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A Review of Patents on "Mozafari Method" as a Green Technology for Manufacturing Bioactive Carriers

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Abstract: Encapsulation and controlled release of bioactive compounds is now an established protocol to enhance the bioavailability and long-lasting efficacy of therapeutic and disease-preventive agents while minimizing or eliminating side effects. Currently, there are a number of products approved by the regulatory authorities for Human or animal use, which contain encapsulated drugs, nutraceuticals, diagnostic agents, cosmetics, or even vaccines. Manufacturing the encapsulation/carrier systems requires special considerations with respect to scalability, environmental issues, and cost-effectiveness. Among the many available procedures for large-scale preparation of micro- and nanocarriers, the "Mozafari method" has proven to be simple to implement and reproducible technique that does not require sophisticated equipment or use of potentially toxic solvents. The mentioned proprietary method and patents in which this method is utilized or incorporated is the focus of the present review article.

Keywords: bioactive agents; carrier vehicle; drug delivery; liposome; nanoliposome; tocosome; solid lipid nanoparticle.

1. Introduction

Encapsulated bioactive materials or natural compounds have been widely utilized in the agricultural, pharmaceutical, and food industries. Encapsulation is a method of enclosing materials into capsules before their delivery into an *in vitro* or an *in vivo* system [1]. Encapsulation involves the incorporation of bioactive compounds such as therapeutic ingredients, nutraceuticals, antioxidants, vitamins, probiotics, polyphenols, enzymes, and vaccines in small vesicles with micron or nano diameters. This method has proved to be an ideal technique for (*1*) production of targeted delivery systems to control the release of the encapsulated compound; (*2*) preserving unstable bioactive compounds from harsh conditions (e.g., high temperature, extreme pH values, and oxygen); (*3*) ease of handling – due to the possibility of changing physical characteristics of the original core material (i.e., the physical change of a substance from a liquid into a solid); (*4*) hiding the undesirable flavors or odors of

certain active ingredients to enhance their acceptance; and (5) increasing the aqueous solubility of the encapsulated compounds [1].

There are different types of encapsulation systems made of different materials such as surfactants, polymers, lipids, or phospholipids. Lipid-based nanocarriers have been successful since 15 of the 21 marketed approved nanomedicines are based on liposomes or lipid nanoparticles. These include AmBisome®, Estrasorb®, DepoDur®, DaunoXome®, Doxil®, DepoCyt®, Marqibo®, Inflexal® V, Visudyne®, Mepact®, Myocet®, Amphotec®, Abelcet®, Diprivan®, and Fungizone® [2]. Having structural units recognized as safe (GRAS), these carriers are well-accepted scientifically for therapeutic purposes [3]. Moreover, lipids' biocompatibility, biodegradability, and versatility properties resulted in their usage as safe delivery systems for humans, with low or non-associated toxicity [4].

Accordingly, one of the most applied encapsulation systems is a liposome, mainly composed of phospholipid molecules [5]. Liposomes are closed, spherical structures with curved double lipidic layers [6]. The main constituents of liposomes are phospholipids, which are amphiphilic molecules containing a hydrophilic head group and two hydrophobic tails composed of fatty acid chains. These characteristics of phospholipids provide liposomes with unique properties in aqueous media and make them an ideal carrier system to be applied in different fields, including food, agriculture, pharmaceutics, and cosmetics. A significant advantage of a liposome is that it can incorporate and release two materials with different solubilities simultaneously. Using liposomes, incorporation of two antioxidant agents, for instance, glutathione (a water-soluble molecule) and alpha-tocopherol (a lipid-soluble molecule), in the same lipid vesicle is achievable [6].

Several methods are used to produce liposomes and nanoliposomes, including conventional and novel preparation techniques. Among the new techniques, the "Mozafari Method" has been highly considered a robust and green technology due to several advantages compared with other techniques [7]. Several scientists have applied this method in many research studies, dissertations, and patents worldwide [8-10].

In this article, different techniques used for manufacturing bioactive colloidal carriers are reviewed and compared, focusing on Mozafari Method as a proprietary green technology. All patents in which this method has been elaborated were investigated to offer a comprehensive and useful scientific and practical insight.

