Skin is the major target as well as a principal barrier for topical/transdermal drug delivery system. There is so many advantages of this system along with some major obstacle like low diffusion rate of drugs across the stratum corneum. A number of devices have been invented to increase the permeation rate of drugs temporarily; among them one simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. The vesicular system is known as one of the most controversial methods for transdermal delivery of active substances in that ethosome is formulated as ethanolic phospholipids vesicles which are used mainly for transdermal delivery of drugs. Because of its high ethanolic content, Ethosomes have higher penetration rate through skin. This article reviews various aspects of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, preparation and patents granted for ethosomes.

Keywords: Ethosomes, Transdermal Drug Delivery, ethanolic liposome, vesicular carrier, skin penetration

INTRODUCTION

In conventional dosage forms; oral route possesses notable advantages like easy administration, non-invasive, least likely to harm the patient, availability of large variety of dosage form, inspite of all these advantages this route can be cost prohibitive and inconvenient due to first pass effect, poor bioavailability, rapid blood level spikes and patient incompliance etc. To overcome these difficulties a novel Transdermal drug delivery involves the application of a drug to the skin to achieve systemically active levels of the drug to treat disease remote from the application site, avoidance of gastrointestinal (GI) incompatibility, variable GI absorption, and first pass metabolism along with reduced frequency of administration, improved bioavailability, improved patient compliance, and rapid termination of drug input. Additionally transdermal delivery can maintain a suitable plasma concentration through a noninvasive zero-order delivery (similar to intravenous administration), which would enhance the efficacy of contraceptive agent with high patient compliance and pharmaco-economic incentives. [1,13].

In transdermal drug delivery Systems (TTS), the skin patches are designed to deliver the therapeutic agent at a controlled rate from the device to and through the skin into the systemic circulation and to maintain efficacious plasma levels of the drug for periods of 1-7 days depending on the particular drug. This route provides a precise amount of drug to be delivered for systemic action. In general, under the most ideal circumstances, only approximately 1 mg of a drug can be delivered across a 1 cm² area of skin in 24 hours. Drugs having melting point above 150 °C and a molecular weight greater than 500 Daltons may reduce this number 10-fold, 100-fold, or even more.

For desired pharmacological action a number of factors influences the rate of delivery of drug across the skin including Penetration, thermodynamic activity of drug, interaction of drug, variation in skin with age, race, anatomical region etc, Whether it is for systemic effects or topical applications, therapeutic agents must first pass through the stratum corneum and epidermis, then enter the dermis layer and exert their
further effects. As skin acts as a good barrier needs a broad range of different enhancing strategies, which involve chemical enhancers, vesicular carriers, iontophoresis, electroporation, acoustical method, microneedle, jet injection etc [2-5].

In the present review, vesicular system of transdermal drug delivery in which Touitou E in 1996 [6] introduced ethosome for the first time for the enhanced delivery of drug into or through skin by the vesicular carrier, which is a novel liposome composed of phospholipid, short chain alcohol (mostly ethanol) at relatively high concentration, and water. Ethosomes could efficiently penetrate skin (because of penetration enhancer) and enhance compound delivery (as they have vesicular structure) to deeper skin strata or systemic effects. As skin acts as a good barrier needs a broad range of different enhancing strategies, which involve chemical enhancers, vesicular carriers, iontophoresis, electroporation, acoustical method, microneedle, jet injection etc [2-5].

The physical and chemical properties of ethosomes make more efficacious than other forms of liposomes for drug delivery to blood stream through the stratum corneum. Zhu et al [8] reported that there were no significant changes in average particle size, distribution, and structure of ethosomes over two years storage that supports stable physical and chemical properties of ethosomes. This has been also proved by another study in which the size of liposomes significantly increased with time, while the average size of ethosomes basically remained constant over four weeks [9]. The physicochemical characteristics of a vesicles strongly affects the effectiveness of ethosomes, in particular its thermodynamic state. Liquid-state vesicles have been found to be more effective in enhancing drug transport as compared to gel-state vesicles [10,11]. In this contrast in the early 1990s a novel series of liquid-state vesicles with elastic lipid membranes were developed [12].

