several cancer types [57]. Flavanones, isoflavones, and lignans have been found to reduce cell proliferation as well as inflammation by impeding the signaling *via* the NF-κB family of transcription factors [58, 59]. Apigenin, a flavone present in parsley, celery, and chamomile, has been found to interfere with the leptin/leptin receptor pathway as well as the STAT family of transcription factors in cancer cells [60]. Crocetin, a carotenoid compound, has been seen to interact with GATA binding protein 4 and MEK-ERK1/2 pathway, thereby exhibiting cardio-protective functions [61]. Cyanidin glycosides from red berries were found to have profound anti-cancer as well as antioxidant functions. It causes suppression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) genes and inhibits mitogen-induced metabolic pathways [62]. Moreover, it could effectively target EGFR in human vulva cells and ErbB2/cSrc/FAK pathway in breast cancer cells, which, in turn, successfully prevents their metastasis [63]. Gingerol inhibits Erk1/2/JNK/AP-1 signaling and results in caspase-dependent apoptosis in solid tumors, as well as hematological malignancies [64]. Kaempferol has been shown to inhibit Src, a protein kinase found to be constitutively active in several solid tumors [65]. Interestingly, most of these phytochemicals also exhibit considerable regulatory effects on epigenetic signaling as well as metabolic reprogramming, which have recently emerged as two hot topics in the field of pharmaceutical research on cancer. Phytochemicals can also modulate epigenetic functions such as DNA methylation, histone modifications, and miRNAs expression [66, 67].

Although several preclinical studies, as well as clinical case studies, have illustrated the therapeutic potential of the above-mentioned phytochemicals, a considerable number of factors act as hurdles on their way to clinical translation. Poor bioavailability, low aqueous solubility, hydrophobicity, and obscure target specificity are some of the major factors that restrict the therapeutic applications of phytochemicals in clinics [68]. Some of the other inhibiting factors include chemical instability in the acidic pH conditions prevalent in the stomach and colon, metabolism by gut microflora, increased oxidation, and first-pass metabolic effects [69]. Furthermore, an active efflux mechanism is present on the epithelial cell surface, which also poses a serious threat to the absorption of phytochemicals *in vivo* [70]. Some studies have shown that glucuronidation and cytochrome P450

activities in the intestine and liver can cause rapid clearance and, hence, has poor bioavailability [71]. Apart from detectable plasma levels, the accumulation of the compound at the target organ is a key factor that determines its efficacy. It is very difficult to predict the bioavailability of a compound from its physicochemical properties. However, some insights can be obtained from Lipinski's "rule of five." A compound will have better bioavailability if it has not more than 5 hydrogen-bond donors, not more than 10 hydrogen-bond acceptors, a molecular mass not greater than 500 daltons, a partition co-efficient log P-value of not greater than 5, and less than 10 rotatable bonds [72]. However, some of the most important phytochemicals, such as curcumin and some of the polyphenols isolated from green tea, including EGCG, do not satisfy these criteria. In addition, most of them possess greater steric complexity, a higher number of chiral centers, and more rigidity, which pose hurdles in their translation into a clinical drug.

Nanotechnology Revolutionized the Biomedical Arena

Nanotechnology deals with the science, engineering, and application of particles with a size in the 10^{-9}m range [73]. Due to the confinement of their size in the atomic and molecular levels, they possess unique structural, chemical, mechanical, magnetic, electrical, and biological properties. Nanoparticles have a large number of surface atoms, surface energy, and a high surface area to volume ratio [74]. These properties enable enhanced interaction of nanoparticles with the cellular substructures and macromolecules, which can positively influence the effective delivery of drugs loaded in the nanoparticles [75]. Moreover, nanoparticles enable surface modification, which further aids in cell-specific and receptor-specific targeting [76, 77]. The discovery of nanoparticles, different synthesis routes, and their application has revolutionized the fields of biosensors, tissue engineering, microfluidics, and theranostics, which is an integration of therapy and diagnostics [78 - 81]. Nanoparticles used for drug delivery are typically in the size range of 50–250nm. The small size of nanoparticles allows for easy cellular uptake, tissue penetration, as well as prolonged circulation in the vasculature [82]. Such particles can be synthesized using a wide array of materials such as metals, ceramics, polymers (synthetic polymers, proteins, carbohydrates, and dendrimers), semi-conductors and quantum dots, lipids, *etc.*, utilizing top- down (from a bulk material) or bottom-up (reduction reactions using a precursor of the salt of the material) approaches [83]. Recently, hybrid nanocarriers, including polymer-protein and metal-polymer nanocomposites, integrating the functionalities from one or more of the aforementioned materials are also gaining interest [84, 85]. The selection of nanoparticles for drug delivery purposes depends on a multitude of factors, among which the most important is the physicochemical nature of the drug. The second main determining factor is the route of administration and the target organ. Drug-loaded nanoparticles must successfully cross the intestinal mucus barrier, which is

a major challenge for administration *via* the oral route. The toxicities of nanoparticles should also be taken into consideration while designing nanoparticles [86, 87]. The release of the drug from the nanoparticles can be triggered by either external (ultrasound or magnetic field) or internal stimulus (enzymatic actions) [88]. The physicochemical characteristics of the nano-drug carrier, especially the nature of the material, its size, shape, structure, surface functionalities, and surface charge, significantly influence the release kinetics of the drugs. For a nanoparticle to be a successful drug carrier, it should satisfy some requirements, which are as follows:

(i) The nanocarrier should be stable during the circulation, and there should not be any premature release of the drug. (ii) The drug should be released either at the tumor site or inside the tumor cells [89]. One of the major drawbacks of nanoparticle-based drug delivery is the uptake of nanoparticles by the mononuclear phagocytic system present mainly in the spleen and liver. However, this may be beneficial for drug delivery to treat inflammatory diseases [90, 91]. To achieve prolonged circulation and successful evasion from the macrophages, the nanocarriers may be grafted to polymers such as polyethylene glycol (PEG), which can significantly improve the pharmacokinetics of the drug. PEGylated nanoparticles, often referred to as "stealth nanoparticles," are found to evade the macrophage uptake more effectively as compared to non-PEGylated nanoparticles [92]. In addition to enhanced circulation, PEGylation is also found to reduce the toxic side effects of the nanocarrier or the drug [93]. Besides PEG, certain other materials may be used for surface coating in order to prevent the agglomeration of nanoparticles, including poly(vinylpyrrolidone) (PVP) and others., as well as natural polymers such as dextran, chitosan, pullulan, *etc.* and surfactants such as sodium oleate, dodecyl amine, *etc.* [94 - 98].

The physicochemical properties of the material used for preparing nanocarriers have a significant amount of influence on the drug delivery kinetics. Numerous substances can be used to synthesize nanoparticles for drug delivery, including albumin, gelatin, phospholipids, polymers, bio-ceramics such as calcium phosphate, hydroxyapatite, dendrimers, carbon-based nanoparticles, and others [99]. Nanocarriers can deliver the drugs *via* two mechanisms, passive delivery or active delivery [100]. Nanocarrier-based drug delivery can reduce the off-target effects, besides enhancing cellular uptake. Both of these features can enhance the therapeutic index of drugs [101]. Nanoformulations of phytochemicals have been shown to improve their bioavailability, in addition to enhancing their target specificity, thereby maximizing therapeutic potential [102]. Fig. (**1**) shows the advantages of nano-drug carriers.

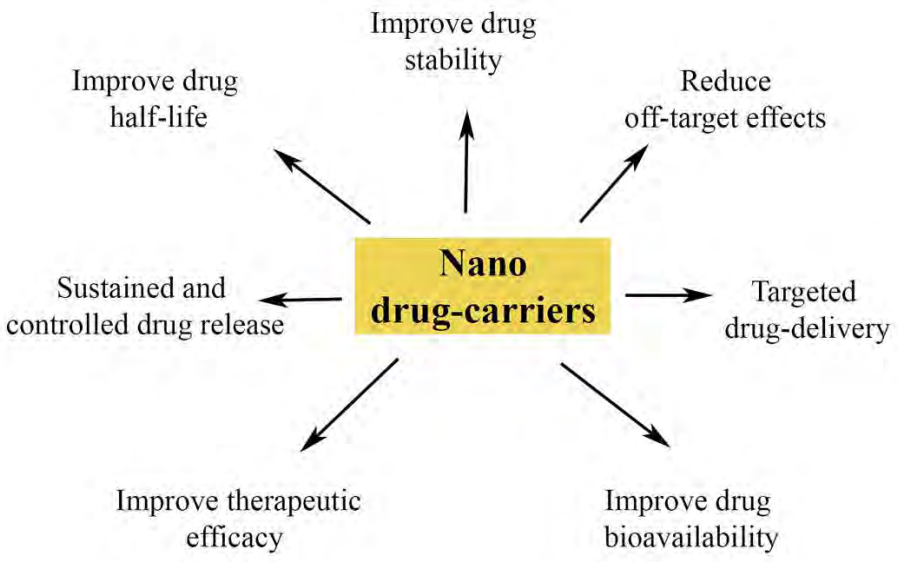

Fig. (1). Features of nano-drug carriers.

An Overview of Nanoparticles for the Delivery of Bioactive Principles from Natural Products

Numerous studies indicate the successful utilization of nanoparticles in theranostic applications. Nanoparticles of different sizes, shapes, and materials can be used for drug delivery. Furthermore, multifunctional nanoparticles are also utilized for contrast-guided and site-specific drug delivery [103]. Nab-paclitaxel, known as nanoparticle albumin-bound paclitaxel, was the first commercially successful nano-drug formulation. It was approved in 2005 for the treatment of metastatic breast cancer [104]. Paclitaxel, an alkaloid that was first isolated from the bark of the Pacific Yew tree, exhibits anti-cancer activity by inhibiting the depolymerization of microtubules during cell division [105]. Secreted protein acidic and rich in cysteine (SPARC) is a protein expressed in solid tumors and often associated with a poor prognosis. SPARC protein can quite effectively bind to albumin, resulting in the uptake of albumin-bound paclitaxel in cancer cells [106]. Therefore, nab technology became a success in hard-to-treat solid tumors such as metastatic lung cancer, breast cancer, and pancreatic cancer.

Polymers are one of the most widely used materials for nanoparticle-based phytochemical drug delivery. Polymeric materials used for drug delivery purposes can be synthetic polymers, carbohydrate polymers, proteins, or copolymers [107, 108]. Pan *et al.* has developed polydopamine-based nanoparticles loaded with curcumin for improving the chemical stability and pH-responsive delivery of this

phytochemical [109]. Lignin is the most abundant aromatic natural polymer that comprises about 25% of straw biomass [110]. Lignin-based hollow nanoparticles have been reported as effective in the delivery of Doxorubicin [111]. Among the biopolymer-based nanocarrier formulations, gelatin nanoparticles have been extensively studied due to their ease of synthesis, non-toxic nature, and enzymatic degradation of the carrier matrix *in vivo* [112]. Cellulose is a linear polysaccharide polymer with many glucose monosaccharide units. Nanoparticles prepared using cellulose and its derivatives have been successfully used for delivering hydrophobic drugs [113]. Dextran is a polymer of anhydroglucose and is another widely used polymer for nanocarrier synthesis [114]. Polymers such as chitosan, PLGA, among others, are preferable choices for delivering phytochemicals due to their biocompatible and biodegradable nature [115]. Chitosan nanoparticles are excellent nanocarriers that are efficient in delivering phytochemicals. Vijayakurup *et al.* have used curcumin entrapped in chitosan nanoparticles for improving the tissue retention of curcumin, thereby enhancing its chemopreventive properties. The study found that the chemopreventive efficacy of curcumin against benzo-pyrene/B[a]P-induced lung cancer increased several folds when curcumin was delivered *via* chitosan nanocarriers. The mucoadhesive properties of chitosan might have been an addition to the improved tissue retention of curcumin in lung tissues [116]. Alginate nanoparticles alone or complexed with other polymers such as chitosan are also excellent nanocarriers of poorly water-soluble drugs [117].

Several studies have successfully shown the benefits of copolymer nanoparticles as a drug delivery agent. PLGA-CTAB nanoparticles encapsulated with curcumin exhibited enhanced anticancer activity in triple-negative breast cancer (MDA- MB-231 cells) as compared to free curcumin [118]. PLGA alone or different copolymers of PLGA with another polymer have been widely researched. PLGA nanoparticles enable surface modifications such that the tumor-targeting ligands can be conjugated for cell-specific targeting [119]. A study reported by Thulasidasan *et al.* has shown that the conjugation of curcumin-loaded PLGA- PEG copolymer nanoparticles with tumor-targeting ligands, such as folic acid, can considerably improve the bioavailability and chemosensitizing efficacy of curcumin towards paclitaxel chemotherapy [120].

Different nanosystems used for drug delivery purposes can be broadly classified into the following categories:

i. Nanoemulsions (NE): Nanoemulsion is a thermodynamically stable isotropic system where two immiscible liquids are mixed to form a single phase through the use of an emulsifying agent such as a surfactant. The average droplet

size usually ranges from 0.1 to 500 nm, and the size of the droplets depends on the nature of the drug particles, mechanical energy, composition, and the nature and relative amounts of surfactants used for preparing nanoemulsion [121].

ii. Nanomicelles: Nanomicelles are self-assembling colloidal dispersions composed of a hydrophobic core and a hydrophilic shell, with a size typically ranging between 10 and 100 nm. These are excellent nanocarriers for solubilizing hydrophobic drugs by entrapping them in a micellar hydrophobic core with a shell composed of hydrophilic side chains, thereby enabling excellent aqueous dispersion. They can significantly lessen drug degradation, reduce adverse side effects and also enhance drug permeation [122].

iii. Nanosuspensions (submicron-sized suspensions): Nanosuspensions consist of hydrophobic drugs suspended in dispersion to improve their solubility and permeability. This, in turn, results in the faster achievement of the required plasma level of the drug. They can be easily administered *via* the intravenous route without any blockade of blood capillaries [123]. Nanosuspensions can also be lyophilized and stored for future purposes. They offer several advantages such as improving the solubility and bioavailability of drugs. The method is suitable for hydrophilic drugs. They can also enhance drug loading, which, subsequently, reduces the dose required to elicit the desired therapeutic action. Further, the physical and chemical stability of drugs becomes considerably enhanced [124].

iv. Nanospheres (drug nanoparticles in a polymer matrix): Nanospheres are colloidal systems that contain therapeutic agents either dispersed within a colloidal matrix or are attached to the surface of the polymer matrix by adsorption or conjugation processes. The matrices used are usually polymers or a combination of two or more polymers, *i.e.* a copolymer. For drug delivery purposes, those polymers that are biodegradable and generate non- toxic degradation products are preferred. Nanospheres can be prepared using different polymers, either synthetic or natural, such as poly (butyl cyanoacrylate) (PBCA), poly (lactic acid) (PLA) or its copolymer poly(lactide-co-glycolide) (PLGA), alginate, chitosan, or combinations of two or more polymers [116, 117, 119, 120, 125, 126]. These polymeric nanospheres enable sustained and controlled release of the drug. They can also be surface-conjugated to ligands to achieve selective targeting.

v. Nanocapsules (encapsulated drug nanoparticles): Nanocapsules are colloidal nano-drug carrier systems with an oily or an aqueous core enclosed by a thin polymer membrane, either natural or synthetic in nature. They have higher dose-loading of smaller volumes and exhibit enhanced site-specific retention. The drugs delivered using nanocapsules undergo rapid absorption and exhibit increased bioavailability. They can be surface-modified using cell-targeting

ligands. Nanocapsules can be prepared using polymers, lipids, or liposomes [127]. The encapsulation of curcumin in polyelectrolyte nanocapsules can improve its neuroprotective activity. Enhanced solubility and antibacterial activity were observed when curcumin was encapsulated by PLGA oily-core nanocapsules [128].

vi. Lipid nanoparticles (lipid monolayer enclosing a solid lipid core): The scarcity of safe polymers and the high cost of available polymers have limited their applications in clinical medicine. This is one of the reasons behind the widespread popularity of lipid nanocarriers for drug delivery [129]. Solid lipid nanoparticles (SLNs) are colloidal emulsions of lipids in the submicron size range composed of solid lipids. SLNs are colloidal carriers in the sub- micron size range composed of a physiological lipid, dispersed in water or in an aqueous surfactant solution. They exhibit controlled drug release at the target site, combined with improved stability of pharmaceuticals. Compared to other carriers, high drug content can be loaded inside SLNs. Both lipophilic and hydrophilic drugs can be delivered using SLNs. The synthesis procedure is devoid of organic solvents, and hence, SLNs are biocompatible. They are suitable for intravenous administration of drugs. Unlike other nanoparticles, SLNs are associated with lower production costs, are easy to scale up and sterilize, and can easily obtain regulatory approval [130]. Thymoquinone, a bioactive compound in *Nigella sativa*, when encapsulated in lipid nanocarriers, showed a six-fold increase in bioavailability as compared to free thymoquinone, as well as better therapeutic effects [131]. Moreover, studies have shown that lipid nanovesicles can significantly influence skin penetration and the absorption of phytochemicals such as curcumin and resveratrol [132].

vii. Nanotubes: Nanotubes are one-dimensional nanostructures prepared using C60 atoms arranged in a long, thin cylindrical structure. Carbon nanotubes (CNTs) can be surface-conjugated to peptides, proteins, nucleic acids, drugs, *etc*. Furthermore, evidence indicates that functionalized CNTs display low toxicity and are non-immunogenic [133].

viii. Liposomes: Liposomes were the first nanoparticle-based drugs to be approved for clinical use in 1995. They are vesicular assemblies that consist of spontaneously formed phospholipid bilayers [134]. Phospholipids, being the main components of the cell membrane, exhibit excellent biocompatibility. Liposomes can vary in size, composition, and charge. They can carry a variety of payloads such as small drug molecules, proteins, nucleotides or plasmids, *etc*. Liposomes can be conjugated to targeting ligands for cell-specific drug delivery. Their properties that include size, lamellarity, bilayer rigidity, charge, and bilayer surface modifications are influenced by lipid type, surface charge, and production method. Moreover, liposomes can be coated with other poly-

mers such as chitosan for the enhancement of their properties and also for bioconjugation with cell- targeting ligands [135]. Chen *et al.* have shown that liposomal curcumin formulation can enhance cisplatin sensitivity by suppressing NADPH oxidase 5 expressions in human epithelial cancer cells [136]. Shankar *et al.* have demonstrated that tryptanthrin, an indolo quinazoline compound isolated from the DCM extract of the leaves of *Wrightia tinctoria*, when encapsulated in liposomes exhibits exceptional chemopreventive effects on non-melanoma skin cancer [137]. The liposomal-quercetin formulation could significantly improve the bioavailability of quercetin. Moreover, the stability of quercetin was very well preserved in liposomes [138]. Phosphatidyl choline-based liposomes were used to encapsulate thymol, and consequently, a significant improvement of anti-oxidant and anti-microbial properties was observed [139]. Rutin encapsulated in liposomal nanoparticles exhibited enhanced anti-oxidant activity in H_2O_2-damaged HUVECs and also improved cell viability [140]. Liposomes are also used for co-loading two or more drugs or phytochemicals. Ramadass *et al.* have demonstrated that co-loading of Paclitaxel and epigallocatechin gallate in liposomes can control the invasive potential of MDA-MB-231 for breast cancer cells [141]. Similarly, Meng *et al.* co-loaded resveratrol and paclitaxel in liposomes for reversing the drug resistance of breast cancer cells *in vivo* [142]. Sesarman *et al.* have shown that co-delivery of curcumin and doxorubicin in PEGylated liposomes favors the anti-neoplastic microenvironment in murine colon cancer [143].

ix. Nano-hydrogels: Hydrogels contain a cross-linked polymer network along with a large amount of water, approximately 70–99%, and hence, resemble tissues. This, thus, gives the hydrogels excellent biocompatibility. Nano- hydrogels have the advantages of both hydrogels and nanoparticulate systems [144]. Stimuli-responsive hydrogels can be used for parenteral drug delivery applications [145]. Pillai *et al.* have reported the use of cross-linked acrylic polymer (FA-CLAP) hydrogel conjugated to tumor-targeting ligand folic acid for site-specific delivery of hydrophobic drugs to cancer cells [146]. Curcumin-loaded poly(N-isopropyl acrylamide) silver nanocomposite hydrogels have been successfully used for antibacterial applications [147].

x. Nanofibers: Electrospun nanofibers, which are highly porous polymer meshes with a large surface-to-volume ratio, are another interesting nanocarriers [148]. Studies have shown that curcumin exhibits controlled release from the electrospun matrix and exhibits excellent antimicrobial activity [149]. Tissue-engineering scaffolds prepared from electrospun nanofibers embedded with phytochemical-loaded nanoparticles are also used for site-specific drug delivery [150].

xi. Noble metal nanoparticles: Noble metal nanoparticles surface-conjugated to

phytochemicals have been shown to improve their bioavailability and therapeutic efficacy. Silver nanocomposite films impregnated with curcumin exhibit superior antibacterial applications [151]. Metal-complexed polymer nanoparticles loaded with curcumin have enhanced anti-bacterial properties and have also been proved to be efficient in treating various chronic diseases [152].

xii. Mesoporous silica nanoparticles: Mesoporous silica nanoparticles have been successfully utilized as drug delivery systems for hydrophobic drugs such as curcumin, which have been shown to improve their bioavailability as well as therapeutic efficacy [153].

Challenges of Bioactive Natural Products Through Nano-Drug Delivery

Although very promising and effective, nano-drug carriers have several limitations that impede their clinical applications. These limitations are applicable in the case of not only phytochemical drugs but also other small molecule drugs. As mentioned earlier, nanoparticles, due to their large surface area to volume ratio, can quite effectively interact with cellular structures and in a multitude of ways, potentially aggravating the toxic side effects [154]. Certain nanoparticles are reported to be capable of inducing cytotoxic and genotoxic effects. Most often, the toxic effects of nano-drug carriers depend on size, charge, surface modification, nature of the material used, among other aspects [155]. The small size enables them to penetrate the epithelial and endothelial barriers and begin to circulate *via* lymph and blood. This allows them to reach different organs quite easily. Studies reported that nanoparticles may trigger mitochondrial damage and platelet aggregation. The use of biologically non-degradable nano-drug carriers may persistently accumulate in the body and can induce chronic inflammatory responses [156]. Hence, nano-drug carriers may raise long-term toxicity concerns. Moreover, due to the small size, nanoparticles may cross the blood-brain barrier, which may be beneficial for delivering drugs to brain cells. However, in other circumstances, this might trigger toxic effects in the central nervous system (CNS) [157].

Furthermore, the concept of mechanisms through which nanocarriers deliver drugs to cells has been controversial. Most often, nanocarriers display safe and effective delivery of drug payload in pre-clinical and *in vitro* studies. However, the same mechanism could not be effectively and safely exhibited in the human body. Unless conjugated to any targeting ligands, most of the nano delivery carriers adopt passive targeting of cells utilizing the enhanced permeability and retention (EPR) effect of tumor cells [158]. However, there is considerable heterogeneity among tumor types and tumors in different stages with regard to the EPR effect, and hence, the pharmacokinetics of drug-loaded nanoparticles is complicated. The EPR effect may

also be influenced by several parameters, including the circulating concentration of nanomedicine, the stability of the carrier and plasma half-life of the drug, location of the tumor, stage of the disease, *etc* [159]. The concentration of the drug that reaches the target site through nanocarriers also depends on the vascularity of the tumor and other microenvironmental parameters. Most importantly, these parameters display patient-to-patient variability in clinical applications [160]. The majority of the multi-functional nanocarriers are sophisticated and aggravates the complexity of drug delivery, which may negatively impact the therapeutic outcome.

Drugs encapsulated in nanocarriers have a complex pharmacokinetic profile. For example, the pharmacokinetic profile of the drug present in liposomes and released from liposomes exhibit significant variability [161]. Small molecule drugs administered using conventional methods exhibit a standard route of absorption, distribution, metabolism, and elimination. However, drug-loaded nanoparticles have to face several hindrances before reaching the target site, for example, the phagocytes present in the reticuloendothelial organs such as the spleen and liver. Moreover, nanocarriers may also be engulfed by circulating monocytes, platelets, dendritic cells, *etc.* [162]. The inter-subject and inter-species variability of the kinetics of drugs encapsulated in nanoparticles is often attributed to the actions of the reticuloendothelial system, also known as the monocytic phagocytic system [163].

Another limitation is the negative effects of protein corona formation on the surface of nanocarriers [164]. Most often, nano-drug carriers administered in the human body have to encounter protein corona effects. A protein corona consists of proteins adsorbed from blood/plasma/intercellular fluids on the surface of nanoparticles. The formation of protein corona can significantly influence drug- release kinetics and also the fate of nano-drug carriers [165]. It can either slow down or accelerate the release of drugs from nanoparticles. This necessitates the development and validation of procedures to quantitate drug release in the presence of the biomolecular corona. Furthermore, the biomolecular corona formation on the surface of nano-drug carriers is considered as a causative factor of the off-target effects of nanomedicines as well as the insufficient drug concentration at the target site [166].

Although surface modification of nanoparticles using PEG can improve the circulation time of nanoparticles by limiting protein corona formation to a certain extent, it can further create complexity in the pharmacokinetic drug profile [167]. In-depth comprehensive research must be conducted to study the fate of drugs encapsulated in nanoparticles, as well as the clearance of nanocarriers from the body. Surface charge plays a significant role in the clearance of nanocarriers.

Drummond *et al*. have found out that uncharged liposomes have a lower clearance rate, as compared to positively or negatively charged liposomes, as a result of a lower rate of opsonization by blood proteins on the surface of neutral nanoparticles [168]. The charged nanoparticles also exhibit an enhanced uptake by the reticuloendothelial system. Surface coating by substances such as PEG can significantly reduce opsonization by blood proteins, thereby reducing the uptake by the reticuloendothelial system, which, in turn, leads to prolonged circulation [169].

Future Prospects

Even though drug discovery from natural sources is often described as "looking for a needle in a haystack," this has always been a continued interest of researchers in the field of phytochemical research. The unprecedented developments in the research of nano-drug delivery have created new hope for bringing phytochemical-based drugs into the clinic. Currently, fascinating developments are occurring in this field of research. To date, one of the areas to have benefitted the most from nanomedical technology is cancer therapy. Doxil, a PEGylated liposome that carries the anticancer drug Doxorubicin, was the first nanomedicine to be approved by the FDA. From then onwards, extensive research is being conducted on the subject of nano-drug delivery systems. The conventional tests and protocols used for the evaluation of drugs and medical devices may not be sufficient for detecting the potential risks of nano-drug carriers. Hence, new science, methods, and protocols are needed for an extensive toxicological evaluation of nano-drug carriers. Since nanoparticles exhibit different physicochemical features that influence their distribution in the human body, both at the organ and cellular level, special attention must be given to the investigation of the pharmacokinetic and tissue distribution parameters. Furthermore, the safety evaluation and risk-benefit analysis should be carried out for each category of nanoparticles using specific toxicology testing models.

Research must give significant attention to the development of nano-drug carriers with uniform size and consistent drug loading and release capacity. The long-term impact of the toxicity of nano-drug carriers on health and the environment, especially non-biodegradable nanocarriers, is an important area of research that requires focus. We believe that the nano-drug research area might witness a huge breakthrough once a better understanding of molecular characteristics of diseases is attained. The advances in *in silico* modeling and simulation tools can be utilized for studying the effects of phytochemical-loaded nanocarriers at the tissue/cellular level. These studies can complement the valuable data provided in preclinical studies. Last but not the least, the degradation of nanocarriers is an important aspect that needs thorough research. The degraded by-products may accumulate in the

cells and can cause disruption to the cellular sub-structures or intracellular organelles. The degradation pathway can also induce deleterious signaling pathways. Hence, for successful drug delivery through nanocarriers, it is crucial to have an in-depth understanding of protein corona, its kinetics, and dynamics for each type and class of nanoparticles used. With these factors considered, it should be presumed that the futuristic application of nanocarriers for the delivery of phytochemical drugs can revolutionize the pharmaceutical industry.

CONCLUSION

The existing literature shows that phytochemicals and compounds of therapeutic values isolated from other natural sources can become excellent lead molecules in drug discovery. One of the limitations of their use in conventional therapeutic regimens is poor solubility in aqueous biofluids, which results in poor bioavailability at the target organ. These drawbacks can be mitigated to a great extent with the use of nano-drug carriers. The current chapter presents an updated summary of the applications and challenges of various nano-drug carrier systems for the delivery of natural bioactive principles. Studies have shown that nanoparticles with an approximate size range of 50–250 nm can very well interact with cellular structures, eliciting a desirable therapeutic effect. Apart from improving the aqueous dispersibility and stability of phytochemical drugs, ligand-conjugated nanocarriers enable cell-specific as well as receptor-specific drug targeting, which leads to the reduction of off-target effects, in addition to the improvement of the therapeutic profile of drugs. Moreover, the stealthy nature of surface-modified nanoparticles, in combination with the controlled release of encapsulated drugs, enables prolonged therapeutic activity *in vivo*. However, surface modifications and the presence of surfactants can critically influence the release of drugs from nanocarriers, which should be sorted out during preclinical studies. Furthermore, studies to assess the effects of nano-drug carriers and their long-term effects on biological systems must be conducted.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We sincerely acknowledge the support provided by the Department of Biotechnology, Government of India, and DST-SERB, Government of India for NPDF fellowship.

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CHAPTER 6

PEGylated Liposomes

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Abstract: PEGylation is a technique that is used to coat the surface of different NDDSs. It may be done covalently or noncovalently with hydrophilic, linear, ionic, non-toxic ether diol polymer such as PEG (polyethylene glycol) to improve their pharmacokinetic as well as pharmacodynamic behaviour. PEG and its several derivatives that possess hydrophilic PEG chains and neutral polymeric nature can produce a protective layer on the surface that prevents interaction with antibodies, therefore, decreasing the immunogenic effects and escaping phagocytosis. The hydration capacity of PEG produces a hydration shield around the PEG-linked DDSs and improves the solubility in serum and blood to protect rapid glomerular clearance and enzymatic degradation. When compared with the conventional liposomes, PEGylated liposomes had improved blood circulation time, higher drug bioavailability, improved drug delivery at the target site as well as bypass reticuloendothelial systems (RES).

The PEG chain's shape, length, weight, types of bond and density would primarily affect PEGylated liposomes efficiency, and currently, researchers have been working relentlessly toward the selection of PEG derivatives and PEG conjugation methodologies. This is to establish the best PEGylation stratagem for a specific biomedical application. The objectives of the present context will be to explicate the principles and strategies of PEGylation, PEG derivatives and the modification used in PEGylated liposomes, their uptake and clearance, the application in various diseases' drug targeting, in herbal medicines delivery as well as the challenges and future approaches to improve PEGylated liposomes in the clinical application of therapeutic purpose. This chapter also discusses the benefits and application of self-emulsifying drug delivery systems (SEDDS) in herbal medicine.

Keywords: Drug delivery, EPR effect, PEG derivatives, PEGylated liposomes, PEGylation, Poly- (ethylene glycol), SEDDS, SMDDS, SNEDDS.

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Swarnlata Saraf, Ram Kumar Sahu & Vivek Dave (Eds.)

INTRODUCTION

Novel drug delivery systems (NDDSs) are well-defined formulations and system-based approaches that are designed to provide the release of drugs or therapeutic agents in a controlled manner to safely deliver them at the targeted site as well as to improve the system's efficacy. Although the exploration and advancements in NDDSs are well discovered in recent years, they often undergo several problems, such as poor blood circulation capability, non-specific interaction, degradation, low hydrophilicity, poor water solubility, rapid glomerular clearance and recognition and neutralization with antibodies and protease enzymes [1, 2]. Due to this, numerous modern methodologies have been endeavoured to evade these problems by augmenting the stealth characteristics to the drug delivery systems (DDSs). One of the most prominent approaches is a surface modification with hydrophilic and neutral molecules of DDSs in which the interaction that occurs between the foreign materials and opsonin is primarily dictated by van der Waals, hydrophobic, electrostatic, and ionic forces [3].

PEGylation is a method used to attach or coat the surface of different drugs, macromolecules and NDDSs either covalently or noncovalently with hydrophilic, linear, non-ionic, non-toxic ether diol polymer such as PEG (polyethylene glycol) molecules to improve their pharmacokinetic and pharmacodynamic properties [1, 4, 5].

The liposome was the first discovered nanocarrier system in the early 1960s, and it immediately gained researchers' attention. In today's era, it is acknowledged as an advanced type of nanocarrier that can target different active moieties (anticancer and antimicrobial agents, peptide hormones, oligonucleotides, enzymes, proteins, vaccines) at the target site while improving their therapeutic efficacy [6 - 8]. Several previous research studies have shown that the liposomal formulations have improved the pharmacokinetics of the therapeutic agent and biodistribution at the target site. To date, various anticancer liposomal formulations, PEGylated [Doxil®/Caelyx® (doxorubicin), Lipo-Dox® (doxorubicin)], non-PEGylated [Myocet®/Evacet® (doxorubicin), DaunoXome® (daunorubicin), Ambisome® (Amphotericin B) and Marqibo® (Vincristine), Visudyne® (Verteporfin)] have been approved and they are commercially available in the market [9, 10].

PEGYLATION

The PEGylation procedure was first introduced by Davis and Abuchowski along with his co-workers in the late 1970s at Rutgers University to improve the therapeutic bioavailability of proteins in polypeptide-based drugs [11]. In the present era, PEGylation signifies a robust and flexible approach that allows the

advancement of developing pharmaceutical products that are still under clinical trials or have been approved and commercially available in the market [12 - 14].

In PEGylation, PEG and its derivatives, due to their hydrophilic PEG chains, neutral and flexible polymeric nature can fabricate the surface protective layers that would enable minimal interaction with antibodies, opsonin's and enzymes that are present in the bloodstream and foreign materials; thus, it helps in decreasing the immunogenic and antigenic effects and making phagocytes stealthier [15]. In addition, due to the PEG molecules' hydration capacity, a high hydration shield that is produced around the PEG-linked DDSs would improve the solubility in body fluids, such as in serum and blood, and protect the attached DDSs from rapid glomerular clearance and enzymatic degradation (Fig. **1**) [16]. The PEGylation scheme has been utilised in various fields by employing different methodologies (physical, chemical, encapsulation/entrapment and enzymatic) to attach PEG molecules at the DDSs surface.

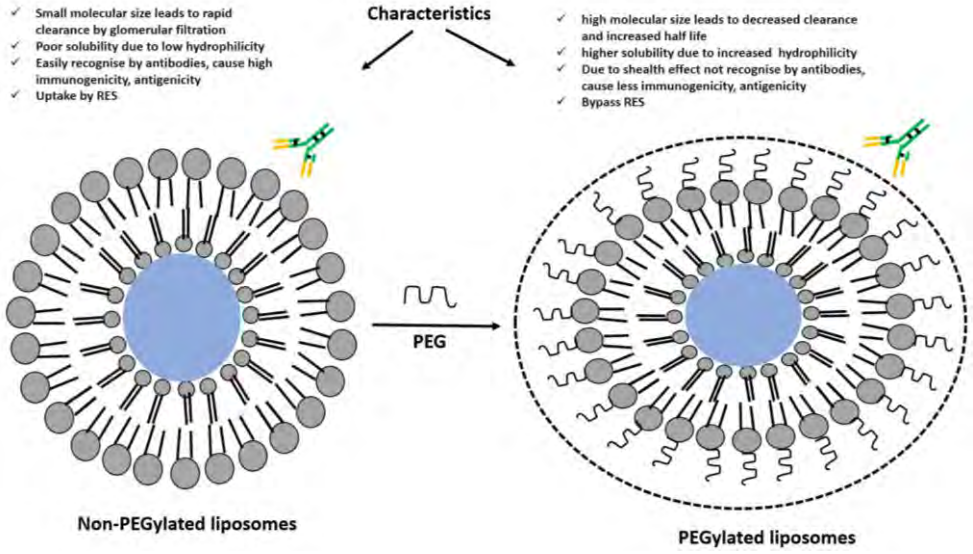

Fig. (1). Characteristics of Non-PEGylated and PEGylated liposomes.

PEGYLATED LIPOSOMES

Liposomes are micro or nanoscopic, spherical lipid bilayered cells that are prepared by single lipids or different types of lipids with cationic, anionic, or neutral charges. The liposomal vesicles can encapsulate various hydrophilic and lipophilic therapeutic agents such as drugs, vaccines (DNA/RNA/oligonucleotides), enzymes, proteins, other biomolecules, macromolecules, herbal extracts; they are still extensively explored as potential DDSs to reduce toxicity and enhance the therapeutic effectiveness and safety [4]. While liposomal formulations are biocompatible, biomimetic thus are able to efficiently deliver the therapeutic agents;

they often suffer from enzymatic degradation, rapid removal from blood circulation, and opsonization. These issues can be easily rectified by surface modification of liposomes as it will increase the physical properties and therapeutic efficacy [17, 18]. The strategy of liposomal surface modification with PEG is commonly known as PEGylation or stealthing or camouflaging. In comparison with conventional liposomes, these PEGylated liposomes have shown bypass reticuloendothelial systems (RES), improved blood circulation residence time, high drug bioavailability, reduced toxicity, and improved drug delivery at the target site (Fig. **1**) [18 - 20]. The stealth liposomes can be achieved by surface modification of liposomes along with polyethene glycol (PEG) and its copolymers [21 - 23].

