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RESEARCH ARTICLE

ELASTIC LIPOSOME MEDIATED TRANSDERMAL DELIVERY OF AN ANTI-HYPERTENSIVE AGENT: NIFEDIPINE

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ABSTRACT

In the present investigation elastic liposomes of nifedipine were prepared for transdermal delivery. Elastic liposomes bearing nifedipine were prepared by rotary evaporation method and characterized for various parameters including vesicles shape, size and size distribution, entrapment efficiency, number of vesicles, and stability. Then entrapment efficiency of different compositions of elastic liposomal formulations was carried out, through which one formulation was selected for the further parameters. Higher entrapment efficiency was found in transferosomal formulaton which was more than liposomal formulation. Also similar trend was observed in number of vesicles present in elastic liposomal formulation were also more than liposomal formulation. The in vitro drug release study was carried out in modified Diffusion cell using Dialysis membrane. The release rate of nifedipine from elastic liposomes was significantly lower than liposomes. *Ex-vivo* study conducted on male albino rats (Sprague Dawley) was also taken as a measure of performance of elastic liposomal and liposomal solution. Skin study showed that elastic liposomal formulation provides higher skin permeation as compared to liposomal solution of nifedipine. (elastic liposome 76.41±0.9, liposome 71.44±0.9) Stability studies showed that there was no change in consistency of elastic liposomal formulation and also drug crystals were not appeared. Hence, the present study reveals that elastic liposomal formulation of nifedipine possesses greater potential to enhance skin permeation, prolong drug release, and improve the site-specificity of nifedipine.

Keyword's: nifedipine. elastic liposomes, Transdermal delivery, stability

INTRODUCTION

The systemic treatment of disease via transdermal route is not a recent innovation. But, in the last two decades, transdermal drug delivery has gained increasing interest. So, transdermal controlled drug delivery systems have been developed in order to avoid hepatic first-pass effect thus improving drug bioavailability and to overcome the side-effects associated with oral route[1,2]. Transdermal drug delivery systems are currently available for the treatment of various diseases such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, depression, anxiety and attention deficit hyperactivity disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal bone loss and urinary incontinence. The application of transdermal system to a wider range of drugs is still limited due to the significant barrier to penetration across the skin which is allied primarily with the outermost stratum corneum layer of the epidermis[3]. The use of lipid vesicles for transdermal drug delivery system for skin treatment has attracted increasing attention in recent years[4-6]. To overcome the above problem, a novel type of highly deformable lipid vesicles called transfersomes has been reported to penetrate intact skin, if applied non occlusively[7-8].

Transfersomes have been defined as specially designed vesicular particles, consisting of at least one inner aqueous compartment surrounded by a lipid bilayer with appropriately tailored properties. Accordingly, transfersomes resemble lipid vesicles, liposomes, in morphology but, functionally, transfersomes are sufficiently deformable to penetrate pores much smaller than their own size. They are metastable, which makes the vesicle membrane ultraflexible, and, thus, the vesicles are highly deformable. Typical transfersomes are, therefore, characterized by at least one order of magnitude more elastic than conventional lipid vesicles, liposomes. Transfersomes for potential transdermal application, contain a mixture of lipids and biocompatible membrane softeners. This optimal mixture imparts flexibility to the elastic liposomal membrane and leads to the possibility of penetration through channels of the skin, which is opened by the carriers[9-11].

Nifedipine is an anti-hypertensive drug belonging to the class of calcium channel blockers. Although the oral route for nifedipine is widely accepted but it is associated with contraindicative manifestations such as gastrointestinal (GI) disturbance, first pass metabolism, less oral bioavailability[12] etc. Transdermal delivery of nifedipine is a better option to overcome problems associated with its oral delivery. Hence, the objective of the present study was to design and evaluate transferosomes of nifedipine that would release the drug over a prolonged period of time thus avoiding first pass metabolism and to improve its systemic availability.

