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DEVELOPMENT, FORMULATION, CHARACTERIZATION AND EVALUATION OF ELASTIC LIPOSOMAL FORMULATION OF CHLORZOXAZONE FOR TRANSDERMAL DELIVERY

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ABSTRACT

Chlorzoxazone (CHZ) is a centrally acting skeletal muscle relaxant. Chlorzoxazone is used to decrease muscle tone and tension to relieve pain and spasm associated with muscoskeletel disorders. CHZ has a half-life of 1- 1.2 hours which is very short and eventually leads to a high dose of 250 to 750 mg 3 to 4 times a day. The aim of the present study is to formulate and characterize a sustained release formulation for the administration of CHZ, to lower its dose and increase bioavailability. Although transdermal administration has shown to deliver the drug to the local sites but major limitation of the transdermal delivery is the slow penetration rate of the drug through skin. Elastic liposomes are an effective tool that can be used to overcome this disadvantage. Elastic liposomes also known as transfersomes are modified lipid carriers that enable drug to reach deeper skin layers and/or the systemic circulation. These vesicular formulations are several orders of magnitudes, more deformable than the standard liposomes and thus well suited for skin penetration. The elastic liposomes were prepared using rotary evaporation sonication method. Particle size and zeta potential were measured by photon correlation spectroscopy. The solid states of the drug in the Elastic liposomes and lipid modification were characterized. Stable chlorzoxazone elastic liposomes having a mean size range of 100 to 200 nm and a zeta potential range of –16 to –21 mV were developed. More than 60% of the chlorzoxazone was entrapped in the EL. The in vitro permeation experiments clearly indicated sustained release of chlorzoxazone with respect to time from elastic liposomal formulations. The prepared elastic liposomes were characterized by differential scanning calorimetry and scanning electron microscopy.

INTRODUCTION

Transdermal administration of drugs is generally limited by the barrier function of the skin. Although transdermal administration offers diverse advantages for non-invasive drug delivery, the drug must transit the lipidic stratum corneum and cross the aqueous epidermal and dermal layers before reaching the target tissues. (1) 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.(2) The transdermal route, besides being convenient and safe, offers several advantages over conventional ones, such as avoidance of GI incompatibility, variable GI absorption, and avoidance of first pass metabolism, improved bioavailability, reduced frequency of administration, improved patient compliance, and rapid termination of drug input. In addition, transdermal delivery can maintain a suitable plasma concentration through a noninvasive

zero-order delivery, which would enhance the efficacy of drug with high patient compliance. (3) Chlorzoxazone (CHZ) is a centrally acting skeletal muscle relaxant. It acts primarily at the level of the spinal cord and subcortical areas of the brain where it inhibits multisynaptic reflex arcs involved in producing and maintaining skeletal muscle spasm of varied etiology. (4) Chlorzoxazone is used to decrease muscle tone and tension to relieve pain and spasm associated with muscoskeletel disorders. (5)

One of the major disadvantages of CHZ is its dose. Its dose is one tablet (750 mg) three or four times daily. If adequate response is not obtained with this dose, it may be increased to 11/2 tablets (750 mg) three or four times daily. As improvement occurs dosage can usually be reduced (5).

Chlorzoxazone is reported to be completely absorbed after oral administration, and the peak

serum concentration is achieved after 1 to 2 h. The elimination half-life is about 1h (6) both these factors lead to high dose of the drug and the present study aims to reduce the dose of CHZ.

MATERIALS AND METHODS

Materials

Soya PC, cholesterol (Ch), sodium cholate, sodium deoxycholate, Sephadex- G-50, 6-carboxyfluorescein, TritonX-100, and phosphotungstic acid were purchased from SigmaChemicals (St Louis, MO). Polyethylene glycol-200, 400, and 4000; Briz-35; sodium chloride; hard paraffin; wax; wool fat; cetostearyl alcohol; white soft paraffin; and copper acetate were purchased from Loba Chemie (Mumbai, India). Ethanol, isopropyl alcohol, butanol,

xylene, chloroform, acetonitrile, glycerol, and hematoxylin and eosin were purchased from E. Merck (Mumbai, India). All other reagents used in the study were of analytical grade. Double distilled water was used for all experiments. Sprague-Dawley rats were used for all the animal experiments.