Properties of PEG

PEG is the most common linear or branched, polyether diol hydrophilic polymer with several significant properties such as biocompatibility, solubility in both water and organic solvents, less toxicity, less immunogenicity, and antigenicity, as well as a safe metabolism and excretion profile. Due to these properties, Food and Drug Administration (FDA) has approved its usage as it is safe for internal use in various fields of biomedical applications [24]. Commonly, PEG is manufactured *via* link-up of repeated ethylene oxide units to produce different types of linear or branched PEG polymers with different molecular weights. For PEGylation rationale, the modifications of PEG *via* the addition of monofunctional monomethoxy group at the terminal end of linear PEG (mPEG) or branched PEG (mPEG2) are made [24].

In-body metabolism of PEG polymers occurs on the basis of their molecular weight *via* alcohol dehydrogenase, PEG polymers with less than 400 Da are converted into toxic metabolites. Meanwhile, longer PEG chain polymers, which are mainly used for liposomes and proteins PEGylation, are not metabolized by enzymatic degradation, yet their elimination from the body takes place. PEGs with 20 kDa molecular weight and PEGs with more than 20 kDa are eliminated by glomerular filtration and by liver uptake, respectively [5, 25].

PEG Linked-Lipids (PEG-Lipids)

Further advancement on stealth liposome formulation technologies arises after the development of PEG-based lipid polymers. These types of lipids in association with PEG would facilitate the formulation of long-circulating PEGylated liposomes with half-lives 15-24 h and 45 h in rodents and humans, respectively [19]. There are several PEGylated liposome products containing PEG-lipids that have reached clinical applications [18]. In PEG-based lipid polymers, PEG polymer of variable chain lengths is attached with the acyl group of lipids through linkers such as

carboxylate ester, amide, disulphide, or ether linkage, phosphate ester, and amide [26 - 31]. During the liposome formulation, the acyl groups would entrench inside the liposome bilayer, and the orientation of PEG chains would confine towards the aqueous environment. The physical and structural fabrication of liposomes such as lamellar or micellar, rate, and the extent of inclusion of PEG-linked lipids are directly influenced by the acyl chain length and arrangement [32 - 34]. In a study, vincristine-loaded liposome was constructed with PEG2000-ceramide (CER) with C8 and C24 carbon length acyl chain. Results had shown that the pharmacokinetics of longer acyl chain PEG2000-CER liposomes, which were loaded with vincristine, were significantly altered with long circulation profiles as well as higher vincristine plasma concentration levels. On the other hand, the partitioning of liposomal formulation with short acyl chain PEG-ceramides took place faster after the intravenous administration as compared to longer acyl chain PEG-ceramides [35]. The magnitude of liposomes' PEGylation was also reduced with a longer acyl chain incorporated with PEG2000-dimyristoylglyceride (80 mol%) as compared to PEG2000- distearoylglyceride (57 mol%) [34]. The selection of linkers also influences the performance of the stealth liposomes. The presence of acetal, vinyl ether, and acetal linkages would expand the hydrolysis susceptibility in body fluids, whereas the negatively charged phosphate linker could possibly trigger the complement system [36, 37]. On the basis of PEG orientation, the linker groups and acyl chains, PEG-lipid polymers are categorized as follows:

PEG-Phospholipids

In the PEG-phospholipids, mainly, the linear chain terminal methoxy group, which consists of PEG chain polymer, is attached *via* covalent bonds to the phospholipids. To construct these PEG-phospholipids, distearoyl phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), and dimyristoyl phosphatidylethanolamine (DMPE) phospholipids are utilised. The PEG chain length and their density with respect to total lipid content would significantly influence the circulation half-life of liposomes [38, 39]. The presence of long PEG chains can offer a better steric barrier characteristic than short PEG chains [40]. Due to PEG addition with phospholipids, a thick stable hydrophilic wall would form in liposomes surrounding [41]. The thickness of this wall would increase as the PEG chain molecular weight increases. It is also observed that by using a combination of both short and long-chain PEG, the wall thickness has significantly increased compared to single-type PEG addition. Furthermore, the circulation time for liposomes has also improved [41 - 46]. In previous research, liposomes with 1,2-distearoyl-sn-glyce- o-3-phosphoethanolamine-PEG were prepared, which possessed PEG chains of 2000 and 500 Da molecular weight, and the results had shown that due to the presence of a thick hydrophilic layer, the liposomes had

resided in the tumour cells for a longer time as compared to conventional PEG-lipid liposomes [41]. Moreover, the PEG's addition level also can affect the rate and the drug release pattern from liposomes [46]. In a study, DMPE-PEG liposomes containing guanosine were prepared and evaluated. It was suggested that if the PEG level in DMPE-PEG increased, the rate of release of guanosine from liposomes would decrease, as well as the release pattern would shift from diffusion-based release to interfacial release [47, 48].

PEG-Non-Phospholipids

Despite PEG-phospholipids being extensively utilised, there are certain drawbacks associated with phospholipids. It is not completely compulsory to conjugate PEG with phospholipids in order to prepare PEGylated liposomes. In lieu of this, a separate PEG derivative class of PEG-non-phospholipids has emerged as a suitable choice. This is to eliminate the complications that come with phospholipids-based PEG [54]. There are several problems that would occur during liposomes usage, such as negative charges, inducing anaphylactic shock and hypersensitivity reactions, inducing platelets activation and aggregation, as well as complement activation [37, 49 - 55]. They also have physiochemical stability issues, are susceptible to enzymatic degradation, and the methods employed for their extraction, synthesis, and storage are costly [56 - 58]. However, the recent new class of PEG-non-phospholipids provides considerable benefits and has resolved every drawback of PEG-phospholipids stated previously [28, 29, 57, 59 - 61].

In the PEG-non-phospholipids category, sphingolipids, fatty acids, and their salt derivatives and conjugate derivatives with other polymers, such as polyglycerols and oxyethylene, have been included [62].

Heyes and colleagues had constructed [PEG2000 linked 1,2-distearyloxypropyl- 3-amine *via* succinimide, amide, and carbamate linkers] and prepared different types of liposomal vesicles that consisted of plasmid DNA and concluded that these new constructs were efficient to form more stable liposomes with low systemic toxicity as compared to PEG-succinoyl diacylglycerol (PEG-S-DSGs) derivatives [28]. Another PEG-non-phospholipid variant is cholesteryl-PEG (Chol-PEG). In general, cholesterol is commonly used in liposomes formulation. In Chol-PEG, cholesterol addition would enhance the hydration of lipids, reduce membrane fluidity, increase lipid bilayer stability, and the encapsulation of hydrophilic drugs [63 - 68]. In many Chol-PEG derivative liposomes, the release of drug contents is performed by cleaving the PEG chain (dePEGylation). For example, PEG-cholesteryl methylcarbamate (PEG-CHMC) and PEG-cholesteryl hemisuccinate (PEG-CHE-

MS) would release the drug from their liposomal formulation in the presence of esterase [69]. Due to this, other PEG variants, which are known as cleavable PEG-CHMC and PEG-CHEMS, have been developed, but they cause a reduction in the circulation half time of liposomes [70]. To rectify this issue, the insertion of a linker (L) has been done between Chol and PEG molecules (Chol-L-PEG), such as the addition of 1,4- diaminobutane linker in Chol-PEG3400 to reduce the serum-induced release of carboxyfluorescein from Chol-L-PEG3400 [71].

Hyperbranched Polyglycerol (hbPG)-Lipids

Hyperbranched polyglycerol (hbPG) is dendrimer-type thermal stable epoxides with many advantages over linear PEG [72]. The hbPG are constructed *via* various epoxides ring-opening polymerization in the presence of cholesterol as an initiator, which fastens the linking with phospholipids or cholesterol [73]. Hofmann and co-workers have synthesized various types of hbPG- lipids *via* conjugation with 1,2-bis-n-tetradecyl glyceryl ether or cholesterol. They have also reported that the structural integrity of lipids would be preserved in these constructs upon liposomes formulation even though no in-vivo studies related to stealth behaviours of liposomes have been reported yet [74].

MODIFICATIONS IN LIPIDS

Liposomes, which were conventionally used, possessed low bioavailability and short circulation time in blood. Not only that, they could be uptaken easily by the RES. To overcome these limitations, the development of the conjugated or coating of the surface was introduced. In this hydrophilic, polymers like (PEG) had been utilised to provide conditional hydrophilicity, avoid macrophages uptake, flexibility, hide immunological recognition and high biocompatibility. There are different types of modifications available for the preparations of the liposomal formulation. The methoxy-PEG-DSPE is the most commonly used PEG lipid derivative with a methoxy terminal. Even though methoxy-PEG-DSPE can extend the circulation, the methoxy group is relatively inactive to react with the ligands in placid circumstances. Thus, it is essential to alter PEG-DSPE with terminal groups by substituting them with a hydroxyl group to build a linkage with certain ligands. The chemical and physical properties of the polymers could be enhanced for the targeted drug delivery system by altering the hydroxyl of the PEG end-group [76, 77]. The frequent end-group modifications of PEG-DSPE derivatives are imparted by applying methylation, carboxylation and amination [75].

Carboxyl-end Group PEG-DSPE Derivative

Carboxyl groups could be introduced at the terminal end of PEG-DSPE block copolymers to enable effortless interaction with target-specific ligands (such as peptide and transferrin) for active delivery at target tissues or cells [78 - 80]. DSPE-PEG-COOH has been synthesised successfully in several reports. In short, DSPE was dissolved in chloroform; methanol was added to PEG-bis (succinimidyl succinate) (PEG-2OSu) dissolved in chloroform, then triethylamine was added. Following that, the reaction mixture was stirred vigorously overnight at room temperature. The products were separated by thin-layer chromatography [81 - 83]. Vaidya *et al*. had developed PEGylated liposomes of arginine-glycin--aspartic acid (RGD), along with DSPE-PEG-COOH as a linker to link up the amine group of cRGD peptide (CNPRGDY[OEt]RC). The RGD peptide–conjugated liposomes had shown higher accumulation at the clotting site with improved thrombolytic potential as compared to the long circulatory liposomes and the plain streptokinase solution [79].

Amino-end Group PEG-DSPE (amino-PEG-DSPE) Derivative

The hetero-bifunctional PEG has an amino group to specifically provide a protective shield through the presence of groups, such as butyloxycarbonyl (Boc) and fluorenylmethyloxy-carbonyl (Fmoc). The active group that reacts with DSPE is the other end of PEG. After the formation of amino-PEG-DSPE, the removal of the shielding groups is allowed. Furthermore, the combination of the amino-PE-DSPE can be made with drugs and ligands molecules. In 1994, Zalipsky *et al.* had synthesized the amino-PEG-DSPE [84]. Firstly, the hetero-bifunctional PEG containing an amino group was selectively sheltered by the Boc group. Then, the addition of the succinimidyl carbonate (SC) group at the terminal hydroxyl end of α-Boc-ω-hydroxy-PEG was done to form urethane (ethyl carbamate) and DSPE's amino group conjugate. Through the acidolytic removal of the Boc group, the primary amine functionality of the PEG was regenerated.

Hydrazide-end Group PEG-DSPE (Hz-PEG-DSPE) Derivative

A hydrazide end group (Hz) onto PEG-DSPE also helps the covalent linkage of the ligands. It has been reported that the oxidized ligands would react with the anchor's hydrazide groups to synthesize Hz-PEG-DSPE [85, 86]. Zalipsky had reported the synthesis of PEG derivatives with heterobifunctional groups with succinimidyl carbonate (SC) group at one end and butyloxycarbonyl (Boc) at the other as protective hydrazide group. The reaction of DSPE's amino group and Boc-PEG-SC had readily produced Boc-PEG-DSPE [87]. Finally, the Boc group was removed by acidolytic reaction yielding a Hz-PEG-DSPE conjugate which was appropriate for linking a range of ligands.

Maleimide-end Group PEG-DSPE (Mal-PEG-DSPE)

In recent times, the modified PEG-DSPE-maleimide group is frequently used in the functionalisation of target-specific delivery systems by permitting appropriate and prompt response after recognition by ligands such as peptide and antibody [88, 89]. For the synthesis of Mal-PEG-DSPE, two methods have successfully been employed [90]. In the first method, N-succinimidyl-3-(N-maleim- do)-propionate would react with the amino-PEG-DSPE in the presence of organic solvents such as dichloromethane, dimethylformamide and triethylamine (TEA) to form Mal-PEG-DSPE. In the second method, DSPE would react with ω-(β-- N-maleimido])-PEG-α-succinimidyl carboxylate (Mal-PEG-SC) and TEA in chloroform [75].

METHODS OF PEGYLATION

There are two methods employed to prepare PEGylated liposomes. The first method is applied prior to liposomes preparations, which requires the addition of PEG-lipids to lipid constituents; this method is known as the pre-insertion method. As for the second method, the mixing of PEG-lipids is done into prepared liposomal dispersion, and it is known as the post-insertion method. The PEG chain length and density would primarily affect the efficiency of PEGylated liposomes. If the PEG molecules used are too short, they cannot avoid protein absorption. On the other hand, if the PEG chains are too long, it can cause a decrease in performance activity [91]. Therefore, PEG molecules with moderate length are utilised for liposome modification. On the molar PEG-lipid/lipid composition ratio, the liposomes surface coverage density, and shape depends on the following conditions:

a. <5% PEG provides <100% surface density coverage with mushroom shape PEG.
b. 5–15% PEG provides ~100% surface density coverage with mushroom- orbrush shape PEG;
c. >15% PEG provides ~100% coverage with brush shape PEG.

Due to PEGylation, the liposomes' residential time in the blood would increase by preventing the interaction between mononuclear phagocyte and liposomes [92, 93], and the accumulation at tumour sites would increase as well [94].

Pre-insertion PEGylation

The addition of PEG-lipid in the lipid phase prior to the formation of the lipid layer, which is further hydrated by the aqueous phase, is known as pre-insertion PEGy-

lation. The thin layer hydration method is the most commonly used technique for the formulation of PEGylated liposomes [95, 96]. This method, however, has a few limitations, such as the required excess amount of PEG-lipids renders the phase environment viscous, making the extrusion process difficult to continue [4]. Another problem is that the modification of liposomal lipid bilayer in interior space could induce steric hindrance and reduce the encapsulation space [97, 98], as well as the presence of these modified PEG-lipids in interior space makes liposomes susceptible for acid/base hydrolytic degradation and drug loading efficiency reduction [4]. Due to the drawbacks mentioned, the pre- insertion method is not suitable for PEGylated liposomes preparation for site- specific targeting with proteins, peptides, antibodies, antibody fragments, and ligands.

Post-insertion PEGylation

To overcome the problems encountered during the PEGylated liposomes preparation *via* the conventional methods, the post-insertion method of PEG-phospholipid that is incorporated into the outer layer of the prepared liposomes was developed and could be utilised in versatile drugs and biological constituents delivery [4, 97, 99 - 101]. Uster and co-workers had developed the post-insertion method in 1996 [99] to insert PEG-phospholipid [MPEG (1900)-DSPE] into prepared liposomes by slowly adding MPEG (1900)-DSPE in the diluted suspension of the prepared liposomes. After that, the spontaneous interaction between the hydrophobic parts and PEG-phospholipids would occur. To prevent the self-assemblization tendency of the amphiphilic PEG-phospholipids, the concentration of these ingredients must be used below their critical micellar concentration (CMC).

Compared to the pre-insertion method, this particular method has numerous advantages, such as it only allows the outer layer modification on the liposomal surface, thus providing sufficient space inside the liposomes for drug or other materials for encapsulation, which is lesser than in the case of the pre-insertion method. It also requires less quantity of PEG-phospholipids. Along with the high quantity of PEG-phospholipids consumption in the pre-insertion method, the incorporation of it in the internal space does not play any significant role in the surface zeta potential change and does not provide homogeneous surface characteristics to form liposomes. Nakamura *et al*., in their work, had prepared irinotecan-loaded PEG-phospholipid liposomes *via* the post-insertion method and had concluded that the prepared liposomes had exhibited superior circulation potential along with less susceptibility for enzymatic degradation of PEG-lipid as compared to liposomes that were prepared by the pre-insertion method [4].

UPTAKE AND CLEARANCE OF PEGYLATED LIPOSOMES

The uptake and clearance of nano-vesicular drug delivery systems (including PEGylated and non-PEGylated liposomes) would primarily occur in the bloodstream *via* phagocytosis through the reticuloendothelial system (RES) cells. This system is mainly present in the bone marrow, spleen, and liver. The cells involved in this system are macrophages, monocytes, dendritic cells as well as the Kupffer cells in the liver. The attributes which induce RES activity could be responsible for affecting clearance, toxicity, circulation capacity, and the pharmacological responses of PEGylated liposomes [102]. After systemic administration, the rapid removal of conventional liposomes is governed by macrophages of the RES. As a consequence, their drug delivery characteristics at the target site are altered due to the featured pharmacokinetics of nano-vesicular systems [19]. Due to the structural modifications of the PEGylated liposomes, their permeability after systemic administration has been enhanced, which in return would further alter the pharmacokinetic and the drug distribution patterns of the encapsulated cytotoxic drugs as compared to conventional cytotoxic drugs [19, 102, 103]. Therefore, advancement in the design of nanocarriers, such as modification/stealth/PEGylation, must be developed to hinder clearance mechanisms, circumvent complement activation and increase the circulation half-life of drugs in the bloodstream [104].

PEGYLATED LIPOSOMES' ACCUMULATION AT TUMOR SITE *VIA* EPR EFFECT

In the tumour progression, a highly vascularized micro-vascular blood capillaries network is established to provide adequate oxygen and nutrients supply for tumour growth and progression. Further excess of angiogenic regulators builds an abnormal, highly disorganized, dilated and leaky micro-vascular network of blood vessels in tumour tissue with poor lymphatic drainage [105]. Due to this favourable pathophysiological milieu, PEGylated liposomes (long-circulating liposomes) loaded with anticancer drugs, which were leaking, had been penetrated and retained especially in tumour sites as compared to conventional liposomes with reduced drug toxicity. This manner of passive drug targeting to deliver chemotherapeutics is known as the enhanced permeability and retention effect (EPR) [105 - 107]. It is proven that the EPR effect is the foremost mechanism of nanocarriers accumulation in tumour tissues [107]. Additionally, surface-modified PEGylated liposomes with hydrophilic PEG chains are also found to be a promising drug delivery system for EPR effect-based tumour targeting (Fig. **2**). They can decrease liposomes uptake by mononuclear phagocyte systems as well as increase their half-life and accumulation [105]. The accumulation of PEGylated liposomes at the tumor site depends on the size, type of tumour, and the extent of tumour progression [108].

One of the well-known examples is Doxil® (PEGylated doxorubicin liposomes), in which several findings have shown that due to the stealth behaviour of liposomes, the half-life of doxorubicin would increase, and its release would only occur in the tumour space in the presence of high ammonia level due to the glutamine lysis metabolism in tumour cells. Doxorubicin that is released from liposomes also can cause damage and cytotoxicity in tumour tissue [106].

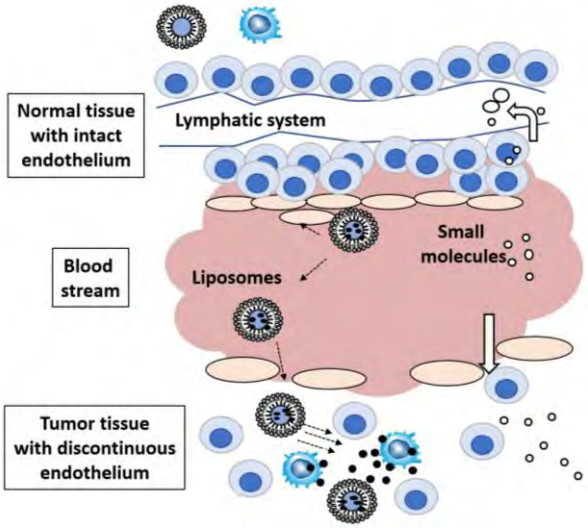

Fig. (2). Surface modified PEGylated liposomes for drug delivery system for EPR effect.

APPLICATIONS OF PEGYLATED LIPOSOMES

Of late, liposomes have been encouraged as a drug delivery carrier for many chemotherapeutic drugs, particularly in cancer therapy. The main obstacle in the case of cancer therapy is multidrug resistance (MDR) that is developed in cancers for anticancer drugs. Nano-vesicular systems, such as liposomes that are loaded with drugs, have proficiently surmounted the MDR problem and improved the therapeutic potential of chemotherapeutics in MDR-resistant cancer treatment. Additionally, PEGylated liposomes could be more accurate in delivering the cytotoxic agents into cancer cells due to their modified surface characteristics, long circulation time, and EPR effect. The ideal example is Doxil® (mPEG2000 coated doxorubicin liposomes), which provides an anticancer effect against cancers, such as breast, ovarian, and AIDS-associated Kaposi's sarcoma [109].

Lately, the surface of Doxil® (PEGylated doxorubicin liposomes) that is coated with glutathione, known as 2B3-101 formulation, has been reported as an emerging approach for safe delivery of doxorubicin in the brain for brain cancer treatment [110, 111]. In another study, Alaaeldin *et al*. have reported that the TS- siRNA (thymidylate synthase siRNA) loaded cationic PEGylated liposomes complex (lipoplex) when co-administered with oxaliplatin (1-OHP) loaded liposomes

against solid tumours have efficiently increased the therapeutic potential of 1-OHP, due to tumour cell growth and inhibited proliferation *via* gene silencing induced by TS-siRNA molecules [112]. In another clinical application, PEGylated liposomes loaded with 64Cu and 177Lu were employed in positron emission tomography (PET) for diagnostic imaging and in internal tumour radiotherapy techniques, respectively [108]. PEGylated liposomes are abundantly used in the targeting of different diseases. Here are some of the examples incorporated, which have been used in the treatment of different diseases (Table **1**).

Table 1. Various therapeutic applications of PEGylated liposomes.

S. No.	Disease	Drug	Mode of action	Cell line	Targeting/Major findings	References
1	Cancer	Mitomycin C/ Promitil®	Selectively inhibits the synthesis of deoxyribonucleic acid (DNA)	Cervix, gastric, pancreatic carcinoma	Folate triggered, 3 fold reduced toxicity, monoexponential plasma clearance	[113]
2.	Human Ovarian cancer	Doxorubicin	Inhibits the enzyme topoisomerase II, breakage of DNA strands, and inhibition of both DNA and RNA synthesis	-	High encapsulation efficiency of ammonium sulfate, drug loading >90%, Particle size~100nm, PDI<0.2, Scale-up technique	[114]
3.	Lung cancer	Nedaplatin (two ammine ligands and the dianion derived from glycolic acid)	DNA damage and triggered apoptosis	human lung, osteosarcoma embryonic kidney, fibroblast cancer	89% entrapment efficiency, vesicles size ~150nm (-ve zeta potential); Vesicles show increased stability, reduced aggregation, Enhanced cytotoxic/antiproliferative	[115]

(Table 1) cont.....

S. No.	Disease	Drug	Mode of action	Cell line	Targeting/Major findings	References
4.	Cervical and hepatic cancer	Dioscin (Dioscorea nipponica, natural steroid saponin glycoside)	↓ reduced expression of VCAM-1 and ICAM-1adhesion molecules	Human cervical and hepatoma cancer	Transferrin receptor-mediated endocytosis, The average particle size of 140.07±1.33 nm, polydispersity index of <0.2, entrapment efficiency (EE), and drug loading (DL) were 88.94±1.02% and 4.16±0.05%, respectively, Increased intracellular uptake, great hemocompatibility, Time-dependent cellular uptake	[116]
5.	Diabetes	Insulin	Impedes lipolysis, proteolysis, and gluconeogenesis pathways	Human colon adenocarcinoma	Folate receptor, Higher stability, increased the resistance in the gastrointestinal tract, Particle size 150−210 nm, negatively charged, 48% drug release, 125I labelled-Insulin loaded liposomes showed better cellular uptake	[117]
6.	Breast Cancer	Exemestane (aromatase inhibitors)	Covalently binds to aromatase as a pseudo-substrate and inactivates the enzyme	Breast cancer cells	Particle size 148.3 ± 8.76 nm, PDI 0.186 ± 0.08, Zeta-Potential -26.8 ± 4.8 mV, Maximum drug release 70.13 ± 2.40%, Reduced acidic degradation, better internalized into the cells and highly permeated through the intestine, increased oral bioavailability	[118]
7.	Alzheimer's Disease	Osthole (coumarin compound)	Inhibition of platelet aggregation	Human neuroblastoma cells, embryonic kidney and human brain endothelial cells	Transferrin (Tf) receptor-based endocytosis with elevated penetration *via* the BBB and augmented drug accumulation and circulation time in the brain	[119]

(Table 1) cont.....

S. No.	Disease	Drug	Mode of action	Cell line	Targeting/Major findings	References
8.	Colon Cancer	Glycyrrhizic acid (GA) (Glycyrrhiza glabra root) and cisplatin (Co-delivery)	GA suppresses the expression of TNF-ά and caspase 3 and cisplatin *via* crosslinking with DNA's bases hamper repair and cause DNA damage	human colon cancer cell lines	Particle size 81.6 nm and Zeta potential − 30.7 mV, 43% increased DNA damage in cancer cells	[120]
9.	Blood	Resveratrol (stilbenoid)	Mimic effects of caloric restriction, exert anti-inflammatory and anti-oxidative effects	-	Erythrocytic hemolysis, vesicles size ~ 85 nm, with -20 mV, PEGylated liposomes preserved the intrinsic antioxidant activity,	[121]
10.	Malignant gliomas,	Chlorogenic acid	Hamper G-6-Pase activity directly, thus decreasing blood glucose level by	-	Immune-stimulating mechanism, excellent entrapment capacity, biocompatibility, decreased expression of Th2 related factors at the tumor site	[122]
11	Epithelial ovarian cancer (EOC)	Doxorubicin (Doxil), carboplatin and paclitaxel	Doxorubicin: DNA damage, Carboplatin: inhibiting replication and transcription Paclitaxel: stimulates the assemblization of microtubules with tubulin dimers and stabilizes microtubules by preventing depolymerization.	-	Observed shortening of the hematologic toxicity and mild non-hematologic toxicities with high maximum tolerance dose	[123]
12	Colon carcinoma	Galbanic acid (Ferula species (Apiaceae))	Inhibition in the activity and expression of MMP2 and MMP9	Colon carcinoma	Combinatorial effect of PLGba on the toxicity reduction, robust tumour growth inhibition effect, controlled hepatotoxicity	[124]
13	Urinary bladder cancer	β-elemene (Rho kinase inhibitor)	Impede cell growth and proliferation, cell cycle arrest, cell apoptosis	Bladder cancer and urinary bladder carcinoma	High entrapment efficiency, good stability, improved bioavailability and anti-tumour effects	[125]

(Table 1) cont.....

S. No.	Disease	Drug	Mode of action	Cell line	Targeting/Major findings	References
14	Alcohol-induced hepatotoxicity	Silymarin (hepatoprotective agent)	Affects the synthesis of RNA and DNA	Human Hepatocellular carcinoma	Efficiently improves the liver function, inflammatory conditions, antioxidant levels, and histological parameters	[126]

APPLICATIONS OF PEGYLATED LIPOSOMES IN HERBAL DRUGS DELIVERY

Theoretically, nanosized herbal drug delivery systems can increase biological activity and solve issues that are associated with plant drugs. Nanoliposomes are able to overcome the disadvantages of standard chemotherapy by increasing the solubility characteristics, therapeutic efficacy, and stability of herbal products. These second-generation liposomes are designed to overcome the cons of the first-generation liposomes; hence multiple PEGylated liposomes have been widely studied for clinical use. There are many existing biocompatible lipids to encapsulate natural goods. Herbal medicine-loaded nanoliposomes, on the other hand, offer hopeful approaches to cancer treatment, Alzheimer's disease, cardiovascular and cerebrovascular disease (Table **2**).

Table 2. Various therapeutic applications of PEGylated Liposomes for herbal drugs.

Types of PEGylated Liposomes;	Phytoconstituents	Plant Source	Preparation method	Diseases	Animal/Cell lines used	Route of administration	Major findings of PEGylated	Ref
LUVs	Salvianolic acid B	Sal*via* miltiorrhiza	Reverse-phase evaporation method	Cardiovascular and cerebrovascular diseases	Beagle dog; RAW264.7 murine macrophage cell line	Intravenous	1. Half-life of pure Salvianolic acid B was enhanced 2. The lower dose was obtained 3. Less chance of side effects	[127]
MLVs	Artemisinin (qinghaosu)	Artemisia annua L.	Thin film-hydration method	Antiparasitic and tumor	CD1 mice	Intraperitoneal	1. Artemisinin's half-life has been raised by 5 times 2. AUC values have been boosted about 6 times	[128]
LUVs	Silymarin	Silybum marianum	Thin-film hydration method	Cirrhosis, hepatitis, and fatty infiltration due to alcohol and toxins	Human hepatoblastoma cell line HepG2	Parenteral	1. Prevent leakage 2. Improved bioavailability 3. Enhanced cellular uptake	[129]

(Table 2) cont.....

Types of PEGylated Liposomes;	Phytoconstituents	Plant Source	Preparation method	Diseases	Animal/Cell lines used	Route of administration	Major findings of PEGylated	Ref
SUVs	Resveratrol	Grapes, peanuts berries, and plums	Ultrasonic dispersion method	Anti-cancer, anti-inflammatory, anti-oxidant, anti-aging, cardiovascular effects and blood sugar reduction	BALB/c nude mice; MCF-7 cells	Intravenously	1. Increased drug deposition at the tumour site. 2. Resveratrol and cytotoxic agents can be co-delivered 3. Prevented capture by macrophages	[130]
LUVs	Plumbagin	Plumbaginaceae Family	Thin-film hydration method	Human promyelocytic leukemia, prostate cancer ovarian, melanoma, breast cancer, lung carcinoma of non-small cells and cervical cancerous cell lines	C57BL/6J mice; B16F1 melanoma cells	Parenteral	1. Higher plasma half-life 2. Slower uptake by the cells of RES 3. No symptoms of toxicity of tissues	[131]
LUVs	Bergamot essential oil	Citrus bergamia Risso	Thin-film hydration method	Neuroblastoma	SH-SY5Y human neuroblastoma cells study	--	1. Eliminate the need for toxic solubilizing agents 2. Decreased dosage of medications 3. Increased activity of anticancer	[132]
LUVs	Ursolic acid	Apple peels, grape skins, cranberry juice, and hawthorn	Thin-film dispersed hydration method	Anti-inflammatory, anti-trypanocidal, anti-viral, anti-oxidant, and anti-tumour agents	Balb/c nude mice; Human oral cancer KB cell line	Intravenous	1. Enhanced solubility and increased bioavailability. 2. Higher human epidermoid carcinoma inhibition	[133]
LUVs	Berberine	Berberis, Coptis, Coscinium spps	Thin-film hydration method	Sarcoma cell carcinoma	**BALB/c mice** Humans Endothelial cells from umbilical veins (HUVECs)	Intravenous	1. Decreased cardiotoxicity 2. Increased therapeutic effectiveness without serious side effects.	[134]

(Table 2) cont.....

Types of PEGylated Liposomes;	Phytoconstituents	Plant Source	Preparation method	Diseases	Animal/Cell lines used	Route of administration	Major findings of PEGylated	Ref
SUVs	Silibinin and glycyrrhizic acid;	Silybum marianum, Glycyrrhiza glabra	Thin-layered film hydration method	Hepatocellular carcinoma	HepG2 cell line, fibroblast cell line	----	1. The biological activity of free drugs was increased by the nano-liposome encapsulation of silibinin with glycyrrhizic acid. 2. Enhanced the stability of the silibinin and glycyrrhizic acid when encapsulated in nanocarrier.	[135]
LUVs	Galbanic acid	Ferula species (Apiaceae)	Thin-film hydration	Anti-cancer and antiangiogenic activities	BALB/c mice, Umbilical vein endothelial cells (HUVECs)	Subcutaneous	1. Effective encapsulation of poorly water-soluble agents and galbanic acid. 2. Decrease the systemic toxicity 3. Boost the circulation time	[124]
MUVs	Silymarin	Silybum marianum	Film hydration method	Alcohol-induced hepatotoxicity	Wistar rats, Human Hepatocellular carcinoma (HepG2 cells)	Oral	1. Increased silymarin bioavailability 2. Increased silymarin solubility	[126]
LUVs	Osthole	Radix Angelicae pubescentis, Cnidium monnieri, Angelica pubescentis, and Peucedanum ostruthium	Thin-film hydration method	Alzheimer's disease	APP/PS-1 mice	Intravenous	1. Increased anti-Alzheimer's disease effects 2. Increased drug deposition in the areas of the lesions 3. Lengthening the drugs' blood circulation time	[119]
LUVs	*Glycyrrhizic acid*	Glycyrrhiza glabra	Thin-layered film hydration method	Colon Cancer	DLD-1 and LIM-2405 human colon cancer cell lines	--	1. Improved anti-cancer efficiency 2. Lessen side effects	[120]
SUVs	Dioscin	*Dioscorea nipponica; Dioscorea zingiberensis*	Film dispersion method	Anti-inflammatory, anti-multidrug resistance, anti-virus, anti-fungal infections hepatoprotective, and lipid-lowering	HeLa cells and HepG2 cells	--	1. Enhanced sustained release of drug 2. Increased the antitumor activity	[136]
LUVs	Salvianolic acid B	Sal*via* miltiorrhiza Bge	Film hydration method	Anti-hyperalgesic activity	Sprague–Dawley albino rats	intraperitoneally	1. Improved and extended the antihyperalgesic activity	[137]

10. Herbal Nanomedicines of SNEDDS and SMEDDS

In the recent era, the use of plant-derived medicines has gotten popular, and they have been directly or indirectly utilised by more than 80% of the world's population. Worldwide, up to 25% share of the pharmaceutical industry comes from phytomedicines [138]. Nowadays, the application of nanotechnology-based delivery systems such as nanoparticles and nanocapsules [139 - 141], liposomes [142, 143], nanoemulsions [144, 145], solid lipid nanoparticles [146], and self-emulsifying drug delivery systems (SEDDS) (same paper) have provided several benefits for the enhancement of phytoconstituent and physiochemical properties (solubility, stability, release), therapeutic potential (biodistribution, bioavailability, and pharmacological activity) as well as to reduce degradation and sustain delivery [147]. Therefore, NDDSs of herbal drugs have a prospective opportunity in improving the therapeutic activity and surmounting associated problems with their delivery [148 - 150].

SEDDS are isotropic thermodynamically stable solution formulations that comprise oils, hydrophobic surfactants, cosurfactants, and drugs that can instinctively form fine oil in water (O/W) micro/nanoemulsions in water in the presence of gentle agitation. Due to their micro/nanoscopic size, they are easily absorbed and taken up by lymphatic pathways and able to bypass the hepatic first- pass effect [150, 151].

The further SEDDS are categorised into two subcategories; SMEDDS (self micro-emulsifying drug delivery systems) and SNEDDS (self nano-emulsifying drug delivery systems), based on the size of droplets present in the emulsion. In SMEDDS, the size of the droplets ranges between 100 to 250 nm, while in SNEDDS, the size of droplets is found to be less than 100 nm [142, 145, 149, 152]. In comparison to conventional nano or microemulsions, due to thermodynamic stability and solubilisation potential, SEDDS formulations can easily enable appropriate oral administration *via* loading of lipophilic drugs into hard or soft gelatin capsules. Currently, there are four drug products based on SEDDS based formulation approaches available in the market, *i.e.*, Sandimmune® and Sandimmun Neoral® for cyclosporine A manufactured by Novartis Pharmaceuticals Corporation, Basel, Fortovase® for Saquinavir manufactured by Hoffmann-La Roche ltd., Basel, Switzerland) Switzerland, Norvir® for Ritonavir manufactured by Abbott Laboratories [153]. Research on SEDDS type delivery systems formulations, however, is currenly ongoing with several phytoconstituents having different therapeutic potentials, such as anticancer, anti-inflammatory, hepatoprotective, anti-inflammatory, anti-aphrodisiac, ulcer protective, cardio-protective, antioxidant, anti-obesity, antiviral, antimalarial, atherosclerotic and antiulcer, to improve their physicochemical properties and therapeutic

efficacy. This is summarised in Table **3**.

Table 3. Improvement of physicochemical properties and therapeutic efficacy of herbal nano-medicines through SEDDS.