MATERIALS AND METHODS

Materials:

Nifedipine was received as a gift sample from Anod Pharmaceuticals Pvt. Ltd. Soya phosphatidylcholine was purchased from Himedia Ltd, Mumbai. Span 80, Chloroform, Ethanol were purchased from S.D Fine chemicals Ltd. Methanol was purchased from E. Merck

Ltd. All reagents used in this study were of analytical grade.

Methods:

Preparation of Elastic Liposomal Formulations

The elastic liposomes were prepared by conventional rotary evaporation sonication method. Different batches of elastic liposomes were prepared using different proportions of surfactant, phospholipids and drug. The accurately weighed amounts of phospholipids and surfactant were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in small quantity of chloroform-methanol mixture. The organic solvent was removed by rotary evaporation under reduced pressure at 40°C. Final traces of solvents were removed under vacuum overnight. The deposited lipid film was hydrated with drug solution in ethanol (7 % v/v) by rotation at 60 rev/min for 1 hr. To prepare smaller vesicles, these were bath sonicated for 10 min.The conventional liposomal formulation (Phosphatidylcholine:Cholesterol, 7: 3), that serve as a control for comparison in the present study, was prepared by the same method as described above.

Drug Loading In Elastic Liposomal Formulations

To determine the maximum amount of drug that could be added to elastic liposomal formulation, increasing amount of drug in concentration range of 4-12 mg was added and formulations were prepared and analyzed by optical microscope. In case of formulations prepared using 4-10 mg of drug, no crystals were observed but formulation containing 12 mg of drug showed crystal formation (shown in fig. 1 and fig. 2). Thus, the maximum amount of drug that could be incorporated in elastic liposomal formulation was found to be 10 mg.

Table 1: Drug Loading in Elastic Liposomal Formulations

S.No.	Amount of Drug (mg)	Observations
1.	4	CNA
2.	6	CNA
3.	8	CNA
4.	10	CNA
5.	12	CA