The various types of vesicular formulations so far have been developed are summarized in table 1.

Table 1. Vesicles developed for Transdermal drug delivery system.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archaeosomes</td>
<td>Archaeosomes are the archaeabacteria lipids containing vesicles which are chemically distinct from eukaryotic and prokaryotic species and are less sensitive to oxidative stress, high temperature, and alkaline pH [14,15].</td>
</tr>
<tr>
<td>Cochleates</td>
<td>Cochleates are the special form of liposomes which are suspended in an aqueous two-phase polymer solution, and allow the logic partitioning of polar molecule-based structures by phase separation. Cochleate are the precipitate of a particle size less than 1μm obtained by the liposome containing two-phase polymer solution treated with positively charged molecules such as Ca²⁺ or Zn²⁺ ions [16].</td>
</tr>
<tr>
<td>Dendrosomes</td>
<td>Dendrosomes have shown excellence results when used as a vehicle for gene delivery compared with other existing synthetic vehicles with advantages such as non-toxic, neutral, biodegradable, covalent or self-assembled, hyperbranched, dendritic, spheroidal nanoparticles which are easy to prepare, inexpensive, highly stable as well as easy to handle and apply [17].</td>
</tr>
<tr>
<td>Dried reconstituted vesicles (DRV)</td>
<td>These are the small, &quot;empty&quot; unilamellar vesicles, containing different lipids or mixtures of them. The small unilamellar vesicles are prepared by mixing with solubilized drug followed by dehydration and then water is added for rehydration that leads to the formation of large quantities of rather inhomogeneous, multilamellar vesicles which need further processing [18].</td>
</tr>
<tr>
<td>Ethosomes</td>
<td>Ethosomal systems delivers a drug more efficiently to the skin, in terms of quantity and depth, than either conventional liposomes or hydroalcoholic solutions and the studies of drug permeation through the skin was demonstrated in diffusion cell experiments. These multilamellar vesicles are composed of soy phosphatidylcholine and about 30% of ethanol [19].</td>
</tr>
<tr>
<td>Immunoliposomes</td>
<td>Immunoliposomes are the modified liposomes established for in vitro and in vivo application with antibodies, Fab’s or peptide structures on the bilayer surface [20,21].</td>
</tr>
<tr>
<td>Immunosomes</td>
<td>Immunosomes are the preformed liposomes prepared by the anchorage of glycoprotein molecules, look like homogenous spherical vesicles (50-60 nm) evenly covered with spikes under the electron microscope. They have structural and immunogen characteristics closer to those of purified and inactivated viruses than any other form of glycoprotein lipids association [22].</td>
</tr>
<tr>
<td>Immune stimulating complex (ISCOM)</td>
<td>ISCOMs are the spherical, micellar assemblies of about 40 nm composed of saponin mixture Quil A, cholesterol, phospholipids and amphiphilic antigens like membrane proteins. ISCOMs already contain a built-in adjuvant, Quilajasaponin, which is a structural part of the vehicle [23].</td>
</tr>
<tr>
<td>Lipoplexes</td>
<td>Cationic lipid-DNA complexes are known as lipoplexes, are efficient carriers for cell transfection with certain drawbacks due to their toxicity. In lipoplexes either cationic lipids or nucleic acids may cause toxicity. [24,25].</td>
</tr>
<tr>
<td>LUVETs</td>
<td>LUVETs are large unilamellar vesicles prepared by extrusion technique [26].</td>
</tr>
<tr>
<td>Niosomes</td>
<td>Niosomes are small unilamellar vesicles made from nonionic surfactants also known as Novasomes. Their chemically they are as stable as archaeosomes [27].</td>
</tr>
<tr>
<td>pH-sensitive liposomes</td>
<td>The pH-sensitive liposomes have been described in four basic classes. The first class most intensively studied, combines unsaturated polymorphic lipids like phosphatidylethanolamines,</td>
</tr>
</tbody>
</table>
with mild acidic amphiphilic in nature and act as stabilizers at neutral pH. The second class includes liposomes composed of permeability enhancer lipid derivatives to encapsulate solutes. A third class of pH-sensitive liposomes is composed of pH-sensitive peptides or reconstituted fusion proteins to destabilize membranes at low pH. The final and most advanced class of pH-sensitive liposomes utilizes pH-titratable polymers to destabilize membranes following change of the polymer conformation at low pH [28].