Preparation of 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 methanol or chloroform-methanol mixture. The organic solvent was removed by rotary evaporation under reduced pressure at 40^{o}C. Final traces of solvents were removed under vacuum overnight. The deposited lipid film was hydrated with 7%v/v ethanol i.e. solution of drug by rotation at 60 rev/min for 1hr. The resulting vesicles were swollen for 2 hr at room temperature to get large multilamellar vesicles. To prepare smaller vesicles, these were bath sonicated for 15 minutes. The sonicated vesicles were extruded through a sandwich of 200 and 450 nm polycarbonate membranes (Millipore, USA). The conventional liposomal formulation (Phosphatidyl choline: Cholesterol, 7: 3) that serve as a control for comparison in the present study was prepared by the same method as described above.

Incorporation of CHZ into Vesicle Dispersion at Saturated Concentration

CHZ was incorporated into all vesicle formulations at saturated concentration to obtain equal thermodynamic activities. To determine the maximum amount of drug that could be added, increasing amounts of CHZ were added during preparation of elastic liposomal formulation. It was assumed that the presence of CHZ crystals would indicate that the formulation was saturated with CHZ. Therefore, all CHZ -loaded vesicle formulations were examined over a period of 14 days using phase contrast microscope (Leica, DMLB, Heebrugg, Switzerland)

In Vitro Characterizations

Visualization of Vesicles

Reconstitution of transfersomes from CHZ formulation after hydration was confirmed by transmission electron microscopy (TEM). Samples were prepared by adding phosphate buffer (pH 7.4) to PTG and shaking the mixture manually for 1 minute. A drop of the sample was placed on a carbon-coated copper grid after 15 minutes and negatively stained with 1% aqueous solution of phosphotungstic acid. The grid was allowed to air

Table-1: Composition of Different Elastic Liposomal Formulations

S.No.	Formulation code	*Soy PC (mg)	Span 80 (mg)
1	**EL-SP1	95	5
2	EL-SP2	90	10
3	EL-SP3	85	15
4	EL-SP4	80	20
5	EL-SP5	75	25
6	EL-SP6	70	30

*Soy PC = Phosphatidylcholine

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

dry thoroughly and samples were viewed on a TEM(Philips, TEM, New Brunswick, Canada).(8) A thin layer of PTG was spread on a slide and a drop of phosphate buffer was added through the side of the cover slip into the cavity slide and again observed. Photomicrographs were taken at 400× and 1000×, respectively, before and after addition of water for both plain and polarized light (Leica, DMLB, Bensheim, Germany).

Table-2: Drug Loading in Elastic Liposomal Formulations

S. No.	Amount of drug (mg)	Observations
1.	4	CAN
2	6	CAN
3	8	CAN
4	10	CA

CNA-crystals do not appear

CA-crystals appear

Vesicle Size, Turbidity, and Entrapment Efficiency Measurement

CHZ (100 mg) was hydrated with 10 mL of saline solution (0.9% NaCl) using manual shaking for 5 minutes. The vesicle size and turbidity after hydration were determined by dynamic light scattering (DLS) method (Malvern Zetamaster, ZEM5002,Malvern, UK) and Nephalometer (Superfit, Mumbai, India), respectively. The entrapment efficiency was determined after separating the unentrapped drug through Sephadex G-50 column. The eluted vesicles were lysed using Triton-X 100 (0.1% vol/vol) and analyzed for drug content. (9) Entrapment efficiency was expressed a percentage of total drug entrapped.

