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FORMULATION, DEVELOPMENT AND EVALUATION OF LIPOSOMAL GEL FOR TREATMENT OF FUNGAL INFECTION

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ABSTRACT

Econazole nitrate (ECZ) is one of the triazole antifungal drugs with poor aqueous solubility and dissolution rate; there is a need for enhancement of solubility. Liposomal gel have emerged as one of the most interesting topical delivery system as it has dual control release system i.e. gel and emulsion. One side the topical applications of the drug offers the potential advantages of delivering the drug directly to the site of action and secondly delivering the drug for extended period of time at the effected site. The major objective behind this formulation was enhancing the topical delivery of hydrophobic drug by formulating Econazole nitrate liposomes. % Entrapment efficiency of optimized liposomes formulation (F-4) was found 73.32±0.58%. Optimized formulation of liposome further incorporated into gel base and evaluated for Viscosity, pH, Drug content, Extrudability and Spreadibility. Viscosity was found 2562±15, 2420±36 and 2345±74 LG-1, LG-2, LG-3 respectively, as the concentration of Carbopol increase the viscosity of formulation decreases. pH of prepared formulation was found 6.9±0.2, 7.0±0.1 and 6.8±0.2 for LG-1, LG-2, LG-3 respectively. The maximum drug content was found in formulation LG-2 (99.45±0.11) select as optimized formulation. The *in vitro* drug release study of prepared formulation LG-2, was found 98.78% after 12 hrs. Prepared gel containing Econazole loaded liposomal formulation was optimized and successfully formulated in the form gel can be use for topical preparation for anti-Fungal effect.

KEYWORDS: Econazole nitrate, Liposomal gel, Evaluation, Antifungal effect.

INTRODUCTION

Transdermal route offers several potential advantages over conventional routes like avoiding putrefaction due to hepatic “first-pass” effect, predictable and extended period of activity,

minimizing side effects, utility of drugs having short half- life, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, reduced inter- and intra-patient variability, and most importantly, it improved patient compliance. But main major problems in transdermal drug delivery are the low penetration rate across the outer most layer of skin (Jain *et al*., 2001). Vesicular systems are drug delivery system to deliver the drug dermally and transdermelly. Liposomes have the possible of overcoming the skin barrier, as the seare bilayered lipid vesicles, consisting primarily of phospholipids and cholesterols (Jain *et al*., 1997).

Liposomes were discovered by Bangham and colleagues (Bangham et al., 1976) and consequently became the most expansively explored drug delivery system. In early 1960's a great knowledge of vesicle derivatives have been experienced for their abilities. Most experimentation, nevertheless, have centered on liposomes, since derivations only add to their basic property. Vesicles are closed, spherical membrane that separates a solvent from the surrounding solvent. Probable use of liposomes in topical drug delivery vehicles for both aqueous and lipid soluble drug has been examined. While it has been optional that the exterior envelop of a liposomes would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped inside the top layer of the stratum corneum cells. Usually liposomes are not expected to penetrate into viable skin, though occasional transport processes. This performance is useful both for local treatment of skin disorders and for cosmetic formulations, but not promising for systemic effect (Jain *et al*., 2004; Kumar *et al*., 2004).

Liposomes are expansively used as transporter for numerous molecules in cosmetic and pharmaceutical manufacturing. Additionally, food and farming industries have expansively studied the use of liposome encapsulation to produce delivery systems that can deceive unbalanced compounds (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield their functionality. Liposomes can entrap together hydrophobic and hydrophilic complex, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets (Benech *et al*., 2002). Because of their biocompatibility, biodegradability, low toxicity, and aptitude to entrap both hydrophilic and lipophilic drugs and shorten site-specific drug delivery to tumor tissues (Johnston *et al.*, 2007), liposomes have enlarged rate both as an investigational scheme and commercially as a drug delivery system. Liposomal encapsulation technology (LET) is the latest delivery method used by

medical investigators to broadcast drugs that operate as curative promoters to the certain body organs. This appearance of delivery system application targeted the delivery of vital combinations to the body. LET is a technique of generating sub-microscopic foams called liposomes, which encapsulate frequent equipment. These 'liposomes' form a barrier around their contents, which is opposing to enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The substantial of the liposomes are, therefore, protected from oxidation and degradation. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the precise target gland, organ, or system where the contents will be utilized (Himanshu *et al*., 2011). Econazole nitrate (ECZ) is one of the triazole antifungal drugs with poor aqueous solubility and dissolution rate; there is a need for enhancement of solubility. Treatment of local skin disorders. liposomal gel have emerged as one of the most interesting topical delivery system as it has dual control release system i.e. gel and emulsion. One side the topical applications of the drug offers the potential advantages of delivering the drug directly to the site of action and secondly delivering the drug for extended period of time at the effected site. The major objective behind this formulation was enhancing the topical delivery of hydrophobic drug by formulating Econazole nitrate liposomes.