System	Phytoconstituents source	Phytoconstituent	Biological activities	Emerging effect	Reference
SMDDS	*Kaempferia parviflora*	Methoxy flavone	Anti-inflammatory, anticancer, anti-aphrodisiac, peptic ulcer protective, antimicrobial, antiallergic and antiobesity.	Improved dissolution rate and oral bioavailability	[154]
	Fruits and vegetables (onions, orange, tea, chamomile and wheat sprouts)	Apigenin	Antioxidant, antitumor, anti-inflammatory, antiviral, and antianxiety	Improved solubility, dissolution and oral absorption of apigenin	[155]
	Curcuma longa	Curcumin	Anticancer, antimicrobial, anti-inflammatory, antiviral, antioxidant	Improved oral absorption of curcumin.	[156]
	Leaves of *Diospyros kaki*	Persimmon leaf extract (active constituents quercetin and kaempferol)	Antioxidant, anti-inflammatory, anticancer and antiallergic	Improved oral bioavailability of active flavonoids	[157]
	Dry rhizome of *Curcuma zedoaria*	Zedoary turmeric oil (ZTO)	Hepatoprotection, tumour suppressive, antioxidant antibacterial	Increased solubility and oral bioavailability of ZTO	[158]
	Root of *Scutellaria baicalensis*	Baicalein	Antiinflammatory, anticancer, antioxidant, antiviral and antiallergic	Increased bioavailability	[159]

(Table 3) cont.....

System	Phytoconstituents source	Phytoconstituent	Biological activities	Emerging effect	Reference
SMDDS	Leguminous plants (soybeans, soy foods and pueraria lobata ohwi)	Daidzein	Anti-hypertension, anti-atherosclerotic and anticancer	Dissolution rate and bioavailability increased	[160]
	sweet wormwood, *Artemisia annua*	Artemisinin (β-Artemether)	Antimalarial	Increased micro emulsifying efficacy, release and antimalarial activity	[161]
	Fruit of *Forsythia suspensa*	Phillygenin	Anti-bacterial, anti-inflammatory, antioxidant, anti-allergy, anti-viral and anti-cancer	Increased dissolution rate and oral bioavailability	[162]
	Herb of *Rabdosia rubescens*	Oridonin	anti-inflammatory, anticancer	Increased absorption and bioavailability	[163]
	Animals and most bacteria	Coenzyme-Q	antioxidant	Increased absorption and oral bioavailability	[164]
SNDDS	Milk thistle Silybum marianum	Silymarin (Flavono-lignans)	Hepatoprotective	Increased bioavailability (49 times) of silymarin	[165]
	seeds and fruits of *Schisandra chinensis*	Wurenchun (Schisandra lignans)	Hepatoprotective	Increased bioavailability (3 times) of wurenchun	[166]
	widely present in fruits and plants	Oleanolic acid (Penta-cyclic Triterpenoid)	Hepatoprotective and antioxidant	Increased bioavailability (2.4 times) of oleanolic acid	[152]

(Table 3) cont.....

System	Phytoconstituents source	Phytoconstituent	Biological activities	Emerging effect	Reference
SNDDS	*Ginkgo biloba*	Ginkgolide Diterpenoids	Cognitive Enhancer	1.5-fold increase in bioavailability of ginkgolide	[167]
	Extracts of *Camptotheca acuminata*	Camptothecin Pentacyclic alkaloid	anticancer	2.2-times increased bioavailability of camptothecin	[168]
	Black seed oil (*Nigella sativa*).	Thymoquinone Terpene	Hepatoprotective	3.87-times increased bioavailability of thymoquinones	[169]
	In grapes, blueberries, raspberries,mulberries and peanuts	Resveratrol Stilbenoid	Antioxidant and anticancer	Approx. 4.31-fold increase in bioavailability of resveratrol	[170]
	Ruta graveolens	Rutin Bioflavonoid	Antioxidant	Approx. 2.3-fold increase in bioavailability of rutin	[171]
	Green leafy vegetables like spinach, kale, and yellow carrots	Lutein Carotenoids	antioxidant	Increase bioavailability of warfarin (10%)	[172]
	Citrus fruits	Hesperetin Flavanone-glycoside	Hypolipidemic	Decreased nephrotoxicity and Increased drug bioavailability	[173]
	In the roots, rhizomes, stems, and bark of Berberis plants	Berberine Benzyl-isoquinoline alkaloids	Antimicrobial	Increased release kinetics of berberine	[174]
	Leaves of *Withania somnifera*	Withaferin A Steroidal lactone	Immunomodulator	Increased drug release	[175]

EMERGING CHALLENGES AND POTENTIAL SOLUTIONS OF PEGYLATED LIPOSOMES

Different polymer-based nanocarriers have elevated the levels of safety profiles, which are FDA-approved. As of now, the linear polymer PEG has demonstrated superiority over several FDA-approved synthetic polymers in various applications.

To enhance the pharmacokinetic properties of the carrier systems, which is the principle of PEGylation, a method of binding PEG molecules to various drugs, polymers, proteins, liposomes and nanoparticles either covalently or noncovalently, has been widely utilised. Despite the wide range of advantages provided by PEGylation, recent research has highlighted several main disadvantages of the system, such as non-degradability and potential accumulations with high molecular weight PEG that require practical-based investigation. The enormous advances in the chemistry of polymers and macromolecular engineering have led to the sense of hydrophilicity, water- solubility, including biocompatibility and several polymer systems such as poly(glycerol)s, poly(vinylpyrrolidone), poly(2-oxazoline)s, poly(amino acid)s and poly(N-(2-hydro-xypropyl) methacrylamide), which are still far from the capacity of PEG but none of them is yet to be approved by the FDA. Further investigations are needed to allow proper evaluation and comparison with PEG, and a detailed study of the polymer structure and its interaction with biological entities is important to identify any PEG alternatives.

There are a few issues that need to be addressed regarding PEG. The first one is that PEG does not completely prevent accumulated absorption by MPS cells. Not only that, the PEGylated liposomes are not biologically and completely inert, with evidence indicating that polymers would still induce the activation of the complement system as repeated injection of empty PEGylated liposomes have changed their pharmacokinetic activity, which would result in lower clinical effectiveness and even unintended side effects.

In treating cancer stealth, liposomes are important for their passive targeting effect, which can lead to preferential accumulation in tumour tissue. This phenomenon, however, is not fully understood. Unfortunately, the kinetics of drug release from liposomes into the interstitial space are not widely known; only the free drug can penetrate the solid tumour, and the ratio of the free/liposome- encapsulated drug in the extracellular fluid of the tumour is difficult to determine. The interaction of stealth liposomes with cell membranes and the release of the drug in the target tissue neighbourhood requires further investigation to address long-term protection, stability and cost-effectiveness; therefore, a novel research is needed.

CONCLUSION

PEGylated liposomes over conventional liposomes provide several benefits, such as long-circulating, enhanced bioavailability, targeted drug delivery as well as enhanced stability, and it is currently used for better chemotherapy. PEGylated liposomes are becoming one of the popular carrier systems for various drugs,

proteins, enzymes, nucleic acid, as well as herbal drugs. PEGylation process with several newer techniques are studied and established till now.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 7

Insulin-Liposomes

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Abstract: Liposomes are utilised as a delivery carrier in insulin therapy for several reasons; an enhancement in the oral absorption of insulin, the ability to selectively target insulin to the hepatic system, and prolong drug action for proper dose regimen. The hepatocytic delivery of insulin can be achieved efficiently through the insulin liposomal method. While treating diabetes with liver-targeted liposomes, it is expected that constraints will arise due to the formulation being supplied by an intravenous route. Furthermore, due to the dilute concentration of insulin in the liposomal formulation, the overall cost of the liposomal insulin would rise. The consequence of encapsulating the drug in the liposomal carrier is improved oral absorption of insulin. Drug action can be continued by giving subcutaneous liposomal insulin. Insulin remains at the site of injection, and the occurrence of a lipid matrix for subcutaneous insulin delivery raises concerns about over-improved antigenicity. The liposomal insulin sustains the role of a delivery system in understanding and treating diabetes using the hepatically targeted liposomal system. This pharmacological aspect has highlighted the role of the liver in the metabolic complications associated with diabetes mellitus.

Keywords: Diabetes, Hepatic, Insulin, Liposomes.

INTRODUCTION

Historically, liposome was first identified in the 1960s by Bangham and his co-workers. In 1965, Alec Bangham had referred to the closed bilayer phospholipids systems as “Bangosomes” which were later named “Liposomes.” The liposomal history is divided into three periods, *i.e.*, Genesis (1968-75), Middle age (1975- 85), and Modern Era (1985 onwards). The use of liposomes in drug delivery was proposed in 1971 by Gregory Gregoriadis. There was a number of problems identified with the first-generation liposomes, and attempts were incorporated to overcome these problems (Table **1**).

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Table 1. Problems of Liposomes that have been overcome.

S. No.	Problem	Attempts made to overcome the problems
1.	The drug molecules which were entrapped had leaked out of the liposomal membrane.	Cholesterol and sphingomyelin were added to the bilayer and resulted in the development of a solid phase instead of a liquid phase.
2.	Fast phagocytosis by the liver and spleen had reduced the distribution of drugs, which resulted in toxicity.	Grafting with PEG was done to develop PEGylated liposomes or "Stealth liposomes'. Moreover, the addition of cholesterol and sphingomyelin had resulted in delayed phagocytosis.
3.	Most of the drugs had required receptor-mediated endocytosis.	PEGylated liposomes were able to overcome this problem.
4.	Difficult to trigger the release of liposomal content.	This was overcome by designing the liposomes that were triggered by pH change, light, heat, and enzyme action.

BASIC STRUCTURE OF LIPOSOMES

Liposomes are artificial spherical-shaped vesicles, which are composed of phospholipids and cholesterol. The small size and its hydrophobic and hydrophilic characteristics make these vesicles extremely utilisable as a drug delivery system. The type of components used for the preparation of liposomes would determine the fluidity and rigidity of the system as well as the charges on it (Fig. **1**).

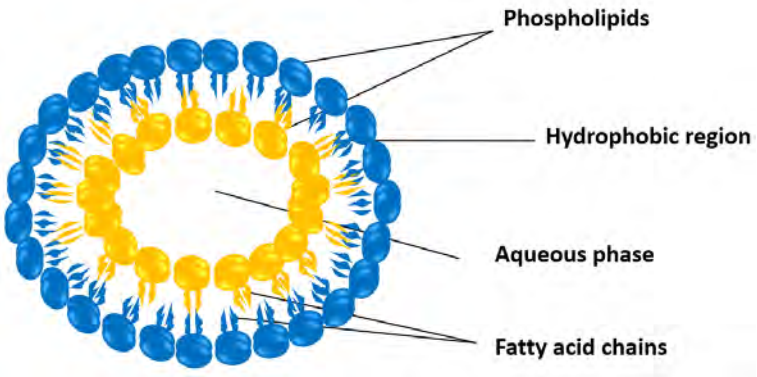

Fig. (1). Structure of Liposomes.

1. Phospholipids are amphipathic in nature. It consists of a hydrophilic (water-loving) polar head along with two lipophilic tails, thereby proving its affinity for both types of drug moiety, *i.e.*, hydrophilic drugs with the aqueous phase and hydrophobic drug moiety incorporated with the lipid bilayer. The abbreviations of

liposomes are shown in Table **2**. Frequently employed phospholipids in the generation of liposomes are mentioned in the following section [1].

Table 2. Abbreviation of Liposomes.

S.No.	Phospholipids	Abbreviations
1.	Phosphatidyl Ethanolamine	PE
2.	Phosphatidyl Choline	PC
3.	Phosphatidyl Serine	PS
4.	Phosphatidyl Glycerol	PG
5.	Phosphatidyl Inositol	PI

TYPES OF LIPOSOMES

1. Liposomes can be categorised based on their functioning and intracellular drug delivery method as follows: Conventional liposomes

2. Cationic liposomes

3. pH-sensitive liposomes

4. Immune liposomes

5. Long circulating liposomes

Liposomes are further classified into different subtypes according to their particle sizes, and these are shown in Table **3** and Fig. (**2**).

Table 3. Liposomes classified into subtypes based on their particle sizes.

S. No.	Liposomes	Particle size	Abbreviations
1.	Unilamellar Vesicles	All Size Range	UV
2.	Small Unilamellar Vesicles	20-100 nm	SUV
3.	Medium Unilamellar Vesicles	---------	MUV
4.	Large Unilamellar Vesicles	>100 nm	LUV
5.	Giant Unilamellar Vesicles	>1 μm	GUV
6.	Multi Lamellar Vesicles	0.5 nm	MLV
7.	Oligo Lamellar Vesicles	---------	OLV
8.	Multi Vesicular	>1 μm	MV

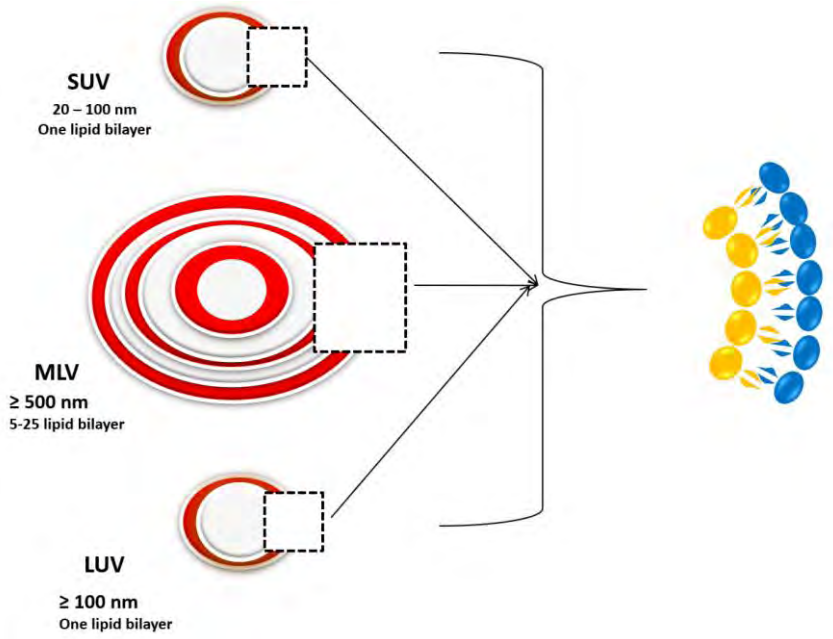

Fig. (2). Pictorial representation of liposomes categorised into subtypes based on their particle sizes.

Special Liposomes

- Bipolar fatty acids
- Antibody directed liposomes
- Methylene X-Linked liposomes
- Lipoproteins coated liposomes
- Carbohydrate coated liposome
- Multi-layered liposome

PREPARATION METHOD FOR LIPOSOMES

The preparation of liposomes can be carried out based on the four basic stages stated below [2]:

Lipid drying from organic solvent

↓

Dispersion of lipid in aqueous medium

↓

Resulting liposomal purification

↓

Analysis and evaluation of formed liposomes

METHODS EMPLOYED FOR THE FORMULATIONS OF LIPOSOMES (FIG. 3)

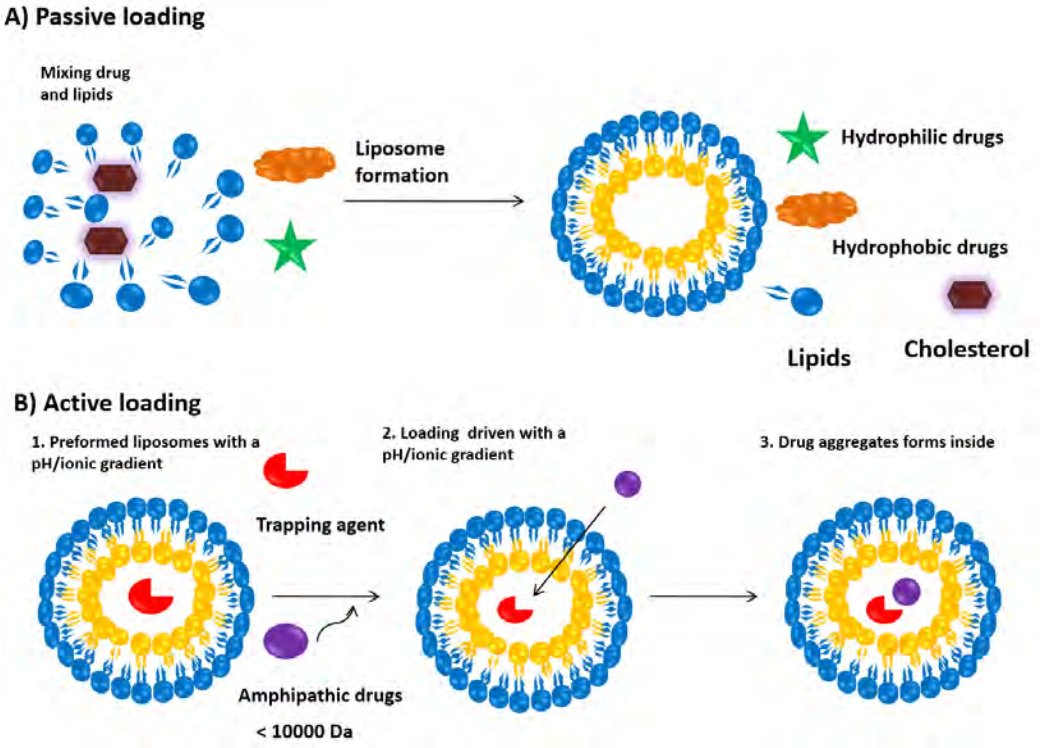

Fig. (3). Preparation of Liposomes.

1. PASSIVE LOADING TECHNIQUES

a) Mechanical Dispersion Method

Lipid is dissolved in non-polar solvent or co-solvent
↓
Removal of non-polar solvent using a vaccum
↓
Deposition of film
↓
Hydration of Solid-Lipid mixture using an aqueous buffer system
↓
Lipid instinctively swells up and hydrates
↓
Liposome formation

Mechanical dispersion can be carried out in the liposomes preparations by the following steps:

- Sonication method
- Using French pressure cell
- Defrost liposomes
- Freeze-dried lipid film hydration
- Micro emulisification
- Membrane expulsion
- Dehydrated reconstituted blister

b) Solvent Dispersion Method:

- **Ethanol Injection: –SUV:** Ethanolic lipid solution is quickly injected into an excess solution of the buffer. This would result in the formation of multi- lamellar vesicles. One of the demerits of this particular method is heterogeneous liposome formations (30-110 nm), which are extremely dilute in nature. Moreover, ethanol removal is difficult due to the formation of azeotropes with water, which results in the inactivation of a number of macromolecules due to the presence of ethanol.
- **Ether Injection -LUV:** Lipids solution is dissolved in diethyl ether or ether/methanol mixture, which is then slowly injected into an aqueous solution, encapsulated at a temperature of 55-65°C or under reduced pressure. The consequent elimination of organic solvent (ether) is carried out through a vacuum, which results in the formation of liposomes. This method is utilized for delicate lipids.
- **Reverse Phase Evaporation Vesicle –LUV:** Lipids along with organic solvent and aqueous solution are mixed. The resultant mixture is sonicated, thus ensuring the development of w/o (water in oil) emulsion. Then, evaporation is carried out to remove the organic solvent. Lipids form a phospholipid bilayer on forceful shuddering, whereby the water droplets collapse, and the formation of large unilamellar vesicles can take place.
- **Stable Plurilamellar Vesicles:** An active ingredient is first poured into an aqueous phase. This is then emulsified by using a polymeric organic solvent, which would result in the formation of primary emulsion. The primary emulsion is then subsequently agitated in an emulsifier containing the second aqueous solution to produce a double emulsion. This can be further obtained through centrifugation and filtration method.
    a. Detergent removal method: The dispersion of micelles is subjected to two different methods for the removal of detergent. These methods include dialysis and chromatography (Column Chromatography).

a. Dialysis: Dialysis is a technique to remove an entire free drug. This can be achieved by incorporating the changes in the dialyzing media. This method has its benefits as it is cost-effective with no expensive equipment utilization. In this case, detergents with high critical micelle concentration (CMC), such as octileglucoside and sodium deoxycholate, are utilised.
b. Column Chromatography: The sample dispersion is passed through the column chromatography (Sephadex G-25 grade). The dry film or sonicated vesicles in the ratio 2:1 form unilamellar vesicles (ULV) with deoxycholate. This can be removed by using column chromatography.

2. ACTIVE LOADING TECHNIQUE

After the completion of drying in the process

The formation of cake takes place using lipids

This resulted in the swelling in the fluid, which leads to lipid bilayer formation

Loading of the drug on pH gradient technique is being done

This ended in the formation of the liposomes (bilayered)

Insulin (Antidiabetic drug)

Insulin is an antidiabetic drug that is administered essentially for type-I diabetic patients in the initial phases. In later stages, this drug is prescribed for patients with type-II diabetes mellitus.

There are different approaches employed in delivering insulin to diabetic patients.

1. Insulin pump therapy has been utilised for patients with type-I diabetes for the last 40 years. However, there are possibilities for errors to occur with this particular system of drug delivery, such as failure in the pump functioning, blockage in insulin infusion set, a problem with the infusion site as well as stability issues with insulin. In case of such errors, the patients will suffer from either hyperglycemia or hypoglycemia and ketoacidosis. Therefore, there is a need for insulin delivery by other suitable methods to deliver the drug efficiently and minimise the occurrence of errors.

2. Syringes are the apparatus with a hollow center, plunger, needle, and removable needle guard. In an attempt to make this system more effective, the syringe sizes of 1 cc, ½ cc, 3/10 cc are provided along with alterations to the needle size to reduce discomfort during injection. However, the alteration adversely affects the onset of insulin action due to the lesser penetration that occured with a smaller needle.

3. Insulin pen is slightly similar to a normal pen, which is specifically designed for patients with weaker eyesight. These pens have the memory to recall their past doses; hence the possibility of overdose and underdose can be avoided. Moreover, these are prefilled, durable, and do not require to be stored in the refrigerator, unlike other insulin preparations. Nevertheless, insulin pens cannot be exposed to prolonged heat and cold after they are opened.

4. Jet injection method is suitable for patients who cannot tolerate needles. The injector contains several doses of insulin, and it is simple to use. However, bruising at the injection site is presented as a side effect of this method.

In the present context, it is necessary to discuss the storage conditions associated with insulin. Insulin is a very unstable drug; therefore, the storage conditions need to be maintained. Listed below are the conditions required for the storage of insulin:

1. Do not use the insulin after the expiration date.

2. Keeping the insulin for a few days at room temperature before use would cause discomfort when it is injected.

3. Do not expose the insulin to excessive cold and heat.

4. Extra insulin must be stored in the refrigerator.

MERITS OF LIPOSOMES

1. Liposomes amplify the effectiveness and therapeutic role of the drug.

2. Incorporating encapsulation in liposomes can increase stability.

3. Liposomes would decrease or reduce the toxicity of encapsulated agents.

4. They are biodegradable, flexible, biocompatible, and non-immunogenic.

5. They reduce the exposure of delicate tissue to a lethal moiety.

6. Specific ligands may be used to achieve targeted action.

Demerits of Liposomes:

1. The overall cost of production is extremely high.

2. Leakage of the amalgamation of encapsulated drugs.

3. Phospholipids may undergo hydrolysis and oxidation.

4. Lesser half-life (t1/2) and low solubility [3].

LIPOSOMES USED FOR INSULIN DRUG DELIVERY

Insulin therapy focuses on the enhancement of oral insulin absorption along with extended insulin action. It also attempts to achieve selective targeting of the drug (insulin) to the liver. All these can be achieved by the insulin liposomal drug delivery system. However, by treating diabetes through liver-targeted liposomes, it is expected that constrain would occur as the formulation is delivered through an intravenous route. Moreover, the overall cost of the liposomal insulin would increase due to the dilute concentration of the insulin present in the liposomal preparation. The entrapment of insulin in liposomes (insulin liposomes) might result in enhanced absorption by the oral route. However, this combination can be used to sustain the insulin action in liposomes that are given subcutaneously (Fig. **4**).

ISSUES RELATED TO ORAL LIPOSOMAL UTILIZATION

Stability and consistency pose a big challenge while administering liposomes orally. The formulation, which consists of liposomes, is a system of vesicles suspended in physiological media. However, the suspension of liposomes over a long duration of time is highly unstable. In a published report on liposomes containing sodium glycocholate, it was suggested that the highest oral bioav-

ailability of 8.5% (non-diabetic rats and 11.0% (diabetic rats) could be achieved. This liposomal delivery containing SCG had a good prospective on oral insulin delivery with better fortification against catalytic deprivation. Furthermore, the shelf-life stability and the ability of the liposomes to remain intact till they reach the target site are the important parameters for commercial utilisation.

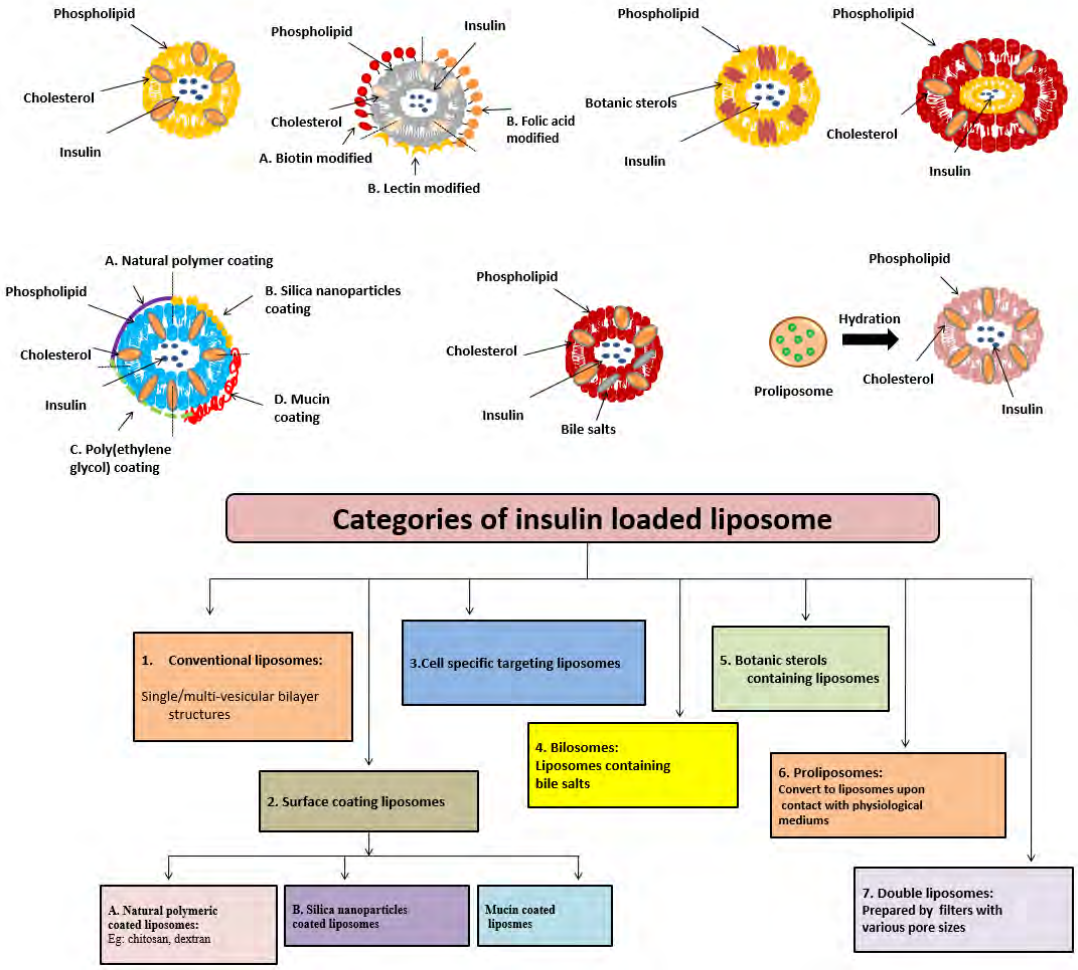

Fig. (4). Entrapment of insulin in liposomes.

Several approaches such as lipid composition alteration, management of particle size, freeze-drying method, and electro-stearic stabilization have been utilized to overcome issues associated with liposomes. In particular, liposomes prepared through the freeze-drying method have good stability prospects.

COMMERCIALLY MARKETED LIPOSOMES

Currently, commercially marketed liposomes are abundant in numbers (Table **4**), which is due to the advantages of liposomal. The modification and enhancement in the action of drug moiety using liposomes play a key role in their commercial and economic outbreak [4, 5].

Table 4. Commercially marketed liposomes.

S. No.	Purpose of Formulation	Drug (Product name)	Manufacturing Company
1.	Solid tumors	Vincristine (**VincaXome**)	NeXstar Pharmaceuticals Inc.
2.	Systemic inflammatory diseases	Prostaglandin E1 (**Ventus**)	The Liposome Company, USA
3.	Asthma	Terbutaline sulphate (**Topex Br)**	Ozone Pharmaceuticals Ltd.
4.	Systemic fungal infection	Nystatin (**Nyotran™**)	Aronex Pharmaceutical Inc.
5.	Bacterial infection	Amikacin (**Mikasome**)	NeXstar Pharmaceutical Inc., USA
6.	Serious fungal infection	Amphotericin B (**Fungizone)**	Bristol-Myers Squibb, Netherland
7.	Serious fungal infection	Amphotericin B (**Abelcet™**)	The Liposome Company, USA
8.	Serious fungal infection	Amphotericin B (**Amphocil™**)	Sequus Pharmaceutical Inc.
9.	Serious fungal infection	Amphotericin B (**Ambisome™**)	NeXstar Pharmaceutical Inc.
10.	Leishmaniasis	Amphotericin B (**Amphotec**)	Sequus Pharmaceutical Inc.
11.	Hepatitis A	Inactivated hepatitis-A Virions (**Epaxal-Berna vaccine)**	Swiss Serum and Vaccine Institute, Switzerland
12.	Breast cancer (metastatic type)	Doxorubicin (**Evacet™**)	The Liposome Company, USA
13.	Kaposi sarcoma in AIDS	Doxorubicin (**Doxil**)	Sequus Pharmaceutical Inc.
14.	Menopausal Therapy	Estradiol (**Estrasorb**)	Novavax
15.	Lymphomatous meningitis	Cytarabine (**Depocyt**)	Pacira Pharmaceutical Inc.
16.	Kaposi sarcoma in AIDS	Daunorubicin citrate (**DaunoXome)**	Galen Ltd
17.	Kaposi sarcoma in AIDS	Daunorubicin citrate (**DaunoXome™)**	NeXstar Pharmaceutical Inc., USA
18.	Chicken pox	Killed Avian retrovirus (**Avian retrovirus vaccine**)	Vineland Lab, USA
19.	Expanding lung disease in infants	Dry protein-free powder of DPPC-PG (**ALEC™**)	Britannia Pharm, UK
20.	Acute promyelocytic leukemia	Tretinoin (**Atragen™**)	Anorex Pharmaceutical Inc.

(Table 4) cont.....

S. No.	Purpose of Formulation	Drug (Product name)	Manufacturing Company
21.	Post-surgical Analgesic	Morphine (**DepoDur**)	Pacira Pharmaceutical Inc.

CONCLUSION

The recent advancement in the drug delivery carriers for insulin has provided an edge to the users. The researchers have developed liposomal insulin for the efficient delivery of drugs to diabetic patients. This delivery system for the drug attempts to unravel the problems associated with the conventional delivery routes. Moreover, the use of chitosan for insulin delivery enhancement can be seen in positive ways. In a matter of time, the advancement and modifications in insulin liposomes can prove to be a revolution in the drug delivery system.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 8

Aquasome: A Promising Novel Drug Carrier

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Abstract: Aquasomes are ceramic nanoparticulate drug delivery systems, and they are utilised for the delivery of antibiotics, hormones, peptides, genes, and proteins. Structurally, aquasomes are self-assembled, consisting of three layers, where the solid core is coated with an oligomeric film. Bioactive molecules or therapeutic agents are adsorbed at the oligomeric film. The structural stability of this nanocarrier is provided by the centre core, whereas the oligomeric film provides protection against dehydration and stabilizes the active biological molecules. Active biochemical molecules with or without modification are incorporated at the oligomeric film by diffusion, adsorption, or copolymerization. It has been established that drug candidates have shown better biological activity and immune response when they are delivered through aquasomes. Insulin, poorly water-insoluble drugs, enzymes, and haemoglobin have been delivered through aquasomes successfully. Therefore, aquasomes provide a new approach to delivering a wide range of therapeutics such as vaccines, proteins, and peptides.

Keywords: Aquasomes, Drug delivery, Nanoparticles, Peptides, Proteins.

INTRODUCTION

Aquasomes are nanoparticulate, self-assembled three-layered (core, coating, and drug) drug delivery systems, which comprise a nanocrystalline solid core that is coated with an oligomeric film in which therapeutically active molecules are adsorbed (Fig. **1**). Van der walls forces and ionic and non-covalent bonds are involved in the self-assembling of ceramic core, coating with oligomers, and drug loading. During the preparation of aquasomes, a coating of sucrose, trehalose, or cellulose is applied at the surface of the ceramic core to obtain the desired sugar ball, and it is used to adsorb the biochemically active materials [1 - 4].

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Swarnlata Saraf, Ram Kumar Sahu & Vivek Dave (Eds.)

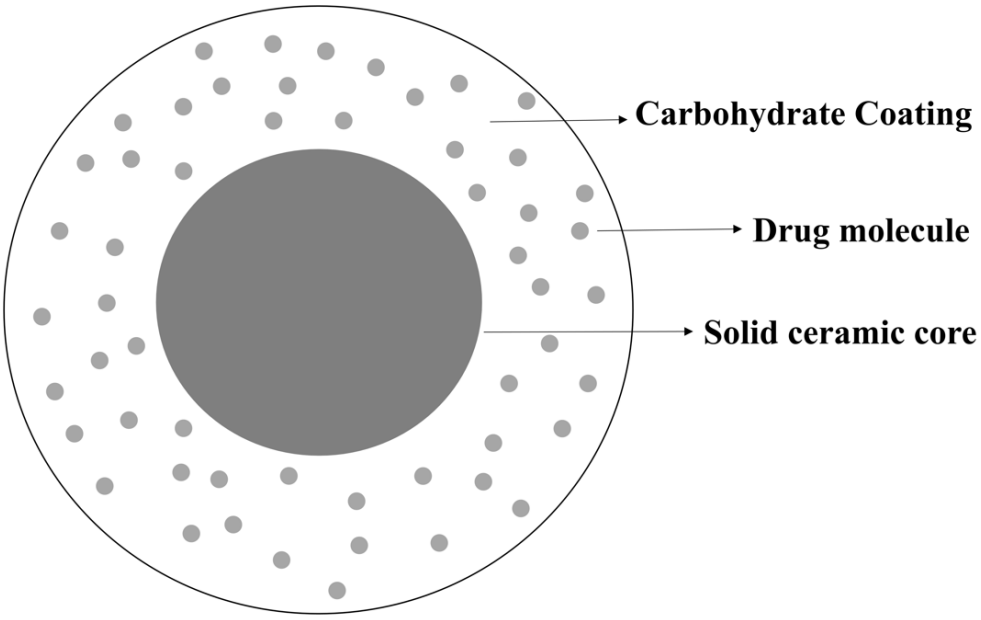


Fig. (1). Structure of the aquasome.

The structural stability of the aquasomes is provided by the ceramic core, whereas the oligomeric film provides protection against dehydration and stabilizes the active biological molecules. To decrease the structural damage of the biologically active material, the ceramic core of the aquasomes is modified with carbohydrates. Aquasomes are also known as bodies of water, and this water-like property of the structure protects the biological molecules that are fragile and need to be preserved. In these carbohydrate stabilized ceramic nanoparticles, therapeutic agents are incorporated by adsorption, diffusion, or copolymerisation. Aquasomes are promising drug delivery systems as they protect the integrity of the therapeutic agents. The film of the solid carrier is treated with carbohydrates, which prevents interactions between the therapeutic agent and solid carrier while at the same time preventing the denaturing of the biologically active material. It also maintains the arrangement of atoms in the drug molecule and provides optimum therapeutic action. These biologically active molecules possess the following qualities:

a. Freedom of bulk movement
b. Specific three-dimensional molecular arrangement
c. Molecular rearrangement due to molecular interactions

All the above qualities should be maintained to achieve the desired pharmacological action. These spatial qualities of the biologically active molecules can be altered by dehydration and decomposition [2, 4]. Many substances like proteins undergo denaturation in an aqueous medium due to the effect of pH, temperature, and salts. They also undergo permanent denaturation when they are desiccated and lose their action. Such types of substances can be delivered *via* aquasomes with natural stabilizers. The natural sugars in the aquasomes maintain a water-like state and prevent any molecular conformations of the biological molecules. Furthermore, these carbohydrates or oligomers possess hydroxyl groups in their structure. These hydroxyl groups interact with the polar groups of the protein and preserve its molecular structure on dehydration [4, 5].

Properties of Aquasomes

1. The large active surface area of the aquasomes helps in the effective loading of the therapeutic agent.

2. The mechanism of drug release from the aquasome is governed by its surface chemistry. It helps in specific targeting as well as drug release in a sustained manner.

