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Elastic liposomes mediated transdermal delivery of verapamil hydrochloride

Jain Neha* [1], Argal Ameeta [2], Gautam Girendra [1]

[1]Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Ajmer, Rajasthan, India

[2]Technocrates Institute of Technology, Pharmacy, Bhopal, M.P., India

ABSTRACT

The aim of present investigation was to formulate and characterize elastic liposomes as a delivery system for transdermal delivery of Verapamil hydrochloride, a drug having low oral bioavailability (approx 20%), short biological half-life and extensive first pass metabolism.Verapamil hydrochloride loaded elastic vesicles were prepared by a slightly modified extrusion method using soya phosphatidylcholine and span 80(edge activator). Prepared elastic vesicles were characterized for various parameters such as vesicle shape, vesicle size and size distribution, entrapment efficiency, elasticity measurements, stability studies and *in vitro* skin permeation studies through excised rat skin (Sprague Dawley) using a locally fabricated Franz diffusion cell. The entrapment efficiency of elastic vesicles was found to be 59.3±3.6%. *In vitro* skin permeation of verapamil hydrochloride through excised rat skin (Sprague Dawley) revealed that elastic vesicles led to an enhanced transdermal flux (50.2±4.52_µg/cm²/h) of verapamil hydrochloride as compared to liposomes (11.6±2.12µg/cm²/h). Decreased lag time (0.9 h) was also observed in case of elastic liposomes. Our results indicate the feasibility of elastic liposomes for transdermal delivery of verapamil hydrochloride for improved skin permeation.

Keywords: Transdermal delivery, Elastic liposomes, Verapamil hydrochloride.

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***Address for Correspondence:**

Neha Jain, Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Ajmer, Rajasthan, India

INTRODUCTION

Skin, the largest sense organ of the body serves as a barrier against physical, chemical and microbial attacks, acts as a thermostate in maintaining body temperature, plays a role in the regulation of blood pressure and protects against the penetration of ultraviolet rays. Natural function of the skin is the protection of the body against the loss of endogenous substances such as water and undesired influences. Skin, effectively the largest organ of the body, is a multipurpose, nonhomogeneous membrane with a complex structure. It contains and protects the internal body organs and fluids and exercises environmental control over the body regarding temperature and humidity. In addition, the skin is a communicating organ, relaying the sensations of heat, cold, touch, pressure, and pain to the central nervous system. Skin has been routinely used as the site for administration of dermatological drugs to achieve a localized pharmacological action in the skin. The skin can also serve as the port of administration for systemically active drugs. The drug applied topically will absorb first into the blood circulation and then be transported to target tissues to achieve its therapeutic concentration. The transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications.[1]

Skin acts a major target as well as a principal barrier for topical/transdermal drug delivery and always remains an attraction of scientists for delivery of bioactives because of its impermeable nature. The skin is divided into 2 main structural layers: the epidermis and the dermis. The outer most layer of the epidermis, the stratum corneum provides a protective barrier that prevents the loss of physiologically essential substances and provides greatest resistance to penetration and is the rate limiting step of percutaneous absorption. The epidermis also has immunologic functions and provides some protection of the skin from ultraviolet light via the pigment system. Low permeability of drugs through stratum corneum, barrier layer of the skin limits the utility of topical drug delivery. 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 attention seeking. Various physical approaches as iontophoresis, microporation, electrophoresis, sonophoresis, pressure wave, magnetophoresis, chemical approaches as chemical penetration enhancers and prodrug approach have been employed to breach the skin barrier. Penetration enhancement with special formulation approache is mainly based on the usage of colloidal carriers. Submicron-sized particles are intended to transport entrapped active molecules into the skin. Various nanosized colloidal and vesicular carriers as liposomes, niosomes, nanoemulsions, ethosomes, elastic liposomes and solid lipid nanoparticles are gaining much more attention of scientists for topical delivery of therapeutics because they are able to cross the barrier nature of skin through different mechanisms.[2-7]

