Expert Opinion

Charged liposomes as carriers to enhance the permeation through the skin

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Introduction: In recent years, there has been increased interest in developing charged liposomes as carriers for transdermal drug delivery. It is necessary to modify the basic composition of the liposomes in order to enhance the penetration properties of the vesicles through the skin. Charged liposomes offer several advantages compared with previous drug delivery systems.

Areas covered: This paper provides a brief overview of the different drug delivery systems that exist which aim to improve the permeation of drugs through the skin, focusing on the use of charged liposomes for transdermal delivery. We propose a classification of such liposomes based on the origin of the charge given to the vesicles.

Expert opinion: Despite the advances that are occurring in the design of charged liposomes for transdermal drug delivery, the long-term stability continues to be a drawback in such systems. The presence of charge on the surface of the vesicles favors the electrostatic repulsion among them, creating a ζ potential positive or negative that prevents their aggregation and flocculation. However, there is loss of the encapsulated drug, which limits the *in vivo* use of these systems. It should be emphasized that charged liposomes are indeed a promising candidate for use in gene therapy and vaccine targeting, in a great diversity of diseases, for which drugs are administered by the percutaneous route.

Keywords: charged liposome, dicetyl phosphate, DOTAP, liposome, stearylamine, surfactant, transdermal delivery

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1. Dermal and transdermal drug delivery

The main barrier for diffusion through the skin is the outermost layer of the skin, the stratum corneum (SC), which is composed of corneocytes (15 – 20 layers) and intercorneocyte matrix. In keratinocytes, cellular membranes account for 50% of the dry mass and consist mainly of phosphatidylcholine (PC) and sphingomyelin. The intercorneocyte matrix is rich in phospholipids (ceramides), cholesterol and fatty acids [1]. The structure of the SC is often depicted in the so-called bricks and mortar arrangement [2] where the keratin-rich corneocytes (bricks) are embedded in the intercellular lipid-rich matrix (mortar). This arrangement is illustrated in **Figure 1**.

For any molecules applied to the skin, two main routes of skin permeation have been defined: the transappendageal (through the sweat glands and across the hair follicles with their associated sebaceous glands) and transepidermal pathways (intercellular or transepidermal pathways). Although the intercellular pathway is widely regarded as the main route of permeation of most compounds, all molecules traverse by a combination of all three routes, the relative importance of which will vary depending on the physicochemical characteristics of molecules [3].

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Article highlights.

- We propose a new classification system of charged vesicles for transdermal delivery: charged phospholipids, coated liposomes and the addition of other charged substances to the vesicle structure.
- Among charged phospholipids, POPG and DMPS have demonstrated important results as permeation enhancers of water-soluble drugs, with the aid of minimally invasive techniques.
- Chitosan and derivatives have shown to be the most used polymers for coating liposomes.
- Other substances added to the vesicle composition are: DOTAP, stearylamine and dicetyl phosphate. Formulations containing DOTAP have shown to be a promising tool for transcutaneous gene therapy and immunization.

This box summarizes key points contained in the article.

Besides its role as a barrier, the skin regulates the flux of water molecules into and out of the body, permitting the influx of a variety of small molecules that are fairly lipophilic (log $P \geq 1.5$) and have molecular mass (MM) < 500 Da [4]. Therefore, drug molecules currently administered via the transdermal route fall within a narrow range of MM and lipophilicity, taking advantage of the natural selectivity of the skin membrane.

A large fraction of drug molecules such as protein and peptide-based drugs lies outside these bounds. The biggest challenge in transdermal drug delivery today is to open the skin safely and reversibly to these high MM hydrophilic drugs.

2. Liposomes as carriers for skin delivery

To increase drug transport across the skin, penetration enhancers as well as other chemical methods have been used. One of the most controversial methods to increase drug transport across the skin is the use of vesicles. Liposomes are lipid vesicles that fully enclose an aqueous volume. They are composed of concentric bilayers formed from self-assembly of amphiphilic molecules. In these vesicles, hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions [5,6].

Many methods for preparation of liposomes are described in the literature [7-10], giving rise to different structures of vesicles (**Figure 2**).

On the other hand, the composition of the vesicles influences their physicochemical characteristics such as size, charge, thermodynamic phase, lamellarity and bilayer elasticity [11]. These physicochemical characteristics in turn have a profound effect on the behavior of the vesicles and hence on their effectiveness in enhancing transdermal drug delivery [12-14]. In this sense, a different behavior has been demonstrated when liposomes are formed with SC lipids, such as ceramides, cholesteroil sulfate and palmitic acid, with respect to the permeation process [15]. Liposomes create a drug reservoir mixing with SC lipids, whilst PC/CHOL liposome promotes lipophilic drug permeation through the skin. In this sense, several authors have demonstrated that SC lipid-based liposomes could deliver a greater amount of aqueous radiolabeled markers to the deeper skin strata, while avoiding systemic absorption and, hence, organ distribution and renal elimination of drug. On the other hand, an important parameter in determining the extent of absorption is the vesicle size. When fluid liposomes made up of unsaturated lecithins were used, a percutaneous absorption was obtained instead of dermal delivery that was reached using SC lipid-based unilamellar liposomes [16].

The rationale for using liposomes in dermal and transdermal drug delivery is many fold. They might act as drug carriers to deliver entrapped drug molecules into or across the skin; they act as penetration enhancers owing the penetration of the individual lipid components into the SC and subsequently the alteration of the intercellular lipid lamellae within this skin layer; they might serve as a depot for sustained release of dermally active compounds; and also, they serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system [17].

3. Enhancement of drug permeation: modified vesicles

Recently, it became evident that, in most cases, classic liposomes are of little or no value as carriers for transdermal drug delivery. Although liposomes have difficulty to pass through the skin, they can exert their action on the surface of the skin acting as a reservoir. In order to facilitate the passage of drugs across the SC, different types of vesicles have been designed. These vesicles provide additional benefits to conventional liposomes [18]. They are discussed below.