3. Structurally, aquasomes are more stable and prevent the degradation of the drug from any environmental challenges.

4. The water-like property of the aquasomes helps in preserving the molecular integrity of the biologically active molecules [4 - 7].

Composition of Aquasomes

Aquasomes consist of core material, coating material, and biologically active material [5 - 7].

Core Material

Aquasomes can be prepared by three types of the core material. They are as follows:

a. Tin oxide
b. Ceramic
c. Calcium phosphate dehydrate (brushite)

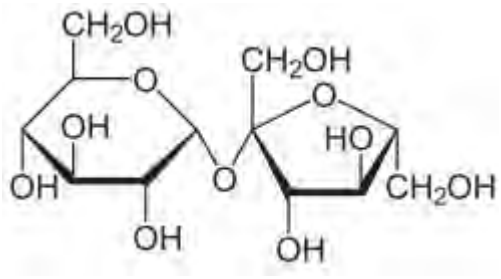
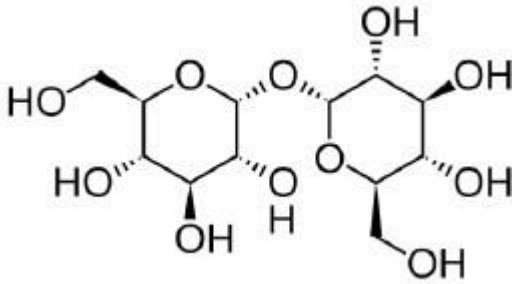
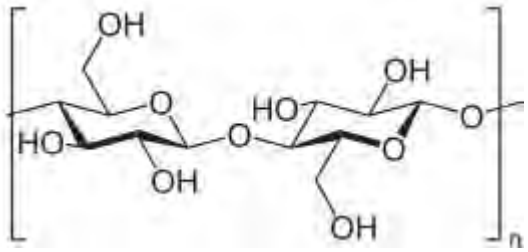
Ceramics are more commonly used as they are structurally more regular and

crystalline. The crystalline structure of the ceramic material ensures its high degree of order. Polymers, such as gelatine, albumin, or acrylate, are also utilised as core materials.

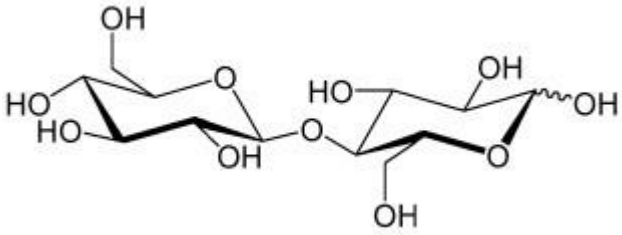
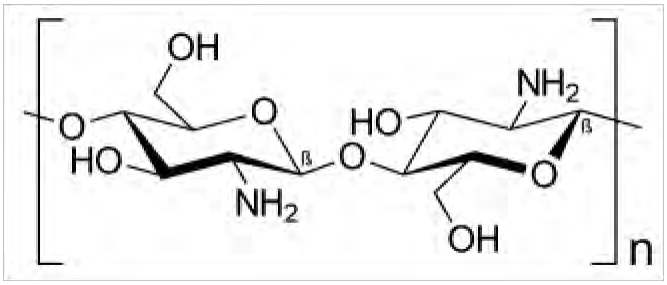
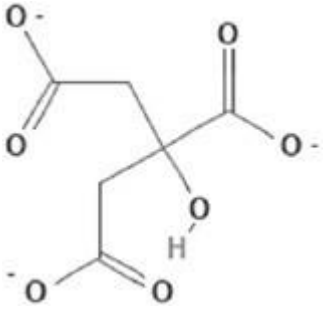
Coating Material

Various coating materials, such as sucrose, cellobiose, and chitosan, are utilised as coating materials. Generally, the therapeutically active substances are adsorbed in the coating material. Coating materials used for the preparation of aquasomes are listed below in Table **1**:

Table 1. List of coating materials used to prepare aquasomes.

Coating material	Structure
Sucrose	
Trehalose	
Cellulose	

(Table 1) cont.....

Coating material	Structure
Cellobiose	
Pyridoxal 5 phosphate	
Chitosan	
Citrate	

Bioactive Material

Aquasomes are predominantly used as bioactive materials to deliver proteins and peptides as they are capable of overcoming various issues related to the delivery

of these molecules (Table **2**). There are several challenges related to the delivery of proteins and peptides such as:

a. Physical and chemical instability
b. Poor bioavailability
c. Side effects

Surface modification of the ceramic nanoparticles with carbohydrate creates a stable film that adsorbs the bioactive materials *via* ionic or non-covalent interactions, which cause minimum denaturation of proteins and peptides [8, 9].

Table 2. Several therapeutic agents delivered *via* aquasomes.

Therapeutic agents	Applications
Insulin	Regulation of blood glucose
Haemoglobin	Oxygen carrier and blood component
Serum Albumin	Used to maintain oncotic pressure
Serratiopeptidase	Enzyme
Hepatitis B vaccine	Effective immunization
Hydroxyapatite	Immunoadjuvant

Preparation methods of Aquasomes

Aquasomes are prepared in three steps (Fig. **2** and **3**)

a. Formulation of the core
b. Coating of the inorganic core with oligomers
c. Drug loading

Formulation of the Core

There are several inorganic materials utilised to prepare the core of the aquasomes, such as brushite, tin oxide, and nanocrystalline carbon ceramics. The fabrication process of the core is selected depending on the material. Brushite, which is also known as calcium phosphate dehydrate and tin oxide, is not usually used to prepare the core as it suffers from stability issues. Upon prolonged storage, brushite is converted to hydroxyapatite, and this affects the stability of the preparation. Due to this, ceramic materials are mainly utilised instead as they are stable and possess specific regular structures. Core fabrication can be done through various methods such as sonication, plasma condensation, and precipitation [4, 6, 7].

Fig. (2). Steps involved in the preparation of the core material.

Coating of the Inorganic Core with Oligomers

Carbohydrates are used to coat ceramic cores. This coating process is carried out by adding carbohydrates (polyhydroxy oligomer) in the aqueous dispersion of the core under sonication. Following that, lyophilisation takes place to help in the irreversible adsorption of polyhydroxy oligomers onto the ceramic core surface. Then, it is centrifuged to remove the excess carbohydrate [6, 7].

Drug Loading

The drug is adsorbed at the coating material. During this process, the coated particles are dispersed onto the solution of the drug (known concentration). The pH of the solution is maintained by the buffer. The dispersion, which contains the drug, and the coated particles, are kept overnight for drug loading. The dispersion can also be lyophilised to obtain the final drug-loaded aquasomes [3, 6, 7].

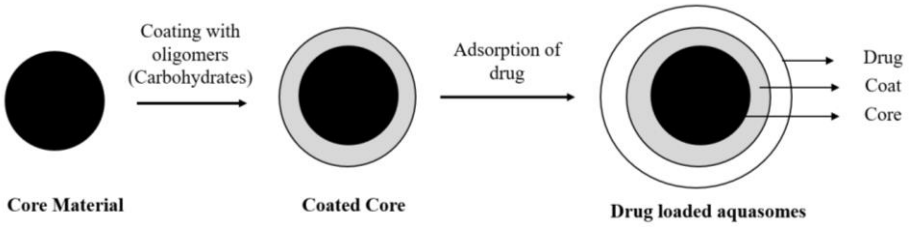


Fig. (3). Formation of aquasomes.

MECHANISM OF SELF ASSEMBLY AND STABILIZATION OF PROTEINS

There are three physicochemical processes that govern the self-assembling of macromolecules in the aqueous medium, which are:

1. Interactions between the charged groups

2. Hydrogen bonding and dehydration effect

3. Structural stability

Interactions between the Charged Groups

Self-assembly of the macromolecules is facilitated by the interaction between the charged groups. The interaction between charged groups will facilitate the self-assembling of macromolecules in the aqueous environment and stabilized the tertiary structure of the folded proteins. The intrinsic chemical groups or adsorbed ions from the biological milieu would lend a charge polarity to a majority of the biological and synthetic surfaces. Due to this, the biologically active molecules would possess both positive and negative charges. The interaction between these various charged groups like phosphate, carboxyl, amino, and sulphate would help in the formation of a long chain. These long-chain will facilitate the self-assembly of the aquasomes, and this is considered as the initial phase of self-assembling [10, 11].

Hydrogen Bonding and Dehydration Effects

The stabilization of the protein structure and base-pair matching is done through hydrogen bonding. Hydrogen bonds which are formed by the molecules are hydrophilic, and they help in the organisation of the surrounding molecules. Hydrophobic materials do not form hydrogen bonding, but they repel water, which helps in organizing the moiety to the surrounding environment.

Structural Stability

Hydrogen bonding and Van der Waal forces play an important role in providing structural stability of the proteins. They are also responsible for maintaining the secondary structures, hardness, and softness of the molecules [1, 10, 12].

APPLICATION OF AQUASOMES IN THE DELIVERY OF PROTEINS AND PEPTIDES

Insulin and Insulinomimetics Delivery

The parenteral administration of insulin using aquasomes with the help of a calcium phosphate ceramic core was reported by Cherian and coworkers. In this present study, different disaccharides were used to coat the ceramic core, and the drug was loaded with the help of the adsorption process. The antidiabetic *in vivo* study for the prepared formulation was tested on albino rats. Apart from cellobiose-capsuled particles, these different formulations of the aquasome had shown a significant decrease in glucose levels. Pyridoxal-5-PO_4-capsuled particles had shown a significant decrease in the blood sugar level as compared to the other formulations. Trehalose or cellobiose disaccharides that were used in aquasomes had been reported to exhibit the intermediate effect. The long effect, however, was due to the

morphological structure of the peptide and the late delivery of the drug from the carrier. Umashankar *et al*. (2010) had also reported that aquasomes with antidiabetic polypeptide k, which was collected from *Momordica charantia* seeds, had shown reduced sugar levels in the blood. The general idea behind the preparation of aquasome is to prepare it by colloidal precipitation, followed by sonication by disodium hydrogen phosphate and calcium chloride at cool conditions. Then, the polymer is covered with cellobiose and trehalose and subsequently loaded with polypeptide-k. Different parameters, such as physical characteristics, structure, load efficiency of the formulation, were considered. The in vitro and *in vivo* studies for antidiabetic by using albino Wistar rats had also been reported. Initially, there was a rapid release of the drug, which was reduced in the second phase, which had helped in the release of the drug to the possible surface release of the polypeptide. An initial faster release rate was observed, followed by a slower release rate in the second phase, which was attributed to possible surface desorption of polypeptide and sustained release rate of the polypeptide from the aquasomes matrix. It was observed that trehalose-coated aquasomes had released polypeptide-k faster than cellobiose-coated aquasomes. The *in vivo* study concluded that oral administration was not effective in producing any change in the serum level. The aquasomal formulation, however, was able to reduce the serum glucose level [6, 7, 13].

Delivery of Antigens

Aquasomes are also reported for the delivery of the antigen. The adjuvants are useful in developing the immune system, enhancing immunity against foreign bodies. This is due to their tendency to change the stereochemistry of the foreign body by adsorption or to protect the functional groups. Kossovsky and his coworkers (1995) had made and tested the efficiency of a ceramic antigen delivery vehicle using organic compounds. The study involved the diamond substrate coated with oligosaccharide (cellobiose) film and an active surface molecule in a water dispersion that was immunologically active. The particle size of the aquosome, which was in the range of 5- 300 nm, had provided conformational stabilization as well as large surface exposure to protein antigen. These aquasomes with the size of 5-300 nm had provided stereochemical stability as well as more degree of surface exposure to new protein antigen. These types of aquasomes are preferred to be used for adsorption and adhesion of cellobiose as they consist of a diamond substrate. Thus, this particular aquosome could produce strong and specific immune responses and could be elicited by increasing the presence and *in vivo* activity of the antigen. In another study, the aquasome was loaded with bovine serum albumin. It was made by constituting hydroxyapatite with the help of the co-precipitation method. Cellobiose and trehalose were used to coat the core, and bovine serum albumin was adsorbed as an antigen onto the coated core. The aquasomes were

structurally spherical, with a diameter of approximately 200 nm. Around 30% of the drug was loaded in aquasomes. The study revealed that the aquasome formulation had higher activity as compared to plain BSA. Hence, the aquasome was suggested to have superior surface immutability, allowing them to inhibit protein production and project to immune cells that had produced a positive immune response. (Vyas *et al*. 2008). The hydroxyapatite ceramic carrier was made with the help of co-precipitation. This carrier had shown better absorption efficiency of immunogens. The carriers were smaller in size, but they possessed large surfaces. These nano-ceramic carriers could act as vehicles for the hepatitis B vaccine for effective release and immunisation, as reported by Vyas and co-workers . This hydroxyapatite core was initially encapsulated with cellobiose, after which the hepatitis B antigens were absorbed over the surface of the core [5, 9].

Delivery of Enzymes

The nanoparticle ceramic carrier system was also used for the oral administration of the acid-sensitive serratiopeptidase. The formulation was prepared by using colloidal precipitation through sonication at room temperature (Rawat *et al.* 2008). In this formulation, the core was covered with chitosan with regular stirring, and the enzyme was absorbed on it. Thereafter, the enzyme absorbed was again covered with alginate gel. The spectral analysis of the prepared aquasome was performed by using transmission electron microscopy (TEM). The percentage drug loading was also calculated and evaluated, including the *in vitro* drug release studies. The reports of the TEM had concluded that the particles were spherical, with a diameter of 925 nm. The drug loading efficiency was found to be 46 percent in the evaluation reports. The *in vitro* drug release was performed using the Higuchi model at 1.2 pH for 6 hours. At pH 7.4, the drug was sustained and completed first-order release of the drug till 6 hours. This aquasome formulation was suggested to have better therapeutic efficacy as it protected the structural integrity of enzymes [14].

CHARACTERIZATION OF AQUASOMES

Structural analyses of aquasomes are performed based on their organisational and morphological properties, particle size distribution, and drug loading capacity. Three layers of aquasomes, *viz*. ceramic core, sugar coating, and drug-loaded aquasomes are examined by utilising the following procedures (Fig. **4**) [7, 12, 15, 16].

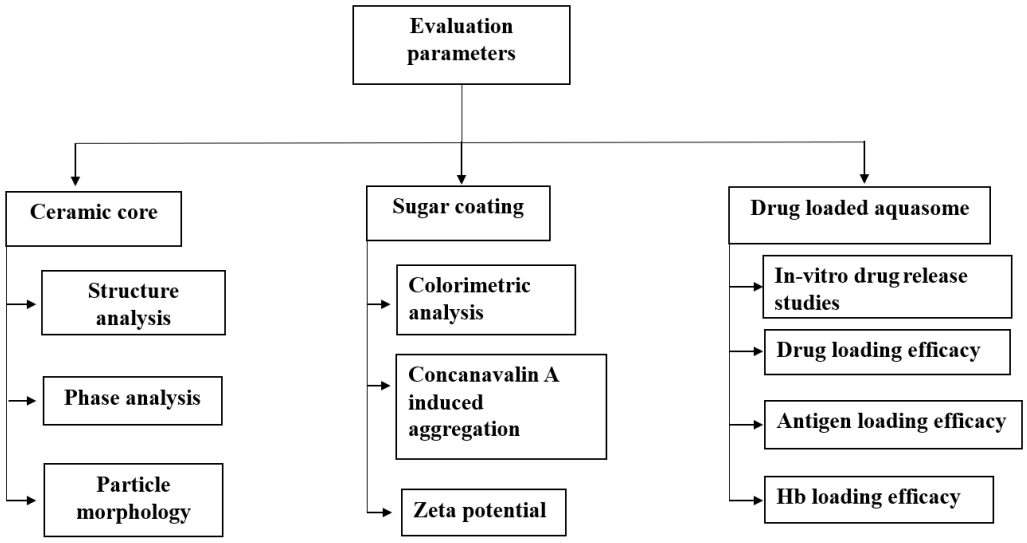


Fig. (4). Evaluation parameters of aquasomes.

Characterization of Ceramic Core

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy method is primarily used for the determination of various functional groups in the ceramic core nucleus. Among various methods, the KBr pallet technique is preferred for the structural integration of chemical structures of the ceramic core. Later, the spectral data is compared with the reference library to distinguish sugar and drug-loaded over the ceramic core [5, 15, 17, 18].

X-ray Diffraction

Crystallinity or amorphous behaviour of the ceramic core is analysed by the X-ray diffraction method. X-ray diffraction, a non-destructive technique, is primarily used to analyse the crystalline/amorphous nature of a compound. It delivers vital information regarding phases and orientations. X-ray diffraction peaks are generated as a result of the transmission of a monochromatic beam of X-rays that are scattered at specific angles from each set of lattice planes in a sample (Fig. **5**). The peak intensities are considered as a fingerprint of atomic arrangements in the sample (ceramic core), which can be compared with the existing diffractogram for further interpretations [5, 9, 12].

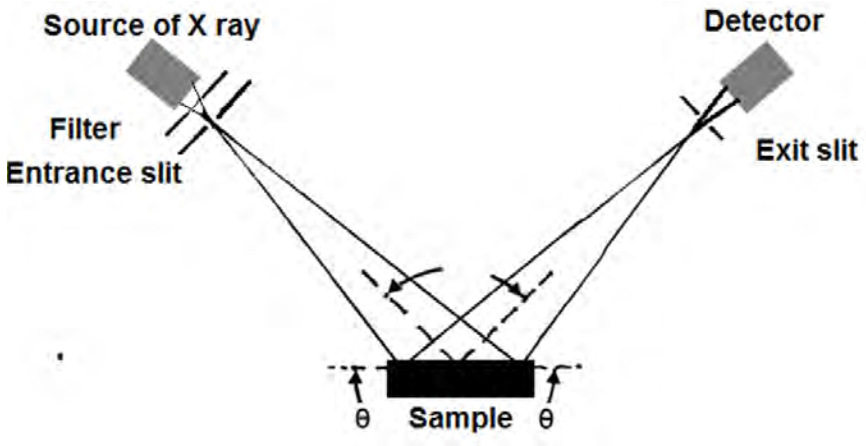


Fig. (5). Schematic diagram of an X-ray diffractometer.

Characterization of Coated Core

Carbohydrate Coating

Sugarcoating over the ceramic core is an important phenomenon in the preparation of aquasomes to bind the hydrophilic compounds. Sugarcoating is performed by concanavalin A-to induce the aggregation process. Moreover, the anthrone method is also used to determine the residual sugar unbound or residual sugar that is remaining after coating.

The zeta potential can be used to evaluate the charge stability of aquasomes. It is used to quantify the magnitude of the electrical charge of the sugar coating. Measurement is taken by applying an electric charge across the sample in a folded capillary flow cell [5, 13, 15].

Glass Transition Temperature

The glass transition temperature is termed as the temperature at which long-chain carbon chains of an amorphous region of aquasomes would begin the transition from rigid to a more flexible state, thereby shifting the temperature at the border of the solid-state to rubbery state on melting the glass. Differential scanning calorimetry studies are used to study the glass transition temperature of carbohydrates and proteins and their effects on aquasomes [5, 13].

Characterization of Drug-Loaded Aquasomes

Drug Payload

The loading efficiency of the drug in aquasome s formulation can be determined

by transferring the required quantity of formulation to a volumetric flask where the drug is dissolved in phosphate buffer solution (pH 6.8). Then, the solution can be diluted with a suitable concentration of sodium hydroxide to obtain a clear solution. The clear solution is centrifuged to obtain a supernatant liquid, where the drug payload is examined by determining the λmax using various spectrophotometric analyses, such as UP-LC and HPLC [7].

In Vitro Drug Release Studies

The *in vitro* drug release kinetics provides information about the safety, efficacy, quality, and drug release pattern from aquasomes. The kinetics of drug release can be evaluated by incubating a known concentration of drug-loaded aquasomes in a buffer of suitable pH at 37.4°C under continuous stirring. At a regular time interval, the samples can be collected and centrifuged for a specified period to obtain the supernatant. After each sampling, the same volume of the fresh medium that is maintained at the same temperature should be replaced. Supernatants collected are then diluted with the suitable buffer solution and evaluated spectrophotometrically at specific λmax of the drug. Then, the percentage cumulative drug release is accessed from the absorbance values [5, 13].

SDS-PAGE

Polyacrylamide gels (PAGE) are formed through the interaction of acrylamide and bis-acrylamide (N,N'-methylenebisacrylamide), which produce a highly cross-linked gel matrix. The gel acts as a sieve in which proteins are separated based on their molecular weight when they are subjected to the electric field. SDS (also called lauryl sulfate) is an anionic detergent, and it is strongly attracted towards an anode in an electric field. On the other hand, PAGE would restrain larger molecules from migrating as fast as smaller molecules. When test compounds are placed simultaneously with reference proteins, the relationship between R_f and mass can be plotted, and the masses of unknown proteins can be estimated. SDS-PAGE can be performed to determine the stability and integrity of protein during the formulation of the aquasomes [5, 13].

FUTURE PROSPECTS AND CHALLENGES

The nano ceramic core of the aquasomes can help with the better delivery of bioactive compounds. Hence, it can be useful in amino acids or peptide delivery. It can also be used as an immunoadjuvant for proteinaceous foreign materials to produce a better immunological response. These aquasomes can increase the pharmacodynamic and pharmacokinetic profiles of bioactive molecules. Therefore, it is important to perform the pharmacokinetics and *in vivo* studies as well as the

toxicity studies to authenticate its safety and efficacy for clinical use in society.

CONCLUSION

Aquasomes can play a vital role in delivering bioactive proteins and peptides. Carbohydrate that is a coating on the structure of the aquasome prevents the interaction between bioactive material and carrier and preserves the structural integrity of proteins and peptides. Aquasomes can also be used as immunoadjuvants as they produce a better immunological response. However, to establish their clinical usefulness and safety, a detailed pharmacokinetic and toxicological study has to be performed. In conclusion, aquasome is one of the best carriers for the delivery of vaccines, proteins and peptides.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 9

Nanoparticle-aided Herbal Drugs: Therapeutic Implications on Cholinergic Dysfunction with Relevance to Alzheimer's Disease

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Abstract: Being a notable form of neurodegenerative disorder, Alzheimer's disease (AD) accounts for the cognitive decline of a wide range of the population globally. Any form of dysfunction to the cholinergic system of the brain marks the onset of cognitive decline and paves the way for progressive neurodegeneration in AD. Alteration in acetylcholinesterase activity, accumulation of beta-amyloid protein, mitochondrial dysfunction, oxidative stress, and neuroinflammation are some of the marked gateways to the pathogenesis of AD. Although nature harbors a wide array of herbal cures to various gateways of cholinergic dysfunction, there exist certain restrictions to efficient delivery and therapeutic action of the phytocompounds *in-vivo*. Despite bearing certain reversible limitations, the application of nanoscience has successfully cleared off several barriers from the drug designing and delivery of herbal extracts and enriched the therapeutic potentiality of the medicinal plants that have been practiced extensively since time immemorial. Several forms of nanoparticles (NP) have been designed to date *viz*., polymeric NP, lipid-based NP, metallic NP, each having their characteristic advantage as drug carriers. In addition to advantages like high drug loading capacity, target-specific drug release, high bioavailability, *etc*., the ability to penetrate the Blood- Brain Barrier (BBB) non-invasively makes the nanocarriers most suitable for delivering herbal drugs targeting neurodegenerative disorders. The present chapter, therefore, discusses the therapeutic qualities of several herbal compounds targeting cholinergic dysfunction and the remarkable milestones set by nanotechnology in amplifying the potentiality of the herbal drugs in the treatment of AD.

Keywords: Acetylcholinesterase, β-amyloid, Blood-brain barrier, Curcumin, *Cuscuta chinensis*, Dendrimer, GFAP, Herbal extract, Liposome, Micelle, Mitochondrial dysfunction, Nanocarrier, Neuroinflammation, Oxidative stress, Pathophysiology, Piperine, Polymeric, Quercetin, Resveratrol, Therapeutic.

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INTRODUCTION

A large group of the population worldwide becomes victim to Alzheimer's disease (AD), whereby the ability to acquire, learn, process, memorize, think, and retrieve stimuli gets tremendously impaired. Dysfunction in brain cholinergic neurons stands as the prime source of neurodegeneration in AD. Altered activity of the enzyme acetylcholinesterase (AchE) leads to the disrupted transmission of acetylcholine (Ach), a neurotransmitter that mediates the cholinergic neurons and is critically responsible for the cognitive behavior of animals [1, 2]. Subsequently, excessive production and aggregation of β-amyloid protein at cholinergic synapses further hinders neurotransmission [3]. Structural and functional aberrations of mitochondria, oxidative stress, and neuroinflammation serve as additional hallmarks to cholinergic dysfunction [4 - 6]. Several therapeutic approaches have come out to tackle this neurodegenerative disorder. However, the best remedy to any form of disorder resides in the lap of nature, and this is true for treating cholinergic dysfunction. Several herbal species like *Withania somnifera, Centella asiatica, Curcuma longa, Cuscuta chinensis*, *etc.*, are reported to be excellent antioxidants, anti-inflammatory, and neuroprotective agents [7]. Despite possessing several qualities, the use of phytocompounds as therapeutic drugs comes along with some major drawbacks such as poor aqueous solubility, bioavailability, and target-specific efficiency, inability to cross BBB non- invasively, and allied toxicity [8]. Nevertheless, the introduction of nanotechnology in formulating, designing, and delivering drugs *in-vivo* helps overcome the limitations of phytocompounds. While the advantages of nanoparticles pave the way for a revolution in the field of medicines, the limitations which come along cannot be overlooked. Nevertheless, the limitations are reversible enough to be dissolved by the implementation of advanced strategies. Numerous studies demonstrated the successful nanoformulation of herbal extracts like curcumin, quercetin, piperine, resveratrol, and the biologically active compound of *Cuscuta chinensis* [9 - 12]. Formulating herbal extracts with polymeric, lipid-based, and metallic nanoparticles resulted not only in their target- specific delivery *in-vivo* but also enhanced their bioavailability and bio-efficiency, thereby accelerating their therapeutic actions in ameliorating cholinergic dysfunction and improving memory and cognition in AD subjects. This chapter hereby discusses the various forms of nanoparticles used as drug-carrier and some of the major milestones set in designing nanoparticle aided herbal drugs targeting cholinergic dysfunction.

ALZHEIMER'S DISEASE AND ITS PATHOPHYSIOLOGY

Alzheimer's disease (AD) has occupied a great platform of attention over the years. Being the most notable form of dementia, a large number of populations worldwide

becomes susceptible to AD. Around 24 million people at present are estimated to suffer from cognitive disabilities worldwide, 60-80% of which is accountable to any form of cholinergic dysfunction resulting in AD [13]. Cholinergic dysfunction is considered to be the central tenet of AD. The cholinergic system is mediated by the neurotransmitter acetylcholine (Ach), which on release at the synaptic clefts binds to mainly two types of receptors *viz*., muscarinic Ach receptor (mAchRs) and nicotinic Ach receptors (nAchRs) of the post-synaptic neuron or neuro-muscular junction to bring forth its effect on behavioral functions [3, 14]. The enzyme acetylcholinesterase (AchE) plays a crucial role in degrading Ach at the synaptic cleft to execute a rapid and short- lasting action of neurotransmission [14]. Any disruption to the structural and functional entities of the brain cholinergic system marks the onset of AD. The major sources of cholinergic dysfunction, as illustrated in Fig. (**1**), revolve around the altered activity of enzyme acetylcholinesterase, accumulation of beta-amyloid protein, mitochondrial dysfunction, oxidative stress, and neuroinflammation.

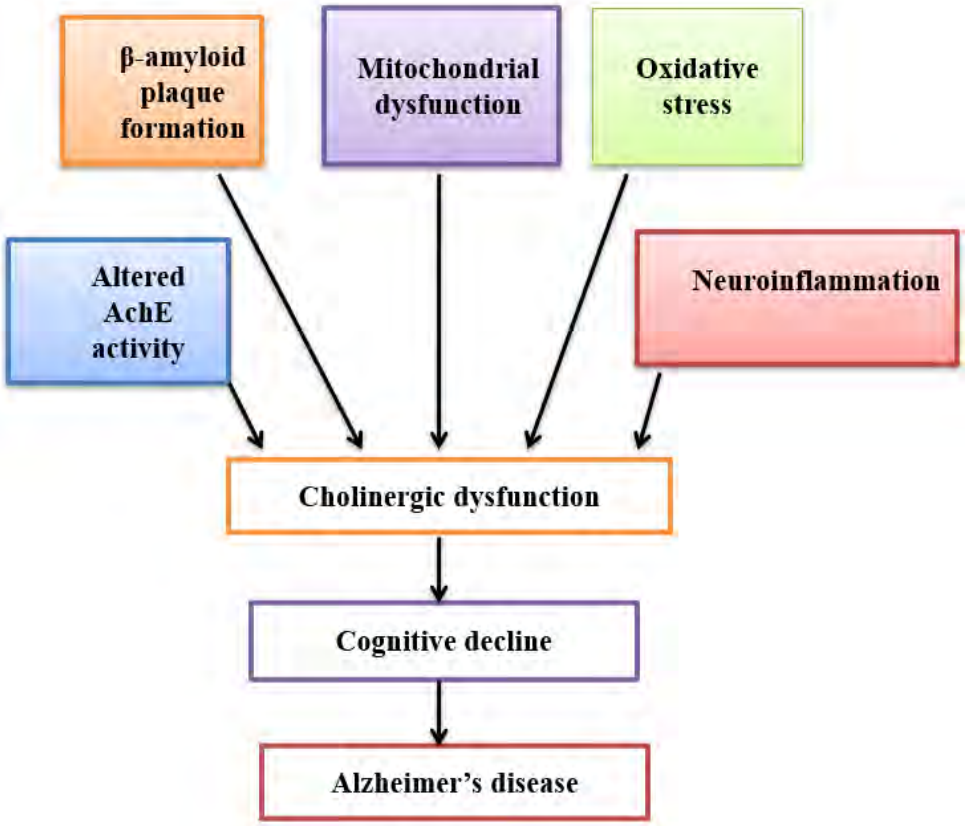

Fig. (1). A schematic representation of the several possible sources of cholinergic dysfunction leading to Alzheimer's disease (AD).

SOURCES OF CHOLINERGIC DYSFUNCTION

Altered Activity of Enzyme Acetylcholinesterase

The enzyme acetylcholinesterase (AchE) plays the crucial role of hydrolyzing the neurotransmitter acetylcholine (Ach) into choline and acetate at the synaptic clefts and serves to be one of the major cholinergic markers [14, 15]. Alteration in the enzymatic activity of AchE is considered one of the major indicators of cholinergic disruption. Excessive increase in AchE activity promotes the production and accumulation of beta-amyloid plaques at the clefts, which is considered a major reason behind the progression of Alzheimer's disease [1, 16]. On the other hand, an excessive decline in AchE activity is reported to be linked with impaired acetylcholine hydrolysis and subsequent accumulation of the substrate acetylcholine at synaptic clefts, which leads to the progression of Alzheimer like neuropathology [2, 17].

Beta-amyloid Accumulation

Abnormal synthesis and accumulation of β-amyloid protein at the synaptic clefts of cholinergic neurons stand as one of the most promising causes of the pathogenesis of AD. Synthesis of the protein by enzymatic cleavage of amyloid precursor protein (APP), when exceeds the rate of degradation results in abnormal aggregation and thus the formation of insoluble plaques, which induce neurodegeneration [3]. According to studies, excessive increase in the enzymatic activity of AchE stands as a major contribution to amyloid formation, whereby the enzyme interacts with β-amyloid and forms insoluble macromolecular complexes of amyloid fibrils at synaptic clefts, which subsequently restricts neurotransmission and leads to allied complexities like mitochondrial dysfunction [3, 18, 19].

Mitochondrial Dysfunction

Mitochondrial dysfunction is considered another major facet playing a significant role in the pathogenesis of AD [4, 20]. Neurons being highly dependent on mitochondria for metabolic energy are amongst the most susceptible to neurodegeneration as a consequence of mitochondrial dysfunction [21]. Reduced expression of mitochondrial biogenesis regulatory factors (PGC-1α, NRF 1, NRF 2, and TFAM), altered expression of factors regulating mitochondrial fission and fusion (Drp1 and Fis1, Mfn1, Mfn2, Opa1, Tomm40), as well as reduced activity of mitochondrial complexes I and IV are found to be predominant in AD brains [22 - 24].

Oxidative Stress

One of the prominent consequences of mitochondrial dysfunction is increased production of Reactive Oxygen Species (ROS), a state referred to as oxidative stress of the cells. Leakage of electrons during Electron Transport Chain (ETC) leads to the generation of superoxide anion (O_2^-) following reaction with oxygen. Further reaction of the generated ROS results in the formation of several other forms of ROS, which brings the cell to the state of oxidative stress [25]. Talking about AD, several reports have demonstrated the key role of oxidative stress in the pathophysiology of this brain disorder [5, 25]. Increased oxidation of protein, lipid, and DNA, decline in glutathione (GSH/GSSG) ratio, and reduced activity of antioxidant enzymes like glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase(CAT) are found to be prevalent in AD brain [26 - 30].

Neuroinflammation

Inflammation marks the cascade of events that eventually leads to the elimination of wounded cells and tissues as well as the root cause of cell injury. It is an essential phenomenon that helps maintain cellular health status. However, an excessive increase in cellular inflammation brings about several deleterious consequences to the cell, resulting in cellular degradation. The rise in neuroinflammation is one the most prominent gateway to the progression of AD- like neurodegeneration [6, 31]. Elevated expression of Glial Fibrillary Acidic Protein (GFAP), Nitric Oxide Synthase (NOS), cytokines (IL-1β, IL-6, IL-12, IL- 23, TNF-α), and several chemokines (CCL2, CCL8, CXCL10) in brain regions of AD subjects marks the progression of neuroinflammation [32 - 36].

Several therapeutic approaches have come across to treat AD, one of which is the use of herbal extracts. Moreover, the introduction of nanotechnology in drug designing and delivery appears to grab much attention not only in the diagnosis and treatment of AD but also in several other chronic diseases.

A LOOK AT THE CONCEPT OF NANOPARTICLE

It was well said by Rudolf Steiner that **"*For every human illness, somewhere in the world there exists a plant which is the cure*."** The use of herbal extracts as the remedy to various diseases dates back to ancient times all around the world. Several plant species treasure biologically active compounds such as terpenoids, flavonoids, alkaloids, *etc.*, which prove to be a potential cure to a large array of diseases, be it physiological, metabolic, or neurological. However, despite being potent drugs, these plant extracts hold certain limitations in exhibiting their efficiency in the recipient's body. Large molecular size, inability to cross lipid

membranes, low absorption are some of the major characteristics of these natural compounds, which lessen their bioavailability and target-specific efficiency [8].

To overcome the crisis of efficient drug delivery system, nanotechnology proves to be a fruitful tool. Though the study and use of nanoscience in foods, textiles, and cosmetic industries date back to many decades earlier, its use in therapeutics and, more specifically, in the drug delivery system is recent [37]. To be precise, nanoscience is the study and use of objects which fall within the range of 1 to 100 nm. However, in the field of medicine, the scale extends up to 1000 nm. Nanotechnological strategies include the use of nanoparticles, microemulsions, liquid crystal system (LC), *etc*., to aid the formulation and delivery of drugs to the target site [8, 38]. The present chapter, however, focuses on the use and advantages of nanoparticles (NPs) in herbal pharmaceuticals.

Nanoparticles (NPs) can be broadly classified into three categories *viz*., polymeric nanoparticles, lipid-based nanoparticles, and metal-based nanoparticles.

Polymeric Nanoparticle

Polymeric NPs form a colloidal system that is prepared from natural or artificial biodegradable polymers. Cellulose, alginate, chitosan, xanthan gum are some of the most widely used natural polymers in the preparation of nanoparticles, while poly-L lactic acid (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA) are among the extensively used synthetic polymers [39]. Due to their smaller particle size (10-1000 nm in diameter) and biodegradable nature, nanoparticles are widely considered as potent vectors for target-specific drug release. Based on their structure and composition, polymeric NPs can be divided into *polymeric nanocapsules* (NCs) and *polymeric nanospheres* (NSs). In NCs, the biologically active constituent of plant extract remains either dissolved in the oily or aqueous core or remains adsorbed in the polymeric film surrounding the core, while in NSs, the drug of interest remains adsorbed in the matrix of the polymeric structure [8, 38]. Enhanced anticancer efficiency was demonstrated by treating human epithelial cervical cancer cells (HeLa) with curcumin-loaded PLGA nanoparticles [40]. Similarly, PLGA nanoparticles encapsulated with curcumin significantly diminished neuroinflammation in rat models by reducing the levels of cytokines IL-6, IL-8, IL-1β, TNF-α [41].

Polymeric nanoparticles can also be prepared as *micelles*, which are composed of amphiphilic polymers assembled to form the hydrophobic core in which the hydrophobic drug is loaded. Small size (100 nm diameter) and hydrophobic drug loading capacity make these micelles a suitable tool for drug delivery [42, 43]. *in-vitro* treatment of PC12 cells with resveratrol-loaded polymeric micelles provided higher protection to β-amyloid induced oxidative stress [44].

