

# Liposomal systems as carriers for bioactive compounds

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**Abstract** Since the revolutionary discovery that phospholipids can form closed bilayered structures in aqueous systems, the study of liposomes has become a very interesting area of research. The versatility and amazing biocompatibility of liposomes has resulted in their wide-spread use in many scientific fields, and many of their applications, especially in medicine, have yielded breakthroughs in recent decades. Specifically, their easy preparation and various structural aspects have given rise to broadly usable methodologies to internalize different compounds, with either lipophilic or hydrophilic properties. The study of compounds with potential biotechnological application(s) is generally related to evaluation and risk assessment of the possible cytotoxic or therapeutic effects of the compound under study. In most cases, undesirable side-effects are associated with an interaction of the liposome with the cell membrane and/or its absorption and subsequent interaction with a cellular biomolecule. Liposomal carrier systems have an unprecedented potential for delivering bioactive substances to specific molecular targets due to their biocompatibility, biodegradability and low toxicity. Liposomes are

therefore considered to be an invaluable asset in applied biotechnology studies due to their potential for interaction with both hydrophilic and lipophilic compounds.

**Keywords** Biotechnology · Carrier · Drugs · Liposome · Delivery

## Liposomes

Liposomes are spherical artificial lipid vesicles with an aqueous core enclosed by one or more lipid bilayers or lamellae, as first reported in 1964 (Bangham and Horne 1964). They are frequently classified on the basis of their size (small, large and giant vesicles), number of bilayers (uni-, oligo- and multilamellar) (Vuilleumard 1991; Vemuri and Rhodes 1995) and phospholipid charge (neutral, anionic or cationic) (Storm and Crommelin 1998).

The properties of liposomes differ significantly depending on their lipid composition, surface charge, size and method of preparation. Thus, the choice of the lipid components determines the rigidity, fluidity and charge of the lipid bilayer and consequently the properties of the liposome. For example, the use of unsaturated species, such as phosphatidylcholines from natural sources (egg or soy phosphatidylcholines), results in the construction of relatively permeable liposomes, but with less stability, while saturated phospholipids with long acyl chains (e.g. dipalmitoyl phosphatidyl choline) form rigid vesicles, but with an impermeable bilayer (Akbarzadeh et al. 2013).

Multilamellar vesicles (MLVs) consist of several concentric phospholipid bilayers interspersed with aqueous compartments whose diameter ranges from 400 to 3500 nm. Large unilamellar vesicles (LUVs) have a single phospholipid bilayer and only one large aqueous cavity with a diameter ranging

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from 200 to 1000 nm. Small unilamellar vesicles (SUVs) are composed of only one phospholipid bilayer and a small aqueous compartment with a diameter ranging from 20 to 100 nm (Yeagle 1993; Akbarzadeh et al. 2013).

Among the various methods reported in the literature for liposome preparation, the most frequently used are lipid hydration, ethanol injection, freeze–thawing and reverse phase evaporation (Akbarzadeh et al. 2013; Patil and Jadhav 2014).

In the lipid hydration method, the lipid or lipid mixture is dissolved in an appropriate organic solvent (e.g. chloroform or methanol) followed by drying with a flow of an inert gas [e.g. nitrogen gas ( $N_2$ )] to ensure complete removal of the solvent and formation of a thin lipid film. This film is hydrated with an aqueous buffer solution and heated at a temperature which is higher than the phase transition temperature of the lipid, with frequent stirring. After heating, the aqueous lipid suspension spontaneously organizes to form vesicles with multiple bilayers (multilamellar). This (re-)organization is driven by the entropy of water molecules and by hydrophobic interactions between the fatty acid chains of lipids.

Once formed, the multilamellar liposomes can be transformed into unilamellar vesicles by different methods (Zawada 2004; Akbarzadeh et al. 2013; Eloy et al. 2014; Madni et al. 2014). For example, MLVs may be sonicated or passed through a membrane with pores of nanometric dimensions (extrusion method) to form unilamellar vesicles that have properties very similar those of natural membranes. Both the sonication and extrusion methods provide the energy required to break the various lamellae that initially formed and reorganize the system into uniform bilayers with controlled sizes of vesicles (Camolezi et al. 2002; Ierardi et al. 2002; Daghestanli et al. 2004; Santos et al. 2002, 2005; Rigos et al. 2008; dos Santos et al. 2009; Bolean et al. 2010, 2011; Simão et al. 2010a, b; Ciancaglini et al. 2012).