Elastic liposomes are at the forefront of the rapidly developing field of nanotechnology with several potential applications in skin delivery. Elastic liposomes differ from commonly used liposomes in that they are much more flexible and adaptable. Elastic liposomes combine phosphatidylcholine with edge activators/surfactants to form flexible membranes. The crucial feature of elastic liposomes in comparision with standard liposomes and other types of lipid suspensions is the flexibility of vesicle membrane and stress dependent adaptability. These liposomes with their characteristic fluid membrane and high elasticity are able to penetrate the narrow pores much smaller than their own diameter. They can deform and pass through narrow constriction without measurable loss. This high deformability gives better penetration of intact vesicles.[8-9]

Verapamil 2-(3,4-dimethoxyphenyl)-5-{[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino}-2-(1-methylethyl) pentanenitrile hydrochloride is a calcium channel blocking anti-anginal agent that regulates high blood pressure by decreasing myocardial contractility, heart rate and impulse conduction. It is used in treatment of hypertension, angina pectoris and supraventricular arrhythmias. Topically and transdermally verapamil hydrochloride may be utilized in peyronie's disease either alone or in combination with trifluoperazine and magnesium sulfate.[10-11] Verapamil hydrochloride on oral administration undergoes extensive first pass metabolism which is responsible for its low bioavailability i.e. 20%. It has a short biological half life of just 4.8 hours, due to its short time of action it is required to administer many times a day decreasing patient compliance. Due to its short biological half life, high hepatic clearance and low bioavailability verapamil hydrochloride seems to be a suitable candidate for transdermal drug delivery. Hence noninvasive delivery via transdermal route utilizing vesicular approach i.e. elastic liposomes could be a better option for effective, sustained delivery of verapamil hydrochloride.

Feasibility of transdermal delivery of verapamil hydrochloride was earlier demonstrated by researchers across excised rat skin and porcine ear skin. Gungor et al developed matrix-type transdermal patches of verapamil hydrochloride and investigated various terpenes as chemical enhancer to improve skin penetration. Microneedles and microneedle rollers mediated transdermal drug delivery of verapamil hydrochloride and amlodipine besylate showed enhanced transdermal flux from porcine ear skin.[12-17]

The objective of present study was to deliver verapamil hydrochloride by transdermal route utilizing novel vesicular approach for enhanced skin permeation. Specially designed vesicles elastic liposomes have shown a great deal of success in transdermal panorama. Elastic liposomes are lipid vesicles consisting of phospholipids and an edge activator that destabilizes the lipid bilayer of the vesicles and increases the flexibility of the bilayer. Due to the flexible nature of membrane these vesicles are capable of squeezing themselves through the intracellular regions of the stratum corneum and penetrate into the deep skin strata. Prepared elastic svesicles were characterized for various parameters such as vesicle shape, vesicle size and size distribution, entrapment efficiency, elasticity measurements, stability studies and *in vitro* skin permeation studies through excised rat skin (Sprague Dawley).

MATERIALS AND METHODS

Materials

Soya phosphatidylcholine (99%), Span 80, Sephadex G-50, Triton X-100 were purchased from Sigma (St. Louis, MO, USA). Verapamil hydrochloride was received as a gift sample from Samarth Life Sciences Pvt. Ltd. (Solan, India). All other chemicals and solvents were of analytical grade and freshly prepared distilled water was used wherever required.

Preparation of vesicular systems

The elastic liposomes were prepared by a slightly modified extrusion method reported by Maghraby et al., 2000.[18] Different batches of elastic liposomes were prepared using different proportions of surfactant, soya phosphatidylcholine and drug. The accurately weighed amounts of soya phosphatidylcholine and surfactant were taken and dissolved in ethanol in a clean, dry, round bottom flask. Ethanol was removed by rotary evaporation above the lipid transition temperature (Rotary Evaporater, Superfit, Ambala, India). Final traces of solvent were removed under vaccum overnight. The deposited lipid film was hydrated with varying concentrations of drug in PBS (pH 6.5) by rotation (60 rpm, 1 hr) at room temperature. The resulting vesicles were swollen for 2 hr at room temperature. These vesicles were extruded through a sandwich of 100 and 200 nm polycarbonate membranes (Milipore, USA). Compositions of different batches of elastic liposomes as shown in Table 1.Conventional liposomes were prepared using Cast film method.