The recombination of polymer with highly branched and multivalent molecules like *dendrimer* sets another milestone to drug delivery [45]. The characteristic multivalent nature of dendrimer makes it highly suitable for loading several drug species at a time, thus targeting multiple purposes in a single treatment [46]. Due to their greater water solubility and biocompatible nature, poly (amidoamine) (PAMAM) dendrimers appear to be the most widely used for therapeutic approaches [47]. *In-vitro* treatment of human ovarian carcinoma cells with anticancer drug conjugates loaded in PAMAM dendrimers showed reduced cytotoxicity than drug conjugates alone [48].

Lipid-based Nanoparticle

The second class of nanoparticles is the lipid-based nanoparticles, of which the solid lipid nanoparticles (SLNs) and liposomes are the most extensively used. The *SLNs* are colloidal particles possessing a solid lipid core. The lipid core is composed of purified triglycerides, diglycerides, monoglycerides, fatty acids and is stabilized by surfactants [38]. The drug of interest remains embedded in the solid matrix of the lipid core, which attributes to the protection of the drug from any chemical degradation. SLNs are spherical in the structure whose diameter ranges between 10-500 nm [8, 38, 43]. Studies demonstrated that quercetin-loaded SLN treatment in aluminium-induced rodents significantly reduced oxidative stress by ameliorating the lipid peroxidation level and enhancing the antioxidant activity of GSH [49].

Liposomes form the group of lipid-based nanoparticles that are vesicular in structure. Ranging between 50-450 nm in diameter, these bilayered nanostructures are composed of phospholipids and steroids. The analogous nature of the liposomal membrane with the living cell membrane makes this nanoparticle remarkably efficient for target-specific drug delivery [38, 43, 50]. A significant reduction in H_2O_2 induced oxidative stress in L929 mouse fibroblast cells was demonstrated by treatment with polyethylene glycol (PEG) encapsulated ginsenoside liposomes, the biologically active component of ginseng [51].

Metal-based Nanoparticle

The use and significant yield of metals as suitable nanocarriers made significant advances in the field of nanoscience. Several metals such as silver, gold, iron, copper, and metal compounds like zinc oxide, titanium oxide showed remarkable potentiality as the carriers of drugs for various therapeutic uses [52, 53]. Polyethylene glycol (PEG) gold nanoparticles loaded with anthocyanin showed remarkable neuroprotection to β-amyloid induced AD-like symptoms in rodents than free anthocyanin [54].

MECHANISM OF NANOCARRIER-DRUG RELEASE *IN-VIVO*

Different species of nanoparticles prefer different ways of delivering the loaded drug to the target site. The preference depends upon the physical and chemical properties of the nanocarriers. The two most convenient mechanisms are *passive delivery and cellular uptake* [39, 55]. Passive delivery is mostly followed by small-sized polymeric nanocapsules and nanospheres in which the drug molecule remains dissolved in the inner cavity of the nanocarrier. Upon reaching the site, the drug molecule readily diffuses from higher drug concentration inside the nanoparticle to lower concentration outside it [39, 56]. On the other hand, uptake of relatively bulky nanocarrier-drug complexes by target cell is mainly carried out by the endocytosis pathway. This mechanism is followed by nanocarriers that are opsonized or tagged with receptor-specific ligands [57]. Opsonized nanocarriers, *i.e.*, the ones tagged with immunoglobulins or complement proteins, upon being recognized by phagocytes, follow the regular phagocytosis pathway of engulfment and lysosome fusion. Subsequently, the nanocarrier-drug complexes undergo degradation by lysosomal enzymes to exert therapeutic effects [57, 58]. While those nanocarriers tagged with receptor-specific ligands are mostly taken up by the target cells through clathrin-mediated or pinocytosis pathway, in which the nanocarrier-drug complex following ligand-receptor recognition gets internalized by clathrin-coated pits to the interior of the cell. After subsequent shedding off of the clathrin coat, the vesicles carrying nanocarrier-drug complex fuse with lysosome and ultimately get degraded by lysosomal enzymes [58, 59].

Advantages of Nanoparticle-based Drug Carriers over Conventional Drug Delivery System

The use of nanoparticles in the formulation and delivery of herbal drugs has come up with several marked advantages in the treatment of chronic diseases like cancer, neurodegenerative diseases, metabolic ailments, *etc.* The physical and chemical properties of these nanocarriers, as discussed below, make them more favorable over conventional drug delivery systems.

Higher Drug Loading Capacity

The potentiality of nanocarriers to carry a wholesome content of drug within reduces the number of therapeutic doses and thus makes the treatment recipient-friendly. Moreover, the multivalent nature of nanodendrimer facilitates high drug loading capacity as well as the capacity to carry different species of drug targeting several purposes at a time [46, 60].

Ability to Carry Hydrophobic Drug

Low aqueous solubility of certain herbal extracts like curcumin stands as a barrier to the therapeutic efficiency of such bioactive compounds. Incorporation of such hydrophobic drugs into the hydrophobic core of nanomicelles facilitates their efficient delivery as well as solubility in the aqueous medium, thereby enhancing their bio-efficiency [61].

Target-specific Drug Delivery

Conjugation of nanoparticles with ligands specific to the receptors located on the target cell surface makes the drug release more specific to the site of treatment, thereby reducing the chance of non-target cells being affected [8].

Higher Bioavailability and Bioefficacy

The easy metabolism of herbal extracts, when used in a conventional way of drug delivery, limits the bioavailability of the drug to brain cells. Nanosizing the bioactive compounds within 1-1000 nm range and recombining with nanocarriers not only enhances their bioavailability but also protects them from getting metabolized on the way before reaching the target site. Curcumin-loaded PEG-PLA nanoparticles yielded a manifold increase in oral bioavailability than free curcumin in an AD mouse model [62].

Minimal Or No Toxicity

Despite being biodegradable, nanocarriers, especially those made of metals and their oxides, might pertain to some level of toxicity while interacting with cells *in-vivo*. However, surface coating and chemical modification of the nanocarriers renders them with less or no toxicity, thus enhancing their biocompatibility [63].

Reduced Therapeutic Dose

Advantages of nanoparticles like higher drug loading capacity, target specific delivery, higher bioavailability of drugs, allows efficient treatment in lesser number of therapeutic dosage [43].

Penetrates Blood-brain Barrier

The efficiency to penetrate the blood-brain barrier (BBB) system stands as the most significant advantage of nanocarriers when it comes to neurodegenerative diseases [64]. The selectively permeable nature of BBB serves to be the biggest hurdle for a large number of drugs to reach the CNS. The large size and hydrophobic nature of certain drugs limit their penetration through the BBB.

Several techniques have paved the way for drugs to cross the barrier; however, these come along with physical and chemical disruption to the BBB. Nevertheless, the technique of nanoparticle aided drug delivery has proved to overcome the allied drawbacks as it not only penetrates the BBB in a non-invasive manner but also allows efficient release of the drug. The relatively smaller particle size and high drug loading capacity of nanoparticles assure their efficient penetration into the BBB by endocytosis and transcytosis pathways, render stability, bioavailability, and high efficiency to the drug [64 - 66].

LIMITATIONS OF NANOPARTICLE-AIDED DRUG DELIVERY

Despite several remarkable benefits, the formulation and delivery of nanoparticle aided drugs bring forth certain limitations which cannot be overlooked. However, these limitations at the manufacturing and pharmacological levels can be overcome under appropriate investigations and strategies.

Large-scale Manufacture

Nanoparticle aided drugs, when formulated at small scales for preliminary laboratory investigations, sufficient reproducibility with product consistency can be achieved. However, upon exhibiting favorable effects, the need for large-scale production of the drug-carrier complex becomes a challenge. Firstly, the multistep manufacturing of nanoparticles at large scales may bring along structural and chemical alterations to the formulation if the key requisites which made the small scale yield successful are not maintained properly. Secondly, large-scale production means the need for more raw materials, advanced manufacturing technologies, and also more labor, which makes the product more expensive [67, 68].

Most of the nanoparticle aided drugs successfully pass the *in-silico* and *in-vitro* screenings, which undoubtedly indicates but does not establish its potentiality as a drug carrier. Toxicity and lesser biocompatibility stand as major pharmacological concerns that reduce the achievability of nanoformulations *in-vivo*.

Toxicity

As discussed earlier, metallic and carbon-based nanoparticles may pertain certain level of toxicity to the host body. Characteristic features like the smaller size, larger surface area, and positively charged moieties on the nanoparticles not only boost up their interaction with cell membranes and ease their cellular uptake but also play an active role in the unwanted accumulation of the nanoparticles and subsequently lead to the generation of Reactive Oxygen Species (ROS) [63, 69]. For instance, human fibroblast cells treated with titanium dioxide nanoparticles showed enhanced

oxidative stress, as indicated by ROS generation and DNA oxidation [70].

Biocompatibility

Lack of biocompatibility stands as a barrier to the application of certain nanoparticle drugs. To be approved as biocompatible, the nanoparticle must exhibit its role in the targeted area of concern in terms of the host's response without causing any adverse effect on the target as well as the neighboring sites. Blood cell damage, including hemolysis and platelet coagulation, and unwanted inflammatory reactions induced by immunostimulation are some of the marked adverse effects of nanoparticle drugs that lower their biocompatibility [71]. Not much is yet known about the mechanism by which nanoparticles bring forth these adverse side effects; however, several studies have demonstrated that the surface charge of nanoparticles plays a crucial role in it. Most of the studies show that positively charged particles have more potential to induce inflammatory reactions than neutral or negatively charged particles [72].

Nevertheless, recent advances in science and technology bring forward certain strategies which can be applied to reduce the manufacturing and pharmacological drawbacks of nanoparticle aided drugs. By devising simple manufacturing procedures and maintaining the key requisites at appropriate levels, a large-scale yield of good quality nanoparticles can be achieved in a cost-effective manner. Moreover, surface modifications, such as lessening the surface area, designing neutral or negatively charged particles, recombining the surface with suitable ligands such as polyethylene glycol, replacing the use of toxic metals with milder ones, are some of the strategies that can be applied to mitigate the toxicity of nanoparticles and render them with biocompatibility [73].

REGULATORY CHALLENGES OF NANOPARTICLE-AIDED HERBAL DRUGS

Any therapeutic product has to face a lot of hurdles throughout its journey from the lab to the market. The challenges for nanoparticle-aided drug formulations, which is a novel field in medicinal science, are numerous. Lack of proper guidelines for clinical assessment and regulations of the nanoparticle drug manufacturing limits the success of the nanoparticle concept in the field of medicine. Moreover, the response of a human body to the administered nanoparticle drug may differ from that of laboratory animal models, and thus the potentiality of the drug formulation gets highly questioned when it comes to the level of human trials [67, 68]. However, the remarkable success of demonstrating the potentiality of nanoparticles as carriers of several drugs, especially herbal products, has geared up the process for devising generalized guidelines to assess the efficiency of the nanoparticle aided drugs for

human applications and also to regulate their yield, quality, and cost to make them commercialized.

NANOPARTICLE-AIDED HERBAL DRUGS AS THERAPEUTICS AGAINST CHOLINERGIC DYSFUNCTION

Several species of herbs possess the outstanding potential to ameliorate and/or cure different forms of cholinergic dysfunction and thus prove to be efficient therapeutics in the treatment of AD. Researchers from several decades have successfully provided a large number of plant species *viz*., *Withaniasomnifera*, *Bacopa monnieri*, *Centella asiatica*, *Convolvulus pluricaulis*, *Curcuma longa*, *Gingko biloba*, *etc*., which contain certain biologically active compounds that can serve as the excellent antioxidant, anti-inflammatory, and neuroprotective agents [7, 74, 75]. However, since nanotechnology has come up as a very recent tool in the field of medicine, its exploration in the delivery of all the documented herbal species and the discovery of more species to treat AD is still in progress. Nevertheless, the recent advances made by nanoscience in accelerating the potentiality of herbal pharmaceuticals to treat AD are as follows (Fig. **2**).

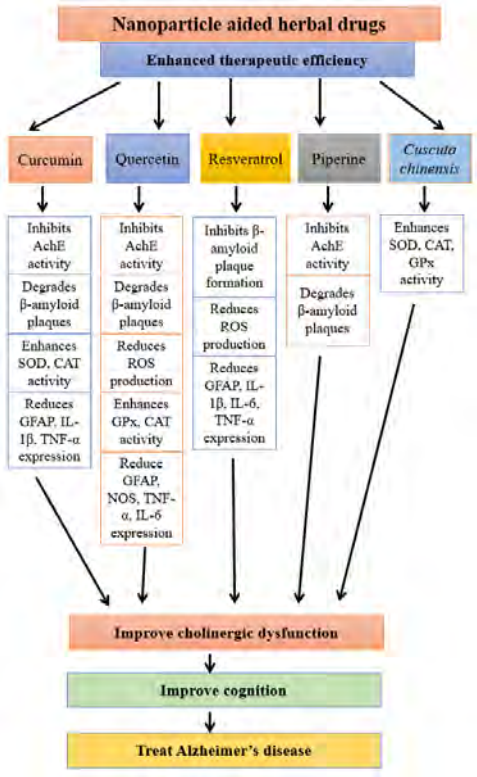

Fig. (2). A schematic representation of the several possible mechanisms by which nanoparticle aided herbal drugs exhibit protection against cholinergic dysfunction, establishing their potentiality as therapeutics to Alzheimer's disease (AD).

Curcumin

Curcumin is a polyphenolic compound found in the rhizome of turmeric *Curcuma longa*. It is hydrophobic in nature and chemically known as diferuloylmethane. The antioxidant and anti-inflammatory benefits of curcumin are decoded long back [76, 77]. Reduction in ROS production, GFAP expression, levels of pro- inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α are some of the major contributions of curcumin towards neuroprotection [7, 74, 76, 78]. However, the hydrophobic nature, low bioavailability, and fast elimination of the compound limit its efficiency as a potent therapeutic. Nevertheless, the introduction of nanoscience in the formulation and delivery of curcumin has served as a milestone in accelerating the potentiality of the compound in the field of therapeutics. Several recent studies have shown that the administration of nanoparticle aided curcumin results in enhanced water solubility and distribution of the compound in rodent brain by about 30 times and enhanced bioavailability, which enables the compound to exert its effects better [8, 79]. Treatment of rodents with curcumin-loaded solid lipid nanoparticles (CSLN) showed a reduction in the activity of acetylcholinesterase and amyloid-beta production [9, 79, 80]. In addition, an increase in the activity of mitochondrial complexes and antioxidant enzymes SOD, CAT, glutathione system, as well as a decreased production of ROS and lipid peroxidation products was several times more by CSLN than the free compound [81, 82]. Treatment with curcumin-loaded lipid- core nanocapsules ameliorated the levels of GFAP, cytokines IL-1β and TNF-α expression in the brain of β-amyloid infused rodents, thereby providing better protection against neuroinflammation than exhibited by free curcumin [9, 83]. Interestingly, the studies also demonstrated significant improvement of cognitive behavior of the animal models upon treatment with nanocarrier-curcumin complex as compared to free curcumin, thus indicating the higher efficiency of nanoparticle-aided curcumin as a therapeutic to AD [9, 62, 83].

Quercetin

Quercetin is a flavonoid found in *Ficus carica*, apple, onion, and broccoli. It is chemically known as 3,3',4',5,7-pentahydroxyflavone. Several studies have demonstrated the neuroprotective potentiality of quercetin as an antioxidant and anti-inflammatory agent [43, 74, 84]. The beneficial effects of quercetin in reversing the cognitive defects in animal subjects also signify the herbal compound as a therapeutic to several forms of neurodegenerative disorder. However, the efficiency of quercetin as an active therapeutic agent has been highly appreciated after recombining the drug delivery with nanoparticle technique. Quercetin-loaded liposome treatment showed a significant reduction of acetylcholinesterase activity, as well as degraded amyloid-beta plaques in AD mouse model [85, 86]. Nano-

particle aided quercetin treatment resulted in enhanced activity of enzymes GPx and catalase, reduced ROS concentration, and H_2O_2 induced neuronal toxicity in brain regions of animal subjects as compared to free quercetin, thereby reducing cellular oxidative stress [43, 85, 86]. The nanomedicine also showed significant protection against neuroinflammation by downregulating the expression of GFAP, NOS, TNF-α, IL-6 in brain regions of animal subjects more efficiently than free quercetin [10, 87, 88]. Interestingly, the improvement of memory and learning abilities was evident more in solid lipid nanoparticle-quercetin (QC-SLN) treated animal models than in those treated with free quercetin [10, 87, 88].

Piperine

The alkaloid derived from the fruit of several species of *Piper* is considered a notable drug to target acetylcholine modulation. The effectiveness of piperine is, however, limited by its insufficient bioavailability; a major drawback that has been able to overcome by the introduction of nanotechnology in its delivery *in- vivo*. Piperine-loaded chitosan nanoparticles and SLNs were demonstrated to not only reduce the acetylcholinesterase activity in AD subjects but also to promote the reduction of amyloid plaques and improve cognitive behavior in AD subjects [12, 89].

Resveratrol

The anticancer, antioxidant, anti-inflammatory properties of resveratrol make it a potential therapeutic against several chronic diseases like cancer, AD, Parkinson's disease (PD). Chemically known as 3,5,4'-trihydroxy-stilbene, resveratrol is a polyphenolic compound obtained mainly from grapes. However, a high tendency to get metabolized, poor aqueous solubility, and bioavailability limit the therapeutic efficiency of the phytocompound *in-vivo* [43, 44]. Increased drug efficiency of resveratrol could be achieved by introducing nanotechnology in the designing and delivery of the phytodrug. In-vivo treatment of rodents with SLN- loaded resveratrol resulted in increased bioavailability of the drug in brain tissues [90]. *in-vitro* treatment of human endothelial cell culture with SLN-loaded resveratrol showed better inhibition to β-amyloid fibril formation and aggregation than free resveratrol [91]. Polymeric micelles carrying resveratrol showed enhanced antioxidant activity against β-amyloid induced oxidative stress by reducing ROS production in PC12 cell culture [44]. Resveratrol conjugated with lipid-core nanocapsules provided better neuroprotection against β-amyloid induced neurotoxicity in rodents by diminishing GFAP expression and improving memory and learning abilities [92]. Similarly, β-amyloid incubated human hippocampal cells showed reduced production of ROS, inflammatory cytokines IL-6, IL-1β,

TNF-α, and GFAP expression upon *in-vitro* treatment with resveratrol loaded lipid-core nanocapsules [93].

Cuscuta Chinensis Nanoparticle (CN-CE)

The antioxidant property of the Chinese native plant *Cuscuta chinensis* is widely famous, whose therapeutic efficiency has been accelerated by the introduction of nanotechnology to the formulation and delivery of the ethanolic compound derived from the seeds of the plant [8]. Nanotechnology solves the limitation of poor aqueous solubility of this phytocompound *in-vivo*. Treatment of rodents with nanoparticles loaded with extracts of *Cuscuta chinensis* (CN-CE) resulted in enhanced activities of antioxidant enzymes SOD, CAT, GPx, thereby accelerating the potentiality of the phytocomponent in countering oxidative stress [8, 11].

CONCLUSION

Nanotechnology in recent years has set remarkable milestones in deducing and appreciating the bio-efficiency of several herbal drugs like curcumin, quercetin, piperine, resveratrol, extracts of *Cuscuta chinensis* as therapeutics for cholinergic dysfunction in AD, as well as several other chronic diseases. However, a lot more is yet to be done in rediscovering the natural assets, which have a tremendous potential to treat neurodegenerative disorders like AD and recombine their drug designing and delivery with nanotechnology to strengthen their therapeutic efficiency. Gold and silver nanoparticles loaded with compounds derived from *Withania somnifera* and *Centella asiatica,* respectively, have been formulated to study their therapeutic contributions. The potentiality of these nano-based herbal drugs as a treatment strategy for cholinergic dysfunction or, more specifically, AD is yet to be demonstrated. Moreover, studies so far have demonstrated the potentiality of nano-herbal drugs as acetylcholinesterase inhibitors, whereas their efficiency as enhancers of the enzymatic activity has not yet been reported. An excessive decline in the activity of acetylcholinesterase equally contributes to the pathomechanism of AD. Therefore, there exists the need for discovering herbs that treasure the ability to enhance the enzymatic activity, as well as exists the demand to design suitable nanoparticle systems for the delivery of such drugs to make the treatment more advantageous.

RECOMMENDATIONS AND FUTURE DIRECTION

Numerous shreds of evidence prevail, which suggest the potentiality of the above-discussed nanoparticle aided herbal drugs in ameliorating cholinergic dysfunction and improving cognitive skills. The remarkable success of the use of nanoparticles as drug carriers, as demonstrated in *ex-vivo* cell cultures and animal models, highly recommends its assessment in the next stages of clinical trials.

However, owing to the novelty of nanoparticles in the medicinal field, there still prevail certain constraints which restrict their assessment in human subjects. Lack of proper guidelines for pharmacological assessment questions the safety and feasibility of these drug carriers in the human body; thus human trials cannot be risked at the present stage. Nevertheless, advances in the development of human-friendly drug nanocarriers and their regulation at laboratory, manufacturing, and clinical stages are progressing at a high pace which seems to recommend their feasibility in human trials and also as potent therapeutics in the near future.

CONSENT OF PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We sincerely acknowledge the support provided by the Department of Biotechnology, Government of India (Sanction Order No.BT/PR17127/NER/ 95/453/2015, dated January 13, 2017).

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CHAPTER 10

Vitamins Based Nanomedicine Approach

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Abstract: With the rapid advances in science and technology in recent years, nanotechnology has gained much attention in all disciplines. Due to its wide applicability in various fields, researchers and academicians are showing great interest in nanotechnology nowadays. Some of its applications include neutraceuticals, nanoemulsions, nanomedicines, nanoencapsulation, and many more. Understanding and controlling matter at the nanoscale is what nanotechnology is all about. Nanomedicine is the most advanced application of nanotechnology. In simple terms, the basic definition of nanomedicine is the application of nanotechnology to repair damaged tissue. The European Medicine Agency (EMA) defines nanomedicine as the application of nanotechnology in the establishment of a medical diagnosis or the treatment/prevention of disease. It exploits the improved and often novel physical, chemical, and biological properties of materials at the nanometer scale. Nanomedicines come in various forms like nanoparticles, liposomes, nanogels, nanoemulsions, nanotubes, and many others, most of which have been approved by various agencies for their diagnostic/therapeutic utility. Nanomedicine has achieved a high success rate due to its widespread applicability and a variety of forms, although the road ahead is not easy. Therefore, even today, many argue about earlier planning to meet all the requirements. Due to the enzymes and chemicals in the gastrointestinal membrane (GI), some vitamins are poorly absorbed. Nanomedicine helps alleviate this problem while increasing the bioavailability of vitamins due to their remarkable absorption and distribution capabilities.

Keywords: Liposomes, Nanocarriers, Nanoemulsions, Nanomedicine, Nanotechnology, Nanotubes, Nutraceuticals.

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INTRODUCTION

The oral route of administration for bioactive agents is considered the best route due to patient compliance. However, some orally administered bioactive agents sometimes undergo enzymatic and chemical degradation in the GI tract, where a decrease in stability, solubility, and absorption is observed [1-3]. Bioavailability is defined as "the rate and extent to which the active substance becomes available in the systemic circulation." Humans need an adequate amount of vitamins, minerals, and carbohydrates daily to maintain a healthy body. Artificial foods containing bioactive ingredients, such as vitamins, minerals, *etc.*, are available in the market. In some cases, these bioactive ingredients have been found to be less stable, soluble and bioavailable. Solubility enhancers, stability enhancers, bioavailability enhancers, and permeation enhancers are needed to improve these factors [4, 5].

It has been found that a colloidal delivery system such as a micro- or nano-sized delivery system improves these factors. Nanosized particles have better absorption than microsized particles due to their smaller size [6]. Nanoformulations improve uptake by enterocytes, which facilitates absorption, while nanocarriers form a protective shield and remain stable in the GI tract, reducing the dose [7]. Nanocarriers can also serve as vehicles for the delivery of various bioactive agents, such as vitamins, nutrients, proteins, peptides, antibodies, *etc.* [8].

The use of self-emulsifying drug delivery systems (SEDDs), mini emulsions, transparent emulsions, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) were found to improve absorption, distribution, metabolism, excretion, and tolerability, to reduce toxicity and increase industrial production rates [9]. Through various pathways, nanomedicine increases the absorption of various agents. For lipids, secretion *via* the bile and pancreas increases, aiding fat digestion [10]. Nanolipids prolong active ingredients in the gastrointestinal tract, thereby increasing absorption. Highly soluble lipids have very good bioavailability because there is no first-pass metabolism [11].

Lipid-based nano-formulations contain lipoids, stabilizers, and water-loving solvents. Vitamins are necessary organic molecules that can act as cofactors and are required for maintaining the health of the body. Vitamins may be taken as dietary supplements to curb obesity, heart disease, brittle bones, ageing, and carcinomas. Vitamins are broadly classified into water-soluble and lipid-soluble vitamins. Vitamin B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B5 (Pantothenic acid), B6 (Pyridoxal), B7 (Biotin), B9 (Folic acid), and B12 (Cyanocobalamine), and C are considered water-soluble vitamins, while vitamin A (Retinol), D2 (Ergocalciferol), D3

(Cholecalciferol), E (Tocopherol), K1 (Phylloquinone) and K2 (Menaquinone) are considered fat-soluble vitamins [12, 13]. Studies suggest that most vitamins taken orally have low bioavailability and are poorly absorbed in the GI tract [14].

To mitigate these problems of absorption, instability, and bioavailability of vitamins, researchers are exploring nanomedicine and developing lipid nanocarriers that vastly increase the oral bioavailability of vitamins. There are various lipid nanocarriers that coat vitamins, such as SEDDs, nanoemulsions, SLNs and NLCs.

VITAMINS DELIVERED ORALLY

Vitamins are an essential part of daily lives to maintain cell regulation, growth, development and metabolism. The human body needs 13 dietary vitamins from food to stay healthy [15].

People who have vitamin deficiencies are first prescribed oral supplements. Few vitamins have low bioavailability, poor GI transport, and low water solubility when taken orally. To minimize these problems, it is necessary to produce supplements that improve absorption [16].

Vitamin A

Vitamin A is a fat-soluble vitamin needed for the maintenance of normal immune function, homeostasis, and proper growth. It is needed for various bodily functions such as normal vision, childbirth, osteogenesis, and maintenance of a good immune system. The most commonly used carotenoid in functional foods is beta-carotenoid due to its important properties as provitamin A and antioxidant [17].

Vitamin B

It is a hydrophilic vitamin that may be considered a cofactor for certain enzymes. Various types of vitamin B are B1, B2, B3, B5, B6, B7, B9, and B12. These vitamins have roles in disease prevention like Alzheimer's and colon cancer, which may occur due to the deficiency of vitamin B1. Taking vitamin B6 is beneficial for patients with heart disease. Cobalamin deficiency may lead to megaloblastic anaemia. The majority of dietary products contain vitamin B12, but bioavailability is lower; it varies from 8 to 12% for dairy sources; however, when tofu and cheese are consumed, bioavailability is 12 to 33% [18 - 22].

Vitamin C

It is a water-soluble vitamin. It is considered one of the most important vitamins that are important for the immune system and iron absorption [23]. Most vegetables and fruits are sources of vitamin C. Ascorbic acid acts as a good antioxidant, reducing stress and free radical formation. Other physiological functions of vitamin C include vascular conductance, reduction of systemic inflammation, reduction of cancer-specific toxicity, *etc.* Intravenous administration of vitamin C is preferred over oral administration due to its low bioavailability [24].

Vitamin D

Vitamin D is a fat-soluble vitamin. It has two forms, namely D1 and D3. Vitamin D2 is produced by UV irradiation of ergosterol in plants. Vitamin D3 forms with the UV radiation of 7-dehydrocholesterol in human skin [25, 26].

Vitamin E

Vitamin E is a fat-soluble vitamin. It acts as an antioxidant and free radical scavenger. It is derived from four tocopherols (alpha, beta, gamma, and delta tocopherols). It has anti-inflammatory effects and is used to treat cardiovascular diseases and cancer [27, 28]. Alpha-tocopherol was found to have the highest bioactivity, whereas delta-tocopherol has the lowest bioactivity [29]. Studies suggest that oral uptake of vitamin E occurs through passive transport across the apical membrane of erythrocytes [30]. Due to its limited water solubility and poor permeability GI, it has low bioavailability. To alleviate these problems, vitamin E is preferably encapsulated in nanoparticles.

Vitamin K

It is a lipophilic vitamin. Blood clotting is one of the main roles of vitamin K. Plant-derived vitamin K1, also known as phylloquinone, acts as a procoagulant and is used in bleeding, while animal-derived vitamin K2, also known as menaquinone, is used in the regulation of blood clotting factors [31, 32].

Lipid-Based Nanoparticles

Due to poor solubility and low bioavailability, lipid-based nano-drug delivery systems, which include SEDDSs, nanoemulsions, microemulsions, NLCs, *etc.*, have been proposed to increase solubility and bioavailability. Studies have shown that bioactive compounds, such as lipids, surfactants, aqueous solvents, and co-solvents (the components of lipid nanoparticles), are biocompatible and less toxic to the human body [33, 34].

SEDDS (SELF-EMULSIFYING DRUG DELIVERY SYSTEM)

The two types of SEDDs, *i.e.*, SNEDDSs (Self-Nano Emulsifying Drug Delivery System), which are generally opaque, and SMEDDs (Self Micro-Emulsifying Drug Delivery System), which are transparent microemulsions [35, 36], significantly alter the bioactivity of active ingredients. The choice of a specific excipient for SEDDS is important to increase bioavailability. SEDDS that are considered 'Generally Recognized As Safe' (GRAS) are prepared with lipids and surfactants. Conventional emulsions can increase hydrolysis and precipitation during long- term storage, thereby reducing stability and absorption. In SEDDSs, however, water is not required. It has been found to improve physicochemical stability, simplify capsule filling, and increase patient acceptance [37].

NANOEMULSIONS

Nanoemulsions are another method for improving the bioavailability of vitamins. These are a heterogeneous mixture of O/W emulsions stabilized by the addition of an emulsifier. Nanoemulsions encapsulate vitamins and nutrients and penetrate lipid-loving structures. They have sufficient strength, easy processing of goods, and improved intestinal digestibility [38 - 40]. The ingredients of nanoemulsions are GRAS or natural products; various surfactants are used in nanoemulsions to reduce toxicity and increase stability, like peptides, caseinate, gums, and starch. Moreover, lecithins and tiny molecules are non-ionic surfactants (spans and tweens) [41]. Due to their small droplets and large surface area, nanoemulsions can combine with the biocomponents of the GI pathway, which makes them a good carrier for bioactive agents because they are better absorbed than conventional emulsions. Studies have shown that nanoemulsions progressively improve absorption when solubilization and gastric emptying time are increased. In addition, they also stimulate lymphatic absorption and block the metabolic process, reducing the effects of efflux transporters [42].

SOLID LIPID NANOPARTICLES (SLNS)

SLNs contain soft lipid-based emulsions that remain rigid at normal temperature and body temperature. SLNs are dispersed nanoparticles composed of crystalline lipids. Due to the unusual properties of their particles, SLNs possess the property of controlled release, enhanced absorption, increased stability, suitability for industrial applications and large-scale production [43].

IMPROVEMENT OF ORAL BIOAVAILABILITY OF VITAMINS USING LIPID NANOCARRIERS

In recent years, research has shown that vitamin deficiency causes many pathological conditions in our bodies. Although many efforts have been made to prevent and treat vitamin deficiency symptoms, it is still believed that this is far from sufficient. Also, conventional vitamin formulations are less bioavailable and often have undesirable effects. The use of lipid nanocarriers has solved these problems to some extent, as they are able to target specific ligands and have better solubility, stability, and bioavailability compared to oral vitamin preparations.

Self-emulsifying Drug Delivery Systems (SEDDs) for Oral Vitamin Delivery

EDDSs are used in the dosage form of capsules or tablets for oral administration. The presence of soybean oil, cremophor EL, and capmul MCM-C8 allow them to convert liquid SEDDSs into solid forms, resulting in a solid powder when mixed with microcrystalline cellulose, which is then compressed into tablets [44]. It has been scientifically documented that the oral administration of vitamin A in the form of SEEDs has a higher C_{max} value (656 mg/ml) compared to conventional tablets C_{max} value (421 ng/ml), suggesting higher bioavailability.

Polysaccharide Materials

Nanocarriers containing high amylose corn and potato starch were used to encapsulate vitamin D3. Ultrasound treatment prolonged the Van der Waals and H-bonds of vitamin D3, resulting in greater thermal stability [45].

The scientific records show that the fusion of cellulose nanocrystals and lecithin into the microsphere increased the stability of *Lactobacillus rhamnosus* ATCC 9595 (an encapsulated probiotic in the intestine) [46].

Protein-based Carriers

The protein-based nanoparticles have a three-layered structure in an aqueous compartment in which the hydrophilic neutraceutical cobalamin is incorporated. They exhibit controlled release behaviour in a stimulated GI environment and *in vivo* conditions. Cobalamin-added nanoparticles increased serum levels of vitamin B12 in rats. Their administration by oral route decreases the concentration of methylmalonic acid more than the free cobalamin formed in the prepared mixture without toxicity, which was observed in 2 weeks. Such nanoparticles can be used to improve the bioavailability of cobalamin when administered orally [47].

Supplements Having Antioxidant Properties

The imbalance between the formation and accumulation of ROS in cells and tissues is oxidative stress, which causes many pathological symptoms. To resolve or reduce this oxidative stress, various oral formulations like flavonoids, vitamin C, carotenoids, *etc.*, are used as dietary supplements. Nanoencapsulation, such as nanogels, nanofibers, nanosponges, nanoliposomes of hydrophobic bioactive food compounds, such as amylose and yeast cells, improved the aqueous solubility, antioxidant and health-promoting properties and reduced the harmful properties of bioactive foods in the gastric fluid [48].

Nano and Microparticles

Due to the lower toxicity, biocompatibility, and biodegradability of natural polymers, the nanoparticles (NPs) made from them are widely used in the pharmaceutical and food industries. Chitosan, cellulose, is obtained by repeating the N-acetyl-d-glucosamine and d-glucosamine subunits through a (1,4)-β glycosidic bond (polymer of natural origin). Due to its important properties, such as improving the solubility of drugs with low hydrophilicity, specific drug targeting mechanisms, preventing damage to the encapsulated compound, and improving absorption, it is more emphasized [49]. When vitamin C (ascorbic acid) is encapsulated with chitosan nanoparticles, ascorbic acid restricts cellular uptake and improves the biocompatibility of chitosan nanoparticles [50].

When ascorbic acid was encapsulated with N-trimethyl chitosan and N-triethyl chitosan, it was found that the particle size also increased with increasing ascorbic acid concentration, but chitosan derivatives improved cellular uptake [51].

Encapsulation of vitamin C with mesoporous silica results in controlled release of the drug and blockade of the GI tract with a reduction in stemness genes [52, 53]. Encapsulation of vitamin C with hydroxyapatite also causes a controlled release of the drug [54]. When ascorbic acid is encapsulated with glyceryl behenate, this nanocapsulation enhances the anticancer effect of vitamin C [55]. Encapsulation of folic acid and ascorbic acid with soybean phosphatidylcholine, cholesterol, and chitosan increases physical stability, antioxidant activity, and improves encapsulation efficiency [56].

Palmitoyl ascorbate and docetaxel were encapsulated in soyabean phosphatidyl choline and cholesterol, and their liposomes were able to penetrate the cells successfully. Studies have shown that the codelivery of the drug has higher efficacy in inhibiting tumour development compared to palmitoyl ascorbate or docetaxel alone [57]. The lipid properties enhance the skin penetration rate of the formulation, resulting in improved bioavailability with higher therapeutic properties of sodium

ascorbate. In addition, the lipid formulation suppresses inflammation to a greater extent when rats are exposed to UVA/UVB than the drug in solution form [58]. Liposomal vitamin C prepared by high-pressure microfluidization showed controlled release and improved bioavailability of vitamin C [59]. Liposomes with vitamin C mitigate the significant tissue changes caused by antimonial drugs [60].

Ascorbic acid with soybean phosphatidyl choline, cholesterol, and Tissue@ 80 low methoxyl pectin liposomes (LMP) increase their stability compared to high methoxyl pectin liposomes or uncoated liposomes. The liposomes containing HMP and LMP increased the absorption of the drug by 1.7 and 2.1 times, respectively, after 24 hours compared to uncoated liposomes [61]. The oil-like microemulsions formulating ascorbic acid using decyl glucoside, propylene glycol, and oils showed improved penetration ability [62].