2. Micro- and Nanocarrier Systems

According to the particle size, encapsulation technology is called microencapsulation (1 µm to 1 mm) or nanoencapsulation (from 10 to 999 nm) [11, 12]. This is while, in some instances, nanoparticles are referred to as structures below the size of 100nm. Micro/nanoencapsulation can help bioactive ingredients be directly delivered to the target site and be protected from adverse environmental factors, thus improving their bioavailability [13]. Over the years, numerous methods have been developed to encapsulate bioactive compounds. These methods are capable of manufacturing micron-sized or nano-sized carrier systems in one or multiple steps [14]. Microcapsules can potentially deliver bioactive ingredients to desired targets. Microencapsulation techniques can be divided into chemical, physicochemical, and physicomechanical procedures [15]. These methods include emulsification, spray drying, spray congealing, fluid bed coating, coacervation, centrifugal extrusion, ionic gelation, pan coating, melt extrusion, polymerization, emulsion solvent evaporation, and liposome/nanoliposome entrapment. Emulsification, coacervation, and supercritical fluid techniques are used for both

lipophilic and hydrophilic ingredients, while emulsification–solvent evaporation, inclusion complexation, and nanoprecipitation- mainly used for lipophilic substances [16, 17]. As a result of recent progress in different aspects of nanotechnology, microcapsules and micro-carriers are gradually being replaced by their "nano" counterparts. The term nanotechnology is originated from the Greek word "nanos", meaning small [18]. In the area of food and pharmaceutical sciences, nanotechnology is being used to understand how physicochemical characteristics of nanoscale materials can change the structure, texture, nutritional value, and quality of drug and food components [19]. The most useful application of nanotechnology in the food, cosmetics, and pharmaceutical industries is encapsulation and controlled release of bioactive materials [20]. The range of applications for nanoencapsulation in these industries has been increasing due to the many benefits of this technology providing for the encapsulated materials. The advantages include protecting the encapsulated materials against environmental, chemical, and enzymatic effects, extreme pH, temperature, ionic variations, and masking undesirable flavors and odors [21].

Nanoencapsulation can be achieved via two main approaches: the top-down and the bottom-up procedures. Top-down procedures include emulsification and emulsification–solvent evaporation, while the bottom-up methods include supercritical fluid techniques, coacervation, inclusion complexation, and nanoprecipitation. Nevertheless, a combination of both procedures is often employed. Nanoencapsulation methods have been used to encapsulate both lipophilic and hydrophilic bioactive compounds [16, 17].

There are various types of materials used for producing micro- and nano-capsules such as carbohydrate polymers (including hydrocolloids), proteins (including gelatin and casein), and lipids (glycerides and phospholipids) which can be employed in the pharmaceutical, food, and nutraceutical sectors. Lipid-based carrier systems have many advantages compared with other encapsulation approaches, such as alginate- and chitosan-based carriers. These benefits include the ability to entrap materials with different solubility alone or combined (water-soluble and lipid-soluble materials), the stabilization of water-soluble ingredients, particularly in high water-activity applications, the possibility of up-scale production using natural ingredients, and the controlled release of core material in the target sites [22]. Obviously, liposomes are important in biological, pharmaceutical, and medical research. Their resemblance to cell membranes makes them ideal for studying certain cell functions and some cellular basis of disease. Since liposomes are one of the most effective carriers for delivering many different types of bioactive ingredients into cells, liposomal products have extremely wide applications [23].

3. Manufacturing Techniques

There are a wide variety of techniques for the production of liposomal formulations. All these techniques require lipids to be combined somehow with an aqueous phase [23]. These methods are categorized into two groups conventional and novel preparation techniques. It should be noted that liposomes and nanoliposomes are formed when the phospholipid ingredients are placed in an aqueous phase, and a sufficient amount of energy (e.g., in the form of mechanical agitation, sonication, homogenization, heat, etc.) is applied [24]. Non-covalent interactions such as van der Waals interactions, hydrophilic-hydrophobic effect, hydration, electrostatic forces, steric and depletion interactions are the main interactions between lipids/phospholipids and the encapsulated material in the structure of the liposome [25].

Coherent selection of a proper technique for the preparation of liposomes, nanoliposomes, and other vesicular carriers depends on the following parameters:

A) Physicochemical characteristics of the encapsulated bioactive materials;

B) The route of drug administration;

C) Physicochemical properties of the medium or solvents in which the vesicles and other components of the formulation are dispersed;

D) Target shelf-life, size, polydispersity index, zeta potential, and release profile of the carriers;

E) Potential toxicity and influential concentration of the encapsulated bioactive ingredients in the formulation [22, 24].