CNA-crystals not appear ; CA-crystals appear

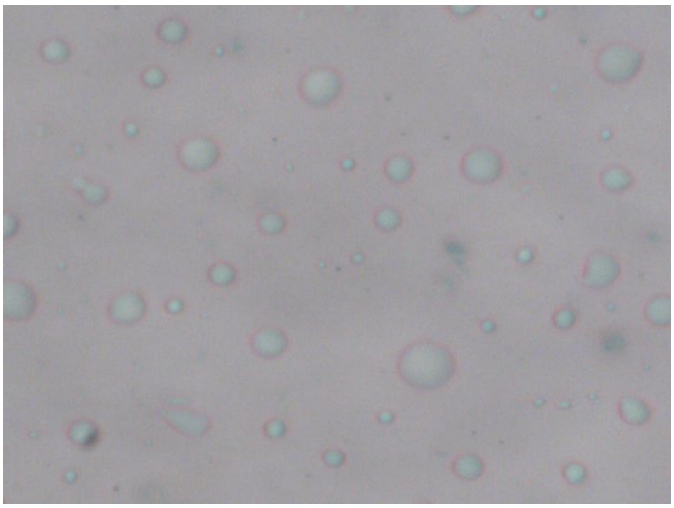

Figure 1: Photomicrograph of Elastic Liposomal Formulation Containing 10 mg Drug

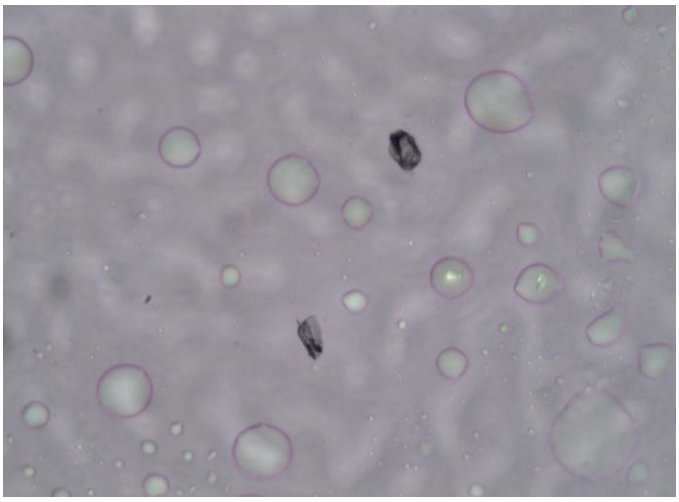

Figure 2: Photomicrograph of Elastic Liposomal Formulation Containing 12 mg Drug

Further formulations (EL-SP1 – ELSP5) were then prepared by incorporating 10 mg of drug to each containing varying ratio of phospholipids and surfactant. The composition of these formulations has been shown in table 1.

Table 2: Composition of Different Elastic Liposomal Formulations

S. No.	Formulation code	*Soya PC (mg)	Span 80 (mg)	Solvent (ml)	
				Chloroform:Methanol	
1	**EL-SP1	95	5	10	5
2	EL-SP2	90	10	10	5
3	EL-SP3	85	15	10	5
4	EL-SP4	80	20	10	5
5	EL-SP5	75	25	10	5

Soya PC - Phosphatidylcholine

****EL-SP - Elastic liposomal formulation containing different concentrations of Span80***

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CHARACTERIZATION OF ELASTIC LIPOSOMAL FORMULATIONS

Vesicle Size and Size Distribution

The vesicle size before sonication was determined by optical microscopy using stage eyepiece micrometer. A total of 100 particles per batch were counted for their size. After sonication the vesicle size was determined by dynamic light scattering method using particle size analyzer (Malvern, Instruments).

Vesicle Shape

Elastic liposomal vesicles were visualized by using Moragagni 268D TEM with an accelerating voltage of 100 kV. A drop of the sample was placed onto a carbon-coated copper grid to leave a thin film on the grid. The grid was allowed to air dry thoroughly and the samples were viewed on a transmission electron microscope.

Vesicles without sonication were also visualized by using an optical microscope. A thin film of elastic liposomes was spread on a slide and after placing cover slip it was observed under the optical microscope[14].

Entrapment Efficiency

For determination of entrapment efficiency, the excess amount of unentrapped drug was separated by the use of the minicolumn centrifugation method..

$$\text{Percentage entrapment} = \frac{\text{Entrapped drug } (\mu g) \times 100}{\text{Total drug added } (\mu g)}$$

Number of Vesicles Per Cubic mm

Elastic liposomal formulation (without sonication) diluted five times with 0.9 % w/v sodium chloride solution and number of elastic liposomes per cubic mm was counted by optical microscopy using haemocytometer by following formula-

$$\text{Total number of transferosomes per cubic mm} = \frac{\text{Counted} \times \text{dilution factor} \times 4000}{\text{Total number of squares counted}}$$

Drug Release Study through Cellophane Membrane

The *in vitro* drug release study was carried out in modified Diffusion cell using Dialysis membrane (Himedia laboratories Pvt Ltd: dry, unwashed, open ended; flat width: 28.46mm; inflated diameter: 17.5mm; Length: 1m). The membrane was soaked in PBS pH 6.8 for 9-12 hrs and it was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter; 4-16 cm^2 area). Then transferosomal gel (which was prepared by using 1% w/v carbopol 934 in distilled water) was spread uniformly on the dialysis membrane. 50 ml of PBS pH 6.8 was taken in a beaker, which was used as receptor compartment. The donor compartment was kept in contact with the receptor compartment. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead. The temperature of the cell was maintained at 37°C. Sample (5 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. Samples were analysed spectrophotometrically at 330 nm and the cumulative % drug release was calculated. The difference between the readings of drug release and control was used as the actual reading in each case.

