

ELASTIC VESICLES AS DRUGS CARRIERS THROUGH THE SKIN

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Abstract

Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: transferosomes and ethosomes. This paper presents the composition, manufacturing and characterization methods, mechanisms of penetration of transferosomes and ethosomes as transdermal delivery systems of active substances.

Rezumat

Administrarea transdermică a medicamentelor este în principal limitată de funcționarea pielii ca o barieră. Sistemele veziculare reprezintă una dintre cele mai controversate modalități utilizate pentru transportul transdermic al substanțelor active. Interesul pentru proiectarea de sisteme veziculare transdermice a fost relansat după descoperirea veziculelor elastice: transferozomii și etozomii. Acest articol prezintă compozițiile, metodele de preparare și caracterizare, precum și mecanismele de penetrație ale transferozomilor și etozomilor ca sisteme de transport transdermic al substanțelor active.

Keywords: transdermal system, liposomes, niosomes, transferosomes, ethosomes.

Introduction

The topical administration of drugs for the local treatment of skin diseases has been used for a long time, but the use of transdermal delivery for the systemic action is relatively new and increasingly used. The rapid development of transdermal delivery formulations in the last years is due to certain advantages of transdermal administration *versus* the conventional oral one [2, 3, 13]:

- it circumvents the fluctuations which appear at gastro-intestinal absorption;

- it increases the bioavailability of drugs because using the transdermal delivery the active principle passes directly into the circulatory system, bypassing the hepatic metabolism;
- it can give a constant, controlled drug input decreasing the variations in drug plasma levels;
- it increases the patient compliance by providing a simplified way of administration, minimum risk of trauma or any other injury of tissue.

In order to design a drug with transdermal administration certain difficulties must be resolved. The major difficulty is the penetration of skin that acts as a two ways barrier, controlling the loss of water, electrolytes and other constituents and preventing the entrance of medicinal or harmful substances from the external environment. The skin is a membranous, flexible, protecting cover, mainly formed by two major layers: an external, unvascularized one (*epidermis*) and an internal, vascularized one (*dermis*). The *stratum corneum* is the uppermost layer of the skin. It represents the final state of stratification and consists of a multi-layered (10-25) structure of keratin-rich corneocytes embedded in a lipid matrix [10, 25]. Its thickness is around 10 μm in the dry state. The perfect barrier properties of the skin are provided by the *stratum corneum*. In order to increase the permeability of the skin for transdermal delivery of drugs several passive as well as active techniques have been proposed: penetration enhancers, supersaturated systems, vesicles, iontophoresis, electroporation, phonophoresis, microneedles, jetinjectors, etc. Despite all the efforts devoted to penetration enhancement, only 10 active substances are currently transdermally administrated. These substances have certain properties, such as: low molecular weight ($<500\text{Da}$), highly lipophilicity and pharmacological activity, effectiveness at low doses: 5-10 mg /day (antihypertensive, antianginal agents, analgesics, steroids and contraceptives).

One of the most controversial methods for drugs' transport across the skin is the use of vesicle formulation as skin delivery systems.

Vesicular structures as skin delivery systems

In the last years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs, because of their certain advantages, e.g. lack of toxicity, biodegradation, capacity of encapsulating both hydrophilic and lipophilic molecules, capacity of prolonging the existence of the drug in the systemic circulation by encapsulation in vesicular structures, capacity of targeting the organs and tissues, capacity of reducing the drug toxicity and increasing its bioavailability [5,14,15,16,20,23,24].

Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more (multilamellar vesicles) concentric bilayers. 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.

Most commonly, the vesicles are composed of phospholipids or non-ionic surfactants. The reason for using vesicles in transdermal drug delivery is based on the fact that they act as drug carriers to deliver entrapped drug molecules across the skin, as well as penetration enhancers because of their composition. In addition, these vesicles serve as a depot for the sustained release of active compounds in the case of topical formulations, as well as rate-limiting membrane barrier for the modulation of systemic absorption in the case of transdermal formulations [14].

Liposomal formulations can be classified in two categories: rigid vesicles – liposomes and niosomes – and elastic or ultradeformable vesicles – transferosomes and ethosomes.

Liposomes

Liposomes are microscopical spherical vesicles mainly composed by one or more lipidic bilayers, separated by aqueous compartments; they represent the most studied nano- and microparticulate systems for pharmaceutical applications.

Mezei and Gulasekharam reported for the first time the effectiveness of vesicles for skin delivery, suggesting that the lipid formulations can enhance the topical release of drugs [5, 15, 20]. Despite all the efforts devoted by several researchers, it was impossible to formulate a liposomal compound that permits the systemical release of an active principle, mainly because the dimension of the liposomes does not allow them to penetrate the *stratum corneum* [7, 23, 24].

Niosomes

Niosomes are vesicles composed of nonionic surfactants. The niosomes have been mainly studied because of their advantages compared with the liposomes: they are quite stable structures and require no special conditions for preparation and storage, they have no purity problems and the manufacturing costs are low [6, 14, 15]. Unfortunately, the performed studies showed that, like liposomes, niosomes are not suitable for

transdermal delivery, because they cannot reach the deeper layers of the skin, being trapped in the superior layers of *stratum corneum*. To overcome this problem, the carried out researches introduced a novel generation of vesicular elastic systems: transferosomes (ultradeformable vesicles consisting of phosphatidylcholine and an edge activator) and ethosomes (ultradeformable vesicles with high alcohol content) [6, 28].