### Table 2: Additives Employed in Formulation of Ethosomes

<table>
<thead>
<tr>
<th>Additives</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 80</td>
<td>Hydrophilic polymer to enhance stability and lengthen half-lives in circulation</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Ensures stability and prevents non-covalent complexation with membrane</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Increases fluidity and mobility of vesicles</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Enhances skin-permeating properties</td>
</tr>
</tbody>
</table>

The vehicle used for the formulation of ethosomal transdermal drug delivery system, have been widely studied. It was suggested that highly elastic vesicles could facilitate an easy drug transport across the skin as compared to rigid membrane vesicles [44]. However, Phosphatidyl choline (PC) [45-48], Span 80, hydrogenated PC, phosphatidic acid (PA) [49], phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols) [50] elastic vesicles were recently reported to be more effective in enhancing the skin permeation of dexamethasone, diclofenac, zidovudine, and norgestrel as compared to PC-cholesterol rigid vesicles [51-55]. Apart from this, it has also been found that ethosomes are well distributed when cholesterol is included in the formulation, and that they are prone to aggregation in the absence of cholesterol. This is because of the cholesterol that stabilizes into a bilayer when ethosomes are maintained in a gel state, and mobility of the vesicles is ensured by the high concentration of ethanol in ethosomes, and that a moderate amount of cholesterol could ensure stability [56-58]. Different types of additives employed in formulation of Ethosomes have been summarized in Table 2.

### Literature Review of Ethosomes

Touitou et al [19] demonstrated the procedure of development of ethosomes by using minoxidil as a model drug and observed that the ethosomal system dramatically enhanced the skin permeation of minoxidil in vitro compared with either ethanolic or hydroethanolic solution or phospholipid ethanolic micellar solution of minoxidil. From this study it was concluded that the ethosomes can efficiently entrap molecules of various lyophilicities.

For the enhanced transdermal delivery Bendas et al [59] prepared ethosomes by the solvent evaporation method [60] using salbutamol sulfate as a model drug and phosphatidylcholine from soybean lecithin, cholesterol, and dicetylphosphate by dissolving in a small volume of diethyl ether: chloroform (1:1) mixture.

For the delivery of a drug having short biological half-life (4-6 hour) i.e. Lamivudine an antiviral drug for treatment of acquired immunodeficiency syndrome (AIDS) and hepatitis [61] prepared ethosomes and deliver it as transdermally using fluorescence marker Rhodamine 123 and soyaphosphatidyl choline (99%) without further purification as per well-known cast film method [62].

By using ethanol injection-sonication technique Ligustrazine- ethosomes were prepared, with entrapment efficiency as an indicator. The ethosome were prepared by 1% (w/v) phospholipid, 0.4% (w/v) cholesterol, and 45% (v/v) ethanol [63].
Maheshwari et al [45] prepared and compare the transdermal potential of novel vesicular nanocarriers: ethosomes and ultradisperse liposomes, containing an anti-fungal bioactive clotrimazole (CLT) and their results suggested that among all types of formulations they prepared ethosomes are the most proficient carrier system for dermal and transdermal delivery of clotrimazole. Ethosomes were prepared by mechanical-dispersion method, as reported and described earlier [9,46,64].

The effect of cholesterol and ethanol on dermal delivery from α-dipalmitoylphosphatidylcholine (α-DPPC) liposomes was observed by Pinto et al [9] using minoxidil (Mx) as a model drug.