Sonication is probably the most widely used method for the preparation of SUVs and can be performed with a tip sonicator or bath sonicator. In the first case, the tip of the sonicator is dipped directly into the solution containing the SUVs, thereby providing very high energy to the lipid system, which can heat the solution. Thus, in order to avoid possible degradation of the lipids, it is necessary to maintain the lipid suspension in an ice bath during the sonication process or alternatively to use a bath sonicator which provides better control of the temperature of the lipid suspension during the process. The main disadvantages of this method are the formation of SUVs with their smaller aqueous cavity – and therefore smaller internal volume – resulting in a lower efficiency of encapsulation, possible degradation of the lipids and encapsulated compounds due to the temperature increase during the process, elimination of large molecules, contamination of the lipid solution with traces of metal (titanium) from the sonicator tip and the presence of MLVs formed together with SUVs (Akbarzadeh et al. 2013).

In the extrusion method, the multilamellar lipid suspension is forced under pressure through a membrane with pores of a predetermined diameters (nanometric scale). This process, as well as the method of freeze/thawing the lipid suspension, results in the formation of unilamellar vesicles which are larger than those formed by sonication; it also has a more accurate size control. The larger size is caused by the reduction of liposome lamellarity due to disruption of the bilayers, which then restructure to form new vesicles, thus increasing the number of vesicles and consequently encapsulation of the compound (Zawada 2004; Akbarzadeh et al. 2013; Eloy et al. 2014; Madni et al. 2014).

In the ethanol injection method, the lipid is first dissolved in ethanol and the solution then injected into an aqueous buffer. In the reverse phase evaporation method, a solution of the lipid and an organic solvent is added to an aqueous buffer solution to form a water-in-oil emulsion that is subjected to evaporation under low pressure, resulting in the formation of the liposome system (Madni et al. 2014).

In the freeze–thawing technique, the lipid film is hydrated with an aqueous buffer solution and subjected to repeated cycles of freezing/thawing, which cause physical disruption of the phospholipid bilayers due to the formation of ice crystals during the freezing process, thus increasing the aqueous solute/lipids ratio, resulting in a better carriage efficiency (Akbarzadeh et al. 2013; Eloy et al. 2014). Anzai et al. (1990) reported a ten- to fifty fold increase in the internal volume of the vesicles when the liposomal suspension was subjected to the freeze/thawing process. These authors suggested that the process induced the fusion of hundreds of small vesicles, which ultimately led to the formation of larger liposomes.

Many conventional methods for the preparation of liposomal vesicles involve the use of either water-miscible/-immiscible organic solvents or detergent molecules. Residual organic solvents may become toxic and degrade active ingredients, thus representing a possible risk to human health (Birbaum et al. 2000). In the case of vesicles carrying proteins, organic solvents or detergents can denature the proteins and affect the membrane properties (Seddon et al. 2004), thus influencing the stability of vesicles (Wagner et al. 2002; Wagner and Vorauer-Uhl 2011; Patil and Jadhav 2014).

### Liposomal systems as carriers for bioactive compounds

Liposomes were initially used as membrane models in biological studies. However, their cell-like structure led to investigations on their efficacy as a tool to carry potent molecules into the human body. Nowadays they are used for the encapsulation of unstable compounds considered to be essential to human health, such as antioxidants, antimicrobials, bioactive

elements, antigenic proteins, thereby preserving their functionality (Madni et al. 2014; Schwendener 2014; Patil and Jadhav 2014). Liposomal vesicles have emerged as one of the novel carrier systems most studied by the scientific community over the past decades and have revolutionized research in many different fields, such as cosmetics, drugs, food, agriculture, with applications in chemotherapy treatments, gene therapy, vaccines, antimicrobial agents, among others (Madni et al. 2014).