Ex-vivo Skin Permeation Study

The *ex vivo* skin permeation study was carried out in a modified Diffusion cell, using rat skin. A section of skin was cut, cleaned and clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter; 4-16 cm^2 area) keeping the dorsal side upward. Then transferosomal gel (which was prepared by using 1% w/v carbopol 934 in distilled water) was spread uniformly on the skin. PBS pH 6.8 was used as dissolution media. The donor compartment was kept in contact with receptor compartment. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead and temperature of the cell was maintained at 37°C. Sample (5 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. Samples were analyzed spectrophotometrically at 330 nm and the cumulative % drug permeated across skin was calculated.

Stability Study

Stability is technically defined as the capacity of particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specification. The optimized elastic liposomal formulations were stored in glass vials at room temperature and in refrigerator (4±2°C) for 60 days. Formulations were evaluated after every 20 days for various physicochemical parameters[15-16].

RESULTS AND DISCUSSION

The conventional rotary evaporation sonication method was used to prepare the transfersomal formulation. Vesicles size and size distribution was determine by optical microscope(less than 1000nm) shown in fig - 3.

To determine the vesicle shape Transmission Electron Microscopic (TEM) study was carried out which reveals the mechanism of skin permeation of elastic liposomes. As shown in Table- 3, the maximum entrapment efficiency obtained was 67.53±1.6 for transferosomal formulation EL-SP3. It was observed that with increase in surfactant concentration, entrapment efficiency increased but up to a certain concentration only (EL-SP3), after that it started decreasing. This may due to the possible coexistence of mixed micelles and vesicles at higher concentration of surfactant resulting in lower drug entrapment in mixed micelles. Similar trend was observed in the number of vesicles and the maximum number obtained was 51.13±1.6 for transfersomal formulation of EL-SP3.

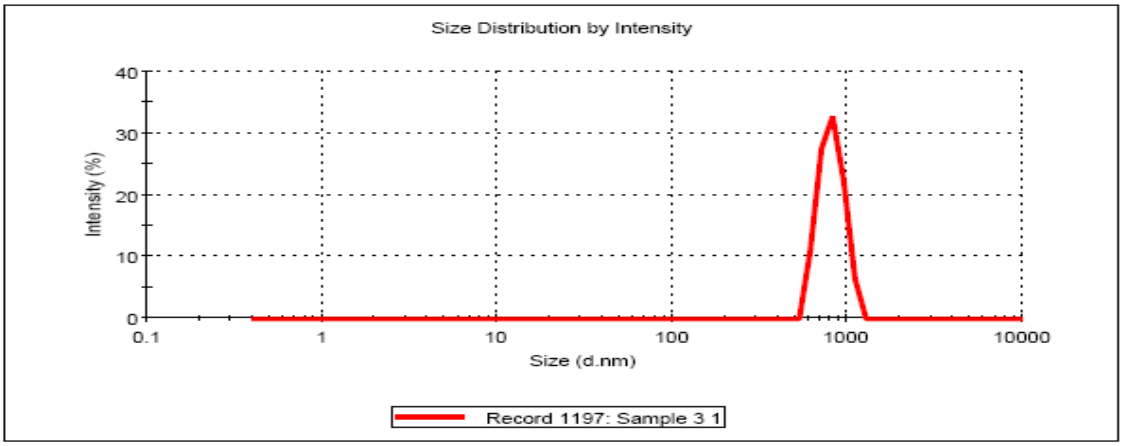

Figure 3: Vesicle Size Distribution of EL-SP3

Table 3: Characterization of Transfersomal Formulations

Formulation Code	Entrapment Efficiency	Number of Vesicles
**EL-SP1	49.46±1.5	46.02±1.1
EL-SP2	53.96±1.3	47.90±1.4
EL-SP3	67.53±1.6	51.13±1.6
EL-SP4	60.40±1.1	43.81±1.2
EL-SP5	58.22±1.0	44.76±1.1
Liposomes	62.30±1.1	49.25±0.6