Table 1: Composition of different elastic liposomal formulations

S. No.	Formulation Code	Soya PC (mg)	Span 80 (mg)
1.	ELS_1	95	5
2.	ELS_2	90	10
3.	ELS_3	85	15
4.	ELS_4	80	20
5.	ELS_5	75	25

Incorporation of verapamil hydrochloride in elastic liposomes

Verapamil hydrochloride was incorporated into vesicular formulations at saturating concentration. To determine the maximum amount of drug incorporated, increasing amounts of verapamil hydrochloride were added during preparation of elastic liposomes. Vesicular formulations were examined over a period of 14 days using Light Microscopy (Leica, DMLB, Switzerland).

Characterisation of elastic liposomes

Entrapment efficiency

Prepared elastic vesicles were separated from the unentrapped drug by Sephadex G-50 minicolumn centrifugation technique. The vesicles were lysed by Triton X-100 (0.5% w/w) and entrapped drug was estimated spectrophotometrically at 279 nm.[19-20]

Vesicles size

Elastic vesicles were visualized using a Philips transmission electron microscope (CM12 Electron Microscope, Eindhoven, Netherlands) with an accelerating voltage of 100 kV. A drop of the sample was placed on to a carbon-coated copper grid to leave a thin film. Before the film dried on the grid, it was negatively stained with 1% phosphotungastic acid (PTA). A drop of the staining solution was added on to the film and excess of the solution was drained off with a filter paper. The grid was allowed to thoroughly dry in air and samples were viewed in a transmission electron microscope.[21]

Vesicle size distribution

The size distribution of elastic vesicles was measured in two sets of triplicates in a multimodal mode, by Dynamic Light Scattering (DLS) technique using a computerized inspection system (Malvern Zetamaster, ZEM 5002, Malvern,UK). Vesicular suspension was appropriately diluted with PBS (pH 6.5) and the measurements were conducted in triplicate.[22]

Turbidity measurements

The turbidity of different elastic liposomes and conventional liposomes were determined using PBS (pH 6.5) as blank (Nephalometer, Superfit, Mumbai, India).

Elasticity of vesicle membrane

The deformability study was conducted for the elastic liposomes against the standard liposome preparation. Briefly, the flux of vesicles suspension through a large number of pores of known size (through a sandwich of polycarbonate filters with pore diameter between 50 and 200 nm, depending on the starting vesicle suspension), was driven by an external pressure of 2.5 bar.[23] The amount of vesicle suspension, which was extruded during 5 min, was measured and vesicle size and size distribution were monitored by DLS measurement before and after filtration. The experiment was performed in triplicate and each sample was analyzed twice. The elasticity of vesicle membrane was calculated by using the following formula [24]

$$D = J \times \left[\frac{r_v}{r_p} \right]^2$$

Where, D, elasticity of vesicle membrane; J, amount of suspension,

Which was extruded during 5 min; r_v, size of vesicles (after passes); and r_p, pore size of the barrier.

Physical stability of elastic liposomes

The drug retentive behavior of vesicles was determined by keeping elastic liposomes at four different temperature conditions, i.e. 4-8ºC (Refrigerator), 25±2ºC (Room temperature), 37±2ºC and 45±2ºC for a period of 5 weeks. The elastic liposomes were kept in sealed ampoules after flushing with nitrogen. Samples were withdrawn periodically and analyzed for the drug content. Stability of elastic liposomes was also assessed by the measuring size and structure using DLS and TEM.

Skin permeation and deposition studies

The *in-vitro* skin permeation of verapamil hydrochloride from elastic liposomes was studied using a locally fabricated Franz diffusion cell. The receptor compartment contained 10 ml PBS (pH 6.5) and was constantly stirred by magnetic stirrer (Expo India Ltd., Mumbai, India) at 100 rpm. Sprague Dawley rat skin was mounted between the donor and receptor compartments. Experiments were carried out for 24h at 37±1ºC, with donor compartments each containing 1 ml of different compositions, (a) elastic liposomes, (b) liposomes, (c) plain drug solution. Samples were withdrawn through the sampling port of the diffusion cell at predetermined time intervals over 24 hr and analyzed. The receptor phase was immediately replenished with equal volume of fresh diffusion buffer. Sink conditions were maintained throughout the experiment. *In-vitro* drug release study from elastic liposomes was repeated with cellophane membrane using the same method as described above.