There are several laboratory-scale and a few industrial techniques for preparing carrier systems. However, most of these techniques are not suitable for encapsulating sensitive bioactive ingredients because of their exposure to harmful chemicals (e.g., volatile organic solvents and detergents), extreme pH conditions, or mechanical stress (e.g., high pressures, sonication, or high-shear force homogenization). Conventional preparation techniques consist of four basic stages: *1*) drying down lipid/phospholipid ingredients from organic solvents, *2*) dispersing these compounds in an aqueous media, *3*) purifying the obtained vesicles, and *4*) analyzing the finished product. However, the remaining organic solvent residues in the vesicles during their preparation could lead to toxicity. Therefore, one of the disadvantages of almost all conventional manufacturing techniques is employing organic solvents in the preparation process. Due to advancements in encapsulation technology, preparing lipid vesicles without using any volatile organic solvent or detergent has become possible in some methods such as the bubble method [26], the polyol dilution method [27], a heating method [28, 29], Mozafari method [7, 30, 31], and microfluidization [32]. Some of the conventional and novel methods for liposome/nanoliposome preparation are listed in Tables 1 and 2, along with their advantages and drawbacks.

3.1. Conventional preparation methods.

Conventional techniques for liposome production mostly involve approaches that are easy to use at a laboratory scale. Some of the most applied conventional liposome preparation methods are described in Table 1. As explained above, organic solvent residues remaining in the lipidic phase and/or aqueous compartments of the liposomes and nanoliposomes during their preparation could result in toxicity. The application of volatile organic solvents in the preparation of phospholipid vesicles is one of the drawbacks of nearly all conventional manufacturing methods.

3.2. Novel preparation techniques.

Recently, unique approaches have been developed for preparing liposomes and nanoliposomes to minimize the concerning issues in conventional liposome preparation techniques. Novel methods appear to be more useful than conventional ones for up-scale production but require some special equipment, some of which are listed in Table 2.

3.3. Mozafari method.

Mozafari method is a modified and improved version of the heating method introduced in 2007 to prepare liposomes and nanoliposomes (in addition to some other carrier systems

such as tocosomes). Tocosome is a colloidal and vesicular bioactive carrier system, and the main constituents are phosphate-group-bearing alpha tocopherols [6]. Nevertheless, similar to nanoliposomes, they can also accommodate sterols, proteins, and polymers in their structure. Mozafari method is regarded as a green technology applied in the preparation of a number of carrier systems on industrial scales without using any organic solvents and at mild temperatures and pH conditions. Therefore, this technique could be conveniently applied to encapsulate heat-sensitive materials such as nutraceuticals, drugs, and vaccines [33]. This method is economical and capable of manufacturing encapsulation systems, with superior monodispersity and storage stability using a simple protocol and one single vessel from small to large, industrial scales.

Recently, the development of functional foods and vaccines has rapidly progressed during the COVID-19 pandemic. As lipidic carriers represent one of the most advanced platforms for nutraceuticals and vaccine delivery, Mozafari Method has acceptable features in this regard.

Mozafari method could be explained in the following three steps [34] (Figure 1):

1. Adding nanoliposomal ingredients to a preheated (40–60 °C) mixture of the active agent and a polyol such as glycerol, propylene glycol, or sorbitol in a heat-resistant vessel.

2. Heating the mixture at 40–60 °C while stirring (e.g., at 1000 rpm) on a hotplate stirrer under an inert atmosphere (such as nitrogen or argon gas).

3. Following the manufacture of the nanoliposomes, the product must be kept at temperatures above the phase transition temperature of the phospholipid ingredients (Tc) under an inert atmosphere to allow the vesicles to anneal and stabilize. Subsequently, the temperature of the nanoliposomal formulation needs to be gradually brought down to an ambient temperature before storage.

The heating method, on the other hand, is composed of the following steps: *i*) Hydrating the ingredients in an aqueous medium for 1–2 h under nitrogen; *ii*) Mixing the dispersion with the material to be encapsulated and adding glycerol; *iii*) Mixing the sample (800–1,000 rpm) at a temperature above Tc of the lipids until all the lipids dissolved; and *iv*) Leaving the product above Tc under nitrogen for 1 h to allow the sample to anneal and stabilize [28, 29].

Table 1. Conventional techniques for liposome preparation (adapted with modifications from References [35, 36]).