3.1 Deformable liposomes (Transfersomes®)

These are the first generation of elastic vesicles introduced by Cevc and Blume [19]. They consist of phospholipids and an edge activator that is often a single chain surfactant, having a high radius of curvature, which destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers [20]. As a consequence, the liposome's lamella structure can be softened, so the vesicle enters more easily into the intercellular lipid pathway of the SC layer and delivers the drug molecules into the deeper layers and through the skin. Surfactants tend to fluidize membranes and make them very elastic, resulting in highly deformable vesicles. El Maghraby *et al.* [21] showed that incorporation of surfactants into vesicles reduced the main T_m, which indicates fluidization of the lipid bilayer. Preparation of deformable liposomes involves methods similar to those used in preparation of traditional liposomes [22-24]. The effects of incorporation of different edge activators on

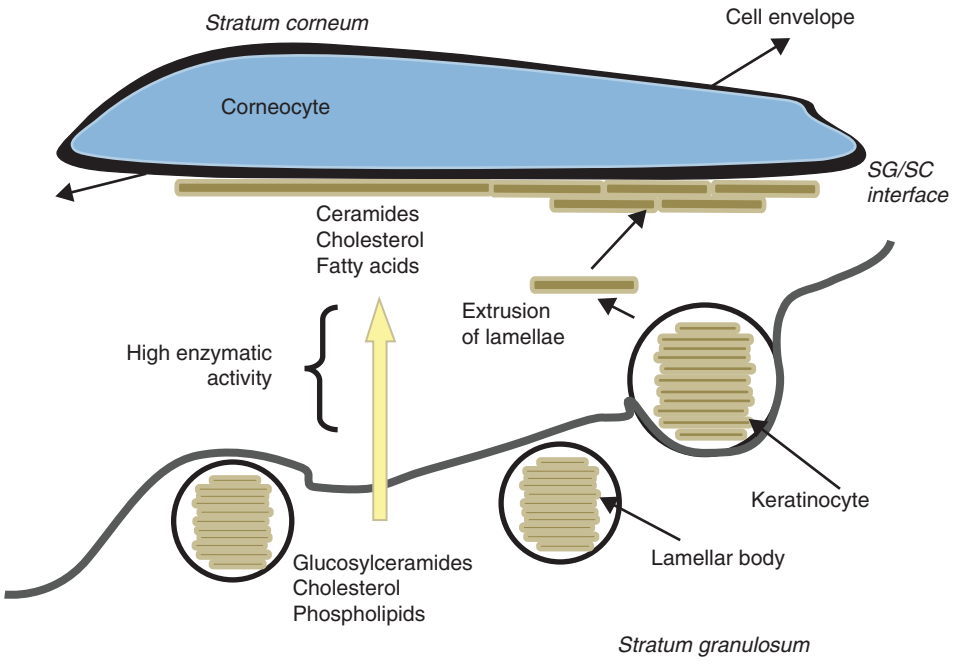


Figure 1. A schematic drawing of the composition of SC and SG.
SC: Stratum corneum; SG: Stratum granulosum.

physicochemical properties (including vesicle size, entrapment efficiency, ζ potential, among others) of deformable liposomes were extensively investigated in several studies [25]. Also, the interaction between edge activators and liposomes was also investigated [21,26].

The difference in skin interaction between occlusive and non-occlusive application is of importance for deformable vesicles. Several authors have suggested that the major driving force for the intact penetration of Transfersomes® is driven by the osmotic gradient across the skin caused by the difference in water content between the skin surface and skin interior. Phospholipid hydrophilicity leads to xerophobia (tendency to avoid dry surrounding). Accordingly, as the vesicles remain swollen to the maximum, those found on the surface of the skin try to follow the local moisture gradient, moving toward the deeper layers [27]. Occlusion would eliminate this osmotic gradient and is, therefore, unfavorable for the actions of the deformable vesicles.

3.2 Niosomes

These vesicles are composed of non-ionic amphiphiles (surfactants) and are similar in function to the liposomes [28]. Niosomes attract much attention because of their advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost and convenient storage of non-ionic surfactants, and large numbers of surfactants available for the design of niosomes. Published works have mainly used Spans and Tweens as non-ionic surfactants together with limited amounts of lecithin and cholesterol [29-31].

Several studies have documented the superiority of niosomes in enhancing permeation of drugs across the SC. In recent years, Paolino *et al.* [32] have shown that niosomes constructed from a new non-ionic surfactant α,ω-hexadecyl-bis-(1-aza-18-crown-6) (Bola-surfactant) show significantly improved percutaneous permeation of ammonium glycyrrhizinate with respect to both the aqueous drug solution. Manosroi *et al.* [33] have evaluated the skin permeation enhancement of diclofenac diethylammonium from bilayer vesicular formulations composed of DPPC and Tween® 61 or Span® 60 mixed with cholesterol and ethanol. In addition, the *in vivo* anti-inflammatory activity evaluated by ethyl phenylpropiolate-induced rat ear edema showed that the drug entrapped in the developed elastic niosomes and incorporated in gel gave more inhibition percentages at 45 and 60 min ear edema inhibition percentages than the commercial emulgel. This result has not only demonstrated the enhancement of transdermal absorption through rat skin, but also the *in vivo* anti-inflammatory effect of the drug when entrapped in the niosomal formulations, as well. Manosroi *et al.* [34] have incorporated gallidermin (Gdm), an antibiotic agent with a potential advantage for the treatment of endocarditis, abscesses and skin infections, into niosomes. Results suggested that Gdm loaded in niosomes and incorporated in gel has a superior topical antibacterial activity because of the high accumulation in the skin with no risk of systemic effect.