Soy PC = Phosphatidylcholine
****EL-SP = Elastic liposomal formulation containing different concentrations of Span80***

Formulation EL-SP3 was subjected to *in vitro* drug release studies using cellophane membrane. After 24hrs nifedipine release from elastic liposomes was 70.42±0.7. This clearly indicated sustained release of nifedipine from elastic liposomal formulations. The mathematical models were used to evaluate the kinetics and mechanism of drug release from elastic liposomal gel. The model that best fits the release data was selected based on the correlation coefficient (r^2) value. According to data the value of r^2 for zero order model was found to be higher than that of higuchi and first order model. So, the EL-SP3 formulation confirmed to show sustained zero order release. In case of korsermeyer peppas the value of n found to be 0.576 i.e. the drug follows non-fickian diffusion (0.45<n<0.89).

The *ex vivo* skin permeation study was carried out in a modified Diffusion cell, using rat skin for each EL-SP3 formulation and liposomes. The value of transdermal flux for EL-SP3 observed was 76.41±0.9. This was higher than that obtained from liposome which was 71.44±0.9. These results indicated that the more amount of drug permeated through skin by elastic liposomes as compared to liposomes.

Table 4: % *Ex-vivo* Skin Permeation study

S.No.	Time (hr)	Elastic Liposomes (EL-SP3)	Liposomes
1.	0	0	0
2.	1	8.62±0.6	6.51±1.0
3.	2	13.17±1.7	7.2±1.5
4.	3	17.68±1.8	8.57±1.0
5.	4	23.67±1.5	11.52±0.9
6.	5	25.25±1.4	13.74±1.0
7.	6	30.02±6.2	16.65±1.1
8.	7	29.9±6.6	26.33±1.1
9.	8	35.61±1.0	27.81±0.9
10.	9	38.76±1.0	31.3±0.8
11.	10	41.54±0.9	32.66±0.8
12.	12	45.86±1.4	39.37±0.8
13.	16	54.71±1.8	48.92±1.1
14.	20	67.57±0.7	60.50±0.1
15.	24	76.41±0.9	71.44±0.9

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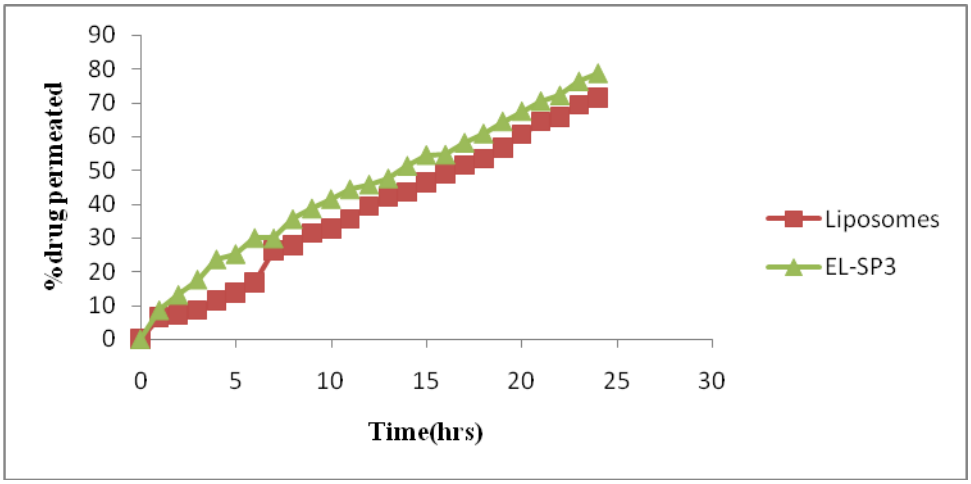

Figure 4: % Drug release of Nifedipine across skin

Table 5: Regression analysis of release data based on best curve-fitting method for EL-SP3 formulation