Palmitoyl ascorbate and polyethylene glycol phosphatidylethanolamine form micelles that exhibit higher antineoplastic activity. Vitamin C in N–acyl chitosan showed controlled drug release at pH 1.3 and pH 7.4 due to the formation of a longer acyl side chain. Alishahi *et al.* reported that nanoparticle formulation of ascorbic acid with chitosan increased drug entrapment by 60% and improved physicochemical properties [63]. Delayed release of ascorbic acid and more stable formulation were also reported. An *in vitro* study was conducted to investigate the release of ascorbic acid from chitosan-based nanoparticles. The study was conducted at 370°C for 100 hours, resulting in the release of 30% ascorbic acid using 0.1M HCl. However, the release profile in PBS medium was 75%. The reason for this rapid release is that the release rate can be altered by reducing the ionic interactions at neutral pH between nanoparticles and complexes. *In vivo* release of vitamin C from nanoparticles was studied and investigated using rainbow trout as a model. Both *in vitro* and *in vivo* results were identical, confirming the conclusions [57].

Zhou *et al.* suggested that the liposomal formulation of doxorubicin (DOX) with palmitoyl ascorbate showed better anticancer activity [59]. It is suggested that the liposomal formulation of ascorbic acid has higher entrapment efficiency with stability, and these are prepared by the double emulsion method [64]. Slow release of the active ingredient was observed *in vitro*. The results of the study suggest that the dynamic high-pressure double emulsion microfluidization technique is a good approach for the preparation of liposomes encapsulating ascorbic acid. Two different types of pectin, namely HMP (high methoxy pectin) and LMP (Low methoxy pectin), were used for the evaluation. The results of evaluations suggest that the profile of the entrapment efficiency of vitamin C was about 50%, which was independent of the molecular weight and pectin concentration. However,

liposomal diameter and polydispersity index increased, while zeta potential decreased with the increasing pectin concentration. TEM and AFM techniques were used to evaluate morphology, which indicated that uncoated liposomes had small and well-distributed particles, while large spherical and irregular particles resulted from coated liposomes. Physical stability was also evaluated at 250°C for 10 weeks with a leakage rate of 10% for coated and uncoated liposomes. The results concluded that better physicochemical stability was achieved with LMP compared with HMP. *In vitro* skin permeation studies also confirmed the fact that LMP allowed better and higher permeation of vitamin C [65]. The entrapment values with LMP coated liposomes are 40.1 ± 4.7 microgram/cm^2 compared to HMP (32.2 ± 5.2 microgram/cm^2) or non–coated liposomes (19.2 ± 3.2 microgram/cm^2).

Tohamy *et al.* designed the included vitamin C-based liposomes and performed the studies to test the higher genotoxic effects of the anticancer drug cyclophosphamide. The toxicological studies were performed on male Swiss albino mice. The cytotoxic effects and clastogenic effects were also assessed [66]. The results showed a significant decrease in the ratio of polychromatic to normal-chromatic erythrocytes using the above-mentioned cyclophosphamide-loaded liposomes and ascorbic acid. An improvement in S-transferase activity was also observed in the group treated with the co-encapsulated liposomes. Further studies demonstrated the anticarcinogenic effect of palmitoyl ascorbate encapsulated in liposomes [67].

DRUG TARGETING

Recent studies suggest that the use of nanotechnology has attracted attention for developing better delivery of drugs to a specific site in the body or a specific cell type [68]. The accessibility of vitamin C and its therapeutic drug targeting is being investigated in numerous studies. Luo *et al.* studied the targeting of Na- dependent vitamin C transport using the nanoparticles approach. The research predicted that the ascorbate conjugated nanoparticles can be utilized for oral drug delivery. The outcome of the study was the conjugation of 20% ascorbate with the polymer PLGA (poly lactic-co-glycolic acid) used [67, 69].

PATENTS AND CLINICAL TRIALS ABOUT ASCORBIC ACID

The use of ascorbic acid in cosmetics or nanoformulations for topical application has shown great potential [70]. In recent years, a number of patents have been filed in the field of cosmetics. In 2004, a single-phase ascorbic acid solution was prepared, claiming that this formulation technique increases the photoprotection, stability, and solubility compared to the conventional dosage form. This patented research includes vitamin C, cinnamon derivatives, and several multiple solvents.

The technique is patented with validity up to 2025 [71]. Another patent filed later consisting of ascorbic acid and its derivatives along with silicone compounds, and essential oil showed enhanced stability of formulation at room temperature [72].

Later in 2008, the method of the liposomal encapsulated ascorbic acid formulation was patented. In this case, the formulation consists of permeation enhancers, a sphingolipid, and ascorbic acid. The resultant formulation (lamellar liposome suspension) had a mean size of 50-290 nm and was later combined with a cosmetically suitable matrix to form a cosmetic preparation. This patent is valid until the year 2031 [73].

Improvement of Oral Bioavailability of Vitamins Using Lipid Nanocarriers

Due to the deficiency of vitamins, certain abnormalities may occur in the human body; to solve this problem, various oral vitamin supplements are prescribed. However, due to the lack of bioavailability, absorption, stability, and some other factors associated with oral formulations, they are not widely used. Studies suggest that the use of lipid-based nanocarriers is far superior to oral formulations. Several properties are associated with lipid nanocarriers, such as modulation of size, surface charge, ingredients, and targeting of specific ligands, and they have improved solubility, stability, and bioavailability.

Self-emulsifying Drug Delivery Systems (SEDDS) for the Oral Delivery of Vitamin

SEDDS is considered the most suitable lipid-based nano-carrier, which enhances the oral absorption of vitamins. This method has varied physicochemical properties and self-assembled nature, which allows the formulation to be prepared in the simplest way. Previous research has suggested the use of this technique for the oral administration of dietary supplements that are poorly soluble in water. The formulation was prepared and administered in gelatin capsules [74]. The formulation containing the dietary supplements was tested in a dispersion test for the spontaneous formation of a microemulsion with a droplet diameter in the range of 25-200 nm. Self-emulsifying drug release systems are important for the formulation and delivery of active ingredients *via* the oral route in the form of tablets and capsules. Lutein, a naturally occurring carotenoid, which is therapeutically used in the treatment and prevention of cataract and age-related disorders, has poor aqueous solubility (approximately 5%). This reduces oral bioavailability and also exhibits wide interindividual variation [75]. In their research, Sato *et al*. proposed the improvement of the transport of lutein into the lymphatic system using solid SEEDS [73]. The experimental work was carried out on rats (thoracic lymph cannulation) to estimate the lutein concentration in the lymph. The result of the experimental work showed that the lutein concentration

was about 100 ng/ml in the control powders after 9 hours, while it was about 250 ng/ml in the SEEDS technique. This indicates improved diffusion of lutein from the intestine to the lymphatic system by the SEEDS technique.

Because of the antioxidant property, vitamin E can stabilize oxygen-sensitive bioactive agents in lipid nano delivery systems. It can also serve as an additive in multiple formulations [76, 77]. TPGS, a non-ionic surface active agent, prevents drug precipitation and enhances supersaturation in lipid nanosystems; it also enhances drug dissolution through H-bond formation [78]. The other approaches available to increase the GIT transport and solubility of vitamins are through micro-emulsification and nano-emulsification techniques. The data published in the literature suggest that vitamins delivered by nanoemulsions have better physical stability in plasma and electrolyte dispersion [79]. Parthasarathi *et al.* studied the effects of droplet size on the absorption of tocopherol by formulating a nanoemulsion with a diameter of 277 nm compared to conventional emulsions with a diameter of 1285 nm [80]. The nanoemulsion coated with saponins exhibited better stability evaluated under conditions of heat, stress, and storage. Vitamin E was administered to rats at a dose of 100 mg/kg and showed a higher Cmax (11.6 *vs.* 2.6 micrograms/ml) and shorter half-life (0.85 *vs.* 1.11 hours). Vitamin E can be obtained naturally from many sources like nuts, vegetables, and grains and can be easily and conveniently administered orally using the nanoemulsion approach [81, 82].

Research suggested the utilization of SLNs as an oral delivery system for vitamins and analogues because they are biocompatible with the matrix and are easily degraded *in vivo*. The naturally occurring carotenoid asthaxanthin is found in shellfish, salmon and shrimp, *etc.* This active constituent is potentially used to treat cancer and various cardiovascular diseases. The potency of this compound is higher than that of tocopherol and β carotene [83]. However, due to being photosensitive, hydrophobic, and oxidative, it is used less for oral formulation purposes. The problem is resolved by researchers by incorporating the asthaxanthin into SLNs containing glycerol and tween 20 [84]. These SLNs have an encapsulation capacity of nearly 89% with a mean diameter of 163-167nm, and the formulation resulted in prolonged drug release when gastric juices were stimulated.

NLCs as drug carriers proved to be a better option for the release of fat-soluble vitamins. Previously vitamin D3, a lipid-soluble vitamin, was encapsulated in NCLs using the hot pressure homogenization technique [85]. These NLCs are more stable with an average diameter of 133nm, which remains unchanged in simulated gastric fluids but shows an increase in size up to 216 nm in simulated intestinal fluids. The activity of vitamin D3 encapsulated in NLCs was demonstrated by *in vitro* digestion

tests, where a release rate of more than 90% was obtained without degradation in simulated gastric fluid.

FUTURE PROSPECTS

Considering the development and benefits of nanomedicines, the critical methods to achieve clinical significance include optimization of formulations, pharmacokinetic parameters, and clinical approval procedures required to commercialise nanomedicines. Therefore, with the advances in science and technology in recent years, ADME evaluations of nanomedicines are of utmost importance. The incorporation of vitamins into nano-based formulations improves the physical, chemical, and biological properties of the formulations at the nanometer scale. Therefore, the evaluation of various parameters is required, which vary depending on the route of administration and distribution. The assessment of vitamins-based nanomedicines depends on whether or not orally administered nanomedicines are present in the gastrointestinal tract in nanoforms. Thus, with proper evaluation and techniques, various nano-based vitamin formulations can be produced in the future with improved properties to meet the global demand for nanomedicines. Recently, D-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS) has been approved by FDA as a safe excipient and is widely used in drug delivery systems. The physicochemical and biological properties of TPGS show multiple merits for its utility in drug delivery like greater biocompatibility, improved drug solubility, enhanced drug permeation, and selective anti-tumour properties. However, due to the many challenges faced by TPGS-based nanomedicines, more detailed research on TPGS properties and nanomedicine-based delivery systems is needed in the future. In the same sense, mathematical optimization can help study the binding kinetics and diffusion of nanoparticles so that targeted nanoparticles can penetrate deep into tumour tissues.

CONCLUSION

Nanomedicine is still in the research process due to its wide applicability and recent discovery. Vitamin-based nanomedicine is very promising, and it is progressing rapidly. Nanomedicine is a novel technique for effective loading of active molecules and surface activation (*e.g.*, antibiotics and functional groups) for active targeting to enhance therapeutic efficacy. The research results are intended to help the less fortunate and point the way to a better future.

CONSENT OF PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 11

Recent Advances in Tumor Targeting Drug Delivery System: Fundamentals of Advanced Pharmaceutical Nanoscience

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Abstract: Cancer is the second leading cause of death worldwide, with the highest morbidity and mortality rates. It is a heterogeneous disease that can occur in any organ or tissue of the body. The current report of the World Health Organization has demonstrated that approximately 1 crore of the world population is affected by different types of cancer, with leading cases of cancer occurring in an Asian population. Despite this, various pathways and proto-oncogenes are responsible for the progression of cancer. As such, it can affect both sexes. Prostate and breast cancer in men and women account for a significant part of cancer cases, respectively. Molecular targeting agents show a pivotal role in drug delivery. Natural compounds such as curcumin, resveratrol, genistein, and lycopene help heal the cancerous tissue efficiently and do not cause any side effects to the neighbouring cells. Targeting the site-specific portion with natural herbs provides better outcomes for cancer patients. A scientist develops various carrier systems for cancer to deliver the active moieties to the regions of the specific site. Delivery of medicament through carrier systems, such as liposomes, niosomes, dendrimers, solid lipid nanoparticles, and carbon nanotubes, provides better therapeutic outcomes due to its site-specific delivery pattern. Thus, various research for the treatment of cancer are currently ongoing. This chapter highlights an overview of various types of cancer barriers and conceptual information of carrier systems.

Keywords: Cancer, Carrier systems, Natural compounds, Proto-oncogenes.

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INTRODUCTION

Cancer is a term that refers to a group of diseases that develop as a result of the uncontrolled growth of malignant cells [1], which tend to invade other body parts. A tumor is a group of abnormal cells and tissue that forms in the human body [2]. According to the World Health Organization, more than 1 crore population was affected by various types of cancer in 2020 (Fig. **1**). The leading causes of cancer patients are found mostly in Australia, as represented in Fig. (**2**). In men, prostate, lung, colon, rectum, and urinary bladder cancers account for the highest percentages of cancer types [3]. Breast cancer, lung cancer, bronchus cancer, colon cancer, uterine corpus cancer, and thyroid cancer are the most common cancers in women [4]. This knowledge shows that prostate and breast cancer in men and women, respectively, account for a significant part of cancer cases. Proto-oncogenes play an essential role in cell division and development in normal environments as a result of cancer proliferation due to a disturbance in cellular relationships [5]. Due to this, various molecular targeting anticancer drugs are developed and are currently under clinical trials. Targeting therapy plays a key role in therapeutic delivery as it reduces the systemic side effects. Molecular targeting agents have played pivotal roles in drug delivery. Natural compounds are able to heal the cancerous tissue very efficiently and do not cause any side effects to the neighbouring cells [6]. There is a variety of effective strategies for the treatment of cancer. The main techniques are surgery, radiation therapy, and chemotherapy. Surgery and radiation therapy depend on the primary tumours and large metastases [7]. Some disseminated tumours, such as breast, prostate, and colorectal cancer, are specifically treated with chemotherapy. A tumour can grow in any part of the human body and, at times, would pose a severe threat to the patient, while some benign tumours are harmless. The tumours can be classified as benign, pre-malignant and malignant. The benign tumours are harmless, whereas pre-malignant [8] tumours do not cause harm to the body, but sometimes it shows a malignant property. The malignant tumours, however, are cancerous and can be fatal to the human body. Various factors such as missing of tumor specificity, drug tolerance to cancer cells and improper drug accumulation to cancer cells would decrease the drug efficacy. However, targeting the tumour with various anticancer agents is a promising technology. Chemotherapy causes side effects to the patient, whereas oral delivery does not show a promising effect on the tumour [9]. Furthermore, a high dose or multi-targeted agent shows promising effects, but it can harm non-cancerous cells. Thus, site-specific targeting plays a key role for cancers patient as it reduces systemic side effects and toxicity. The most common strategies used for drug delivery are self-assembly, PEGylation, stimulus resilience, increased permeability across the biological membrane, drug retention at the tumour site, and prodrugs [10]. It has been shown that targeting the tumour cells with natural compounds is safer than synthetic agents [6].

Various natural products are under clinical trials currently for the ailment of multiple cancers, as shown in Table **1**. Different natural products have demonstrated anticancer activity by modulating different signalling pathways (Fig. **3**). There are several natural products that act on signalling pathways (Fig. **3**), such as sulforaphane (Wnt (β-catenin and NF- kB), lycopene (PI3K/Akt, IGFR1, NF-kB, AR, cyclins/CDKs, and Wnt (β-catenin)), resveratrol (AR, STAT3, IGFR1, Cyclin/CDKs, Wnt (β-catenin), NF- kB, P13K/Akt and Notch-1), curcumin (mTOR, STAT3, PI3K/Akt, HIF-1 and NF-kB), genistein (Erβ, Wnt (β- catenin), FOXO3 and NF-kB) and EGCG (PI3K/AKT, VEGFR1/R2, MAPK, NF-kB, IGF/IGF1, COX-2 and EGFR/HER2). Various carrier systems have been developed and are under clinical trials, which could bring out potential outcomes in cancer treatment. Moreover, various organic and inorganic nanoparticles comprise highly promising candidates for the development of drug delivery. This chapter highlights an overview of various types of cancer barriers and conceptual information of carrier systems.

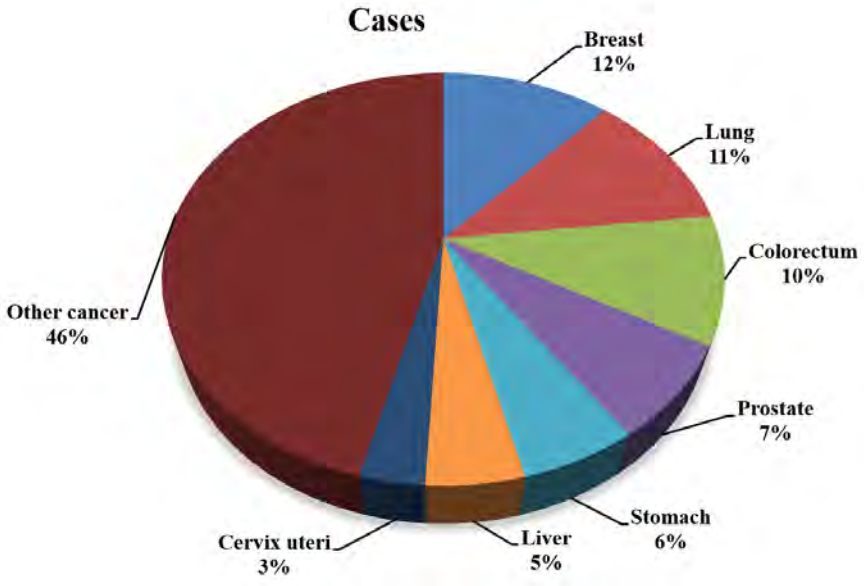

Fig. (1). Worldwide cancer-related incidence.

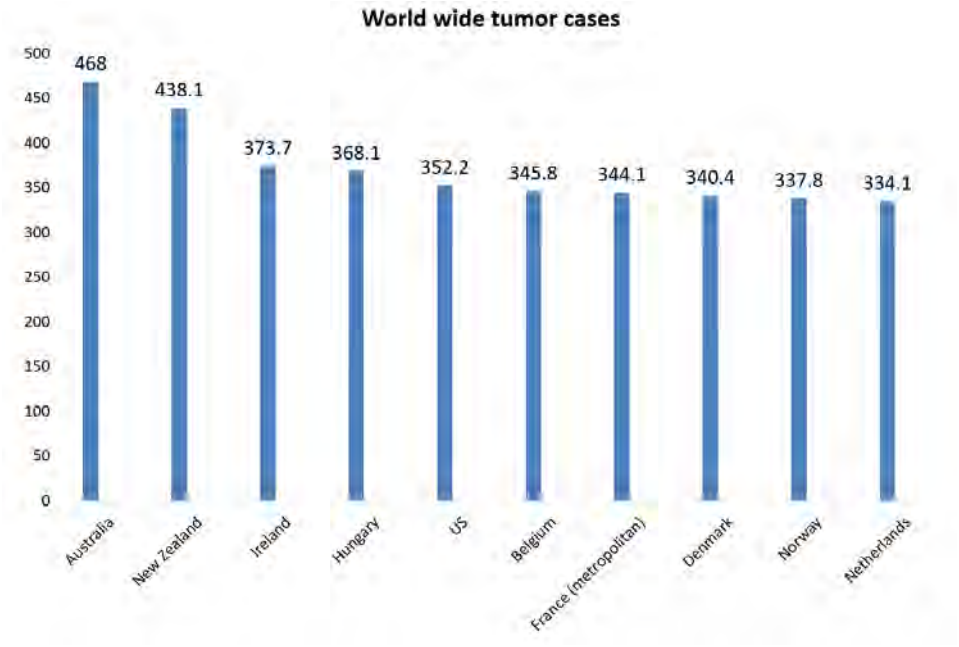

Fig. (2). Country-wise cancer-related incidence.

Table 1. Clinical trials data for various types of cancers.

S.No	Title	Types of Cancer	NCT No. Phase and Status
1.	A Pilot Study Conducted for Breast Cancer on Young Women.	Female Breast-Cancer	NCT03913936, Not Applicable, Completed
2.	Promoting Cancer Screening in Minnesota for Medicaid Recipients	Colorectal-Carcinoma	NCT03275987, Not Applicable, Completed
3.	Immuno Prost Trial in Prostate Cancer Using Nivolumab with DNA Repair Defects	Prostate Cancer	NCT03040791, Phase 2, Recruiting
4.	Combination therapy of Niraparib-Osimertinib for Lung Cancer treatment	Lung Cancer	NCT03891615, Phase 1, Recruiting
5.	For High-risk Endometrial Cancer Adjuvant Sequential & Concurrent CarboTaxol with Radiotherapy	Endometrial Cancer	NCT03935256, Phase 2, Recruiting
6.	Resilience and Equity in Aging, Cancer, and Health (REACH)	Gastric Cancer	NCT04674267, Not Applicable, Not yet recruiting
7.	To impact the Needs and Quality of Life of Patients, use an APP in Post Oral Cancer Surgery.	Oral Cancer	NCT04049968, Not Applicable, Completed

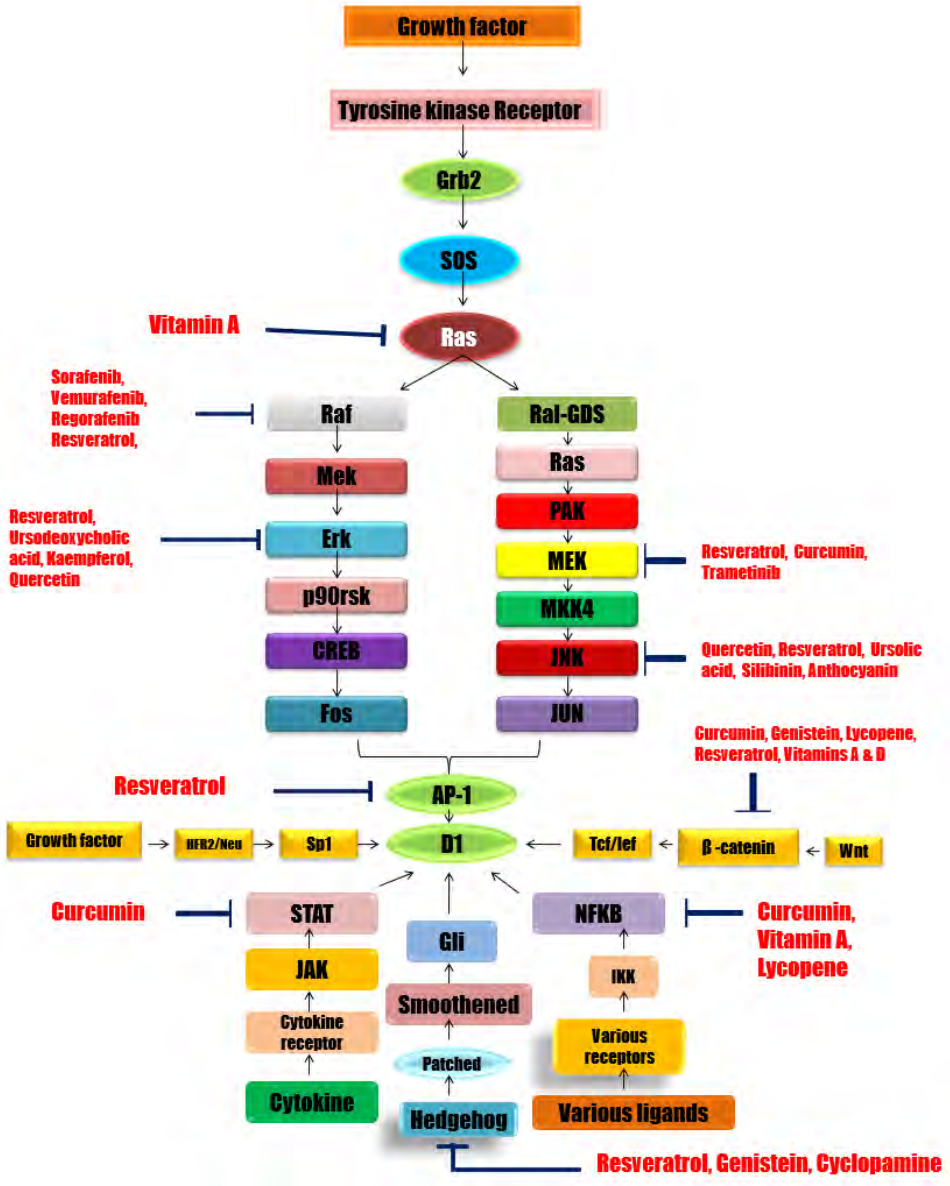

Fig. (3). Impact of various natural compounds upon selected growth-promoting signaling pathways.

DIFFERENT APPROACHES OF TUMOR TARGETING

Targeting the active moieties to the tumour region has a significant advantage to the cancerous patient [11]. An anticancer agent can be targeted through various targeting mechanisms such as passive targeting, active targeting, and receptor-mediated targeting (Fig. **4**).

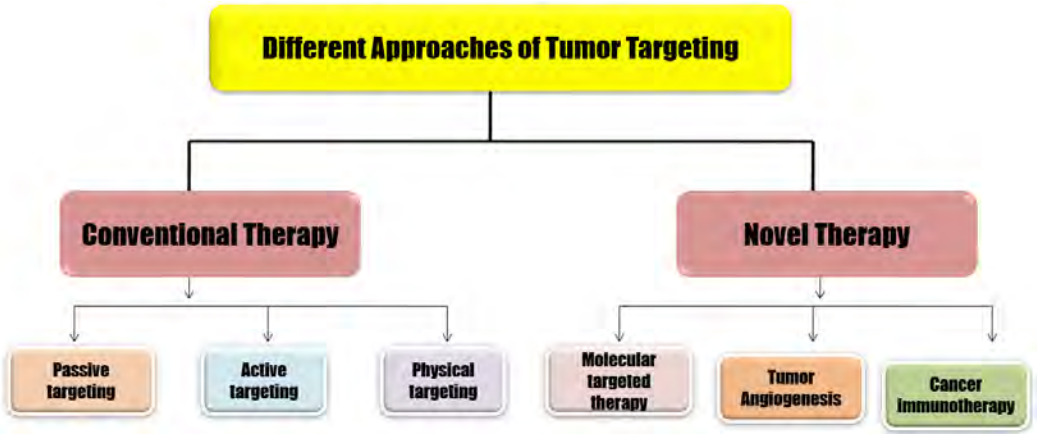

Fig. (4). Different approaches of tumor targeting. Cancer can be treated *via* conventional as well as novel therapy. The novel therapy shows better outcomes as compared to the conventional. The novel therapy involves molecular targeted therapy, which targets the active moiety of the tumor tissue and has potential benefits and lesser side effects on the non-cancerous tissue.

Conventional Therapy

Passive Targeting

The natural bio-distribution of the drug delivery carrier is commonly utilised due to its preferable site, as well as the excessive permeability throughout the proximity of tumours [12]. It can be done by the retention of drugs, phagocytosis of the particulate carrier through mononuclear phagocytosis systems (MPS), and specific reticuloendothelial localization in the organs [13]. Additionally, some usual properties of the microenvironment of the tumour are micro-acidosis and moderate hyperthermia [14]. The specific target capacity of the passive targeting method is comparatively small, and most of it is associated with a selective non- specific location of the therapeutics in normal tissues, which must be taken into consideration whenever these treatments are used [15]. The structural features of tumour cells are coherent with passive targeting. The introduction of innovative vessels to supply cancer nutrients and metabolite accumulation is indeed a necessary attribute for tumour growth [16]. This process begins once the tumour is around 1-2 mm large in size. The major release of pro-angiogenic variables is preceded by optimum growth in the cancerous population of cells, leading to disruptive development in newly established vessels. The vascular bed of the tumour comprises infantile and perilous vessels, and it has been marked by either a random orientation without even a defined pyramid of vessels and distinguishable arteries, capillaries, and venous structures [16]. Passive targeting is based on the physicochemical property. The nanoparticle would show prolonged action when the formulation is administered through an intravenous route. The enhanced

permeability and retention (EPR) effect provides better therapeutic action and highlights stealth characteristics to the tumour site (Fig. **5**). The EPR effect increases the gathering of macromolecules. This is because the vascular within a tumorous zone is leaking, and the lymphatic system is dysfunctional [17]. The activities behind the EPR mainly consist of absorption and convection. The major factors that affect the EPR efficacy usually involve extracellular matrix composition, vessel architectural features, phagocyte infiltration, colloidal carrier factors, interstitial fluid composition, and necrotic domain presence [18].

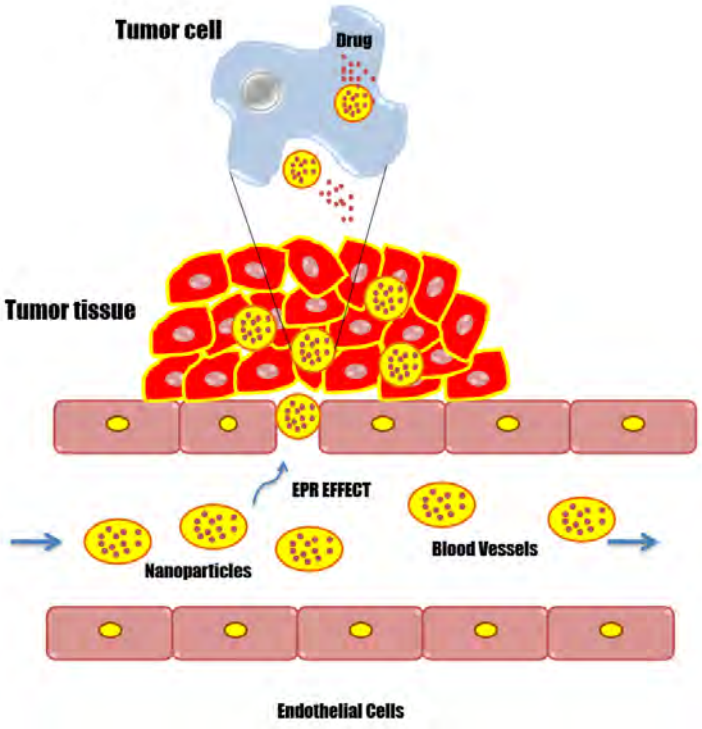

Fig. (5). The enhanced permeability and retention effect provide better therapeutic outcomes in drug delivery. As it targets the tumor tissue *via* a passively targeting mechanism and provides stealth characteristics. The figure illustrates the release of drug-loaded nanoparticles in tumors and provides the potential to the tumor tissue.

Active Targeting

Successful targeting method has been commonly used to enhance the therapeutic delivery of nanoparticles. Active cellular targeting must be intended to increase the tumour site of the nanocarrier by boosting its targeting potency and continuing to enhance its availability at the target location, mostly as a complementary method to the passive target nanocarrier [19]. In the 1980s, active targeting was first coined by covalently adding a particular antibody to the liposome surface (named

immunoliposome) to recognize a selected antigen on the target cell [20]. Active targeting is mostly employed to target the site *via* a colloidal carrier system. Several ligands have been identified for the active targeting of nanoparticles. Thus, ligands bind to a specific receptor and produce a therapeutic response. For this reason, different types of ligands have been used, as shown in Table **2**.

Table 2. Ligands for successful targeting of delivery channels for nanoparticles.

Category	Expression of ligand	Advantages/Disadvantages
Aptamers	AS-1411, GBI-10	High precision, small volume, high price
Peptides	RGD, IL4RPep-1	Easy processing, small size/ peptide-cleavable
Polysaccharides	Hyaluronic acid	Can be used in liver tissue as a polymer receptor backbone for nanoparticles.
Proteins	Antibodies, Transferrin	High precision, large scale, low stability.
Small molecules	Folate, anisamide phenylboronic acid	Relatively small, less expensive in normal tissues; targets are also expressed.

Novel Approach

Molecular Targeted Therapy

Targeted therapy plays a crucial role in therapeutic drug delivery. Targeted therapy helps deliver the system into a cancerous cell [21]. The penetration in the tumour epithelium would be better if the size of the carrier was smaller. The cell targets have always been genetically altered in the tumour cells; therefore, they are crucial for cancer progression as well as survival [22]. The oncoprotein or tumour suppressor genes targets are mainly active throughout the different signalling pathways, and they are mainly gene fusion products [23]. Table **3** displays the function of molecular targets and endorsed agents for the treatment of cancer.

Table 3. Molecular targeted therapy in anticancer.

Molecular Targets	Types of Cancer	Multiple Pathways	List of Targeting Agents
EGFR	Lung Cancer (Non-Small Cell)	Formation of PI3K/AKT signalling leads to lung carcinoma.	Various agents act on particular targets, such as erlotinib, gefitinib, afatinib, and neratinib.

(Table 3) cont.....

Molecular Targets	Types of Cancer	Multiple Pathways	List of Targeting Agents
VEGF and mTOR	Renal Cancer	Responsible pathways include VEGF and Mtor.	VEGF inhibitors: Sorafenib, sunitinib, and pazopanib. mTOR inhibitors: temsirolimus and everolimus.
HER2	Female breast Carcinoma	Activation of Ras/Raf/MAPK andPI3K/Akt pathways	The utilisation of monoclonal antibodies, such as trastuzumab and pertuzumab, leads to the suppression of breast cancer. Furthermore, lapatinib, afatinib, and neratinib also show promising outcomes for breast cancer.
FLT3-IT	Acute myeloid leukaemia	The occurrence of C-Myc, ERK, STAT and AKT pathways.	Sorafenib, daunorubicin, cytarabine are some of the therapeutic agents which reduce myeloid leukaemia.
VEGFR	Liver Cancer	Responsible pathways Ras/Raf/MEK/ERK	Various multi-kinase inhibitors such as sorafenib produce promising results. Moreover, some drugs like dovitinib act on receptors and reduce hepatic cancer cells.

THE PHYSIOLOGICAL BARRIER FOR DIFFERENT TYPES OF CANCER

The micro-environmental factors of the tumour usually pose hurdles to therapy. The deficient flow of oxygen by the defective vasculature and the uptake of oxygen by the tumour cells would result in a hypoxic environment [24]. Oxygen deprivation and activation of HIF-1 would mediate tumour cell tolerance to hypoxia by increasing the import and utilising cytoplasmic glucose (glycolysis and anaerobic lactate conversion) while reducing mitochondria, the primary source of oxygen consumption. The induction of VEGF leads to angiogenesis, while carbonic anhydrase induction of IX and XII contributes to ECM acidification [25]. The release by cancer and stroma cells of cytokines and angiogenic factors produce a complex network of interactions that control connective tissue plasticity and tumour perfusion [26]. The genetic, biochemical, and physiological factors which control these barriers are specifically meant for novel molecularly targeted agents. Despite their immense ability, passively and aggressively targeted NCs must overcome many physiological challenges to achieve better effectiveness in the clinic [27]. Local distribution directly into the diseased compartment of the therapeutics is an enticing technique as bypassing systematic administration would be able to overcome those hurdles. There are many body parts, such as the brain, bladder, peritoneum, lung and eyes, that are considered uncommon as they can be accessed internally for therapeutic administration (Fig. **6**) [28].

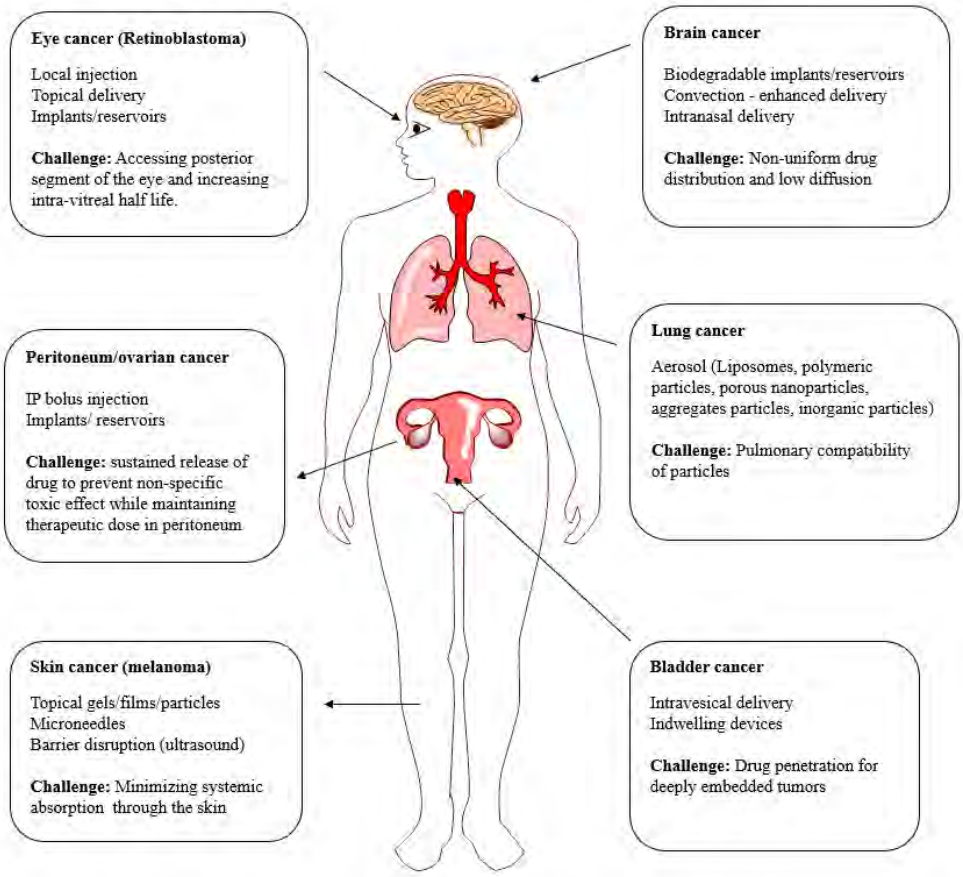

Fig. (6). Biological and technological obstacles to local drug delivery effectiveness. Local administration for some cancers is a promising method for selective delivery of medications. However, there are still some biological and technological obstacles unique to cancers that need to be addressed by this approach's clinical success.