However, even though niosomes exhibit good chemical stability during storage, aqueous suspensions of niosomes may exhibit problems of physical instability such as aggregation,

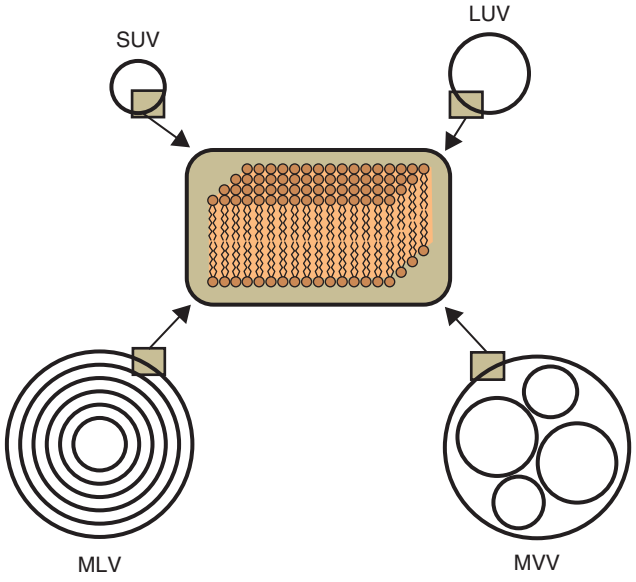


Figure 2. Schematic illustration of different structures of liposomes as a function of the preparation method.
LUV: Large unilamellar vesicle; MLV: Multilamellar vesicle; MVV: Multivesicular vesicle; SUV: Small unilamellar vesicle.

fusion, leaking of entrapped drugs or hydrolysis of encapsulated drugs, thus limiting their shelf life. The latest approach in the field of vesicular delivery is tended through the formation of 'proniosomes' which are converted to niosomes on hydration [35-37].

3.3 Ethosomes

Ethosomes are primarily composed of water, ethanol and phospholipids. They were developed by several authors, showing enhanced skin delivery [38-41]. The use of different proportion of ethanol in formulations has been postulated by several authors. Liposomal formulations containing up to 10% ethanol and up to 15% propylene glycol [42] and the use of high ethanol content have been described in literature: 30, 45 and 90% [43,44].

The presence of ethanol into liposome formulations has certain effects on the physicochemical characteristics of vesicles, as described by several authors. With respect to the size, ethosomes show a smaller size than liposomes, when both are obtained by preparation methods not involving any size reduction steps [45]. This reduction in vesicle size could be explained as a result of incorporation of high ethanol concentration. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease [38,39]. The effect of phospholipid concentration on the size of ethosomal vesicles was also investigated [38,46]. Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol, which allows for better solubility of many drugs [47].

Ethosomes were reported to improve *in vivo* and *in vitro* skin delivery of various drugs, such as hormones [48], antiviral drugs [49] and minoxidil [39]. Contrary to deformable liposomes, ethosomes were able to improve skin delivery of drugs both under occlusive [38,45,48] and non-occlusive conditions [45,46].

Ethanol is known as an efficient permeation enhancer and is added to the vesicular system to prepare the elastic vesicles. It can interact with the polar head group region of the lipid molecules, resulting in the reduction of the melting point of the SC lipids, thereby increasing their fluidity, and cell membrane permeability. The high flexibility of the vesicular membrane from the added ethanol permits the elastic vesicles to squeeze themselves through the pores that are much smaller than their diameters [50].

Other vesicular carriers are used in transdermal drug delivery, such as invasomes. These vesicles are composed of PC, ethanol and terpenes. Terpenes have shown to enhance the percutaneous absorption of many drugs because they increase drug permeation by disrupting lipid packaging of SC and/or disturbing the stacking of the bilayers [51]. They provide high amounts of drug in the SC and deeper skin layers, indicating that the incorporation of a single terpene into invasomes could provide efficient nanocarriers of drug. They are a promising tool for delivering the drug to the skin.

4. Charged liposomes as carriers for transdermal delivery

Phospholipids are the main components of liposomes. Some of them are amphiphilic lipids consisting of a rather polar 'head group' and comparably apolar residues. In the solid state, the molecules tend to orientate in a distinct way, which is that lipophilic tails and hydrophilic head groups take a separate, packed arrangement. When they are hydrated, they do not exhibit abrupt transitions from the solid to the liquid state, but do undergo 'intermediate' states, also known as 'mesophases' or 'liquid crystals', where properties of solid crystals and liquids can be observed, as well. Phospholipid membrane phases are commonly grouped into crystalline, gel and fluid liquid crystalline membrane phases. A generalized sequence of thermotropic phase transition for phospholipids that exhibit limiting hydration is indicated in **Figure 3**. Not all of these phases and transitions necessarily appear for a single phospholipid. This depends on the molecular structure of the particular phospholipid.

The addition of water-soluble solutes into liposome dispersions may alter the interaction between the phospholipid molecules at the bilayer surface. Thereby, osmotic gradients change the bilayer properties of liposomes such as elasticity, permeability and partition coefficients, perhaps by altering the area per lipid at the membrane surface.

Also, in a lipid bilayer, some of the constituting lipid molecules may carry charge due to dissociation of their