The main advantage of these ultradeformable vesicular systems is the elasticity of the bilayer, given by the surfactant molecules in the case of transferosomes and ethanol in the case of ethosomes; this elasticity allows them to squeeze through channels in the *stratum corneum* that are less than one-tenth the diameter of the vesicles.

Transferosomes

Transferosomes are a special type of liposomes, consisting of phosphatidylcholine and an edge activator. The concept of transferosomes was introduced in 1992 by Cevc and coworkers. These vesicular transferosomes are several orders of magnitude more elastic than the standard liposomes and thus well suited for the skin penetration [6, 8].

Composition. From the composition point of view, a transferosome is a self adaptable and optimized mixed lipid aggregate. Transferosomes are vesicles composed by phospholipids as the main ingredient (soya phosphatidylcholine, egg phosphatidylcholine, dipalmityl phosphatidylcholine, etc), 10-25% surfactants for providing flexibility (sodium cholate, tween 80, span-80), 3-10% alcohol as a solvent (ethanol, methanol) and hydrating medium consisting of saline phosphate buffer (pH 6.5-7).

Manufacturing method. Phospholipids, surfactants and the drug are dissolved in alcohol. The organic solvent is then removed by rotary evaporation under reduced pressure at 40°C. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the appropriate buffer by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated at room temperature to get small vesicles.

Characterization. Visualization of transferosomes can be performed using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Particle size and size distribution can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). The drug entrapment efficiency by transferosomes can be measured by the ultracentrifugation technique. Vesicle stability can be determined by assessing the size and structure of the vesicles over time and

drug content can be quantified by HPLC or spectrophotometric methods. *In vitro* drug release can be measured using a diffusion cell or a dialysis method [16, 29].

Mechanism of penetration. Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of *stratum corneum*. At present, the mechanism of enhancing the delivery of active substances in and across the skin is not very well known. Two mechanisms of action have been proposed [18 - 21]:

1. Transferosomes act as drug vectors, remaining intact after entering the skin
2. Transferosomes act as penetration enhancers, disrupting the highly organized intercellular lipids from *stratum corneum*, and therefore facilitating the drug molecules penetration in and across the *stratum corneum*.

Cevc and coworkers proposed the first mechanism, suggesting that deformable liposomes penetrate the *stratum corneum* because of the transdermal hydration gradient, normally existing in the skin, and then, crossing the *epidermis*, enter in the systemic circulation [6].

The recent studies propose that the penetration and permeation of the vesicles across the skin are due to the combination of the two mechanisms. Depending on the nature of the active substance (lipophilic or hydrophilic) and the composition of the transferosomes, one of the two mechanisms prevails.

Ethosomes

Ethosomes are deformable liposomes with high alcohol content (up to 45%). It is proposed that the alcohol fluidizes the ethosomal lipids and *stratum corneum* bilayer lipids thus allowing the soft, malleable ethosomes to penetrate [1, 4]. They have been introduced for the first time by Touitou in 1996. The ethanol from ethosomes' composition plays the same role as the surfactant from the transferosomes, namely disorganizing the lipid bilayer, conferring a ten times higher deformability to the particles [12, 17].

Composition. Ethosomes are composed mainly of phosphatidylcholine, high concentration of hydroalcohols or hydroalcohols, glycols and water [26]. Phosphatidylcholine can be: phosphatidyl soya phosphatidylcholine, egg phosphatidylcholine, dipalmityl phosphatidylcholine, hydrogenated phosphatidylcholine. As alcohols, we can use ethanol or isopropyl alcohol, and as polyglycols propylene glycol and transcitol.

Manufacturing methods. The ethosomes can be prepared from soybean phosphatidylcholine (Phospholipon 90), ethanol, drug and distilled

water. Phospholipon 90 and the drug should be dissolved in ethanol. Water has to be added in small quantities and the preparation mixed by mechanical stirring under controlled conditions [9, 11, 25, 27].

Characterization. The methods for characterization of the ethosomes are the same as the ones for transferosomes [16, 22, 29].

Mechanism of penetration. The mechanism of penetration of the ethosomes in and through the skin is not yet completely elucidated. Two simultaneous mechanisms of action have been proposed: ethanol has a fluidization effect on the ethosomal lipids and ethanol has a fluidization effect on the *stratum corneum* lipids.

Because of the use of ethanol in the preparation of the ethosomes, the deformability of the prepared vesicles is increasing. Besides, the high alcohol content is expected to partially extract the *stratum corneum* lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes [16, 25]. The ultradeformable vesicles can forge paths in the disordered *stratum corneum* and finally release drug in the deeper layers of the skin. Therefore, a path through the skin can be expected to result, permitting the fusion of ethosomes with the cells from the deepest skin layers [25, 26].

Conclusions

The use of the transdermal route has been well established in the past, and, because its inherent advantages, new methods for transdermal delivery are continuously developed. The introduction of ultradeformable vesicles, transferosomes and ethosomes, was an important step in relaunching the researches regarding the use of vesicles as transdermal drug delivery systems.

In comparison to other transdermal delivery systems, the use of elastic vesicles has certain advantages: they allow enhanced permeation of drug through skin; their composition is safe and the components are approved for pharmaceutical and cosmetic use; they can increase the transdermal flux, prolonging the release and improving the site-specificity of bioactive molecules; they can accommodate drug molecules with a wide range of solubility.

Hence, enhanced delivery of bioactive molecules through the skin by means of an ultradeformable vesicular carrier opens new challenges and opportunities for the development of novel improved therapies.

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