By using soya phosphatidylcholine (PC) (99%), phosphotungstic acid, Rhodamine Red-X 1, 2 dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (RR), ethanol and methotrexate (MTX) as model drug Dubey [46] prepared ethanolic liposomes for Dermal and transdermal delivery of an anti-psoriatic agent (MTX).

Dubey et al [64] prepared and evaluated novel ethanolic liposomes of Melatonin Cast film method using Soya PC (2.0% w/w) as lipid excipients and concluded that the ethosomes provides an enhanced transdermal flux, lower lag time, higher entrapment efficiency and low skin irritancy potential, thus, this approach offers a suitable approach for transdermal delivery of melatonin.

Paolino et al [47] prepared and evaluated various ethosomal suspensions made up of water, phospholipids and ethanol at various concentrations for their potential application in dermal administration of ammonium glycyrrhizinate. 1-3% (w/v) Phospholipon 90®, 30-45% (v/v) ethanol, active molecules as described and water to 100% (w/v) were used to prepare ethosomes colloidal suspensions as reported by Touitou [19].

Fang et al [65] used 5-aminolevulinic acid-photodynamic therapy (ALA-PDT) to treat Psoriasis and prepared ethosomes using phosphatidylethanolamine and ethanol according to the thin-film hydration method [66] that was his previous study in which 5-aminolevulinic acid-encapsulated liposomes were compared to ethosomes for the skin delivery for photodynamic therapy.

Verma et al [49] used ethanol with a commercially available lipid mixture, NAT 8539, to improve the topical delivery of cyclosporin A (CyA) and the size of vesicles were found to be 56.6 to 100.6 nm in diameter depending on the amount of ethanol added in the formulation.

Liu et al [67] investigate the pharmacokinetics of the ligustrazine ethosome patch and antimyocardial ischemia and anti-ischemic reperfusion injury effect by applying ethosomal patch in rats and found that prepared ligustrazine ethosome patches could improve drug absorption and bioavailability. Because ligustrazine degraded by first pass effect upon oral administration while several ligustrazine preparations via transdermal administration have been reported to improve its bioavailability and safety [68-70].

Liu et al [71] prepared ethosomes by ethanol injection-sonication and formulation as a patch of ligustrazine and evaluated in vitro and in vivo.

In another research Dubey et al [50] prepared ethanolic liposomes to deliver an anti-HIV agent (Indinavir), using phosphatidylcholine as lipid polymer and ethanol as penetrating agent.

Godin et al [72] successfully permeate Bacitracin an antimicrobial agent through cellular membrane using ethosomes as a vehicle and support the previously suggested mode of action of these soft vesicles. For this study Fluorescent phospholipid (rhodamine red dihexadecanoylglycero-phosphoethanolamine, RR) and Phospholipon-90 were used.

Elsayed et al [73] prepared deformable liposomes (prepared by conventional mechanical dispersion method) and ethosomes (according to Dayan [74]) to investigate the possible mechanisms by which deformable liposomes and ethosomes could improve skin delivery of the model hydrophilic drug, ketotifen fumarate (KT), under non-occlusive conditions. According to the results observed deformable liposomes were found to be the best way to deliver Ketotifen across the skin.

Verma et al [75] prepared ethanolic liposomes i.e. ethosomes using Econazole nitrate as a model drug to treat skin infections caused by various species of pathogenic dermatophytes by cold method [55].

### Table 2: Additives employed in formulation of Ethosomes

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples of lipid excipients</th>
<th>Uses and importances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmitlyphosphatidyl choline, Distearlyphosphatidyl choline</td>
<td>Help in the formation of vesicles</td>
</tr>
<tr>
<td>Polyglycol</td>
<td>Propylene glycol, Transcutol RTM</td>
<td>Acts as a skin penetration enhancer</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Ethanol, Isopropyl alcohol</td>
<td>Provides softness to the vesicle membrane and enhances skin penetration</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>Stabilizes the vesicle membrane</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine-123, Rhodamine red, Fluoresceinsothiocynate (FITC), 6- Carboxy fluorescence</td>
<td>Dyes are used for the characterization of vesicles</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carbopol D934</td>
<td>Used in the ethosomal gel type formations</td>
</tr>
</tbody>
</table>
Zhang et al [76] prepared ethosomes according to [19] by using Lipoid S 100-phosphatidylcholine (PC) from soybean lecithin and 5-fluorouracil as a model drug and observed the penetration of ethosomes in hypertrophic scar (HS) and skin was analyzed by ethosomes labeled with rhodamine 6GO (a fluorescent agent) and confocal laser scanning microscopy (CLSM).