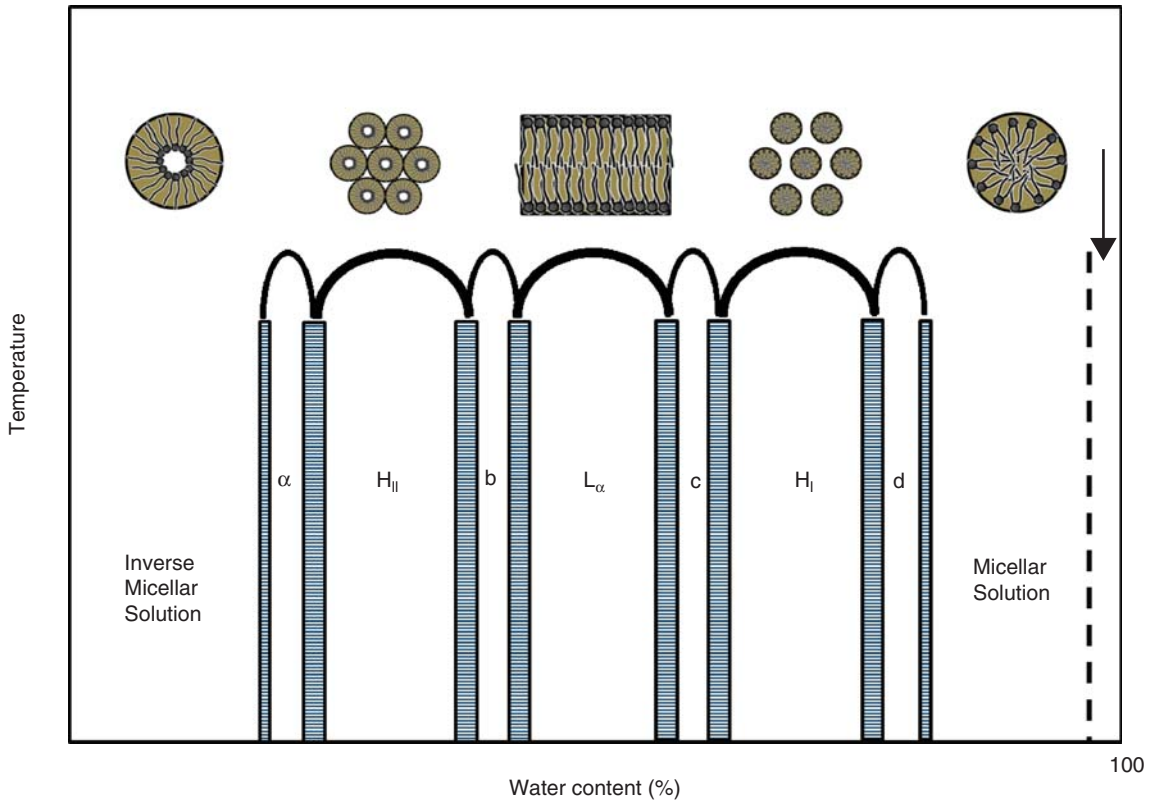


Figure 3. Schematic drawing of the structures of the lamellar and non-lamellar phase transitions of lipids.

ionizable groups, and the charge amount could depend on pH. Therefore, characterization of the surface charge properties under various conditions is of fundamental importance for liposome applications [52,53].

The important role of water in the membrane structure is explained by the fact that hydration of lipids leads to significant changes in thermodynamic parameters of their main phase transition depending on head group structure. The ordering of the water molecules at the membrane surface may be the reason of the main part of dipole potential at the membrane boundaries and the hydration forces. In this sense, Zellmer *et al.* [54] treated non-occlusively human SC with DMPC liposomes. They observed that vesicles did not penetrate into SC but the lipid can penetrate and change the enthalpy of the lipid-related transitions of the SC. In addition, other studies revealed that, depending on composition, vesicles may produce an enhancing effect, their lipid components may penetrate deep into the SC, or may fuse and mix with skin lipids to lose their structure [55].

However, the limited stability of liposomes during storage and administration restricts their application and development. Many attempts have been made to enhance the stability of liposomes. In addition to the thermodynamical state of the liposome membranes, many researchers have outlined that drug penetration can be influenced by modifying the surface charge of liposomes. Successful results were obtained by modifying the surface charge of liposome by using several strategies: charged phospholipids, coating liposomes with polymers and addition of charge inducer agents. Some of these compounds are reported in **Table 1**.

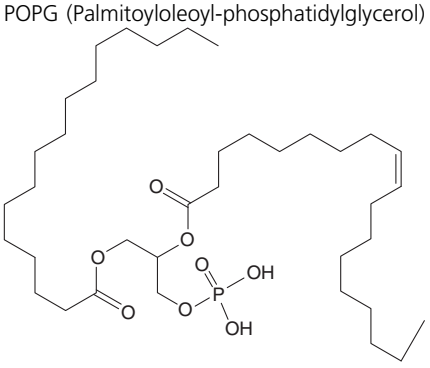
4.1 Charged phospholipids

The lipid molecules of liposomes are arranged in a head-to-tail and tail-to-head geometry across the bilayers. In addition to the outer surface of a liposome in contact with the external aqueous medium, there is an inner surface in contact with the aqueous medium inside vesicles. When molecules are added externally to the liposomes, they rapidly adsorb onto the outer surface of vesicles. If the bilayer is permeable to the molecules, they will migrate across this structure and adsorb onto the inner surface. By symmetry, the adsorbed molecules on the inner and outer surfaces of the liposome are oppositely oriented. In this sense, Liu *et al.* [56] investigated this phenomenon by using the triphenyl organic cation, malachite green, loaded in liposomes composed with different ratios of negatively charged POPG and zwitterionic POPC. They found that the dye adsorption increased linearly with the fraction of negatively charged lipids in the bilayer. Similarly, the transport rate constant for crossing the bilayer increased linearly with the fraction of negatively charged lipid in the bilayer.

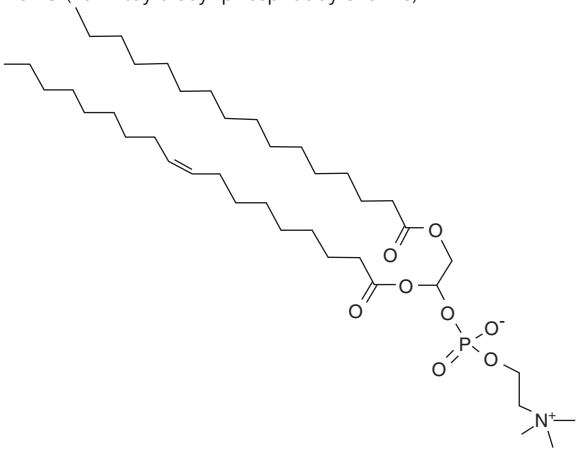
The enhancement of drug permeation by using a minimally invasive method combined with the application of negatively

Table 1. Chemical structures of several charged compounds used in the formulation of charged liposomes.

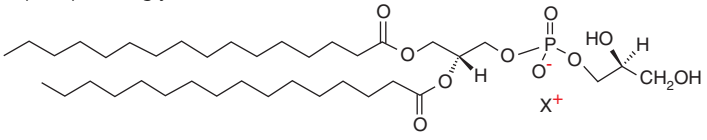
POPG (Palmitoyloleoyl-phosphatidylglycerol)



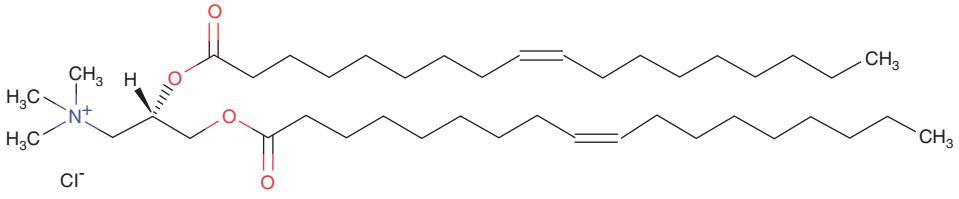
POPC (Palmitoyloleoyl-phosphatidylcholine)