Ocular Cancer

There are intricate physical and biochemical obstacles to the human eye. The anterior part of an eye consists of the conjunctiva, ciliary body, aqueous humor, cornea, iris, and lens. Similarly, the posterior portion mainly consists of the retina, choroid, vitreous humor, and optic nerve [29]. Usually, the traditional dosage forms would provide drug administration by the topical route to the anterior portion of the eye, such as 8.7% suspensions, 62.4% solutions, and 17.4% ointments, accounting for over 90% of ophthalmic drugs sold [30]. Much of the drug loss is contributed by tear turnover from lacrimal secretions. A stable eye with a tear volume of approximately 7–9 μL has a turnover rate of 0.5–2.2 μL/min. The total volume of major formulations is approximately 35–56 μL during topical administration, in

which the excess volume would drain into the systemic circulation through the nasolacrimal duct [31]. Epithelial close junction, reflex blinking, metabolism of ocular tissues, tear turnover, nasolacrimal drainage, efflux pumps are some of the anterior section barriers. Various nanocarriers have been developed and are currently under clinical trials (Table **4)** for ocular delivery, including microemulsions, nanosuspensions, liposomes, dendrimers, niosomes and discomes, cubosomes, nanomicelles as well as nanoparticles.

Table 4. Nanocarriers in Clinical trials

Nanomaterials	Type of Therapy	Status	Registered number of Clinical Studies
Various artificial tear grades, including liposomal spray method.	Dry eye	Completed	NCT02420834
Liposomes	Dry eye	Unknown	NCT02992392
Microemulsion	Dry eye	Completed	NCT02908282
Nanoparticles	Cataract management	Completed	NCT03001466

Brain Cancer

Neurodegenerative disease is caused due to the morbidity and mortality of neurological conditions, such as psychiatric disorders, pains, and infections. Delivery of drugs in tumour tissue *via* brain cells, however, poses major difficulty [32]. Administration of a drug to the brain cells has some difficulties as the majority of chemical moieties are removed from the blood-brain barrier (BBB). The BBB had been previously observed by Paul Ehrlich's developed model in which almost all peripheral organs had been stained upon intravenous infusion of water-soluble dye, except the brain and spinal cord [33]. The carrier-mediated transport mechanism plays an important part in the transport of polar molecules to the CNSS, such as glucose, amino acids, and nucleotides (Table **5**). The use of the multiple BBB carrier structures would provide the brain with efficient methods to carry medications and nanoparticles filled with drugs [34]. Levodopa is an example of a drug that has utilised carrier proteins for transportation through BBB. Levodopa acts as a precursor to lipid-insoluble dopamine, which has been used for the therapy of Parkinson's disease. Levodopa consists of alpha-amino and carboxyl groups which are integral for the BBB to undertake a substantial neutral amino acid carrier.

Table 5. Regulation of the penetration of substrates through the BBB endogenous transporters.

Carrier System	Abbreviation	Alignment	Substrate
Hexose carrier	GLUT-1	Transport the agent from blood to the brain.	Facilitative bidirectional D glucose
Novel Transporter for Organic Cation	OCTN2	Blood to the endothelium, endothelium to the brain.	Carnitine
Novel Transporter as Organic anion	OAT1	Transport the agent from the endothelium to the brain.	17-β-Estradiol-D-17-β-glucuronide
Novel Transporter as organic anion carrying polypeptide	OATP1	Transport the agent from endothelium to the brain.	Pravastatin, glucuronide conjugates, aldosterone, opioid agonists (N-tyrosinated peptides).
Thyroid hormone carrier	MCT8	Transport the agent from blood to brain.	Hormone thyroid T3

Lung Cancer

Lung cancer occurs when the cells in the lungs become irregular. Lung cancer cells would migrate to another area or organ in the body *via* the blood or lymph system. Lung cancer can be categorised into two types: small cell carcinoma of the lung (SCLC) and non-small cell carcinoma of the lung (NSCLC). It is extremely difficult to transmit the formulation into the pulmonary tissue [35]. Due to the low water solubility of many chemotherapeutic agents, it is recognized as a significant limitation of the pulmonary delivery of anticancer drugs. This limitation can be minimized *via* novel delivery systems such as polymeric particles, liposomes, aggregates particles, porous nanoparticles, and inorganic particles. Due to their protection and ability to provide controlled drug release in the lungs, liposomes are selected as drug carriers for inhalation. These carriers can capture a wide range of therapeutic molecules for delivery to the peripheral airways in large quantities (Table **6**). Liposomes can be administered into the body through the following devices such as pressurized metered-dose inhalers (pMDIs), dry powder inhalers (DPIs), soft mist inhalers (SMIs), and medical nebulizers [36].

Table 6. *In vivo* tests of aerosolized liposomal formulations and their conditions for lung cancer therapy in animals and humans.

Active Moieties	Available Devices	Liposomes Used	Model	Side Effects	Required Dose
Camptothecin	Nebulizer-Aerotech II	DLPC	Animal	-	81 μg/kg appropriately used for 30 min *via* inhalation.
PTX	Nebulizer-AeroMist	DLPC	Animal	Fierceness	Administered dose 5 mg/kg for 30 mins.
DOX-liposomes	Nebulizer-Collison jet	EPCChol, DSPEPEG	Animal	As compared to free drugs, very few adverse effects were shown.	Combination of inhalation (14 μg/kg) and *i.v*. (2.5 mg/ Kg).
IL-2	Nebulizer-Puritan Bennett twin jet	DMPC	Human volunteers	Very few	Required doses of 1.5, 3.0, and 6.0 for 8-84 days.
Cisplatin	Nebulizer-PARI LC Star jet	DPPC	Human volunteers	A sensation of harshness, nausea, and vomiting,	Increase from 1.5 mg/m2 until DLT for 1 – 4 consecutive days every 1 – 3 weeks.
DOX + antisense oligonucleotides	Nebulizer-collision attached with four port	DLPC	Animal	No side effect was found	DOX 2.5 mg/kg and 0.125 mg/kg antisense oligonucleotides every third day *via* inhalation.

APPROACHES FOR DRUG DELIVERY CARRIERS FOR THE TREATMENT OF CANCER

Inorganic and Organic Nanoparticles

Nanoparticles, either organic or inorganic, are deemed as highly promising candidates for the development of drug delivery mechanisms for cancer treatment (Fig. **7**). Drug delivery methods, which are based on nanoparticles are more preferred than conventional anticancer medications because they can decrease the systemic side effects that patients experience under typical chemotherapy by ensuring that the cytotoxic levels of the drugs are still available at the tumour sites [37]. Different physicochemical properties may be present in the NPS used for drug delivery, such as different sizes (1 nm to 100 nm), surface, form, and soft materials (organic and polymeric), or hard to use (inorganic) material [38].

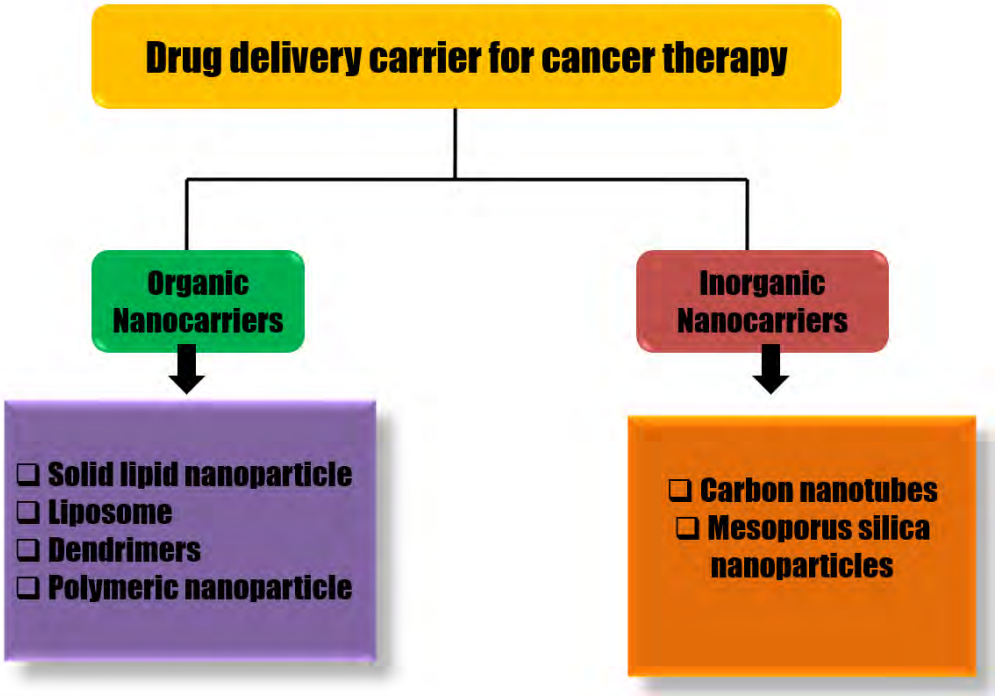

Fig. (7). The figure highlights the classification of nano-carriers used for cancer therapy.

Organic Nanoparticles

In the field of cancer treatment, a natural or synthetic polymer that is formed through organic-based nanomaterials has been commonly used due to their excellent properties such as biological stability and degradability. Stable lipid nanoparticles, dendrimers, liposomes, polymeric NPs, polymer-drug conjugates, and polymeric micelles can loosely be classified into different forms [39].

Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are lipid nanocarriers with a solid nucleus that can hold both hydrophilic and hydrophobic medicines. They can be made up of biocompatible materials; therefore, they are one of the preferred drug delivery options. Besides, surface modifications of SLNs can provide specific characteristics such as adhesiveness or targeting capabilities. SLNs have been researched for the production of better compositions/formulations of therapeutics or cosmetic applications, or nutraceutical applications [40].

The new polyethylene glycol (PEG) and chitosan copolymer grafted with folate were synthesized with solid lipid nanoparticles (SLN), which were developed by Rosiere *et al*. (F-PEG-HTCC). After pulmonary transmission, the coated SLN

reached the folate receptor (FR)-expressed in vitro HeLa and M109 HiFR cells and *In vivo* M109 tumours. Half of the gross paclitaxel inhibitory concentrations in M109-HiFR cells were substantially decreased in vitro by the coated SLN (60 *vs*. 340 nM, respectively). They demonstrated that FR was involved in these improvements, especially in 109-HiFR cells. Future studies will review the anti-tumour efficacy and toxicity, local and systemic, of coated SLN in animal models. He also discussed in detail the possible capability of SLN to resolve resistance processes as well as the mechanisms of entry into FR-expressing lung cancer cells and tumours [41]. In MDA-MB-231 cell lines, polyethylene glycol (PEG) binding with docetaxel (DTX) and gemcitabine (GEM) (DTX-PEG-GEM) demonstrated marginally higher cytotoxicity of DTX and NPs compared to MCF-7 cell lines (breast cancer cell lines), which could be attributed to higher OATP1B3 carriers in MDA-MB-231 cells, as stated in the literature by Kushwah *et al.* Additional uptake mechanisms involved in the case of NPS can be attributed tothis cellular uptake [42].

Dendrimer

As a drug carrier, dendrimers have excellent properties such as monodispersity, nanoscope scale, multiple periphery functional groups, tunable size, reproducibility and biocompatibility [43]. The cancer-controlled medicine can accommodate the inner voids of the dendrimers or be able to operate according to the specifications on the surface of the dendrimers. Targeting ligands may be added to the surface of the dendrimers for greater cancer cell selectivity, which would aid in the reorganization of cancer cells without damaging normal cells.

Aleanizy *et al*. had successfully demonstrated neratinib encapsulation and a sustained release profile of G4 PAMAM dendrimer. Comparative in vitro experiments found that these neratinib-loaded TZ-targeted dendrimers were more selective and had higher antiproliferation activity than neratinib alone and dendrimer-loaded neratinib against SKBR-3 cells. Neratinib loaded in plain and dendrimer grafted with trastuzumab were successfully packed. Improved cellular absorption of trastuzumab conjugated dendrimers and a stronger cytotoxic activity than plain neratinib dendrimers were shown. These results had reflected the ability of TZ-conjugated dendrimers to target cytotoxic drug carriers [44].

The PAMAM-drug-trastuzumab conjugates, especially for HER-2 positive SKBR-3 cells and very low toxicity to HER-2 negative MCF-7 cells, had been shown in the analysis by Marcinkowska *et al*. As predicted, trastuzumab from both conjugates accumulated rapidly in the HER-2-positive cell line SKBR-3, while a high level of PAMAM-ptx-trastuzumab was observed for HER-2-negative MCF-7 cells. Our findings confirmed that high selectivity of HER-2-positive cells,

PAMAM-doc-trastuzumab, and PAMAM-ptX-trastuzumab conjugates, had indicated the efficacy of the target agent trastuzumab. The studied conjugates thus offer a positive approach to enhance the efficiency of the targeted supply of drugs like docetaxel or paclitaxel [45].

Liposomes

Artificially prepared vesicles consisting of a lipid bilayer are called liposomes. Liposomal medications can be packed into liposomes and utilised to treat cancer and other diseases. Liposomes are one of the unusual drug mechanisms that can be used to monitor and control the delivery of drugs. Liposomes are delivered orally, parentally, and topically in cosmetics and hair technology for continuous- release and diagnostic purposes, and they are good gene transporters with numerous liposome-administered medicines that have been licensed [46].

Kushwah *et al*. have suggested that functionalised liposomes (DTX-AA-P- G-Liposomes) loaded with docetaxel (DTX) anacardic acid (AA) have been produced. High cell absorption and cytotoxicity of DTX-AA-PEG-liposomes have been demonstrated in in vitro experiments. In the case of AA-modified liposomes, effective nuclear co-localization has been observed. Compared to Taxotere®, DTX-AA-PEG-Liposomes have exhibited higher DTX bioavailability. With minor side effects, DTX-AA-PEG-liposomes have demonstrated possible tumour targeting. Moreover, compared to free DTX, formed liposomes have displayed comparatively higher cell inhibition and apoptosis in MCF-7 cells [47]. Jain *et al*. have reported that chlorine e6 is a photosensitizer containing chlorine-based porphyrin that is primarily used for the treatment of cancers, such as neck and head, early-stage lung cancer, and topical skin cancers. The chlorine e6 molecule exhibits heavy fluorescence in acetonitrile at an emission wavelength of 665 nm when excited at an excitation wavelength of 400 nm. The linearity of the fluorescence concentration plot is observed over a concentration range of 50 to 1000 ng/mL. The developed and validated method is successfully applied to the estimation of encapsulation efficiency over generated stealth liposomes [48].

Inorganic Nanoparticles

Various inorganic materials with interesting compositions and unusual chemical and physical properties have also been investigated as NPs for cancer therapies in addition to organic NPs used in the field of cancer therapies [49].

Mesoporous Silica Nanoparticles

Inorganic mesoporous like silica nanoparticles are entrants to the field of all available nanoparticles due to their unusual and superlative properties. A brief

overview of the most recent advances in the synthesis of nanoparticles of mesoporous silica and their use as drug delivery nanocarriers is offered. The excellent properties of mesoporous silica nanoparticles for biomedical applications have led to the manufacture of new, advanced multifunctional materials for a large range of biotechnological applications [50]. Gautam *et al*. developed an S-MTN@IG-P nano-platform which demonstrated greater drug loading (~20 percent) and enhanced drug release (~60 percent). In both colon cancer cells (HCT-116 and HT-29), which were triggered by the chemotherapy action of imatinib and the photothermal and ROS output effects of graphene oxide, a major cytotoxic effect was shown. The *In vivo* analysis also showed that S-MTN@IG-P could aggregate significantly in the tumour region and suppress metastasis under NIR irradiation without any biocompatibility concerns. They concluded that the above study cumulatively showed beneficial effects of S- MTN@IG-P in the productive chemophototherapy of colon cancer [51]. For combinational tumour therapy, a multifunctional nanoplatform based on mesoporous silica nanoparticles (MSNs) was developed by Cheng *et al*. As an antitumor drug, doxorubicin (DOX) was selected and biologically absorbed into MSN mesoporous. The dramatically improved tumour cell absorption efficiency and mitochondrial disruption ability of DOX@MSN-ss-DTPP&DTCPP nanoparticles in tumour environments were demonstrated in *in vitro* experiments in which DOX@MSN-ss-DTPP&DTCPP nanoparticles demonstrated the favoured cytotoxicity for alpha-3 positive cervical carcinoma (HeLa) cells [52].

Carbon Nanotubes

Carbon nanotubes (CNTs) are a family of fascinating carbon allotropes that make them productive materials, such as electronics, optics, and therapeutics for use in various nanotechnology disciplines. They demonstrate different properties such as electricity and heat power, and high conductivity. Their beauty can be due to the fact that the bonding pattern between the atoms is exceedingly stable with high extreme aspect ratios. For selective doxorubicin (DOX) transmission, Liu *et al*. formulated hyaluronic acid (HA)-modified amino single-walled carbon nanotubes (NH2-SWCNTs) developed to boost breast cancer therapy. HA, which specifically binds to the CD44 receptor, was coated non-covalently with NH2- SWCNTs by simple electrostatic adsorption. In the cancer cell spheroid assay to inhibit the growth of cancer cell spheroids, SWCNTs-DOX-HA had shown notable findings. Multifunctional SWCNTs-DOX-HA complexes had stronger effects than SWCNTs-DOX in inducing growth inhibition and apoptosis. The migration and production of cancer cell spheroids for MDA-MB-231 cells were also significantly inhibited by SWCNTs-DOX-HA [53].

CONCLUSION

Over the past few decades, tremendous research has been done for the treatment of cancer. Cancer is recognized as the second leading cause of death worldwide and has affected every population group. The delivery of medicament to specific sites is a key challenge due to various barriers posed by several types of cancers, such as ocular cancer, brain cancer, and lung cancer. Therefore, delivering the medicaments *via* a carrier system provides pivotal benefits for the treatment of cancer. This chapter helps understand the targeting mechanism and its barriers as well as the carrier system for various cancers.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 12

Niosomes: A Revolutionary Progress in the Field of Pharmaceutical Sciences

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Abstract: Niosomes are widely used nowadays as a novel drug delivery system. The amphiphilic nature of the niosomal drug delivery system provides an added advantage for a large number of newly discovered drugs. In this context, the bilayered structure of the niosomes helps in modifying various parameters involved in drug encapsulation. The niosomal drug delivery system also helps in altering the bioavailability, enhancing the skin permeability, and improving the drug interaction at the targeted sites. The present chapter focuses on the factors that influence niosomes formation, advantages and disadvantages of niosomal drug delivery systems, the formulation of niosomes in herbal products, their future prospects, commercial benefits as well as availability.

Keywords: Drug Delivery Systems, Encapsulation, Niosomes, Vesicular Delivery.

INTRODUCTION

Niosome is one of the most promising systems in drug delivery technology. It was first reported as a non-ionic surface active agent by researchers who were working in the cosmetic industry before it was transformed into a vesicle. Niosomes are formulated through a combination of non-ionic surfactants with cholesterol moiety with a bilayered structure. The bilayered structure is obtained by using the energy from the physical mixing or heat, which is amphiphilic [1].

In niosomes, the water-loving head remains in close contact with the aqueous solvent, while the lipophilic part is oriented away (Fig. **1**). Niosomes formation is governed by several factors such as the surface charge, particle size, and various

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attractive and repulsive forces, including Vander Waal forces, entropic repulsion as well as electrostatic interactions.

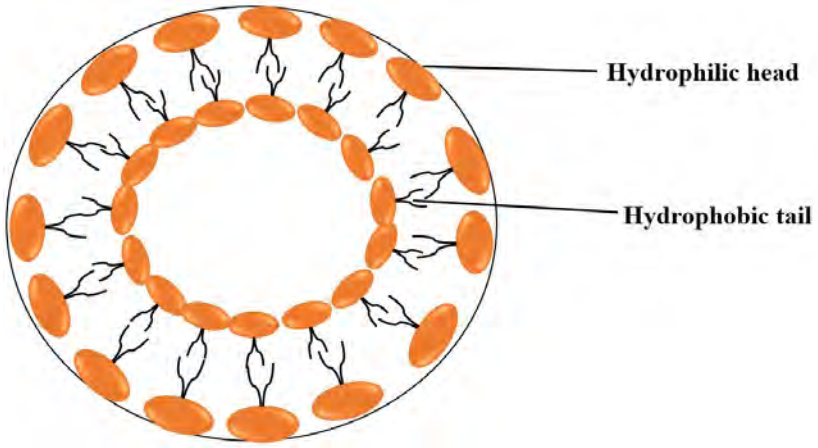

Fig. (1). Structure of Niosomes.

Niosomes can accommodate a wide range of drug molecules due to their amphiphilic nature. As a stable carrier, niosomes are widely utilised for the preparation of controlled release systems for different kinds of drugs. The stability of the niosomes depends on several factors, namely the nature of the encapsulated drug, temperature, type of surface-active agent, the presence of charged particles, and polymerisation. The therapeutic efficacy of the niosomal formulation is improved by delaying the drug clearance, protecting the drug from biological factors, and targeting drug delivery.

When preparing niosomes, the selection of surfactants depends on several parameters. These parameters include biodegradability, non-immunogenicity, and biocompatibility. As with other drug delivery systems, niosomes also have a few notable disadvantages, such as leaking, aggregation as well as fusion, which must be addressed to achieve successful outcomes [2].

Niosomes are utilised as a drug delivery carrier for many pharmacologically active agents used in the treatment of many diseases. In novel drug delivery, niosomes help in increasing the absorption rate of poorly absorbable drug moieties. Drugs that are delivered in the niosomal formulations show a remarkable increase in their bioavailability. Although the evolution of the niosomal drug delivery techniques still requires more exploration, they have shown potential for future applications in the pharmaceutical field.

CLASSIFICATION OF NIOSOMES

Based on their size, niosomes can be classified as follows (Fig. **2**):

- Small uni-lamellar vesicles (25-50 nm)
- Multilamellar vesicles (>50 nm)
- Large uni-lamellar vesicles (>100 nm)

There are several special types of niosomes; aspasomes, ethosomes, discomes, proniosomes, elactic niosomes, and polyhedral niosomes.

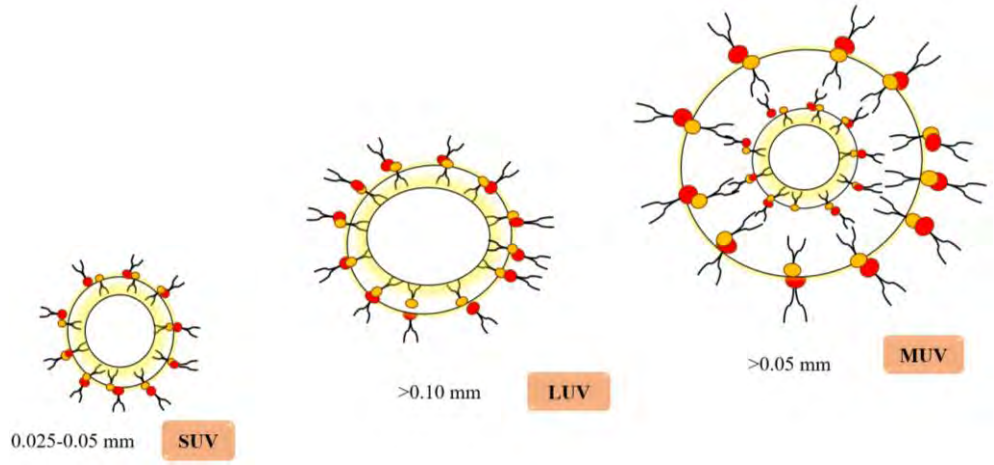

Fig. (2). Types of niosomes based on their size.

THE ADMINISTRATION ROUTES OF NIOSOMES

The route of drug administration is often implied by the disease. The nature of the drug to be encapsulated and the targeted site, however, pose challenges to the formulation scientists. Table **1** highlights the various routes of niosomal delivery.

Table 1. Various routes of niosomal delivery.

Sr. No.	Route of drug administration	Details
1.	Intravenous route	Niosomes provide a better and more stable formulation. The drugs administered through the intravenous route have a prolonged action along with improved bioavailability.
2.	Intramuscular route	Niosomes can be administered through the intramuscular route, and an example of it is the flu antigen intramuscular formulation.

(Table 1) cont.....

S. No.	Route of drug administration	Details
3.	Dermal and transdermal route	Many researchers have developed transdermal formulations for various drugs. NSAIDs administered by this route have resulted in fewer gastric disturbances. For example, clomipramine, when given through this route, has shown no first-pass metabolism with enhanced bioavailability. Fluconazole and lopinavir-like drugs also have been administered through this route in the form of niosomal gels. In addition, celecoxib transdermal patches have also been formulated and evaluated.
4.	Oral route	Niosomes also can be administered orally, such as tenofovir, cefdinir, paclitaxel, ginkgo biloba, and lornoxicam. The use of a niosomal drug delivery system has resulted in enhanced bioavailability due to better permeation and increased duration of action. The niosomal oral delivery is also used to control the drug release rate.
5.	Ocular route	Topical ocular delivery is ideal for delivering drugs with poor bioavailability (*i.e.*, 1-3%) in the anterior chamber of the eye. Drugs such as naltrexone, tacrolimus and prednisolone acetate are given in the form of niosomes or discomes. By using this route, niosomal drugs can be retained for an extended time.
6.	Pulmonary route	The administration of drugs *via* this route has many advantages, including superior therapeutic effects, sustained drug delivery, drug targeting, and improved permeation. Glucocorticoids are formulated as niosomes and delivered *via* the pulmonary route.
7.	Nasal route	The nasal route is preferred in cases where the drugs have to bypass the liver. Calcium channel blocker drug, such as diltiazem, has an oral bioavailability of 30-60%, but it undergoes first-pass metabolism. The nasal route provides the advantage of bypassing the liver; therefore, its bioavailability is enhanced.

THERAPEUTIC APPLICATIONS OF NIOSOMES

1. Niosomes are used for the delivery of proteins and peptides.
2. Anticancer drugs, such as paclitaxel, can be easily delivered using niosomes.
3. Niosomes provide a better option for the delivery of antigens and vaccines.
4. Haemoglobin can be enclosed and delivered in niosomes.
5. Zoduvidine-like drugs also can be given through a niosomal drug delivery system for the treatment of HIV-AIDS.
6. In the management of psoriasis, niosomes can be delivered *via* the dermal and transdermal routes.
7. With suitable adjustment of pH, niosomes are an excellent carrier for targeted drug delivery.
8. Niosomal delivery *via* the nasal route provides an alternate for brain drug delivery.

The literature review also suggests that there are some novel formulations prepared by using niosomes. This strongly signifies the importance of niosomes in drug delivery. The details of the research work are highlighted below (Table **2**).

Table 2. Details of research work focused on niosomes

S. No.	Formulation Types	Brief Detail	References [3 - 13]
1.	Elastic niosomal gel	The protease enzyme, papain, was incorporated in elastic niosomal gel, which resulted in better penetration than nanosphere. The enhanced absorption and treatment of the scar in animals were reported, which proved the utilisation of niosomes as permeation enhancers.	Manosroi *et al*. (2012)
2.	Liponiosomes (new biocompatible structure)	The combination of liposomes and niosomes was made, known as a liponiosome, which possessed a biocompatible pH-sensitive structure. This combination was used to deliver multiple chemotherapeutic agents. The results had shown increased drug entrapment, pH-sensitive sustained drug release, smooth globular surface morphology, and small size of vesicles.	Naderinezhad *et al.* (2017)
3.	Nanoparticles	Chitosan-based nanoparticles were employed to deliver certain drugs to the brain *via* the nasal route. The formulated nanoparticles had shown improved bioavailability of the drugs.	Casettari and Illum (2014)
4.	Smart Niosomes	Temozolomide (an oral alkylating agent) -loaded niosomes were formulated by using the thin-film hydration method. The prepared formulation had resulted in a 3-fold increase in the drug concentration in the cerebral spinal fluid. The surface modification helped achieve higher permeation, enhanced entrapment efficiency (79%), and smaller particle size (220 nm).	De *et al.* (2018)
5.	Magneto Niosomes	This type of noisome was developed by using a pluronic L64 surface-active agent along with cholesterol. The pluronic niosomes were used as carriers for the delivery of the anti-cancer drug doxorubicin HCl. The formulation was tested by using tumor cell lines (MDA-MD-231 and MCF-7). The results had shown significant time and dose reduction compared to the conventional formulation.	Tavano *et al.* (2013)

(Table 2) cont.....

Sr. No.	Formulation Types	Brief Detail	References [3 - 13]
6.	Niosomes	Niosomal formulation encapsulating trioxolone was prepared and evaluated for anti-leishmanial effect. The activity of the formulation was observed against *Leishmania tropica.* The cytotoxicity of the formulation and the drug were measured by using an MTT assay (assessing cell metabolic activity). The results of the conducted study had proven an increase in the Th1 cytokinase profile and a decrease in the Th2 cytokinase profile.	Parizi *et al.* (2019)
7.	Niosomes (Selenium+ Glucantime)	Niosomal formulation of selenium with glucantime was prepared. MTT assay, gene expression profile, and macrophage modelling were carried out. The results of the study had highlighted the potent effect of the anti-leishmania drug and the enhanced lethal activity with no cytotoxic effect.	Mahshid *et al.* (2019)
8.	Nanovascular gel (Act-loaded niosomes)	Acitretin was formulated in the form of nanovascular gel (act-loaded niosomes) to observe the activity of the drug for the treatment of psoriasis. The results had shown an enhanced *ex-vivo* permeation profile (up to 30 hours) compared to simple act-gel. This niosomal combination had resulted in a remarkable increase in the drug deposition in the epidermal and dermal layers.	Hashim *et al.* (2018)
9.	Niosomes (ether injection method)	Stavudine was encapsulated in the niosomes for a prolonged duration of action. The drug had a very short half-life; hence the niosomal formulation could prolong the duration. The formulation was prepared by using the ether injection method, and it was used in the treatment of HIV-AIDS. The formulation had shown good potential specifically for treatment with a prolonged release profile.	Shreedevi *et al.* (2016)
10.	Benazepril-loaded niosomes	Benazepril has extremely poor oral absorption, which prevents the development of an effective oral formulation. Therefore, an attempt was made to overcome this problem by formulating benazepril-loaded niosomes. The homogenously dispersed vesicles were prepared with 80-97% entrapment efficiency. This provided a good alternative to the conventional formulation for the delivery of such drugs with poor oral absorption.	Radhi *et al.* (2018)
11.	Rifampicin-loaded niosomes	Rifampicin-loaded niosomes were prepared by sonication technique, and the formulation was used for the treatment of tuberculosis and leprosy. The drug was poorly water-soluble in nature. The results of the study had revealed a stable formulation [63] with small-sized niosomes and a good drug release profile.	Khan *et al.* (2019)

The literature suggests that there are numerous formulations available that utilize niosomes as the drug delivery carrier. Drugs with poor oral absorption and less permeability can be easily delivered by encapsulating them in the form of niosomes. Moreover, the niosomal formulation that is applied topically has shown enhanced retention and prolonged activity of the drugs. Therapeutically, niosomes are among the best drug carriers available commercially.

Advantages and Disadvantages of Niosomes

Advantages	Disadvantages	Factors Involved
Can be used for controlled drug delivery	In some cases, surfactants may cause toxicity	Type of surfactant
Niosomes are osmotically stable.	Sterilisation problem	Hydrophilic-lipophilic balance
Targeting drug delivery	Short shelf-life of aqueous suspensions of niosomes	Geometric features of amphiphilic molecules
High compatibility and low toxicity	-	Thermodynamic features
Various routes of administration are possible with niosomes.	-	Gel-liquid transition temperature
No specific handling and storage conditions required	-	Nature of the drug
Can be used to achieve enhanced skin permeation	-	Excipients or additives

NIOSOMAL DRUG DELIVERY FOR HERBAL PRODUCTS

Herbal and natural products have been in great demand in recent years. Researchers have developed many options to deliver herbal drugs through novel drug delivery systems. Niosomes are established as a suitable method for the delivery of natural products with some added advantages. Curcumin is one of the main constituents of turmeric; however, it has poor water solubility, stability issues, a higher metabolic rate, and low bioavailability, which makes it difficult to manufacture curcumin formulations. These problems can be overcome by delivering the drug in the form of a niosomes-based formulation. By using this method, curcumin can be efficiently delivered for the treatment of various cancer cases (lung, prostate, breast, pancreatic blood, and colorectal).

The review of the literature also suggests that apart from curcumin, many herbal products can be delivered using niosomes. Paeonol, a herbal product with anti-inflammatory, anti-allergic, anti-diabetic, and anti-cancer properties, has been delivered by using the ethosomes system introduced by Ma *et al.* [14]. The stability and aqueous solubility issues can easily be overcome by this method. Agarwal

et al. [15] have also formulated morusin (prenylated flavonoid) -loaded niosomes with anti-bacterial and anti-tumor activity. Drug targeting was achieved by bypassing the reticuloendothelial system with this formulation. Insan and Jufri [16] had reported enhanced topical permeation of the green tea extract by using a niosomal system. Jin *et al.* [17] had also formulated niosomal ginkgo extract formulation, which had shown an increase in oral bioavailability. Waddad *et al.* [18], on the other hand, had prepared an intravenous formulation of morin hydrate by using niosomes, and it had displayed the ability to cross the blood-brain barrier.

IMPORTANT PATENTS RELATED TO NIOSOMES

Numerous niosomal formulations are available on the pharmaceutical market. These formulations have been patented before being commercially utilised worldwide. Some of the patents existing for niosomes are as follows (Table **3**):

Table 3. Some of the patents existing for niosomes.

S. No.	Company/Patent Inventor	Title of Patent	Patent No.
1.	Liposome Technology, Inc.	Liposome drug delivery method and omposition	US4873088A
2.	The Liposome Company Inc.	Steroidal liposomes	US4891208A
3.	Bayer Aktiengesellschaft	Ketoprofen liposomes	US5741515A
4.	Imarx Therapeutics, Inc.	Method for delivering bioactive agents using cochleates	US6403056B1
5.	Wm. Marsh Rice University	Temperature-sensitive polymer/nano-shell composites for photothermally modulated drug delivery	US6428811B1
6.	Jun Yang	A stent having cover with drug delivery capability	US20020143385A1
7.	Liposome Technology, Inc.	Liposome drug delivery method and composition	US4873088A

FUTURE PROSPECTS

Niosomes are widely used drug delivery systems in the pharmaceutical industry. There are large numbers of options available with the niosomal system. They can be used to encapsulate and deliver different pharmacologically active drugs, such as anti-HIV, anti-inflammatory, anti-viral, and anticancer drugs. Niosomes also provide better bioavailability, enhanced permeation, high aqueous solubility, targeted drug delivery, and most importantly, they are safer than ionic drug carriers.

ECONOMICAL PROSPECT OF NIOSOMES

Niosomal formulations have a wide variety of materials with different economic values. The ability of niosomes to modify the physical and chemical aspects of the drugs is a crucial aspect which sustains its marketed values. Some marketed niosomal formulations are listed in Table **4**.

Table 4. List of marketed niosomal formulations.