Preparation method	Preparation procedure	Advantages	Disadvantages
Bangham Method	- Dissolution of lipids in an organic phase - Removal of the organic solvent, usually via evaporation, to form a lipid film - Hydration of the dried lipid film using an aqueous media such as a buffer	-The first method introduced for liposome preparation using basic laboratory equipment	- Use of toxic organic solvents - Production of large particles with no control on size - Poor encapsulation efficiencies of hydrophilic materials - Time-consuming - Sterilization issue
Detergent Depletion Method	- Formation of detergent-lipid micelles - Removal of the detergent to form liposomes	- Relatively mild process - Can be used for the preparation of a wide variety of vesicle types -Homogeneous liposomes can be obtained	- Low final concentration of liposomes can be obtained - Low entrapment of any hydrophobic compound - Remaining detergent in the formulation can cause toxicity - Time-consuming
Injection Methods	- Dissolution of lipid ingredients into an organic phase - Injection of the lipid solution into an aqueous media	- Simple method	- Forming heterogeneous liposomes

Preparation method	Preparation procedure	Advantages	Disadvantages
Emulsification Method	- Dissolving lipids in an organic solvent - Mixing both organic and water phases together	- Higher encapsulation efficiencies compared to the injection methods	- Organic phase is not removed soon afterward
Reverse-Phase Evaporation Method	- Dissolving the lipids in an organic solvent - Adding a small volume of the aqueous phase - Sonicating the solution to produce inverted micelles - Removing the organic solvent using a rotary evaporator and formation of a viscous gel	- Simple design -Acceptable encapsulation efficiency	- Not suitable for fragile molecules such as peptides due to the contact of the encapsulated compound with an organic solvent.
Heating method	- Hydrating the ingredients in an aqueous medium for 1–2 h under nitrogen - Mixing the dispersion with the material to be encapsulated and adding glycerol - Mixing the sample (800–1,000 rpm) at a temperature above Tc of the lipids until all the lipids dissolved - Leaving the product above Tc under nitrogen for 1 h to allow the sample to anneal and stabilize	- No toxic solvents or detergents required - No need for sterilization - Possibility of scale-up manufacture	- Requirement for high temperatures in some instances.

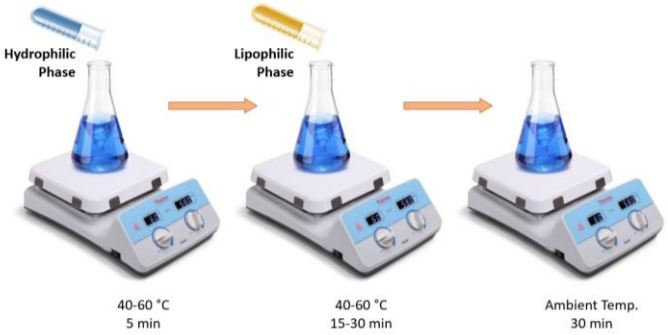

Figure 1. Schematic illustration of Mozafari method for preparation of carrier systems including liposomes, nanoliposomes, and tocosomes. The hydrophilic phase may contain a polyol as co-solvent/dispersant and water-soluble bioactive compounds, while the lipophilic phase contains lipid/phospholipid ingredients and may contain lipid-soluble bioactive compounds.

Table 2. Novel techniques for liposome preparation (adapted with modifications from References [35-37]).

Preparation method	Preparation procedure	Advantages	Disadvantages
Freeze Drying of Monophase Solutions Method	- Dissolving a phospholipid in t-butyl alcohol to form an isotropic monophase solution - Freeze drying the solution	- Long-time storage - Smaller and more uniform particle size - Used for both lipophilic and hydrophilic drugs	- Increased sucrose concentration affects the polydispersity of liposome particles
Microfluidic Channel Method	- Dissolving a stream of lipid in alcohol passing between two aqueous streams in a microfluidic channel - Mixing at the liquid interfaces and thus liposomes forming	- Size and size distribution of the liposomes are controlled - Used for drug encapsulation immediately before use - Eliminating shelf-life limitations of the current liposome preparation techniques	- High-pressure treatment may damage the structure/function of the drug and other bioactive compounds
Membrane Contactor Method	- Pressing lipid at temperatures above the melting point passing the molten phase through the membrane	- Producing solid-lipid particles - Simple design - Controlling the size - Scaling-up abilities	NA