1,2-Dihexadecanoyl-sn-glycero-3-[phospho-(1-glycerol)]



DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,Ntrimethylammonium)



DOSPER (1,3-Di-oleoyloxy-2-(6-carboxy-spermyl)-propylamid)

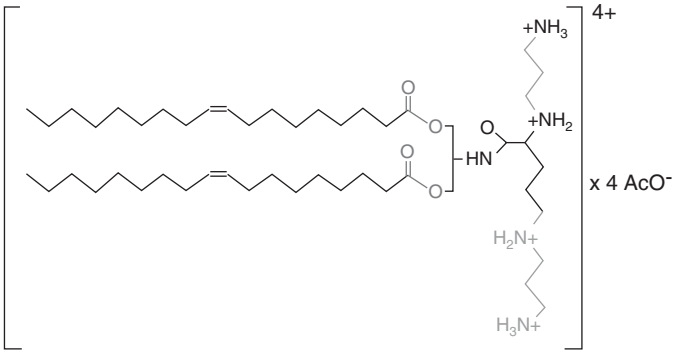
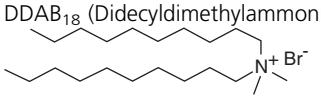
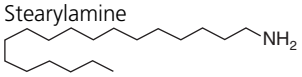


Table 1. Chemical structures of several charged compounds used in the formulation of charged liposomes (continued).

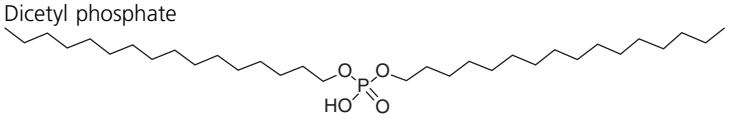
DDAB$_{18}$ (Didecyldimethylammonium bromide)



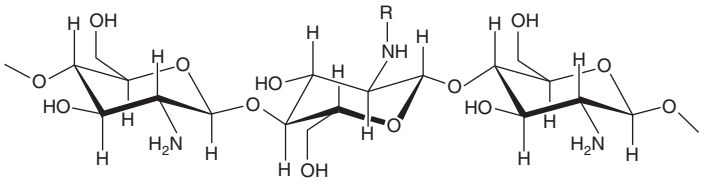
Stearylamine



Dicetyl phosphate



Chitosan



R = H or $COCH_3$

charged liposomes has been described by several authors. **Figure 4** shows the behavior of charged vesicles in contact with the SC.

Murthy *et al.* [57] reported the phenomenon of prolonged permeabilization of mammalian epidermis after incorporating an anionic lipid, dimyristoyl phosphatidylserine (DMPS), in the SC lipid domain with the aid of electrical pulses. The perturbation of SC lipids by joule heating associated with applied electrical pulses was previously demonstrated [58,59]. It is likely that the exogenous lipids such as DMPS get incorporated in the SC lipid lamellae due to the phase transition of SC lipid brought about by the joule heating and the incorporated DMPS retard the reformation of the SC barrier. This synergistic application of anionic lipid formulation and electroosmosis offers a promising non-invasive technique to deliver insulin transcutaneously.

Sen *et al.* [60] prepared lipid vesicles using DOPG and DOPC. When the lipids were mixed with the transport target molecule, the electroporation-induced transport through porcine epidermis was increased as compared to that without the lipids. They demonstrated that the enhancement in transport was dependent on the size and the charge of the transported molecule.

4.2 Coated liposomes

The lipid lamellae of the SC contain a high ratio of negatively charged lipids that are expected to interact with cationic liposomes. Transfer of some of the bilayer components of the liposomes to the skin is then possibly induced. Recent studies showed the efficacy of the ionic polymers in improving skin compatibility of drug formulations and enhancing the penetration of bioactive compounds.

There has been much interest in studying the structures resulting from the self-assembling of liposomes with natural or synthetic charged polyions because of their possible applications in the enhancement of drug delivery across the skin. Several authors have postulated that in the case of charged liposomes, the short-range attractive interaction (and hence the aggregation) is promoted by the addition of adsorbing oppositely charged polyions. This peculiar mechanism produces interesting and counterintuitive phenomenon that has been termed 'charge inversion' [61,62]. This effect occurs when more polyions than necessary to the complete charge neutralization adsorb at the surface and the sign of the overall surface charge changes. This is due to the strong lateral correlations among the adsorbed polyions [63] from which a gain in free energy results, avoiding each other and residing as far away as possible to minimize their electrostatic attraction. When the charge of the polyion coating is almost completely neutralized by the liposome surface charge ('isoelectrical point' or charge neutralization), the short-range attractive potential arising from the non-homogeneous charge distribution promotes the aggregation of complexes.

Several polymers such as carboxymethyl chitin, chitosan [64,65], poly(vinyl alcohol) [66] and Eudragit® EPO [67] have been used for preparation of polymer-coated liposomes to enhance the permeation properties of drugs. **Figure 5** shows an illustrative scheme of this coating process.

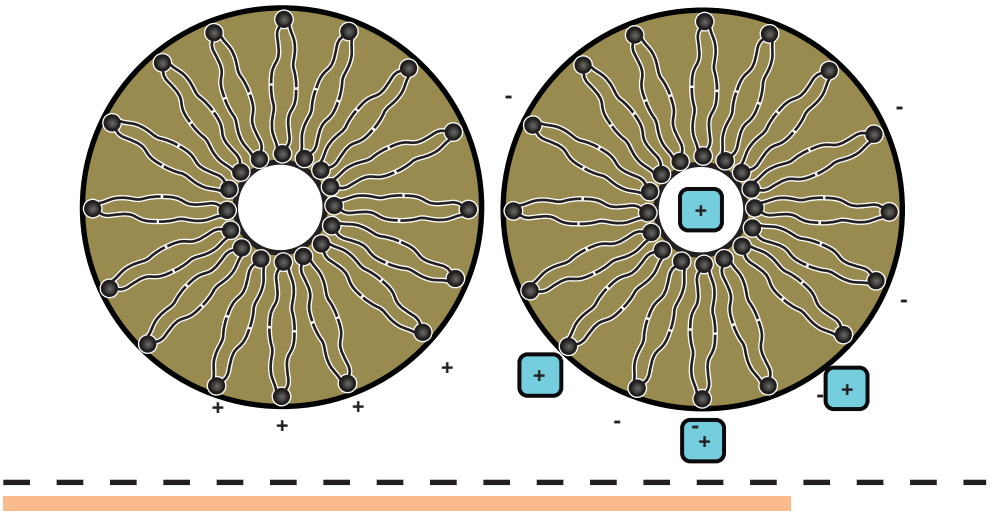


Figure 4. Schematic illustration of charged vesicles formulated with charged phospholipids. Left: positively charged phospholipids. Right: negatively charged phospholipids.