The amount of verapamil hydrochloride remained in the skin was determined at the end of the *in-vitro* permeation experiment (24 hr). The skin was washed 10 times using a cotton cloth immersed in methanol. A sample of skin was weighed and homogenized in 1ml of methanol for 5 min with an electric stirrer. The resulting solution was centrifuged for 10 min at 7000 rpm. The supernatant was analyzed for drug. The cumulative amount of drug permeated per unit area was plotted as a function of time, the steady state permeation rate (Jss) and lag time (T_L, hr) were calculated from the slope and X-intercept of the linear portion, respectively.

RESULTS AND DISCUSSION

Elastic liposomes combine phosphatidylcholine with edge activators/surfactants to form flexible membranes. These liposomes with their characteristic fluid membrane and high elasticity are able to penetrate the narrow pores much smaller than their own diameter. The slightly modified extrusion method reported by EI Maghraby *et al.,* 2000 was used to prepare verapamil hydrochloride elastic liposomes. Span 80 was selected as edge activator because it is biocompatible and pharmaceutically acceptable. Various formulation variables which may affect the formulation i.e. lipid: surfactant ratio was studied in order to produce optimized drug loaded vesicles. Optimum ratio of soya phosphatidylcholine and surfactant was found to be 85:15 w/w for the preparation of elastic liposomes.

For optimization of drug amount, the elastic liposomes were prepared using varying concentrations of drug, followed by morphological characterization and determination of entrapment efficiency. The maximum concentration of verapamil hydrochloride that could be incorporated into elastic liposomes was found to be 15mg with percentage entrapment efficiency of 59.3±3.6%. On further increasing the amount of drug, precipitated drug crystals were observed, this precipitation of drug crystals may be due to the saturation of the vesicles. Further, a lower entrapment of verapamil hydrochloride in elastic liposomes may be due to the hydrophilic nature of the drug.

Table 2: Drug loading in elastic liposomal formulations

S. No.	Amount of drug (mg)	Microscopic observation	Entrapment efficiency (%)
1.	5	NO	51.1±2.6
2.	10	NO	53.7±3.1
3.	15	NO	59.3±3.6%.
4.	20	O	57.4±1.8

NO- Not observed, O- Observed

Various parameters of elastic liposomes were evaluated like vesicles size, vesicle size distribution, turbidity measurements, entrapment efficiency, elasticity of vesicle membrane, physical stability, skin permeation and deposition studies.

Entrapment efficiency of different elastic liposomal formulations was investigated by minicolumn centrifugation method. Formulation ELS_3 showed maximum entrapment efficiency of 59.3±3.6% so it was selected for further evaluation parameters. The entrapment efficiency first increased up to 15% w/w concentration of surfactant, after which decrease in entrapment efficiency was observed. The entrapment efficiency is highest at 15% surfactant concentration. The influence of surfactant concentration on the entrapment efficiency has been shown in Table 3.

Table 3: Entrapment efficiency of different elastic liposomes

S. No.	Formulation code	Surfactant concentration (%w/w)	Entrapment efficiency (%)
1.	ELS_1	5	45.9±1.9%
2.	ELS_2	10	53.2±2.7%
3.	ELS_3	15	59.3±3.6%
4.	ELS_4	20	51.4±3.2%
5.	ELS_5	25	42.1±4.3%

The size of vesicle was measured for formulations extruded through polycarbonate membrane and the results were expressed as the average vesicle size. Average vesicle size of elastic liposomes was found in between 100-150 nm.Vesicle size of optimized ELS_3 formulation was found to be 126±10. As the surfactant concentration increases, the size of vesicles increases. Visualization by TEM revealed the unilamellar, spherical shape of elastic liposomes. Polydispersity index of these formulations were found in the range of <0.1(0.06) suggesting homogeneous nature of elastic liposomes.