In Vitro Skin Permeation Study

The in vitro skin permeation of CHZ from different formulations was studied using locally fabricated diffusion cell. The effective permeation area of the diffusion cell was 2.303 cm2. The receptor compartment contains 22.5ml PBS (pH 6.8). Albino abdomen rat skin was mounted between the donor and receptor compartments. The donor compartment was maintained at 37±1 ^{o}C with constant stirring at 125 rpm. The elastic liposomal formulation (2 ml) was applied to the epidermal surface of the rat skin. Samples were withdrawn through the sampling port of the diffusion cell at predetermined time intervals over 24 hrs and analyzed. The receptor phase was immediately replenished with an equal volume of fresh buffer. The in vitro drug release study of elastic liposomal formulation was repeated with a cellophane membrane by using the same method as described above. (10) Experiments were conducted to optimize the amount of CHZ that can be incorporated into the vesicles and to optimize the elastic liposomal formulation.

Table-3: Entrapment Efficiency of Different Elastic Liposomal Formulations

S. No.	Surfactant concentration (% w/w)	Entrapment efficiency (%)
1.	5	48.13±2.9
2.	10	55.21±3.3
3.	15	61.17±3.6
4.	20	42.11±2.5
5.	25	41.36±2.4
6.	Liposomes	57.42±2.7

Stability Studies

The formulation EL-Sp8033 was selected for stability studies based on its in vitro characterization. The formulations were stored in glass tubes covered with aluminum foil at 30 ± 2°C and 4 ± 2°C for 2 months and observed visually under microscope for change in consistency, liquid crystalline structure, and appearance of drug crystals. (11)

Statistical Analysis

Statistical significance of all the data generated was tested by analysis of variance (ANOVA) followed by studentized range test. A confidence limit of P G .05 was fixed for interpretation of the results using the software PRISM (Graphpad, San Diego, CA).

RESULT AND DISCUSSION

Different batches of elastic liposomes were prepared with Span 80 and Phosphatidylcholine using conventional rotary evaporation sonication method (12, 13). Span 80 was selected as edge activator surfactant because it is biocompatible and pharmaceutically acceptable (14). Phosphatidylcholine is used as bilayer forming agent. In the present study 7% v/v ethanol was used as hydrating agent because ethanol is known to extract stratum corneum lipids and alter the barrier property of intracellular lipoidal route, thereby allowing higher drug permeation. Previous finding demonstrated that elastic liposomes followed this route for skin permeation (15). Further, in the present study menthol was added as permeation enhancer (1.0 % w/v) in optimized elastic liposomal formulation. The compositions of different elastic liposomal formulation are summarized in Table-1. These elastic liposomal formulations were colloidal dispersions having average diameter in range of 100-200 nm.

In optimized elastic liposomal formulation (EL-SP3) different amount of drug in concentration range of 2-12 mg was added and formulations were prepared. In case of formulations prepared using 2-8 mg of drug, no crystals were observed but in case of formulation prepared using 10 mg of drug crystals were observed. The maximum amount of drug that could be incorporated in elastic liposomal formulations was confirmed to be 8 mg.

Table 4 – Turbidity of Elastic Liposomal Formulations

S.No.	Formulation code	Turbidity
1	EL-SP1	15.2
2	EL-SP2	19.7
3	EL-SP3	27.1
4	EL-SP4	23.4
5	EL-SP5	24.2
6.	Liposome	25.3

All the elastic liposomal formulations were in the size range of 4 to 5µm before sonication and after sonication the vesicle size was in the range of 100-200 nm. For better skin permeation the vesicle size must be in the range of 100-200 nm. (Figure-1)