MATERIAL AND METHODS

Material

Econazole nitrate was procured from pharmaceutical company as a gift sample. Soya PC and cholesterol was obtained from HiMedia Laboratories Ltd, Mumbai, India. Dichloromethane were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Methods

Preparation and characterization

Liposomes were prepared by rotator evaporation method given by Touitou *et al.*, The accurately weighed amounts of phospholipids and surfactant were taken in a clean, dry, round-bottom flask in different ration as given in table and this lipid mixture was dissolved in minimum quantity of ethanol and Dichloromethane mixture in ratio of 1:1 (5ml). The round bottom flask was rotated at 45º angle using rotator evaporator at 40°C in order to make uniform lipid layer. The organic solvent was removed by rotary evaporation under reduced

pressure at the same temperature (40°C). Final traces of solvents were removed under vacuum overnight. The prepared lipid film in the inner wall of round bottom was hydrated with 1% w/v of drug solution in phosphate buffer pH 7.4 followed by rotating the flask containing mixture of drug by rotation at speed of 60rev/min for 1 hr. After complete hydration of film, the prepared formulation of liposomes was subjected to sonication at 4°C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The prepared formulation was stored at 4°C in closed container till further use for analysis.

Table 1: Formulation composition of Econazole loaded liposomes.

Formulation code	Soya PC: cholesterol (% w/v)	Drug (% w/v)	Dichloromethane: Ethanol (ml)
F-1	1:1	1.0	1:1 (5ml)
F-2	2:1	1.0	1:1(5ml)
F-3	3:1	1.0	1:1(5ml)
F-4	4:1	1.0	1:1(5ml)
F-5	1:4	1.0	1:1(5ml)
F-6	1:3	1.0	1:1(5ml)
F-7	1:2	1.0	1:1(5ml)

Preparation of Gel Base

Carbopol 934 (1-3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker (Wertz *et al.,* 1986). This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate liposome containing gel in which previously prepared liposomal suspension was added. liposomes preparation corresponding to 1% w/w (100mg of drug in 1g of gel) of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Characterization of Liposomes

Entrapment efficiency

Entrapment efficiency of Econazole liposomal formulation was determined using centrifugation method. The entrapment efficiency of Econazole in liposomes vesicle was determined by ultracentrifugation, 10mL of liposomes formulation were collect in test tube (Ishida *et al.,* 2002). The amount of drug not entrapped in the liposomes was determined by

centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 224nm using UV spectrophotometer.

$$\%\ Entrapment\ Efficiency\ = \frac{Therotical\ drug\ content - Practical\ drug\ content}{\text{Therotical drug content}} \times 100$$

Vesicle size

Microscopic analysis was performed to determine the average size of prepared liposomes (Alam *et al.*, 2008). Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the flowing formula.

$$Average\ Diameter\ = \frac{\Sigma n.d}{\Sigma\ \text{n}}$$

Where n = number of vesicles; d = diameter of the vesicles

Characterization of Liposomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical liposome based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Zheng *et al.*, 1992).