Preparation method	Preparation procedure	Advantages	Disadvantages
Dense Gas Techniques	- Using the substance in the region surrounding the critical point, mostly carbon dioxide	- Replacing many organic solvents - Using non-flammable, non-toxic, non-corrosive, inexpensive, environmentally acceptable material - Recovering solvent easily	- Need for sterile operating conditions and one-step production
Mozafari Method	- Adding the ingredients to a preheated (<60 °C) mixture of the active agent and glycerol - Mixing and heating the ingredients at a mild shear force (e.g., 1000rpm) under an inert atmosphere - Annealing the formulation before storage	- No degradation of the lipid ingredients and encapsulated bioactive compounds - Requires single vessel - Possible up-scale production	NA

4. Recent Patents on Methods for Liposome Preparation (2010-2020)

Lipid-based carriers, including liposomes, nanoliposomes, solid-lipid nanoparticles (SLN), archaeosomes, and vesicular phospholipid gels [22], currently hold an outstanding position among drug delivery systems. In clinical applications, liposome-encapsulated drugs have been proven to be most useful for their ability to reduce the side effects of the encapsulated drugs compared with the non-encapsulated drugs. Due to their application as experimental biomembrane models since the 1960s, lipid dispersions in water are currently one of the most methodically studied lyotropic liquid crystalline structures. Consequently, the mechanisms of formation of bilayer-lipid-vesicles are well elaborated, and a variety of methods for their manufacture have been developed [8, 26, 34, 37]. Among the conventional methods for liposome preparation, the oldest technique (based on dissolving the liposomal ingredients in organic solvents) is currently known as "thin-film hydration" [22, 34, 35]. A common limitation of conventional methods includes dissolving phospholipid solution in organic solvents prior to dispersion in an aqueous phase. However, the toxicity of organic residue is a major problem in their pharmaceutical application. The use of a volatile organic solvent may also affect the chemical structure and stability of the encapsulated drugs. On the other hand, exposures to detergents, sonication, or high shear-force homogenization, employed in conventional methods, are not suitable for processing fragile drug compounds. The drawbacks of the conventional liposome preparation methods also include being complex, time-consuming, and high cost for large-scale manufacture. Since industrial-scale liposome production has become a reality, several increasingly attractive procedures have extended the range of liposome manufacture techniques. Some of these new methods represent advancements of the conventional techniques allowing for scale-up, better reproducibility, and enhanced process control. Different preparation techniques of lipid-based carrier systems mentioned in recent patents are listed in Table 3.

Table 3. Recent patents on methods for liposome preparation.

Patent number	Patent Title / Inventor(s)	Reference
US2010202928 US8715591	Microfluidic apparatus to control liposome formation Gaitan *et al*. (2014)	[38]
WO200232564 EP1334765 JP2002535796 US2004099976	Process for producing liposome and apparatus thereof Otake *et al*. (2004)	[39]
US2010239521	Method for the preparation of micro-and nano-sized carrier systems for the encapsulation of bioactive substances	[14]

Patent number	Patent Title / Inventor(s)	Reference
	Mozafari (2010)	
US2010247620	Method for co-encapsulation of combination drugs and co-encapsulated combination drugs product Castor & Corp (2014)	[40]
WO2011105835	Method and apparatus for preparing novel liposome Hwang *et al*. (2011)	[41]
WO2013059133 AU2012326370	Method for lyophilizing liposomes Cabral-Lilly *et al*. (2012)	[42]
US9592198B2	Microfluidic liposome synthesis, purification and active drug loading Hood & De Voe (2014)	[43]
CN106345542B	A kind of micro-fluidic chip and preparation method thereof preparing liposome for multi-emulsion method Yuying *et al*. (2016)	[44]
WO2018127016A1	Light-responsive liposome, preparation method and application thereof Guanjing *et al*. (2017)	[45]
CN108939090B	Liposome, preparation method and application Liangcheng *et al*. (2018)	[46]
CA3089529A1	A novel blank liposome with ginsenoside rg3 or its analog as membrane materials and preparations and uses thereof Wang *et al*. (2019)	[47]
CN111544393A	Preparation method of drug-loaded liposome Ting & Ning (2020)	[48]
CN112401009A	Liposome, preparation method and application thereof Jian *et al*. (2020)	[49]

5. Patents Incorporating "Mozafari Method"

A particularly robust and simple technique for liposome preparation without using organic solvents, detergents, high-shear-force procedures, extreme pH values, etc., is the Mozafari method. The method can also be used for the manufacture of other carrier systems, including nanoliposomes, tocosomes, niosomes, solid-lipid-nanoparticles, and vesicular gels [7, 34, 35]. In this method, heating and stirring of the aqueous lipid dispersion may occur simultaneously [7, 14]. Temperature and mechanical agitation provide adequate energy for the formation of stable liposomes. The particle size can be controlled by the phospholipid selection as well as the duration of the overall process. Bioactive agents (e.g., vaccine candidates, diagnostic agents, drugs, nutraceuticals, genetic material) can be added at several stages, which provides flexibility to the invented method to allow the encapsulation of a wide variety of molecules and compounds.