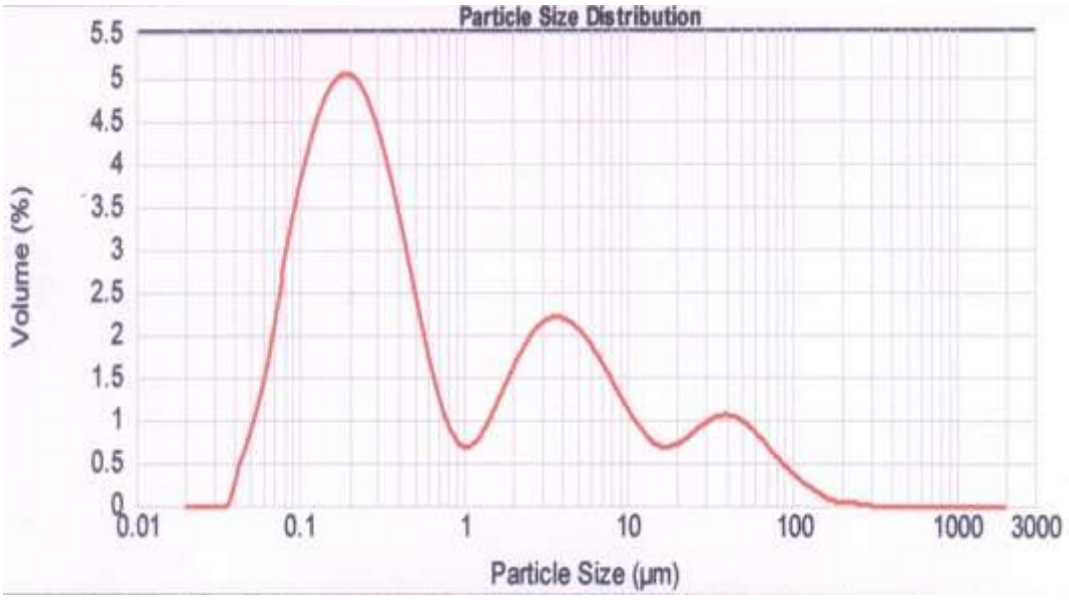

Figure-1: particle size distribution of optimized elastic liposomal formulation.

Table 5 - % Amount of CHZ Permeated across Cellophane Membrane from Different Formulations

S. No.	Time (hr)	EL-SP3	Liposome	Drug solution
1.	0	0	0	0
2.	0.5	1.31±0.2	2.43±0.5	25.73±0.4
3.	1	3.1±0.5	4.82±0.7	45.48±0.7
4.	1.5	4.52±0.3	5.87±0.4	79.45±1.6
5.	2	6.45±0.8	7.89±0.2	85.97±1.8
6.	3	9.83±0.7	17.5±1.3	93.71±1.3
7.	4	14.23±0.5	19.6±1.7	99.65±2.5
8.	5	18.9±1.3	24.25±1.1	
9.	6	30.24±1.5	36.71±2.6	
10.	7	42.72±1.8	49.73±2.3	
11.	8	58.28±2.5	65.76±2.5	
12.	12	70.75±2.2	75.21±2.4	
13.	16	77.24±2.6	80.43±3.2	
14.	20	81.37±3.4	86.59±2.9	
15.	24	84.73±3.9	89.14±3.0	

Entrapment efficiency

The entrapment efficiency of CHZ in elastic liposomes was calculated as percentage of total drug entrapped into the vesicular formulation and determined by using Sephadex G-50 minicolumn centrifugation method. Results are shown in Table-3.