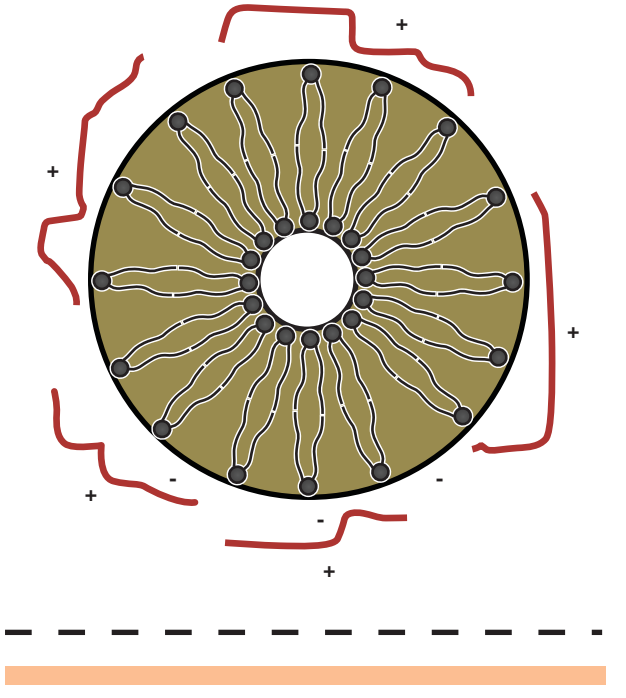


Figure 5. Schematic illustration of vesicles coated with a positively charged polymer, such as chitosan.

4.2.1 *Chitosan*

Chitin is a linear cationic heteropolymer of randomly distributed 2-acetamido-2-deoxy-β-d-glucose residues with β (1→4) linkage. Higher water solubility is expected at an acetylation value lower than 50%. Many derivatives of chitin are described in the literature with this purpose [68,69]. Among them, carboxymethyl chitin (N-CMC) has been used for skin delivery serving as coating material for liposomes [70].

Chitosan is a product derived from *N*-deacetylation of chitin in the presence of hot alkali. The degree of deacetylation and the degree of polymerization, which in turn decides MM of polymer, are two important parameters dictating the use of chitosans for various applications.

It is a natural-sourced cationic polymer with unique biological properties, including favorable biocompatibility and mucoadhesiveness, and has been extensively studied in drug delivery research [71]. However, chitosan is water insoluble under physiological pH value, which largely constrains its application. When the MM of chitosan is decreased by physical, chemical or enzymatic depolymerization, an excellent improvement of its water solubility is achieved as a result of the decrease in intramolecular hydrogen bonds [72].

Chitosan seems to be an optimal candidate to be combined to liposomes [73]. As a cationic biopolymer, it showed the ability to improve skin compatibility of skin formulations and enhancing effect on the penetration of drugs [74]. By combining chitosan and liposomal characteristics, specific, prolonged and controlled release may be achieved. Takeuchi *et al.* [75] showed that the chitosan-coated liposomes were formed via ionic interaction between the positively charged chitosan and negatively charged dicetyl phosphate (DP) on the surface of the liposomes. However, the authors do not show the hydrogen bonding and hydrophobic interaction between chitosan and neutral lipid. In addition, turbidity studies revealed that the coating of DPPC liposomes with chitosan did not significantly modify the main phase transition temperature of DPPC at tested chitosan concentrations [76].

Chitosan and derivatives are able to enhance the paracellular permeability of hydrophilic and macromolecular drugs by transiently opening the tight junctions in the

epidermal barrier [77]. This mechanism can be exerted because at skin pH value (5.5), chitosan is protonated. So, it can interact with anionic components of glycoproteins on the surface of the epidermal cells and with fixed negative charges in the interior of the tight junction, which leads to trigger the opening of the tight junctions, facilitating the transport of hydrophilic compounds.

Chitosan coating resulted in a particle size increase and a more positive ζ potential of liposomes, forming a more stable system, as was recently described by Mady and Darwish [76]. Other studies have been focused on the investigation of the effect of chitosan concentration and lipid type (high purity and low purity) on the characteristics of chitosan-coated liposomes and their interactions with drug. Results showed that polymer bridging caused flocculation at low polymer concentration whereas at high concentration, the adsorbed chitosan molecule led to steric stabilization. Also, leuprolide entrapment efficiency decreased when chitosan was added to liposomes, showing the disturbance of bilayers. In addition, the leakage of leuprolide from low purity liposomes was larger than that from high purity liposomes, because low purity lipid possessed more negative charge and formed thicker adsorptive layer by stronger electrostatic attraction with chitosan [64].

With respect to the control of drug release, chitosan coating has a significant effect on drug release behavior. Appropriate combinations of the liposomal and chitosan characteristics may produce liposomes with specific and prolonged release of drugs, such as doxorubicin [76], steroid hormones [78], acyclovir and minoxidil [67]. Also, the association of chitosan to liposomes is expected to affect also the release rate of glycolic acid from the vesicles by decreasing it; in fact, this compound is very small and hydrophilic and for these reasons it is very problematic to control its diffusion rate from lipidic bilayers.

Chitosan-EDTA was identified as an interesting gelating agent for topical formulations. Therefore, it has been formulated with liposomes as coating agent. The introduction of EDTA on backbone of chitosan converts this cationic polymer to an anionic polymer which displays strong mucoadhesive properties which can be explained by the hydrogen bond formation of its carboxylic acid groups with the mucus gel layer [79]. Biruss and Valenta [78] have suggested that chitosan-EDTA acts by disrupting the intercellular tight junctions [80] and has been proposed as a promising vehicle especially for skin because of its broad spectrum against bacteria. This antimicrobial activity can be explained on the basis of the mechanism of action, which included a high binding affinity to magnesium and calcium [81]. These bivalent cations are essential components in the outer membrane of bacteria and chitosan-EDTA has a chelating effect on these ions.