Table 4. Recent patents have elaborated Mozafari method as a technique for liposome preparation.

Patent number	Title	Application	Reference
WO2013087083A1; US9636414B2	Particles comprising single-stranded RNA and double-stranded RNA for immunomodulation	The useful carriers for RNA include lipidic carriers such as cationic lipids, liposomes, and micelles. In spite of different methods utilized for liposome preparation, Mozafari method, as a novel method, is employed to produce materials for human use.	[50]
WO2012095660A2	Method using fluorinated amphiphiles	Method of inhibiting the insertion of one or more membrane proteins into a lipid bilayer. It also relates to a method of inserting a predetermined number of membrane proteins into a lipid bilayer liposome. Lipid bilayers could be prepared using Montal & Mueller or Mozafari method.	[51]
US8663599B1	Pharmaceutical composition of nanoparticles	A pharmaceutical composition of pH-sensitive liposome nanoparticles for lodging in a target tissue cell *in situ* of an animal subject, the nanoparticles comprising a proton-releasing photosensitive compound that releases protons upon photolysis, wherein the compound is in vesicles of the liposomes. New methods such as the Mozafari method and extrusion produce liposomes for human use.	[52]

Patent number	Title	Application	Reference
US20150190843A1	Method of preparing silica-coated nanodiamonds	This patent involves contacting a nanodiamond encapsulated in a liposome with a silica precursor and reacting the silica precursor to form a silica coating on the nanodiamond. Liposomes were fabricated by sonicating phospholipids in water. Liposomes can also be prepared by the Mozafari method.	[53]
WO2013192190A1; US20150150245A1	Liposomal formulations	Liposomal formulations comprising pesticides, nematicides, or herbicides are provided to control pests and weeds. Pesticides and herbicides can be entrapped in lipid vesicles produced by any method, including heating, Mozafari, or extrusion methods.	[54]
US9889208B2	Lipid-based drug carriers for rapid penetration through mucus linings	Different methods for the manufacture of mucus-penetrating lipid nanoparticles are described along with their applications. The nanoparticles include lipid molecules, one or more PEGylated lipids, and additional ingredients that stabilize the particles chemically and physically. Sonication is considered a harsh preparation method as it can damage the structure of the encapsulated drug. Robust and safe methods such as the Mozafari method and extrusion are employed to produce materials for human use.	[55]
US20160270400A1	Liposome-attractant formulations	Liposomal-attractant formulations comprising pesticides or nematicides are regarded for the control of pests. The liposomal-attractant formulations can be applied to pre- or post-emergent crops and to the soil, plant media, plants, plant tissues, and seeds to treat or control pest or nematode infections of humans and animals. The Mozafari method, extrusion, polyol dilution, bubble, and heating method can be used for the manufacturing of liposomes.	[56]
US20160324986A1	Compositions and methods for providing active telomerase to cells *in vivo*	Liposomes were employed for delivering to target cells in a subject, nucleic acids for expressing telomerase reverse transcriptase and/or telomerase RNA components. The therapeutic genes were encapsulated in the liposomes according to any of the well-known encapsulation techniques. These methods include the Mozafari method, sonication, freeze/thaw, evaporation, and extrusion through membrane filters.	[57]
US9801862B2	Immunosuppressive treatments, formulations, and methods	Inventive methods and formulations have been provided for immunosuppressive therapy for dry eye and other related immune-mediated eye diseases. The formulations comprise one or more immunomodulatory drugs such as liposome-encapsulated rapamycin and tacrolimus. Mozafari method is regarded as a safe method for preparing liposomes to entrap rapamycin and tacrolimus.	[58]
WO2015110777A1	Method for controlling the movement of a polynucleotide through a transmembrane pore	According to this patent, lipid bilayers can be employed as biosensors to detect the presence of different substances. Suitable lipid/phospholipid bilayers include liposomes and nanoliposomes. It is offered to use sonication, extrusion, or the Mozafari method to form liposomes.	[59]
US20180360755A1	Target-specific delivery of therapeutic agents	Isolated peptides targeting cardiovascular disease are described. Targeting peptides are encapsulated in delivery vehicles which are but not limited to liposomes, micelles, non-liposomal nanoparticles. Liposome formation is done by sonicating, extrusion, or Mozafari method.	[60]
US20160120806A1	Nanocrystals formed in a microenvironment	The methods of forming nanocrystals in a microenvironment and the compositions formed, including pharmaceutical compositions, are discussed. The therapeutic properties of many drugs are improved by incorporation into liposomes. New methods such as extrusion and the Mozafari method are employed to produce materials for human use.	[61]
WO2017218824A1	Anti-guanosine antibody as a molecular delivery vehicle	Guanosine-targeted nanocarriers for encapsulating an active agent and delivering it to extracellular guanosine and DNA are discussed. The Mozafari method, extrusion methods, the polyol dilution method, the bubble method,	[62]