S. No.	Drug Used	Preparation Method	Advantages
1.	Glipizide and Metformin HCl	Thin-film hydration method	Release and encapsulation of amphoteric anti-diabetic drug
2.	Ammonium glycyrrhizinate	Thin layer evaporation method	Transdermal delivery of a drug
3.	5- Fluorouracil	Thin layer evaporation method	As a drug delivery carrier
4.	Acyclovir	Film hydration method	Increases bioavailability
5.	VIP (vasoactive intestinal peptide)	Probe sonication method	For brain targeting
6.	Cefpodoximeproxetil	Film hydration method	Reduces drug toxicity
7.	Ketoprofen	Thin-film hydration method	Increases entrapment efficiency
8.	Ribavin	Thin-film hydration method	For hepatic targeting
9.	Indomethacin	Lipid hydration method	Achieving sustained anti-platelets effects
10.	Urokinase	Film hydration method	For thrombolytic diseases
11.	Fluconazole	Ether injection method	Stability improvement
12.	Rifampicin	Handshaking method	Pulmonary (lung) targeting
13.	Gadobenate	Ether injection method	Diagnostic imaging purpose
14.	Insulin	Film hydration method	For oral delivery of peptide drug
15.	Gentamicin	Thin-film hydration method	For ophthalmic delivery of drug
16.	Vincristine	pH-mediated drug uptake process	For neoplastic therapy
17.	Methotrexate	Lipid layer hydration method	For psoriasis (local infection)
18.	Rifampicin	Reverse phase evaporation method	Release time prolongation achieved
19.	Diclofenac sodium	Lipid film hydration method	Anti-inflammatory effect
20.	Transferrin	Sonication method	Drug targeting

CONCLUSION

Niosome is a novel drug carrier that has shown great potential in drug delivery systems. The past research, which has focused on drug encapsulation in niosomes, suggested that it can be used for the delivery of numerous categories of drugs, such as anti-inflammatory, anti-neoplastic, anti-HIV, anti-infective, anti-viral, and steroidal drugs. Moreover, niosomes have several prominent advantages in drug delivery, including better bioavailability, enhanced permeation, high aqueous solubility, and organ targeting. Lastly, the safety of most of the niosomal formulations is better than the ionic drug carriers.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 13

Infectious Diseases: Pharmaceutical Nanoscience Targeted Drug Delivery

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Abstract: Infectious diseases are one of the greatest challenges of our new era. Due to their high incidence and outbreak rate, they can affect human health. Furthermore, the use of conventional drugs to treat infectious diseases is gradually being exhausted due to increasing rates of resistance. Herbal medicines and natural ingredients may also be a good resource for drug production. Several innovations, including the development of nano-drug delivery systems, have new mechanisms of action and various loadings for herbal and non-herbal treatments; this helps decrease the pathogenicity of infectious diseases. In addition, these nano-drug delivery systems provide a good opportunity to improve the efficacy of herbal and non-herbal treatments. They have also been used to deliver target medicinal agents, increase solubility, improve bioavailability, extend half-life for herbal and non-herbal treatments, increase stability, minimize adverse effects, and tissue engineering. Nanocarriers are advanced engineering tailors that control the physicochemical properties of nanoparticles for infectious diseases, leading to targeting by passive or active mechanisms. In this chapter, we highlight the advances in nanocarriers loaded with herbal and non—herbal agents for treating infectious diseases.

Keywords: Herbal treatment, Infectious diseases, Nanoparticles, Non-herbal treatment, Targeted drug delivery.

INTRODUCTION

Previous studies have revealed that infectious diseases are disorders that result from contact with an infectious organism, such as viruses, bacteria, worms, fungi, prions, or other parasites. Prions are known to be infectious proteins that are transmitted in several ways, including the inheritance or consumption of contaminated meat and other biological products [1]. The term infectivity describes the ability of an organism to spread, survive, or multiply in the host, while disease infectivity is a relative comparison by which a particular disease is transmitted to other hosts.

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Moreover, a pathogen can be transmitted in several ways, such as physical contact, vector organisms, body fluids, contaminated food, objects, or airborne inhalation [2].

The manifestation and severity of the disease can be promoted by exposure to any pathogen, as well as the ability of the pathogen to harm a particular host and the ability of certain hosts to be resistant to the pathogen. Therefore, clinicians classify microbes or infectious microorganisms as either opportunistic pathogens or primary pathogens, depending on the status of host resistance; primary pathogens were presumed to cause disease because of their activity or presence in the healthy and normal host, whereas organisms that were suggested to cause infectious disease in low-resistant hosts were classified as opportunistic pathogens. The opportunistic disease is thus associated with a decline in host resistance. This may occur as a result of genetic defects (including chronic granulomatous disease), exposure to ionizing radiation contact, ingestion of antimicrobial drugs, or treatment with immunosuppressive chemicals (as in poisoning or cancer chemotherapy), or as a result of an infectious disease with immunosuppressive effect (particularly in HIV disease, measles, or malaria). For infectious diseases, it is helpful to determine whether the outbreak is sporadic (episodic manifestation), endemic (regular cases frequently occur in a given area), epidemic (numerous conditions in a given area), or pandemic (a global epidemic) [3].

INFECTIOUS DISEASES' CLASSIFICATION DEPENDING ON THE ROUTE OF TRANSMISSION

Infectious Diseases of Digestive System

Several studies have been conducted on infectious diseases of the digestive system, including infectious diarrhea, cholera, bacillary and amebic dysentery, typhoid and paratyphoid fever, poliomyelitis, viral hepatitis, and several others.

Respiratory System Infectious Diseases

Every day, the respiratory system is exposed to numerous infectious diseases such as pulmonary anthrax, epidemic meningitis, SARS, pneumonic plague, measles, pulmonary tuberculosis, diphtheria, scarlet fever, whooping cough, rubella, epidemic parotitis, and especially influenza.

Infectious Disease Transmission *via* Contact

Transmission of infectious diseases by direct contact has high pathogenicity, such as rabies, leprosy, anthrax, avian influenza, brucellosis, and acute hemorrhagic

conjunctivitis. In addition, gonorrhea, AIDS, and syphilis can be considered among these types of infections due to their transmission through sexual contact.

Vector-Borne Infectious Diseases

Kala-azar, dengue fever, glandular plague, malaria, epidemic hemorrhagic fever, epidemic, endemic typhus, and filariasis are examples of vector-borne infectious diseases.

Blood-Borne Infectious Diseases

This class of infectious diseases includes a variety of diseases, such as AIDS and viral hepatitis. Accordingly, these infectious diseases have been found to be transmitted through a variety of routes. For example, a plague has been reported to be transmitted by fleas, while AIDS or HIV infection is transmitted through blood, sexual contact, and vertical mother-to-foot transmission; the pneumonic plague is transmitted through the respiratory tract; anthrax transmitted by contact, and respiratory infectious disease is transmitted through pulmonary anthrax.

Water-Borne Infectious Diseases

Water-borne was recognized over the years as the main vector for infectious diseases, including schistosomiasis and leptospirosis, and these were recorded to be transmitted *via* contaminated water and might cause an infection [4].

TREATMENT OF INFECTIOUS DISEASES

Herbal Treatment of Infectious Diseases

Herbal Treatment of Bacterial Origin Infectious Diseases

Prospects for Plant Natural Products to Control Infectious Diseases

Human health is considered a precious jewel, and the most vital challenge is to protect it from the diversity of the available infectious diseases owing to their rising incidence and outbreak rate [5]. Various innovations, including the evolution of drugs possessing new mechanisms of action, inhibition of microbial growth, as well as the availability of new drug categories, can alleviate the disease by inhibiting the production of a virulence factor. This category of drugs is considered a source of antibiotic adjuvants so that antimicrobial resistance may increase its power and delay [6, 7].

The Synergy of Natural Products Discovered for the Treatment of Infectious Diseases

The need for structurally and chemically complex molecules is one of the interesting fields of infectious disease treatment [8].

1- Up to date natural plant products have lower potential and selectivity than available natural microbial agents, with some exceptions through a constant activity of microbial growth inhibition. For example, acylphloroglucinols from S Johns Wort species (Hypericum spp.) have been found in methicillin-resistant Staphylococcus aureus (MRSA) isolates with a confirmed MIC (minimum infective concentration) in the range of 0.5–1 g/mL.

2- The preparation of natural plant products is done with several single compounds rich in antivirulant properties. For example, epigallocatechin gallate, as the main component of green tea catechins, was a promising non-bactericidal antiviral agent against Streptococcus pneumonia.

3- Vancomycin potential has been actively explored in biofilm-associated MRSA infections with American witch hazel bark and leaves, called hamamelitannin [9 - 15].

4- The finding of incredibly successful synergies between different compounds in the plant extract is a starting point for infection prevention. These synergies will improve efficiency and reduce the tendency to develop resistance. Artemisinin and its source plants, *A. annua,* are clear examples and can be helpful in a rat model for malaria infection. The procedure with a dosage of pure artemisinin equivalent to whole plant material was compared to oral administration of dried leaves [16 - 18].

The Antimicrobial Activity Mechanisms for Natural Products

The mechanisms of action of herbal medicines are generally associated with cytoplasmic membrane dissolution, electron transfer, proton motive force (PMF) destabilization, active transport, and coagulation of cellular material. It was found that not all of these mechanisms are effective at specific targets and that specific sites may be affected due to other mechanisms. Fig. (**1**) shows the sites where the components of herbal medicines consider bacterial cells as targets of action [19].

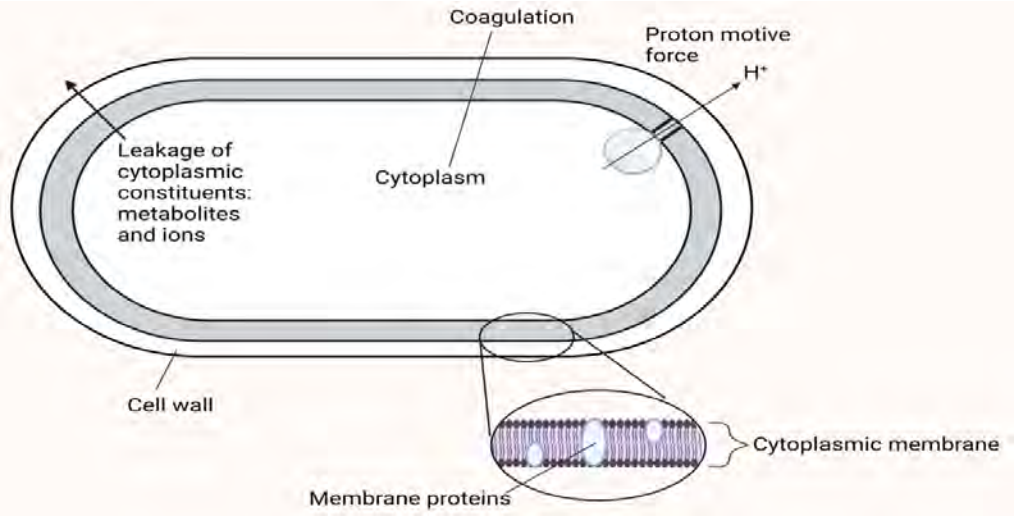

Fig. (1). Bacterial sites where plant medicines were active.

The antimicrobial activity of essential oils is essential for the involvement of lipids of the membrane of bacterial cells that disrupt cell structures and make them more permissible, including hydrophobic components. In addition, essential oils contain chemical compounds that act on cytoplasmic membrane proteins. Similarly, cyclic hydrocarbons could act on ATPases, which are known to be located at the cytoplasmic membrane and surrounded by lipid molecules. Similarly, the distortion of lipid-protein interactions by lipid-hydrocarbons and direct communication between lipophilic compounds and hydrophobic proteins is very likely. Thus, the discovery of the essential oils that promote pseudomycelial growth indicates that these enzymes can be used to synthesize the structural components of bacteria [20 - 23].

Herbal Treatment of Viral Origin Infectious Diseases

To date, effective immunization for most of the known virus types appears to require further research, and in addition, approval for clinical practice has been secured for only a few antiviral drugs. Natural products and herbal treatments represent an essential resource for the development of new antiviral drugs. As a result, recognizing the antiviral mechanisms of action of natural products on viruses to influence the viral life cycle, including viral entry, replication, assembly, and release, as well as targeting virus-host specific interactions, became essential [24 - 27]. Herbal treatment of infectious diseases originating from a virus is shown in Table **1**.

Table 1. Antiviral Effectiveness Related to Selected Viruses from Numerous Natural Ingredients Available and Herbal Therapy [28].

Virus	Natural ingredient (s)	Proposed mechanism(s)
Hepatitis C virus	Flavonolignans from *Silybum marianum*/ silymarin	The antiviral influence may be linked with flavonolignan antioxidant purposes.
	Curcumin	HCV replication inhibitor by deleting the pathway of Akt-SREBP-1
Coronavirus	*Houttuynia cordata* water extract towards SARS-CoV	SARS-CoV 3CL protease inhibitor; viral polymerase inhibitor
	Amentoflavone isolated from *Torreya Nucifera* towards SARS-CoV	SARS-CoV 3CL protease inhibitor
Herpes simplex virus	Proanthocyanidin-augmented extract from Myrothamnus flabellifolia Welw. towards HSV-1	Viral adsorption and exposure reduction
Respiratory syncytial virus	Resveratrol towards RSV-induced inflammation	Minimizes the inflammation of viruses through IFN-γ downregulation during RSV infection.
Influenza virus	Aqueous extract from dandelion (*Taraxacum officinale*) towards IFA	Inhibition of viral RNA NP amounts and operations of polymerase
	Extract from *Pelargonium sidoides* roots towards IFA	Removal of viral permeability and virus release; Removal of viral clotting and NA operation

Abbreviations:
HCV: hepatitis C virus, IFA: influenza A virus, NA: neuraminidase, NP: nucleoprotein, RSV: respiratory syncytial virus, SARS-CoV: severe acute respiratory syndrome coronavirus.

Viral Infectious Diseases and the Herbal Products with Antiviral Activity

A virus is an organism that cannot exist without the context of a cell. It uses the environment and cellular properties of the surrounding host cell to develop. Accordingly, various vaccines have been used to reduce the ability of a virus to replicate by attacking it in a way that necessarily reduces its impact on infected cells. Although some vaccines, including human immunodeficiency virus (HIV) and hepatitis C (HCV), have been or can be regulated to get rid of important pathogenic viruses such as polio, smallpox, and mumps, other viral diseases have been shown to be difficult to control by the old vaccine system. The manifestation of viral resistance and a therapeutic issue resulting from the prolonged use of antiviral agents have resulted in medical problems. This is often the case when treatment involves the concurrent use of multiple drugs, and the cost of these treatments is relatively high [29 - 31]. Drug-resistant viruses present a difficulty for the evolution of novel antiviral therapies. The development of novel antiviral

drugs whose treatments target the reproduction process itself presents a significant challenge.

If we understand the molecular mechanisms of viral invasion and replication, we can develop targeted antiviral drugs for the different phases of the viral cycle. However, in principle, any significant viral molecule is likely to be a target for a drug in viral reproduction [32]. Targeting viral molecules is more accurate and less toxic. However, it creates a limited number of viruses and poses a greater risk of resistant viruses. Drugs targeting cellular molecules may have a wider range of antiviral activity and a lower risk of developing viral resistance.

For thousands of years, herbal remedies have been utilized to eradicate the symptoms of many different human diseases, including infectious diseases. In addition, these naturally active compounds, which have more properties than normal combinatorial chemistry with increased chemical diversity and biochemical specificity, have great potential for finding new active structures against a broad range of test targets. In terms of the assay, plant-derived products are usually small molecules with pharmaceutical properties that are biologically active. They are specifically designed to be absorbed and metabolized by the body. The expansion costs of orally-active drugs are now likely to be far lower than the expansion costs associated with biotechnological or highest-value chemical compounds. Consequently, successful new antiviral drugs show that natural products are more promising compared to conventional medicinal plants.

At the same time, the structures of herbal medicines can be chemically modified and enhanced by understanding the mechanism of action, the link between structure and activity (SAR), molecular modelling, metabolism of medicinal products, and combination studies in chemistry. To uncover virus-specific targets, these compounds, when selectively interacting, must exhibit antiviral activity. Assessment of the cytotoxicity of the antiviral compound plays an important role in determining the chemotherapeutic agent when it exhibits toxicity that cannot persist long-term or acutely against the host. At the same time, it is expected to contribute to new drugs produced from these herbal agents by establishing the toxicity and effectiveness of these herbal medicines and conducting clinical trials.

Moreover, the advancement of new technological platforms, such as computer-aided drug design, biochip, RNAi techniques, high-throughput screening, and techniques, are expanding the possibilities for new drug discovery [33]. Progress has been made in the use of plant-derived agents, especially plant-based, that are effective against HIV, influenza, and various natural products that can help prevent and/or ameliorate the disease (Table **2**) [33].

Table 2. Anti-HIV and Anti-influenza Activity for Selected Natural Products

Compounds	Origin of plant	Activity/Target
Terpenoids Vaticinone	Vatica cinereal	Reduced replication
Flavonoids Baicalin	Scutellaria baicalensis	Reverse transcriptase infection/entry, replication
Flavonoids Biflavonoids (Ginkgetin) tetrahydroxyflavone	*Ginkgo biloba* L. *Scutellaria baicalensis*	Influenza virus sialidase influenza virus sialidase
Alkaloids Thalimonine Indole alkaloid	*Thalictrum simplex* L. *Uncaria rhynchophylla*	Influenza virus replication influenza virus replication
Lignans Rhinacanthin E, F	*Rhinacanthus nasutus*	Influenza virus

NON-HERBAL TREATMENT AND STRATEGIES TO OVERCOME ASSOCIATED PROBLEMS

Studies regarding existing routine treatments have found multiple drug resistance in a variety of bacterial isolates. Therefore, to meet demand, pharmaceutical companies have to modify the molecular structure of their existing products or reintroduce antimicrobial functions previously removed to bacterial resistance mechanisms. Otherwise, natural science cannot be ignored in the face of continued demand for new antimicrobials [34, 35].

HERBAL AGENTS AND ANTIMICROBIAL DRUGS INTERACTION

In terms of the antimicrobial effect on pathogens, there is a synergy between herbal medicines and synthetic antibiotic medicines same as that of plant extracts and essential oils. Probable drug-drug interactions that have motivated researchers to test these potentials are widely detected. However, associations between synthetic and herbal products need to be highlighted since combinational *in vitro* may not provide the same results in humans due to differences in pharmacokinetics and dosages [36], as shown in Table **3**.

PARADIGMS OF COMBINATION BETWEEN HERBAL AGENTS AND ANTIMICROBIAL DRUGS

1- A separate combination of garlic extracts with ampicillin and various effects on *Klebsiella pneumoniae* were observed. Although growth inhibition was observed in Proteus species by the combination of clove extract and tetracycline, these herbal extracts showed prolonged activity against antibiotic-resistant microorganisms acting

either uniquely or in combination with antibiotics used in traditional treatment [34].

Table 3. Antimicrobial Properties of Herbal Compounds [39].

Category	Subcategory	Kinds	Mechanism of Action
Alkaloids	-	Berberine	The cell wall and/or DNA interpolation
	-	Piperine	
Phenolics	Simple phenols	Catechol	Privation of substrate
		Epicatechin	Interruption of membrane
	Flavonoids	Chrysin	Adhesin binding
	Flavones	-	Complex with cell wall
		Abyssinone	Enzyme inactivation
			HIV reverse transcriptase inhibition
	Flavonols	Totarol	-
	Phenolic acid	Cinnamic acid	-
	Tannins	Ellagitannin	Protein binding
			Adhesin binding
			Reduction of enzyme
			Privation of substrate
			Complex with cell wall
			Interruption of membrane
			Complexing of metal-ion
	Quinones	Hypericin	Adhesin binding, complex with the cell wall, enzyme inactivation
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)
Lectins and polypeptides	-	Mannose-specific agglutinin	Decoy is designed to block the fusion of viral infections or to adsorb to a cell surface.
		Falxatin	Formation of disulfide bonds

(Table 3) cont.....

Category	Subcategory	Kinds	Mechanism of action
Terpenoids, essential oils	-	Capsaicin	Break down the structure of the cell membranes
Polyacetylenes	-	8s-heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol	-

2- Confirmation of the synergistic interaction of *Pelargonium graveolens* oil with norfloxacin against B. *cereus* and S. *aureus* was done, demonstrating the ability of the oil to enhance its antimicrobial activity *in vitro* assays [37].

3- It was shown that when commercial oils of *Melaleuca alternifolia*, *Thymus vulgaris*, and R. *officinalis* were used in high concentrations together with ciprofloxacin against *K. pneumonia and S. aureus*, and in mixtures with amphotericin B against *C. albicans strains*, the synergism of R. *officinalis* in combination with drugs tested *vs.C. albicans strains* and *S. aureus* mostly showed antagonistic profiles [38].

TARGETING DRUG DELIVERY FOR INFECTIOUS DISEASE TREATMENT

The usefulness of treating bacterial infections with conventional antibiotics has become a critical issue in today's world as resistance rates increase and the development of new drugs decreases. The mechanism of action of antibiotics is known to be DNA damage, inhibition of protein synthesis, and cell wall biosynthesis. However, antibiotic resistance has evolved in microbes through numerous mechanisms. It can rise from pre-existing forms, organisms, and variants, but it can also be caused by mutation or DNA shift. Resistance of microbes to antibiotics can be developed by mutation(s) through numerous mechanisms. It can affect the targeting or permeability of antibiotics, increase their efflux, or increase an antibiotic inactivating enzyme as well as bypass an enzyme pathway [40 - 42]. Accordingly, targeted delivery of antimicrobial agents to the site of infection, especially in intracellular infections, using nanoparticle systems is considered a stimulatory way to treat infectious diseases.

Non-antibiotic Treatment for Infectious Diseases

As alternatives to antibiotics, studies have shown that problems with drug resistance can be reduced through the following major classes:

Phage Therapy and Bacteriophages

Due to the extremely accurate and exclusive properties of new antimicrobial chemotherapies, renewed attention is currently being paid to the possible replace-

ment or supplementation by the use of bacteriophages (phages) in the control of bacterial strains resistant to traditional antimicrobial drugs. Furthermore, phages are recognized as structures with biological activity because they do not require metabolic equipment and are intracellular parasites that require a bacterium to replicate by using the biochemical machinery of bacterial cells *via* genetic material [43].

Bacteriocins

These bactericidal peptides, separated in the environment by a wide variety of bacteria, were discovered by Gratia in 1925 [44], as shown in Table **4**.

Table 4. Potential Antibiotics Developed from Bacteriocins (A chosen sample occupied from a study of Gillor *et al.* [45]).

Bacteriocin	Strain of producer	The contagious condition
Mersacidin	*Bacillus subtilis*	Strain of vancomycin-resistant
Lanthiopeptin	*Streptoverticillium cinnamoneum*	Herpes simplex virus
Epidermin	*Staphylococcus epidermidis*	Dermal infections

Killing Factors

This concept is used to identify the characteristics of bacterial cells that are released to destroy sibling cells during hunger. The biological world has been fascinated by this phenomenon, which is equivalent to the cannibalism of higher animals. For example, *B. subtilis* is preferable to examine; it has a series of "cannibalism" genes that trigger lysis in its sister cells when nutrients are scarce [46 - 50].

Quorum Quenching

It was deciphered that cells of microbial origin can be contacted by messenger molecules, analogous to cells in the higher organism. Several inspections found that cells in contact with microbial origin cells play a crucial role in virulence initiation. Two cross-talk processes have been predicted; the primary type of quorum sensing that involves the identification of an indicator *via* the cytosolic transcription factor and the mediation of an indicator *via* a membrane receptor in other categories. The microbe could be detected by a quorum-sensing device or a combination of both, *i.e.*, hybrid. [51, 52].

Antiviral Techniques

Pathogens create mechanisms that allow them to resist removal by their host and invade and attack host tissues as well as destroy host cells [53]. Many new alternatives under development respond by virulence, which facilitates the battle with the immune system.

Inhibiting Toxins and Activation of Secretion Processes

Once established, these agents may be chemical inhibitors or antibodies [54], as shown in Table **5**.

Table 5. Products of toxins suppressor (effector proteins) and secretion systems (chemical suppressors and antibodies) Revised from a study [54].

Name of product	Type	Bacterium	Target
Bezlotoxumab	Monoclonal antibodies	*C. difficile*	Toxin B
CHIR-1	Tiny molecules	*H. pylori*	Type 4 secretion systems
Compounds 1-9	Tiny molecules	*p. aeruginosa, B. pseudomallei*	Type 2 secretion systems
Compounds 7086, 7832, 7812	Small molecules	*Yersinia pestis*	Type 3 secretion systems
MED14893	Monoclonal antibodies	*S. aureus*	α – hemolysin
Raxibacumab	Monoclonal antibodies	*B. anthracis*	Cellular receptor anthrax toxin
Salicylidene acylhydrazides	Tiny molecules	*Pseudomonas, Salmonella, Chlamydia, Yersinia, Shigella*	Type 3 secretion systems
Shigamab	Monoclonal antibodies	*E. coli*	Stx-1, Stx-2

Targeting Biofilms and Adhesion

These new methods are being constructed, which are capable of collapsing biofilms, thereby circumventing the formation of biofilms [55].

Regulating, Targeting, and Signalling

Quorum-sensing is an intercellular signalling mechanism that controls the virulence of many bacterial pathogens.

Vaccines

In recent decades, “prophylaxis against pathogenic multidrug-resistant infections”

has become increasingly important due to the high economic and environmental costs of antibiotic treatments as clinical targets to promote novel antimicrobial agents using passive immunization and vaccination [56].

THE MODULATION OF MICROBIOME

In humans, the microbiome is the account of microorganisms in the body of human beings while all their genomes are collected by the microbiome.

Microbiome Usefulness in Medicine

At present, the most inspiring application of medicine is the treatment of recurrent *C. difficile* infections, an anaerobic, poison former, sporulating, and Gr+ve bacilli that tend to be the leading cause of diarrhea with which antibiotics and pseudomembranous colitis are associated [57].

A. **Prebiotics:** These polysaccharides (for example, fructooligosaccharides, inulin) are not absorbable. They have a beneficial impact on the health of the hosts, inspiring human gut microbiome biodiversity [58].
B. **Probiotics:** These are live microorganisms used in sufficient quantities to provide a health benefit to the host, maintain an adequate microbiome, and prevent the development of pathogenic bacteria [59].
C. **Fecal microbiota transplantation (FMT):** Fecal matter is used to obtain the intestinal microbiome of a healthy person affected by a patient, eliminating *C. difficile* and other microorganisms [60].

Nanoparticle Drug Delivery Systems as Targeted Therapy for Infectious Diseases

In recent research, it was discovered that antibiotic-loaded nanoparticles (NPs) can enter host cells *via* endocytosis and then release the payloads to destroy intracellular microbes. Instead, several NPs were tested for their ability to be deposited in the lungs and other organs. In addition, intratracheally administered NP antibiotics were found to enter the systemic circulation through the alveolar- capillary barrier and accumulate in extra-pulmonary organs, such as the spleen, liver, bones, and kidneys [61 - 63].

Developing Roles of Nano-Techniques in Antimicrobial Actions and Infectious Diseases Treatment

The development of many antimicrobial agents using multiple NPs has been shown to be effective. Both the surface and the interior of NPs can be associated with various types of hydrophobic and hydrophilic antibiotics or supported by

encapsulation. By maintaining suitable nanocarriers, the key pharmacokinetic properties of antibiotics are achieved, including release control, enhanced solubility, and targeted delivery [64, 65].

Nano Techniques for Infectious Disease Control and Vaccination

Researchers have developed particle systems in recent years that offer many advantages for vaccination because the sizes of bacteria and viruses that the immune system distinguishes include almost the micro-and nanoparticles. To increase uptake *via* the mononuclear phagocytic system (MPS), immune presence of antigens, and activation of antigen-presenting cells (APs), the chemical structure, size, surface properties, and loading can also be adjusted. This category can be administered through the mucous membranes, which would be especially necessary for vaccination in developing countries [66 - 68].

Nanomaterials to Control Infection: Nanoantibiotics

Nano-sized materials, which exhibit antibacterial behavior or increase the safety and efficacy of antibiotics administration, are referred to as “nanoantibiotics”. Unlike several currently used antimicrobial compounds, antimicrobial nanoparticles have no known acute or immediate side effects. It is strongly recommended that nanoparticles inhibit the biological pathways formed in various types of microbes, as well as the number of mutations that must take place to increase tolerance to nanoparticles' antimicrobial activities. Since antimicrobial nanoparticles are cost-effective, the synthesis of antibiotics would be associated with them, and they would be reasonably stable for long-term storage as well as an extended expiration date. In addition, antibiotic delivery using nanomaterials offers several advantages: 1) improvement in solubility, 2) relatively uniform and controllable distribution in target tissues, 3) controlled and sustained release, 4) minimization of side effects, 5) improvement in patient compliance and 6) enhancement of cellular internalization [64, 69 - 73].

Nanomaterials with Antimicrobial Effect

Antibacterial NPs are characterized in modern pharmaceutical science by being composed of metals and metal oxides, nanomaterials with carbon-based inherent antibacterial substances, and nanoemulsions with surfactant-based surfactants. The large surface area to volume ratio and the chemical and physical properties of many nanomaterials are expected to contribute to successful antimicrobial activity. Recent research has also shown that natural bacteria do not enhance antimicrobial resistance to metal nanoparticles. Accordingly, the mechanism of antimicrobial nanomaterials includes: 1) damage to cellular and viral components through photocatalytic of reactive oxygen species (ROS), 2) the obstruction of energy

transport, 3) adaption of bacterial cell wall/membrane, and 4) inhibition of DNA synthesis and enzyme activity [69, 74], as shown in Table **6** [75].

Table 6. Nanomaterials with antimicrobial effect.

Nanomaterial	Clinical and Industrial Applications	Antimicrobial Mechanism
Nanoemulsion	Inhaler for antimicrobials; nasal sprays; antibiofilm agent; delivery agents for vaccination	Membrane disruption; disruption of the spore coat
Fullerenes	Potential disinfection applications	Eradicating cell membrane solidity; increasing activity of infiltrating neutrophil
Chitosan	Disinfectants of the drinking water; immobilizing bacteria; biomedical products microbiocidal.	Improved permeability and disruption of the cell membrane; trace metals chelation; alteration of enzyme activation.
Nitric oxide-releasing nanoparticles	Diabetic foot care and injury infection	Nitric oxide release and production of ROS

Effective Antimicrobial Drug Delivery and NP Materials

The benefits of NP-based administration of plant and non-plant-based antimicrobial drugs include prolonged half-life, increased solubility of the poorly water-soluble drug, and prolonged and stimulatory drug release, which reduces dosing frequency. Moreover, synergistic, combination and resistance-overcoming effects can be achieved by co-administering antimicrobial drugs using NP carriers and targeting drug delivery with antimicrobial activity [76, 77].

Liposomes for Delivering Antimicrobial Agents

Liposomes remain the most commonly used carriers by pharmaceutical companies for the delivery of herbal and non-herbal drugs. This may be related to their structural similarity to cell membranes and the fact that they can easily attach to infectious bacteria. It is possible to encapsulate hydrophilic and hydrophobic antimicrobial agents in phospholipids of the bilayer and aqueous core, respectively [78]. Lipid-based nanocarriers for the delivery of antimicrobial agents are outlined in Table **7** [75].

Solid Lipid (SL) NPs for Targeting Drug Delivery

In pharmaceutical studies, SLNPs have been found to have a mixture of the advantages of typical solid NPs and liposomes, not just their drawbacks. Research suggests that SLNs can be effectively administered *via* various routes of adminis-

tration, including topical, parenteral, nasal, pulmonary, and ocular routes of administration, including enhancing drug delivery with the antimicrobial agent in addition to bioavailability.

Table 7. Lipid-Based Nanocarriers for Antimicrobial Drug Delivery.

Type of Nanocarrier	Composition	Target Microorganism	Encapsulated Antibiotics	Pathways Toward Successful Treatment Efficacy
Solid lipid	STC, SPC, and SA	Gr +ve bacteria, Gr - ve bacteria, and mycoplasma	Ciprofloxacin hydrochloride	Extended drug release
-	SDC, and GB	Fungi	Ketoconazole	Extended drug release; elevated physical stability
Liposomes	Chol, DSPG, and HSPC	Gr -ve bacteria	Amikacin	Prolonged stay within fluid compartments minimizes removal from the kidney and prevents excretion.
-	Chol, PC, and PG	*Mycobacterium avium*	Streptomycin	Targeted formulation utilizing drug encapsulation; enhanced antimicrobial activity by preventing microbial multiplication.

Abbreviations
Chol: cholesterol, HSPC: hydrogenated soybean phosphatidylcholine, PC: phosphatidylcholine, GB: glyceryl behenate, SDC: sodium deoxycholate, DSPG: distearoyl phosphatidylglycerol, SPC: soybean phosphatidylcholine,SA: stearic acid, STC: sodium taurocholate, PG: phosphatidylglycerol

Unlike polymeric NPs and liposomes, SLNPs that can be inhaled are quite safe, have a high degree of drug incorporation, and are less prone to environmentally harmful organic solvents. Table **11** outlined SLNPs studied for antimicrobial drug delivery [79 - 84].

Polymeric NPs for Drug Delivery

Polymeric nanoparticles used for the drug delivery of herbal and non-herbal origin offer numerous advantages: 1) well tunable properties (for example, zeta-potentials, particle size, and drug release profiles) by changing the lengths of polymer, organic solvents, and/or surfactants, used for nanoparticle preparation, 2) stability of structures in various biological fluids, storage and manufacturing conditions (such as ultrafine milling and spray drying), and 3) use of a specific functionalized surface for drug conjugation and/or ligand targeting. It was found that the system of nanoparticles (NPs) (lectin-conjugated gliadin NPs) bound to the carbohydrate receptors of microbes, and the antimicrobial agents were released, for example, H. pylori. Two main categories of polymeric nanoparticles, amphiphilic

block copolymers, and linear polymers, have been discovered for antimicrobial drug delivery (such as polymethyl methacrylate and polyalkyl acrylates) [85].

Drug Delivery Utilizing Dendrimers

Recent studies on targeted delivery of drugs of plant and non-plant origin have identified dendrimers as polymers with hyperbranching properties as well as specific nanoarchitecture and minimal polydispersity, built up layer by layer around a core, resulting in branch points (drug conjugation ability), high size control and surface functionality [86]. Other numerous antimicrobial agents are effectively combined in dendrimer nanoparticles to improve solubility and, thus, therapeutic efficacy, as shown in Table **8** [75].

Table 8. Nanocarriers with Polymer-Based Drug Delivery of Antimicrobial agents.

Type of Nanocarrier	Polymer	Target microorganism	Encapsulated antibiotics	The mechanism for enhanced therapeutic effectiveness
Dendrimers	PAMAM	*Escherichia coli*	Nadifloxacin and Prulifloxacin	Enhanced water solubility modifies the action of these agents to increase access into the bacteria's membrane.
	PLCP	Plasmodium falciparum	Artemether	Improved stability of drugs; solubility improvement; extended circulation half-life of drugs
Solid NPs	GPAA	Staphylococcus aureus and Bacillus anthracis	N-sec-butylthio β-lactam, Ciprofloxacin	Enhanced bioavailability; significantly enhanced therapeutic effect due to the conjugation of the antibiotics with GPAA
	PIHCA	Salmonella typhimurium	Ampicillin	Drug concentration in the liver and spleen were increased; more efficient drug cellular absorption by macrophages; and lower mortality rate in test animals.

Abbreviations
GPAA: glycosylated polyacrylate, PIHCA: polyisohexylcyanoacrylate, PLCP: pegylated lysine-based copolymeric dendrimer, PAMAML polyamidoamine.

Drug-Resistant Microorganisms and Biofilms Treatment

A combination of more than one antimicrobial agent in the appropriate nanoparticles for synchronous delivery is another promising technique to overcome antibiotic resistance. Furthermore, an integrated system of antibiotics and antimicrobial nanoparticles (such as silver nanoparticles) has been used as a promising method to improve antimicrobial activity and likely reduce resistance

to established antibiotics. For example, in the presence of silver nanoparticles, the activity of antibiotics such as erythromycin, kanamycin, ampicillin, and chloramphenicol against Gram-negative and Gram-positive bacteria was enhanced [87].

CONCLUSION

In summary, polymeric nano-drugs can dramatically increase biological activity and overcome problems caused by herbal and non-herbal drugs. However, there are still obstacles to the development of effective therapies in this field. Attempts to regulate biological systems with nanomaterials represent some of the obstacles to translation into the drug. New challenges in the development of nanotechnology-based drugs are the numerous synthesis methods that should be used for rapid mass production of these novel drugs and the production of multifunctional systems that meet many biological and therapeutic needs. It is a new challenge for researchers to develop nano-drug delivery systems containing both natural and non-natural substances.

CONSENT FOR PUBLICATION

Not Applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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Swarnlata Saraf

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Vivek Dave

Vivek Dave, presently working in the capacity of Associate Professor at Central University South Bihar, Gaya, Bihar has commendable experience and skills coupled with sound theoretical knowledge. Besides his past experiences with many pharmacy colleges, he also worked for more than 8 years at the Banasthali University, Rajasthan soon after pursuing his Ph.D. from Banasthali University itself. During his Ph.D., he worked on the design and evaluation of aceclofenac and esmolol HCl containing ocular drug delivery system. He did his Masters in Pharmaceutics on Aceclofenac ethosomes for enhanced transdermal drug delivery system. His expertise in working with the formulation and development of novel drug delivery systems like PEGylated liposome, ethosomes, nanoparticle, quantum dots has shaped the leading scientific high impact research and book publications of international repute. Currently, he is working on nano-formulation for therapeutic managements of various diseases like Alzheimer's, inflammation, and cancer, etc. He has been the recipient of several awards in various conferences, seminars and conventions, including a prestigious award, "Best Sustainability Award" on Smart Shoe sole in the 2019's International Conference on Future Healthcare and Economic Development from Medical Device Innovation Center (MDIC), National Cheng Kung University, Taiwan. Dr. Dave is also acting as a regular reviewer of journals of national and international repute of different publishing houses, namely Wiley, Springer, Elsevier, BMC, etc.