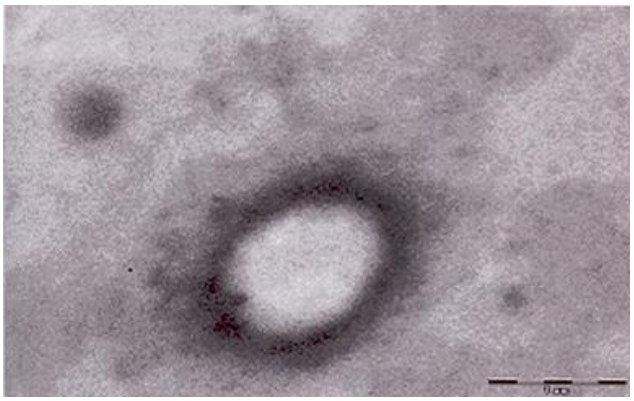

Figure 1 TEM (Transmission Electron Microscopy) Photograph of Elastic Liposome (X 80,000)

Turbidity measurement of different elastic liposomes was done by using Nephalometer. Maximum turbidity was found in the formulations containing lipid surfactant concentration of 85:15 w/w suggesting fair population of vesicles. Low turbidity values of different elastic liposomes suggesting that vesicles might have ruptured or converted into mixed micells which have been reported less deformable in nature. These mixed micelles also have less skin permeability as compared to elastic liposomes.

Table 4: Effect of phospholipid to surfactant ratio on turbidity of drug loaded elastic liposomes

S. No.	Formulation code	Phospholipid to surfactant ratio (%w/w)	Turbidity (N.T.U.)
1.	ELS_1	95:5	16.9±4.1
2.	ELS_2	90:10	19.3±3.5
3.	ELS_3	85:15	26.2±5.2
4.	ELS_4	80:20	20.8±4.6
5.	ELS_5	75:25	15.4±6.3

The crucial feature of elastic liposomes in comparison with standard liposomes and other types of the drug loaded lipid suspensions is the flexibility of vesicle membrane and stress dependent adaptability. 'Elasticity' is the measure of flexibility of the vesicles to penetrate the skin spontaneously and minimize the risk of complete vesicle rupture in the skin. This feature of elastic liposomes is chiefly due to the presence of surface active agent in appropriate proportion and in our case it was found to be 65.4±5.5. Elastic vesicles with this flexibility may proficiently approach the deep subcutaneous layers for effectual delivery of drug and thereof sustained action. Maximum elasticity was observed for formulation containing 15% w/w surfactant concentration. Further increase in surfactant concentration decreased elasticity may be due to the conversion of vesicles to mixed micelles.

Table 5: Effect of surfactant concentration on elasticity of drug loaded elastic liposomes

S. No.	Formulation code	Surfactant concentration (%w/w)	Elasticity
1.	ELS_1	5	29.6±3.4
2.	ELS_2	10	36.8±1.9
3.	ELS_3	15	65.4±5.5
4.	ELS_4	20	39.7±4.3
5.	ELS_5	25	31.4±2.8

Stability studies of verapamil hydrochloride formulations suggested that there was no significant difference in shape lamellarity and particle size of vesicles stored at refrigeration temperature and room temperature for five weeks suggesting their stability under given conditions. But, under condition of elevated temperature, a significant alteration in morphology, vesicle size as well as increased drug leakage was observed. This may be due to the effect of temperature on the gel to liquid transition of lipid bilayers. Hence loss of drug from vesicles was observed. Refrigerated conditions were found to be best for storage with negligible drug leakage at all times.