Patent number	Title	Application	Reference
		and the heating method can be employed to produce liposomal carriers.	
AU2017286733B2	Antibody-mediated autocatalytic, targeted delivery of nanocarriers to tumors	DNA-targeted nanocarriers encapsulate an active agent and deliver it to extracellular DNA are discussed. The nanocarriers, for example, polymeric particles, liposomes, and multilamellar vesicles, have targeting moiety that targets DNA. Carrier manufacturing methods include the Mozafari method, extrusion methods, the polyol dilution method, the bubble method, and the heating method.	[63]
US20180360758A1	Nanoliposomes comprising corticosteroids as medicaments and methods to prepare them	A nanoliposome comprises at least one outer lipid bilayer and at least one corticosteroid encapsulated by at least one lipid bilayer. The size of the nanoliposome is between 1 0 - 1 000 nm or 50 - 150 nm. These nanoliposomes are used as a medicament and for use in the treatment of cardiovascular disease. Methods to prepare such liposomes include extrusion methods, sonication methods, and the Mozafari method.	[64]
US20200087724A1	Method for attaching one or more polynucleotide binding proteins to a target polynucleotide	New techniques for attaching one or more polynucleotide binding proteins to a target polynucleotide are described along with characterization thereof. The methods have been adapted for manufacturing lipid bilayers by clamping liposomes. The techniques necessitate stable, giant unilamellar liposomes and the fabrication of small apertures in materials having a glass surface. Liposomes can be prepared by the Mozafari method, sonication, or extrusion.	[65]
US20210180124A1	Coupling method	The patent describes a novel method of determining the presence or absence of an analyte and its characterization. As part of the invention, the analyte is coupled to a membrane, which is preferably a lipid bilayer. These lipid bilayers can be employed for *in vitro* analysis of membrane proteins by single-channel recording. Alternatively, it can be utilized as a biosensor to detect the presence of a range of materials. These lipid bilayers can be formed using the Mozafari method, sonication, or extrusion.	[66]
US20210147904A1	Methods for delivering an analyte to transmembrane pores	A novel technique for delivering an analyte to a transmembrane pore in a membrane is described. Lipid bilayers can be employed as biosensors to detect the presence of different substances. The methods of preparation of the bilayers have been discussed. Mozafari method is suggested as a proper technique.	[67]
US20210292376A1	Mutant csgg pores	It relates to mutant forms of the outer membrane-located lipoprotein CsgG, particularly modifications at one or more positions Tyr51; Asn55; and Phe56. The method requires stable, giant, and unilamellar liposomes. Liposomes can be formed by Mozafari method of sonication or extrusion.	[68]
US20210205447A1	Cytotoxic immunostimulating particles and uses thereof	The invention relates to immunomodulating cytotoxic particles comprising immunostimulating RNA comprising a cytotoxic nucleotide or cytotoxic nucleotide having pharmaceutical uses. The useful carriers include lipid-containing carriers, cationic lipids, liposomes, and micelles. Mozafari method is employed as it does not damage the encapsulated drugs.	[69]
US20210269872A1	Mutant pores	The invention relates to mutant forms of the outer membrane-located lipoprotein CsgG and analyte detection and characterization using said mutant CsgG. The method requires stable, giant, and unilamellar liposomes which can be produced by the Mozafari method, sonication, or extrusion.	[70]
AU 2021105510	Compositions and green technology methods for the fortification of solid and semi-solid food products	A new method and several formulations are described for the fortification of solid-type and partially solid-type foods. The encapsulation technology was based on the Mozafari method as a green, environment-friendly procedure.	[71]