Chourasia et al [77] prepared nanosized ethosomes vesicles by the method reported elsewhere with some modifications [64] using Soya phosphatidyl choline as a major excipient and Ketoprofen as a model drug and the results of the in-vitro release study through the skin revealed higher transdermal flux with ethosomal formulation compared to hydroalcoholic drug solution.

Lodzki et al [78] used Cannabidiol (CBD) as a model drug candidate for treatment of rheumatic diseases and prepared ethosomes the method reported elsewhere with some modifications [19] and the kinetic profile of CBD's plasma concentration shows that steady-state (SS) levels were reached at about 24 h and lasted at least until the end of the experiment (72 hrs). The in vivo studies were conducted using male CD1 nude mice 8-9 weeks old.

Fang et al [79] prepared ethanolic liposomes with the aim to develop and evaluate liposomal formulations encapsulating tea catechins, which possess antioxidant and chemopreventive activities using anionic surfactants such as deoxycholic acid (DA) and dicetyl phosphate (DP) in the liposomes in the presence of 15% ethanol increased the (+)-catechin permeation by five to seven-fold as compared to the control. The in vitro release and skin permeation were determined using a Franz diffusion cell along with a cellulose membrane or female nude mouse skin.

Buspirone was used as model drug to treat Menopausal syndromes in women by formulating ethosomes as a drug carrier and it was observed that the ethosomal buspirone transdermal system could be considered as a promising delivery system for the treatment of menopausal syndromes. The study also shows an enhanced skin permeation in-vitro, good bioavailability and efficient pharmacodynamic responses in animals [80].

**Mechanism of Drug Penetration [47, 81]**

The enhanced release of active drug ingredient from the ethosomes can be described by an interaction between ethosomes and skin lipids. The mechanism for this interaction is not yet cleared but two types of effects have been assumed while drug release from ethosomes. First part of the mechanism is assumed due to the 'Ethanol Effect' whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. After this effect a second type of mechanism comes under influence i.e. 'Ethosome Effect' that includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin. The drug absorption probably occurs in two phases as discussed hereunder:

Ethanol effect: Topical ethanol solutions are also used as penetration enhancers [47,82-84] and can also be used in transdermal preparations in combination with Labrasol as a cosurfactant [102]. Ethanol penetrates easily into intercellular lipids and increases its fluidity and decrease the density of lipid multilayer of cell membrane.

Ethosomes effect: The ethanol used in the preparation of ethosomes increases cell membrane lipid fluidity results in increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers i.e. Stratum corneum, where it got fused with skin lipids and releases the drugs into deep layer of skin [49].

**Methods For Characterization of Ethosomes Visualization**

This is very important to visualize the ethosomes and this can be done by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [85]. The visualization studies by electron microscopy reveals that the ethosomal formulation exhibits vesicular structure ranging from 300-400 nm in diameter.
Mean size is measured by dynamic light scattering (DLS) and structure changes are observed by TEM [96]. The stability studies can be carried out by storing the ethosomal formulations at two different temperatures i.e. 4°C and 25±2°C. The drug content in ethosomal formulation can be estimated by using high performance liquid chromatographic [74] for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation.

**Penetration and Permeation Studies**

After the release of drug from ethosomal formulation it reaches to the skin, and the depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM) [97].