The first report on the liposomal encapsulation of a therapeutic agent appeared in 1971 (Gregoriadis et al. 1971). A key advantage of liposomes in general, and liposomes-based delivery systems in particular, is their versatility and plasticity. The composition of the liposome and the method for its preparation can be chosen to achieve desired features, such as lipid composition and size, charge, size distribution and entrapment and location of different compounds. Depending on the chemical properties of the liposome, hydrophilic compounds (proteins, peptides, nucleic acids, carbohydrates) can be entrapped within the aqueous core, thereby increasing penetration of these compounds through physiological lipophilic membranes, whereas lipophilic compounds (linker molecules, antigens, adjuvants, lipopeptides) can be intercalated inside the lipid bilayer, thereby increasing their solubility in the aqueous body fluids (Fig. 1) (Rahman et al. 1974; Schwendener 2014).

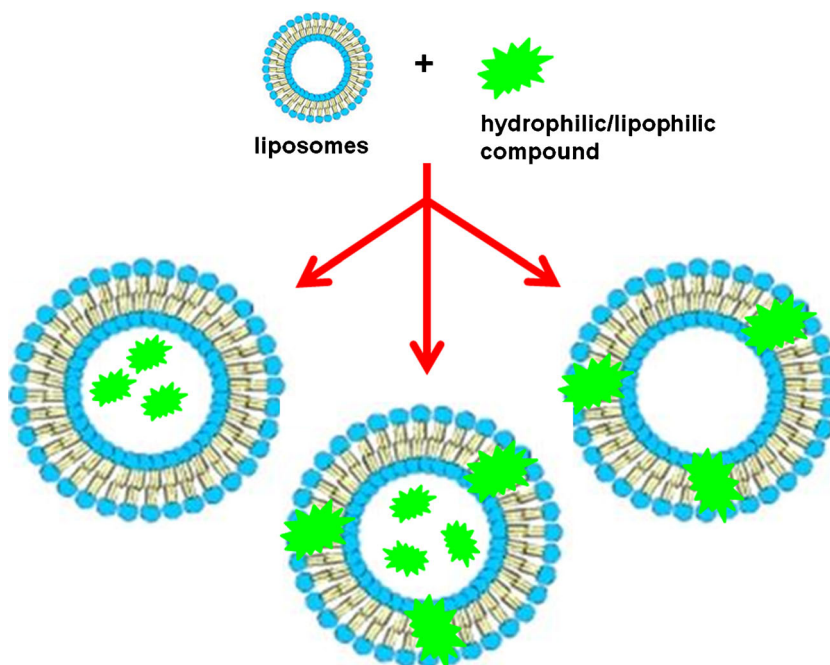
The enormous versatility of liposomes makes them highly valuable carrier systems for vaccines. The liposome's ability to induce immune responses to incorporated or associated antigens was first reported by Allison and Gregoriadis (1974, 1976). Since then, liposomes and liposome-derived nanovesicles have become important carrier systems, and

interest in developing liposome-based vaccines remains remarkably high. Antigens or adjuvants can be attached to the liposome surface either by adsorption or stable chemical linking (Torchilin 2005; Watson et al. 2012). Co-formulations containing different types of adjuvants or antigens can be combined with specific liposome properties for the development of liposomal vaccines for individual applications (Schwendener 2014). Liposomes provide antigens or adjuvants with greater protection from external degradation caused by enzymes (Chaize et al. 2004), and because they are prepared from natural materials or their synthetic derivatives, they are biocompatible and biodegradable (Rooijen and Nieuwmegen 1980).

Depending on their specific properties of the carrier system, including size, composition and surface properties of the liposomes, these systems also possess the ability to overcome biological barriers, such as mucosa and skin, and they provide controlled and slow release of antigens, as well as improved antigen stability and presentation to immunocompetent cells. Together with the ability to induce strong immune responses provided by co-formulated adjuvants, liposome-based vaccines provide the fundamental properties required for the development of modern vaccine formulations (Schwendener 2014).

Immunoliposomes, which are formed from the conjugation of antibodies directly to the lipid bilayer of the liposome, are able to deliver the load specifically to the target tissues and safeguard other normal tissues from toxic effects. Liposome-encapsulated drugs have been shown to have a much higher efficacy than other conventional drug delivery systems. The main advantage of modern liposomes lies in their sustained

**Fig. 1** Liposomal systems as carriers for bioactive compounds. Depending on the chemical properties, hydrophilic compounds are entrapped within the aqueous core of liposomes, whereas lipophilic compounds are intercalated inside the lipid bilayer. This feature makes it possible to produce liposomal systems with only one type of molecule incorporated, or more complex systems with different compounds encapsulated



action, enhanced bioavailability, high cellular uptake and targeted delivery of therapeutic agents. It is predictable that these delivery systems will be increasingly applied in the near future with success, leading to major improvements in the field of vaccine development (Madni et al. 2014; Schwendener 2014).