4.3 Addition of charged compounds

Figure 6 shows the linkage of these compounds to the vesicle bilayer, giving a net positive (left) or negative (right) charge.

4.3.1 *DOTAP*

Kitagawa and Kasamaki [82] studied the delivery of retinoic acid to skin by using cationic liposomes containing N-[1-(2,3-dioleoyloxy)propyl]-N,N,Ntrimethylammonium (DOTAP) and consisting of double-chained cationic surfactant, PC and retinoic acid. These results suggest the potential of the use of the cationic liposomes for intradermal delivery of lipophilic drugs.

Liposomes have been widely used to enhance transfollicular delivery of low/high MM and hydrophilic/lipophilic compounds. Ham *et al.* [83] developed the transfollicular adriamycin delivery system using cationic liposomes including DOTAP that improved the delivery amount and penetration of drug into the follicles and skin. In addition, to accelerate delivery, iontophoresis was combined with the cationic liposome, suggesting that the combinative system has a significant synergistic effect on transfollicular delivery of adriamycin. As this drug itself is a cationic molecule, when iontophoresis was combined with cationic liposomes, it showed excellent transfollicular delivery. Also, this process was enhanced by the increased positive charges of the cationic liposome, adding multication additives, such as DOSPER, spermine and protamine.

Song and Kim [84] examined skin surface charge changes with low-molecular-weight heparin cationic flexible liposomes (flexosomes), prepared using DOTAP and Tween®. In this study, the surface charge of skin with flexosomes has decreased from 7.5 to -13.7 mV in 12 h, whereas the surface charge of the intact skin was constant at -10 mV, suggesting that cationic flexosomes could pass the SC as intact structures.

The skin is an attractive target tissue for somatic gene. In this sense, the promoter elements of tissue-specific genes, including keratin genes, have been identified and can be used to target the expression of genes delivered into the skin. In addition to treating skin disorders, cutaneous gene delivery can be used to express gene products with systemic effects.

Although the charge of the liposome may not influence the penetration efficacy through the SC, the charge may influence the interaction with DNA thus influencing its penetration. Hong-Yu *et al.* [85] developed a formulation for topical skin gene delivery that utilized naked plasmid DNA. The *in vivo* successes with Transfersomes led to their introduction as carriers for non-invasive gene delivery [86]. *In vitro* transfection efficiency of plasmid DNA was assessed by the expression of green fluorescent protein. It was also tested for *in vivo* transfection efficiency and its retention time within the organs, by applying the complexes on hair-removed dorsal skin of mice, non-invasively. It was found that genes were transported into several organs for 6 days once applied on intact skin, suggesting promising properties for non-invasive gene delivery.

In spite of the literature concerning the formulation of cationic liposomes for topical pDNA delivery [86-88], the interest for cutaneous siRNA therapy is profound [89]. Several

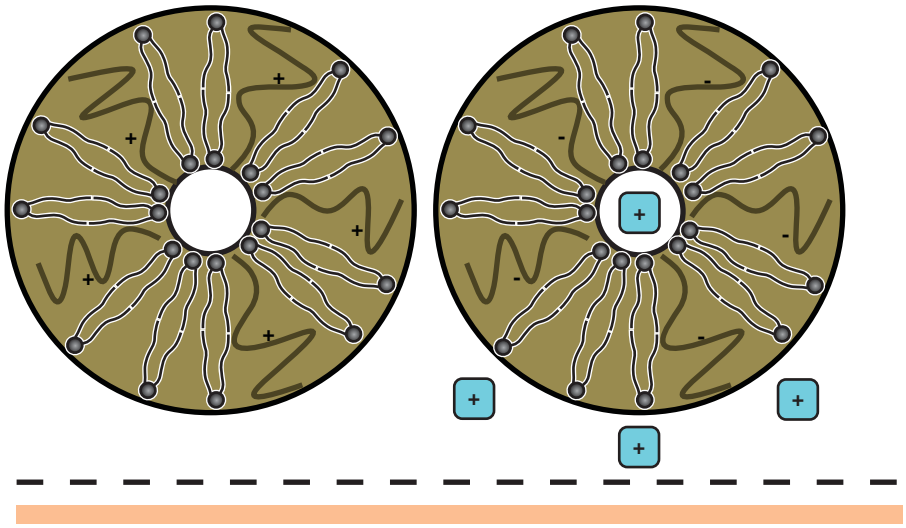


Figure 6. Schematic illustration of vesicles that are formulated with a charged compound, enclosed into the bilayer structure. Left: positively charged phospholipids. Right: negatively charged phospholipids.

penetration-enhancing techniques in combination with liposomal formulations have been described [90]. Geusens *et al.* [91] develop a new lipid-based nanosome that enables the effective delivery of siRNA into human skin. The major finding is that ultraflexible siRNA-containing liposomes, prepared using DOTAP, cholesterol, sodium cholate and ethanol (SECosomes), penetrate into the epidermis of freshly excised intact human skin and are able to enter the keratinocytes.

The transcutaneous route is particularly attractive for immunization, because the skin is highly accessible and has unique immunological characteristics. The presence of professional antigen presenting cells in the epidermis and dermis mediates the immune response following cutaneous immunization [92]. However, the SC acts as a barrier for diffusion and thereby forms a major obstacle to transcutaneous immunization, for example, vaccination through intact or pretreated skin. Currently, the main challenges for cutaneous immunization are to enhance the transport of antigens across the skin barrier and to improve the immunogenicity of topically applied subunit vaccines [93]. Among them, cationic liposomes may allow them to be widely used as non-invasive delivery agents for vaccine antigens into the skin [94]. When formulating vaccine antigens with liposomes it is critical to determine the compatibility of the antigen with the liposome. A major concern with the use of liposomal delivery systems for vaccine antigens is toxicity to cells of the immune system. Cationic lipids are highly toxic to phagocytic cells such as macrophages [95]. This toxicity may be due to destabilization of the lysosomal membranes by the cationic lipids [96].