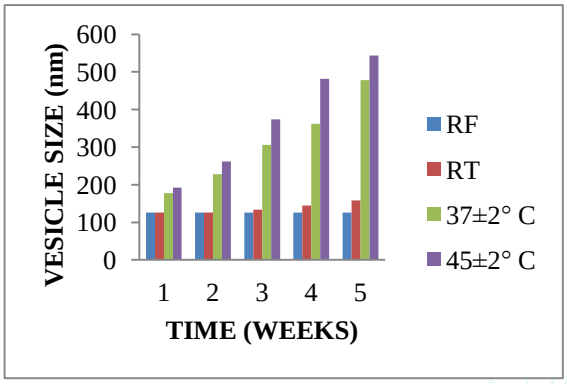

Figure 2: Effect on vesicle size of elastic liposomes after storage

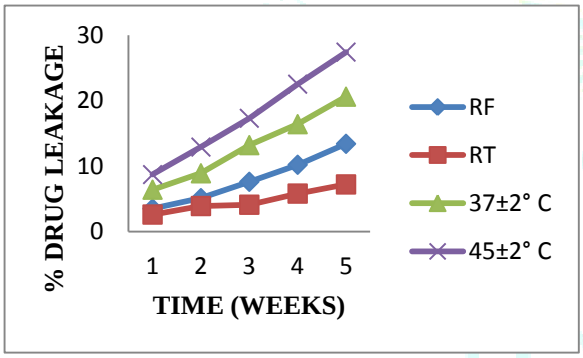

Figure 3: Effect of temperature on drug leakage from elastic liposomes

Skin permeation potential of verapamil hydrochloride loaded elastic liposomes was investigated by studying various parameters as transdermal flux across excised rat skin, lag time and skin deposition of the drug. The cumulative amount of drug permeated per unit area was plotted as a function of time and steady state transdermal flux was observed. The steady state transdermal fluxes for different elastic liposomes were observed between 15.1±4.2 μg/h/cm² and 50.2±4.52 μg/h/cm². Verapamil hydrochloride loaded elastic liposomes containing 15% w/w Span 80 revealed a transdermal flux of 50.2±4.52 μg/cm²/h and lag time of 0.9 h. Conventional liposomes and plain drug solution provided significantly lower flux values and longer lag time of 11.4±1.23 μg/cm²/h, 2.3 h and 3.14±0.88 μg/cm²/h, 2.9 h; respectively as compared to elastic liposomes.

Verapamil hydrochloride loaded elastic liposomes led to better percent skin drug deposition (5.61±0.4) as compared to conventional liposomes (1.78±2.5) and plain drug solution (1.19±1.6), thus providing sustained delivery of verapamil hydrochloride. Our results were analogous to that of available reports on transdermal delivery of verapamil hydrochloride, as reported earlier by Kaur *et al*., 2014 (49.96μg/cm²/h). Kaur *et al* investigated the effect of stainless steel solid microneedles and microneedles rollers on percutaneous permeation of verapamil hydrochloride and amlodipine besylate through porcine ear skin and suggested improved permeation of both the drugs.

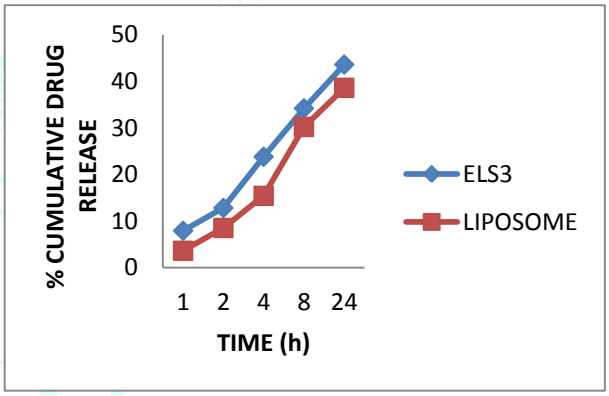

Figure 4: % Cumulative Drug Release from elastic liposomes for 24 hours

Table 6: Composition and characterization of verapamil hydrochloride loaded elastic liposomes

S.No.	Parameters	Elastic liposomes PC:S (85:15w/w)	Liposomes	Plain drug solution
1	Vesicular shape	Spherical, unilamellar	Spherical, multiilamellar	–
2	Vesicular size (nm)	126±10	434±7.0	–
3	Polydispersity index	0.055	0.058	–
4	% Entrapmemt efficiency	59.3±3.6%	39.3±1.8	–
5	Deformability index	65.4±5.5	17.6±2.4	–
6	Transdermal Flux across rat skin (μg/cm²/h)	50.2±4.52	11.4±1.23	3.14±0.88
7	Lag time (h)	0.9	2.3	2.9
8	Percent skin drug deposition	5.61±0.4	1.78±2.5	1.19±1.6

Values represent mean±SD (n = 3).

CONCLUSION

In the present investigation skin permeation profile of verapamil hydrochloride through elastic liposomes was observed. Enhanced transdermal flux and decreased lag time of verapamil hydrochloride via elastic liposomes concluded that problems associated with verapamil hydrochloride i.e. low oral bioavailability, hepatic first pass metabolism and short biological half life could be sorted out via elastic liposomes. Enhanced delivery of bioactive molecules through the skin and cells by means of aforementioned carriers opens numerous challenges and opportunities for novel improved therapies.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest in relation to publication of manuscript.

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