Thus, liposomes are promising delivery systems mainly because of their biocompatibility, biodegradability and low toxicity in the human body (Benech et al. 2002; Saddi et al. 2008; Shehata et al. 2008; Akbarzadeh et al. 2013; Gao et al. 2014). Any compound, irrespective of its solubility, can be entrapped inside a liposome, resulting in a slow degradation during transport to the target, minimal side-effects and greater stability (Ghalandarlaki et al. 2014).

Despite the many advantages of using liposomes, including safety and biocompatibility, a major drawback is their instability in plasma. After intravenous administration, they are rapidly captured by the mononuclear phagocytic system (MPS) and removed from the blood circulation. This behavior has been explored for the efficient delivery of antiparasitic drugs for treating infections in these cells (Aqil et al. 2013). In this context, for example, plants from genus *Piper* have been investigated as a source of new natural products with potential antitumoral, antifungal, antiplasmodial, antioxidant and tripanocidal properties (da Silva et al. 2014), as well as antimicrobial, antipyretic, aromatic, emetic, diuretic, hallucinogen, ornamental and insect repellent roles, among other applications (Parmar et al. 1997; Wadt et al. 2004; Ferreira et al. 2010). Many components have been isolated from *Piper* plants, such as terpenes, phenylpropenoids, lignans, neolignans, steroids, benzopyrans, chalcones, flavones, other phenolic compounds and a series of alkaloids (Parmar et al. 1997; Wadt et al. 2004; Felipe et al. 2007).

Some species, such as *P. chaba*, *P. claussonianum*, *P. longum*, *P. sanguineispicum* and *P. tuberculatum* have shown antileishmanial activity due to the presence of compounds such as propanoic acid, lignans, terpenes, esters and amides (Carrara et al. 2013). The 3-(3,4,5-trimethoxyphenyl) propanoic acid extracted from *P. tuberculatum* Jacq. has been found to have potential leishmanicidal activity (Facundo et al. 2008; Ferreira et al. 2010; Barros et al. 2013). Leishmaniasis is endemic in more than 98 countries and territories. Not only do trusted treatments cause many side-effects in patients, but there is still the problem of parasites developing resistance to the drugs (Kaur and Rajput 2014). In addition, there is a lack of knowledge regarding the action mechanism of the drugs, and current treatment regimens are expensive (Alvar et al. 1997; Laguna et al. 2003; Chakravarty and Sundar 2010; Kaur and Rajput 2014).

In a recently published study, Cury et al. (2015) introduced synthetic analogs of 3-(3,4,5-trimethoxyphenyl) propanoic acid into liposomes and subsequently demonstrated their antileishmanial activity in cultures of *Leishmania*

*amazonensis* promastigotes. The liposomal systems carrying the drugs were standardized, and the composition of the liposomes favored their interaction with the parasite cells. The compounds loaded into the liposomes led to an efficient reduction of the *L. amazonensis* culture at drug concentrations that were 20-, 40- and 100-fold lower than those used in the initial tests (with the drug dissolved in DMSO). Depending on the analog used, the carrier systems reduced the *L. amazonensis* culture by 50–80 %; the liposomes without drugs did not affect culture viability (Cury et al. 2015).

Liposomal systems have already been used to carry various compounds for the treatment of leishmaniasis, such as liposomal amphotericin B (AmBisome®). This system has the potential to become a first-line drug in the treatment of visceral leishmaniasis (VL), but its high cost currently limits its use (Alvar et al. 1997; Laguna et al. 2003). This formulation also has a longer half-life time, which reduces the possibility of the emergence of resistance (Chakravarty and Sundar 2010). Studies in India have reported that a single dose of AmBisome® is effective and safe for the treatment of VL, indicating that a reduced number of doses is efficacious, thereby reducing the costs of the treatment (Sundar et al. 2010).

Liposomal systems containing negative surface charges may result in a better delivery of drugs to the target cells. Akaki et al. (2001) evaluated the surface charges of infective forms of a number of parasites, including *Leishmania*, and observed that the parasites had positive charges in the membrane region which makes the initial contact with the host cells. Tempone et al. (2004) reported that negatively charged liposomes were efficient drug delivery systems to macrophages, inducing a decrease in *Leishmania* growth.