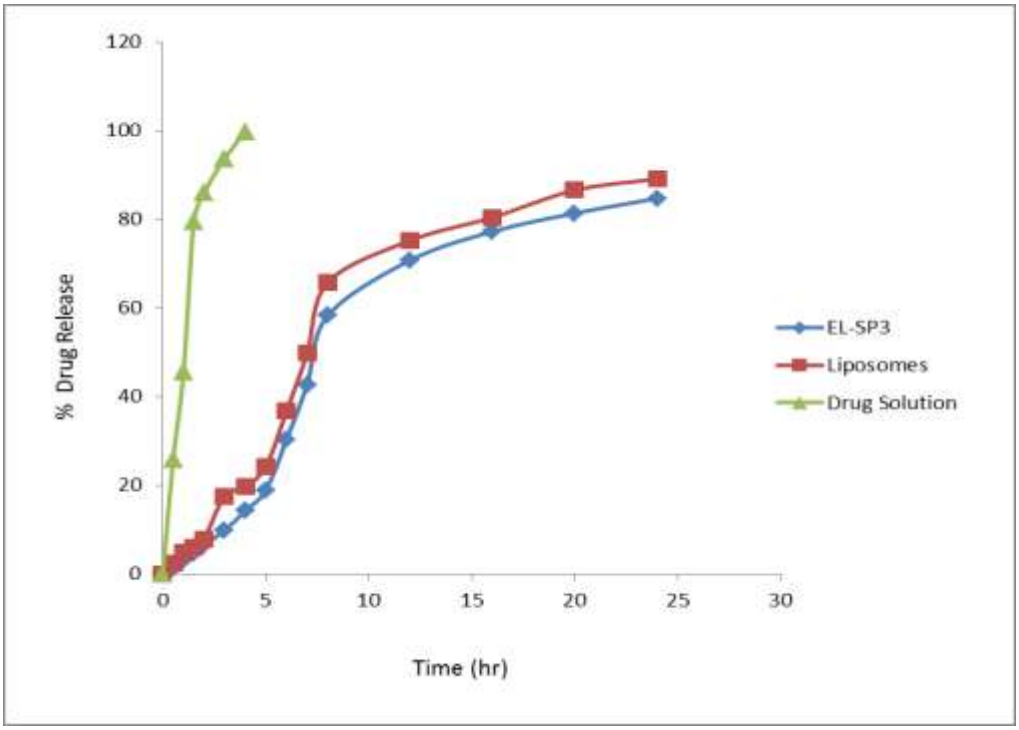

Figure- 2: % Drug release of CHZ across Cellophane Membrane from Different Formulations

The maximum entrapment efficiency obtained was 61.17±3.6% for formulation EL-SP3

Turbidity measurements

Transformation of elastic liposomes to mixed micelles is concentration dependent process and was governed mainly by progressive formation of mixed micelles within the bilayer. To support the above fact, turbidity measurements were performed. The results of turbidity measurement studies (Table-4) support the fact that micelles were formed at higher concentration of surfactant.

In vitro Drug Release Studies through Cellophane Membrane

Formulation EL-SP3 was optimized and found to be suitable for further studies. Elastic liposomal formulation was subjected to in vitro drug release studies using cellophane membrane for the optimization of drug concentration that could be incorporated into the vesicles, the liposomal formulation was subjected to in vitro drug release through cellophane membrane and the % amount of drug permeated was calculated. Values of % amount of CHZ permeated across cellophane are summarized in the Table-5 and Fig.2

In vitro Skin Permeation Studies

Skin permeation studies were carried out using rat skin. Table- 6 and Figure-3 shows % amount of CHZ permeated across rat skin as a function of time from different elastic liposomal formulations. Permeation of CHZ from vesicles increased with

Table-6: % Amount of CHZ Permeated across Rat Skin for Different Formulations

S.No.	Time (hr)	EL-SP3	Liposomes	Drug soln.
1.	0	0	0	0
2.	0.5	2.36±0.1	2.17±0.2	5.67±0.8
3.	1	7.63±0.6	4.58±0.3	12.32±1.2
4.	1.5	13.56±0.8	8.72±0.5	23.4±2.6
5.	2	19.88±0.8	12.8±0.7	40.75±2.8
6.	3	28.83±0.9	18.34±0.8	57.83±3.2
7.	4	36.5±1.0	23.56±1.1	75.37±4.6
8.	5	43.62±1.3	30.74±1.0	87.8±4.8
9.	6	52.15±1.4	34.5±1.2	95.17±5.6
10.	7	58.92±1.7	42.97±1.5	
11.	8	66.7±1.9	54.24±1.7	
12.	12	75.33±2.3	61.47±1.9	
13.	16	78.49±2.5	65.35±2.0	
14.	20	80.16±2.4	70.24±1.8	
15.	24	82.4±2.8	73.48±2.2	