**Transition Temperature**

The transition temperature (the transition temperature is the temperature at which an amorphous solid becomes soft upon heating or brittle upon cooling. The glass transition temperature is lower than the melting point of its crystalline form) of the vesicular lipid systems can be determined by using differential scanning calorimetry [98].

**Statistical analysis**

The statistical analysis of the experimental results can be performed by ANOVA. Differences were considered statistically significant at p<0.05. All the data values should be represented as mean ± standard deviation of 3 measurements. Moreover, the in-vitro dissolution data can also be compared using a model independent analysis involving determination of similarity factor f2, which is a measure of similarity in two drug release profiles. The following equation is used to calculate the similarity factor [99]

$$f_2 = 50 \log \left[ \left( \frac{1}{n} \right) \sum_{t=1}^{n} \frac{W_t}{(F_t - T_p)^2} \right]^{0.5} \times 100$$

**CONCLUSION**

Ethosomes is a carrier system offers new therapeutic dimension and opportunities in while approaching transdermal route to deliver a drug with more patient compliances. Ethosomes has initiated a new area in vesicular research for transdermal drug delivery that provides a better skin permeation compared to the liposomal drug delivery systems. The drugs having low drug diffusion rate across the stratum corneum have been considered to be the major limiting factor in case of transdermal drug delivery system which can be bypassed by formulating as ethosomes. Recent advancements and further research in this area will allow a more effective approach in dermal/transdermal delivery of drugs.
### Table 3. US Patents Granted For Ethosomes

<table>
<thead>
<tr>
<th>Patent publication number</th>
<th>Inventors</th>
<th>Title</th>
<th>Date of patent Granted</th>
<th>Patent description in brief</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 55,40,934</td>
<td>Elka Touitou</td>
<td>Composition for applying active substances to or through the skin</td>
<td>Jul. 30, 1996</td>
<td>The present invention relates to the cosmetic and or medical composition for topical application to the skin, in which essential ingredients used in this are phospholipid, a lower aliphatic alcohol of two to four carbon atom instead of this propylene glycol may also be optional, water and a compatible active ingredient. The alcohol used from 20 to 50% alone and upto 70% along with glycol.</td>
<td>6</td>
</tr>
<tr>
<td>US 57,16,638</td>
<td>Elka Touitou</td>
<td>Composition for applying active substances to or through the skin</td>
<td>Feb. 10, 1998</td>
<td>The present invention relates to the cosmetic and or medical composition for topical application to the skin, in which essential ingredients used in this are phospholipid, a lower aliphatic alcohol of two to four carbon atom instead of this propylene glycol may also be optional, water and a compatible active ingredient. 0.5 to 10% phospholipids, 5 to 35% C₃ or C₄-alcohol, 15 to 30% ethanol, that contain at least 20% but not more than 40% by weight of ethanol and upto 20% propylene glycol.</td>
<td>50</td>
</tr>
<tr>
<td>US 2009/0047234 A1</td>
<td>Elka Touitou, Biana Godin, Shaher Duchi</td>
<td>Composition for nasal delivery</td>
<td>Feb. 19, 2009</td>
<td>The invention relates to the administration of least one active pharmaceutical agent by intranasal route. The formulation includes phospholipids (0.2-10%), one or more C₂-C₄ alcohol (12-30%) and water</td>
<td>100</td>
</tr>
<tr>
<td>CN101273971</td>
<td>Liping Liu, Yimin Li, Lijuan Hu, Minggao Shen, Yang Jin</td>
<td>Ethosomes preparation of antimycotics pharmaceutical and method for preparing the same</td>
<td>Oct 01, 2008</td>
<td>Description is not available</td>
<td>101</td>
</tr>
</tbody>
</table>

### REFERENCES

34. Klibanov A L, Maruyama K, Torchilin V P, and Huang L, Amphipathic polyethylene glycols effectively


50. Touitou E, Composition of applying active substance to or through the skin, US patent, 57,16,638, (1996).


60. Liang W, Levchenko T S, Torchilin V P, Encapsulation of ATP into liposomes by different methods: optimization of the procedure, J Microencapsul, 21,