Barros et al. (2013) evaluated the antileishmanial activity of the lupane-triterpene 3 $\beta$ ,6 $\beta$ ,16 $\beta$ -trihydroxylup-20(29)-ene, which has been shown to have various pharmacological properties (anti-inflammatory, anti-ulcerogenic and antileishmanial activities) when loaded into liposomes and delivered using this liposome carrier system. These authors concluded that this lupane–liposomal system can be considered a promising therapeutic regimen, conferring antileishmanial activity to infected macrophages. Studies have shown that epiisopiloturine is potentiated and its antischistosomal effect prolonged when encapsulated into liposomes. These benefits can be attributed to the properties of extended delivery, with increased availability of the active principle (Guimarães et al. 2014).

The use of peptides as new drugs and insecticides is associated with both strengths and weaknesses. The peptides and proteins present in animal secretions and poisons have co-evolved with their prey, or biological system, so that their mechanism of action is perfectly adapted. However, for biotechnological applications, they should be protected from hydrolysis and environmental changes, or even be preserved until they reach the specific target, in order to prevent any



(adverse) reaction(s) other than the desired effect. Thus, active substances (from plants and/or animals) may be transported to specific molecular targets through nanostructured systems of natural or synthetic membranes, which can also preserve and increase the half life of the molecule (Salay et al. 2011; Barbosa et al. 2011).

The liposomal systems of SUVs also deserve attention as biosensing and delivery systems. Such systems can be used as biomimetic models for studies of interaction with various hydrophobic drugs and membrane proteins, with potential applications in the field of immunology (vaccines and drugs targeting specific cell membranes). Liposomes associated with proteins can be used to study the activity of photosensitive dyes applied during photodynamic therapy (Bolfarini et al. 2012; Longo et al. 2012; Rocha et al. 2012; Bicalho et al. 2013; Faria et al. 2015), as well for studies related to the ability of proteins to prevent, treat and/or act as nanosensors for various diseases (Daghastanli et al. 2004; Santos et al. 2006; Zucolotto et al. 2007; Migliaccio et al. 2008; Colhone et al. 2009, 2015; Calderon et al. 2009a, b; Perinoto et al. 2010; Paulovich et al. 2011; Barbosa et al. 2011; Salay et al. 2011; Barros et al. 2013; Fotoran et al. 2015).

Various types of liposomal delivery systems have been widely studied in recent years, in association with compounds for systemic and/or topical application in various therapeutic fields (e.g. cancer treatment, epilepsy, osteogenesis, treatment of viral and bacterial infections, among others) as well as for many other biomedical applications. These systems have very important physicochemical properties that can lead to highly optimized cell specificity, thus minimizing various limitations related to the administration of the drug alone. Their biological interaction is directly related to the chemical affinity for the delivery systems, which has led in recent years to the development of vehicles with greater biological potential as these favor higher biological uptake and are therefore associated with adequate pharmacokinetics. The attributes of liposomal delivery systems therefore minimize the side-effects usually present when many therapeutic drugs are used in their native therapeutic form, which can limit their applications.

## Conclusions

Among the various benefits reported with the use of liposomal carrier systems, we highlight (1) the increased effectiveness of such therapies due to the gradual and controlled drug release through matrix degradation; (2) increased time of the drug in blood circulation; (3) significant reduction in toxicity of therapeutic regimens; (4) widely varying composition of the carriers; (5) ability to modify the carrier system to reduce its instability and drug decomposition (e.g. biological degradation by enzymes, pH and others). These features lead to a safer

administration of the drug (e.g. without causing local inflammatory responses) and greater convenience to the patient (e.g. lower number of doses). The potential for drug targeting to specific targets is another advantage that has attracted the interest of researchers in various therapeutic fields. The potential for liposomes to combine with both hydrophilic and lipophilic compounds possibly extends the applications of liposomal systems and is a key reason motivating the development of this scientific field which is still underexplored.

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## Compliance with Ethical Standards

**Declaration of conflict of interest** The authors report that they have no conflict of interest to declare.

**Ethical approval** This article does not contain any studies with human or animal subjects performed by the authors.

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