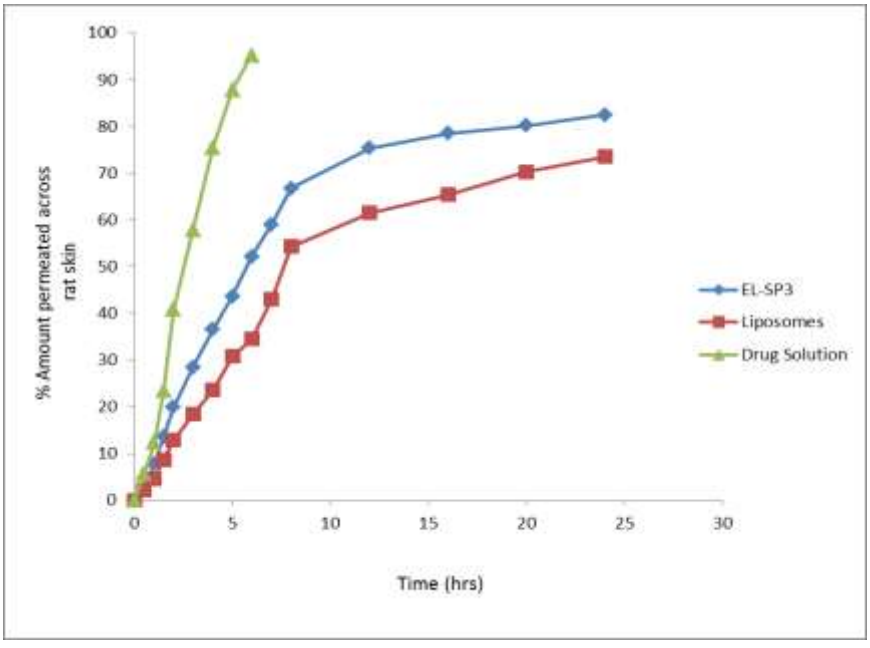

Figure-3: % Amount of CHZ Permeated across Rat Skin for Different Formulations

Table-7: Transdermal Permeation Parameters of Different CHZ Elastic Liposomal Formulations across Rat Skin

Formulation Code	Transdermal Flux(mg/cm2/h)	Lag Time (h)	% Skin Deposition	Enhancement Ratio (ER)
EL-SP1	18.19±1.5	2.1	14.19±2.35	6.2
EL-SP2	16.19±3.5	1.8	12.19±1.64	5.6
EL-SP3	28±2.67	0.7	18.19±2.43	9.6
Liposomes	4.19±1.35	2.5	7.19±1.35	1.44
Plain drug	2.89±1.27	2.9	2.99±1.35	------

increase in the concentration of surfactant and after reaching a maximum it decreased. This is in accordance with the entrapment efficiency, elasticity and turbidity measurement data. Permeation of CHZ from EL-SP3 vesicles was maximum after 24 hrs across rat skin as compared to other formulations.

The in vitro skin permeation of drug is mainly assessed by its flux, Jss (µg/cm^2/hr). This was calculated from the slope of the linear steady portion of % cumulative amount released vs. time (hrs) plot. Another important parameter is diffusion lag time (LT) for the drug to reach the receptor compartment. All these skin permeation parameters for CHZ have been calculated and reported in Table-7.

The value of transdermal flux for EL-SP3 observed was 28±2.67µg/hr/cm^2. This is about 10 times higher than that obtained from drug solution (2.89±1.27 µg/hr/cm^2).

Skin retention studies were carried out with the objective of determining the depot effect of elastic liposomes in the deeper layer of skin. Figure-4 compares the skin deposition of CHZ after the application with optimized elastic liposomal formulation, liposomal formulation and drug solution. The amount of drug deposited is 6.1 times higher in case of elastic liposomes (18.19±2.43%) than drug solution (2.99±1.35%) and 2.5 times higher than liposomal formulation (7.19±1.35%), which could be attributed to the difference in the mechanism of drug transport across the skin from vesicles and drug solution.

CONCLUSION

Elastic liposomal formulation developed for transdermal delivery of lovastatin possessed better skin permeation potential, better stability, and higher entrapment efficiency than liposomal formulation. However, detailed in vivo studies are further suggested.

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