The use of Transfersomes to formulate antigens in transcutaneous immunization has also been reported in a few studies. Formulations prepared with soybean PC, Span® 80 and ethanol were loaded with hepatitis B surface antigen (HBsAg). Similar immune response was induced as compared to those obtained by intramuscular injection of the same dose of alum-adsorbed HBsAg [97]. In contrast, elastic cationic liposomes made of PC, Span 80 and DOTAP did not improve the immune response when loaded with diphtheria toxoid. Transcutaneous immunization of all formulations resulted in substantial antibody titers only if microneedle pretreatment was applied [98].

4.3.2 *Dicetyl phosphate and stearylamine*

The bilayer membranes mostly consist of either natural or synthetic phospholipids, although other double-tail surfactants such as dialkyl quaternary ammonium compounds are also used in pharmaceutical applications. In addition, minor amounts of cholesterol, or single-tail surfactants, may be added to modify specific characteristics such as the membrane permeability or electric charge density [99].

Recently, Villasmil-Sánchez *et al.* [100] have investigated the effect of the charge inducer agent on the ζ potential values between negatively and positively charged liposomes. In accordance to several authors, they have concluded that DP incorporated in liquid-crystalline PC bilayers is randomly distributed on the plane of the bilayer. Furthermore, the distribution of this negatively charged phospholipid between the two halves of the bilayer is uniform. However, this pattern was not reproduced with liposomes containing stearylamine (SA), where a possible asymmetrical distribution of SA in the bilayer was obtained. In addition, several authors have reported the capacity of SA to escape easily from the lipid bilayer and protect its hydrocarbon chain from the hydrophilic environment: SA is organized in micelles, undergoing a rapid segregation into the medium, which change the surface charge density [101].

Several studies have demonstrated that positively charged liposomes have a remarkable effect in enhancing the

penetration of drugs across the skin because the SC is negatively charged and favors the electrostatic attraction for positively charged liposomes [82]. For this reason, SA, a positively charged lipid, has been included in numerous liposome formulations. On the other hand, several studies have been developed by using DP as an anionic surfactant that favors the ionic interaction with the drug, which is partially positively charged in the formulations, as was recently demonstrated by Manosroi *et al.* [34]. The anionic niosomes loaded with Gdm gave a high entrapment efficiency of drug due to the effect of charge interaction between the anionic niosomes and cationic charge of the drug at pH 5.4. This study has demonstrated that chemical degradation of drug at high temperatures was not only protected, but also its antibacterial activity existed with more sustained release effects, showing greater cumulative amounts and fluxes in the skin without risk of systemic effects.

Temoporfin (mTHPC) is a very potent second-generation synthetic photosensitizer. Topical application of mTHPC would be of great interest for the photodynamic therapy of basal-cell carcinoma or psoriasis. In order to increase topical delivery of mTHPC, Dragicevic-Curic *et al.* [102] have prepared neutral, anionic (DP) and cationic (SA) flexosomes and investigated their penetration enhancing ability. The results obtained were in agreement with those of Katahira *et al.* [103] and Montenegro *et al.* [104] who found that the skin permeation of drugs incorporated in positively charged liposomes was higher compared to negatively charged liposomes.

On the other hand, Puglia *et al.* [105] demonstrated *in vivo* a higher sustained release of methyl nicotinate from neutral and negatively charged liposomes with respect to positively charged liposomes, which was in accordance with results from Katahira *et al.* [103]. They proposed that the electrostatic interaction between the negatively charged skin surface and the positively charged liposomes could promote the drug permeation and its consequent rapid depletion by the bloodstream in the vascularized section of the skin.

Surfactant properties of DP contribute to the permeation enhancement of drugs from elastic vesicles: increasing the permeation rate of different model drugs through the skin [106,107] or delivering the highest amount of drug in the viable epidermis [13].

4.3.3 Other charged substances

The influence of charge and lipid concentration on the *in vivo* percutaneous absorption of a model compound, methyl nicotinate, from $DDAB_{18}$ (didecyldimethylammonium bromide) liposomal vesicles has been investigated by Puglia *et al.* [105]. Methyl nicotinate was chosen as the model compound as it was capable of causing cutaneous erythema, the intensity and duration of which was proportional to the amount entering the living epidermis over time. The extent of the erythema was monitored by reflectance spectrophotometry, a non-invasive technique. *In vivo* findings showed an interesting enhancement drug permeation when positively charged liposomes were used, giving rise to a more pronounced erythematous effect.

5. Expert opinion

The rationale for using liposomes in dermal and transdermal drug delivery is many fold: as penetration enhancers owing the penetration of the individual lipid components into the SC, as depot for sustained release of dermally active compounds and as a rate-limiting membrane barrier for the modulation of systemic absorption. Considering that the composition of the vesicles has a great effect on the effectiveness in enhancing transdermal drug delivery, many promising strategies have been described in the literature to enhance the drug permeation across the skin.

The limited stability of liposomes during storage and administration restricts their application and development (*in vitro* and *in vivo*), although some attempts have been made to improve stability. The addition of charged agents to the overall structure of the lipid vesicles constitutes an interesting strategy to improve the stability of formulations. The use of charged phospholipids or the incorporation of charged substances to the vesicle surface, such as SA or DP offers many advantages with respect to the coating process. First, the preparation process is easier, requiring fewer stages in the process. Also, more reduced sizes are obtained. However, besides these differences, all the systems designed offer a proper mechanism to enhance the penetration of drugs, the permeation differences among them being nonsignificant.

Despite the advances that are occurring in the design of charged liposomes for transdermal drug delivery, the long-term stability continues to be a drawback in these systems. The presence of charge on the surface of the vesicles favors the electrostatic repulsion among them, creating a ζ potential positive or negative that prevents their aggregation and flocculation. However, there is loss of encapsulated drug, increasing the size of vesicles and destruction of structures over time, limiting the *in vivo* use of these systems.

The high potential of charged liposomes as promising candidates for use in gene therapy and vaccine targeting to treat a great diversity of diseases for which drugs are administered by the percutaneous route should be emphasized.

Declaration of interest

The authors declare no conflict of interest and have received no payment in preparation of this manuscript.

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