Formulation	Zero order		First order		Higuchi		Korsemeyer Peppas	
	N	R^2	n	R^2	N	R^2	N	R^2
EL-SP3	5.732	0.991	2.009	0.988	11.48	0.989	0.576	0.885

STABILITY STUDY

Table 6: Visual and Microscopic Appearance of Elastic Liposomal Formulations after Storage

Parameteres	Time (in days)	Room Temp/ Refrigrated Temp.	Observations
Consistency of liposomal formulations	20	(1)	No Change
	20	(2)	No Change
	40	(1)	No Change
	40	(2)	No Change
	60	(1)	Increased
	60	(2)	Increased
Appearance of drug crystals	20	(1)	Not appeared
	20	(2)	Not appeared
	40	(1)	Not appeared
	40	(2)	Not appeared
	60	(1)	Not appeared
	60	(2)	Not appeared

1. Room temperature 2. Refrigerated temperature (4±2°C)

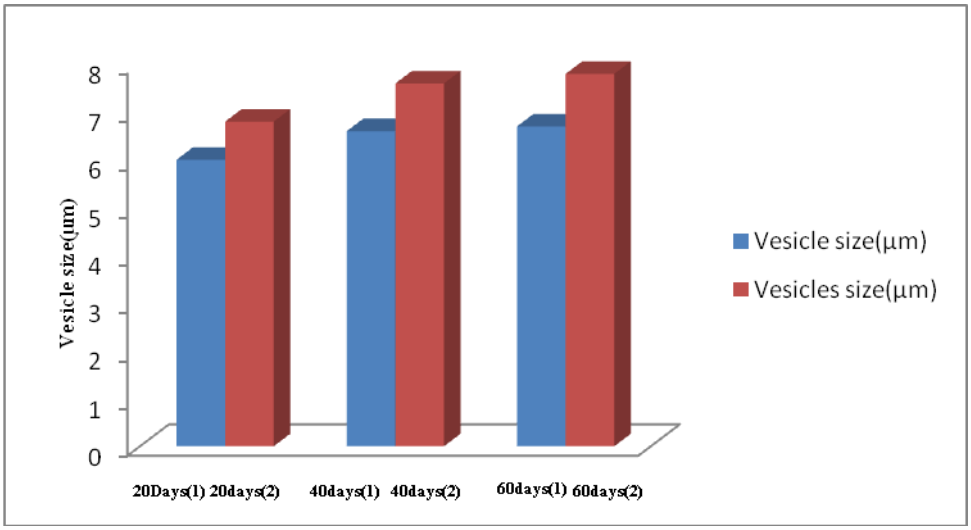

Figure 5: Effect of Storage Temperature on Vesicle Size

Table 7: Effect of Storage Temperature on Vesicle Size

Parameters	Time (in days)	Storage temperature	Observation
Vesicle size (µm)	20	1	6.0±1.9
	20	2	6.8±1.2
	40	1	6.6±1.7
	40	2	7.6±1.8
	60	1	6.7±0.9
	60	2	7.8±2.6

1. Data are represent of mean ± SD (n=3)

1. Room temperature 2.Refrigerated temperature (4±2°c)

Effect of storage temperature on morphology

The formulations were observed in glass vials for a period of 2 months and were observed at definitive intervals visually and under optical microscope for change in consistency and appearance of drug crystals. Results are shown in Table 6.

Effect of storage temperature on size and size distribution

Elastic liposomal formulations were also characterized for size at regular interval time intervals for the period of two months. Results are recorded in Table-7 and shown graphically in Fig - 5.

CONCLUSION

The results of the present study showed that Transdermal delivery can maintain a suitable plasma concentration through a noninvasive zero-order delivery, which would enhance the efficacy and improve the permeability of nifedipine. Thus, deformable lipid vesicles, transfersomes, are better alternative as a drug delivery system for nifedipine over oral route because of avoidance of first pass metabolism thus leading to improved bioavailability of drug and rapid termination of drug input. It creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

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