Consequently, the drug can be added: (*i*) initially, along with the liposome ingredients and the aqueous medium; (*ii*) after the heating and agitation has been initiated; or (*iii*) after the termination of the heating and stirring step, i.e., after the carrier system has completely formed. The last protocol is suitable for temperature-sensitive materials. The method is fast and efficient since it avoids using carrier materials to be hydrated separately before heating and avoids exposure to volatile organic solvents. It is completely suitable for large-scale liposome production for pharmaceutical, cosmetic, nutraceutical, and food industries [7, 14, 34, 35]. Taking the Mozafari method as a model Greentech procedure, all patents in which this technique is incorporated, utilized, or cited are listed in Table 4.

As a result of the innovations and progress in liposomology, several liposomal drug products have been approved and are in clinical use, while more formulations are in the final clinical trials [72]. Trends in the increase of the number of patents on the Mozafari method are depicted in Figure 2.

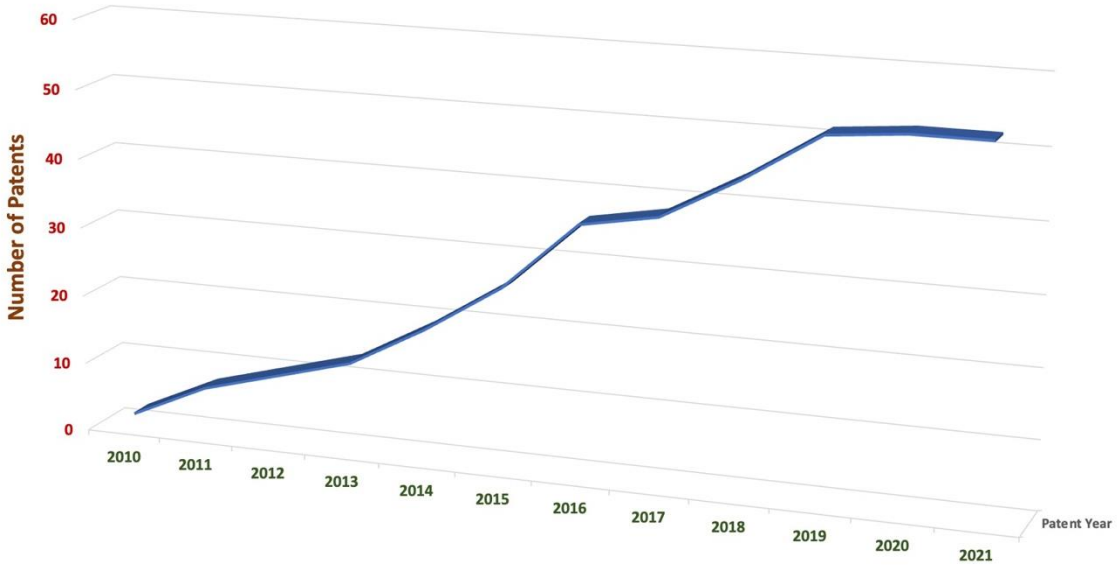

Figure 2. Number of patents on Mozafari method since 2010. The data source was Google Patents.

6. Perspectives

Encapsulation and controlled release of bioactive compounds have enabled researchers and industries to add novel aspects to conventional drug formulations, vaccines, diagnostic agents, and food products. Encapsulated drugs have been proven to be most useful for reducing the side effects compared to free drugs and targeting them to the precise location where their effect is needed *in vivo* and *in vitro*. This has resulted in an overall increase in the therapeutic index by increasing efficacy and bioavailability while reducing or eliminating toxicity. This review article presents innovations on the methods of preparation of encapsulation systems, emphasizing green-tech approaches. As a most simple and robust preparation technique, the article is focused on the Mozafari method developed in our laboratory. The method has been utilized to formulate encapsulated therapeutic agents, vitamins, antioxidants, and nutraceuticals, to name but a few by other research groups as well. The success of lipidic encapsulation systems, for instance, to reach the market - after being approved for human and animal use - is due to the novel production methods allowing for facile scale-up, satisfactory reproducibility, and process control. These characteristics allow a coherent design approach to achieve clinical objectives and point to an increase in the variety of novel and increasingly

sophisticated lipid-based carriers in the future. It is envisaged that the nutraceutical share of the food and drug market will exceed the current rate of around 500 Billion US Dollars.

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Conflicts of Interest

The authors declare no conflict of interest.

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