Drug content

Accurately weighed equivalent to 100 mg of topical liposome gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 224 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.,* (1956). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadibility show lesser time to spread.

$$Spreadibility\,(g.cm/\sec) = \frac{Weight\ tide\ to\ Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ take\ nto\ slide}$$

***In-vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 224nm.

RESULTS AND DISCUSSION

Prepared formulations of liposomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of liposomes were examined under trinocular

microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, UK) and found that average vesicle size of optimized formulation F-4 was 165.58±0.45nm. Zeta potential was -39.85±1.2. It was observed that the vesicles size of liposomes was increase with increasing the concentration of phosphotidylcoline and similarly vesicle size was decease with increasing the concentration of cholesterol due to its surfactant action. There was no significant difference in average vesicle size was observed with increasing the drug concentration. % Entrapment efficiency of optimized liposomes formulation (F-4) was found 73.32±0.58%. It was observed that the percent drug entrapment was decrease with increasing the concentration of polymers and on increasing the time of sonication. It is due to the leaching out the drug from vesicles on increasing the mechanical force by sonication and size reduction of size liposomes on increasing the concentration of surfactant due to their surfactant action. Optimized formulation of liposome further incorporated into gel base and evaluated for Viscosity, pH, Drug content, Extrudability and Spreadibility. Viscosity was found 2562±15, 2420±36 and 2345±74 LG-1, LG-2, LG-3 respectively, as the concentration of Carbopol increase the viscosity of formulation decreases. pH of prepared formulation was found 6.9±0.2, 7.0±0.1 and 6.8±0.2 for LG-1, LG-2, LG-3 respectively. The maximum drug content was found in formulation LG-2 (99.45±0.11) select as optimized formulation. The *in vitro* drug release study of prepared formulation LG-2, was found 98.78% after 12 hrs.

Table 2: Entrapment efficiency and Average vesicle size.

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F-1	56.65±0.32	215.56±0.36
F-2	69.98±0.45	220.23±0.45
F-3	65.58±065	225.65±0.21
F-4	73.32±0.58	165.58±0.45
F-5	65.58±0.45	256.23±0.56
F-6	69.98±0.25	278.85±0.32
F-7	63.32±0.36	245.56±0.14

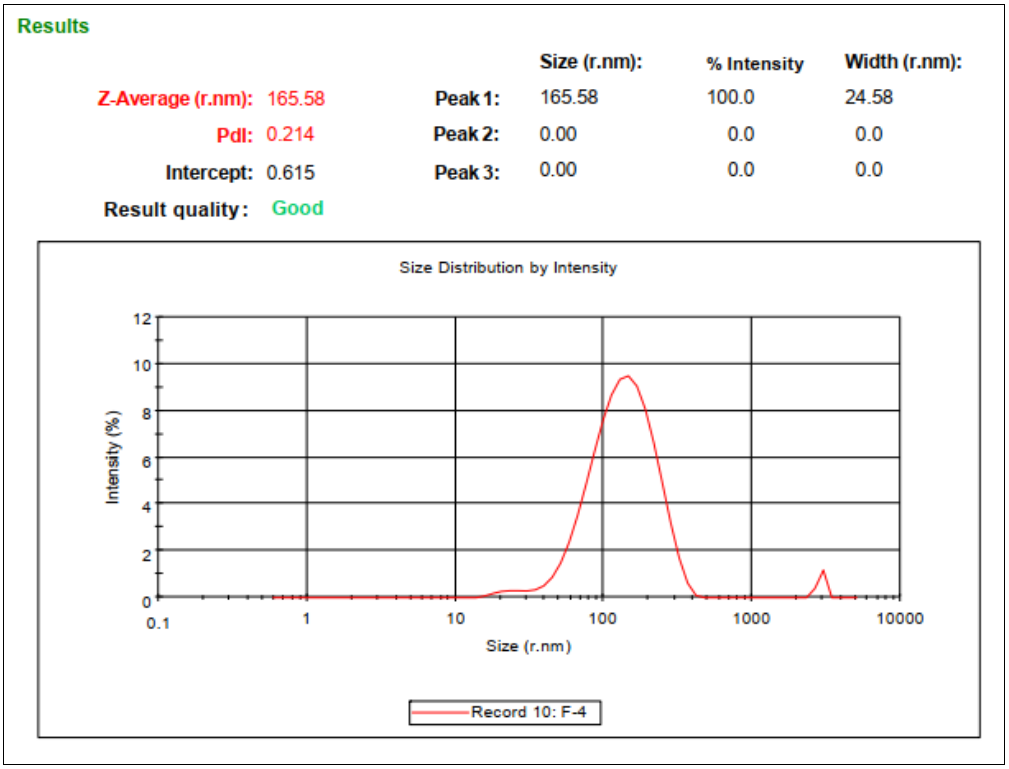

Figure 1: Graph of Average vesicle size (nm) of optimized formulation F-4.

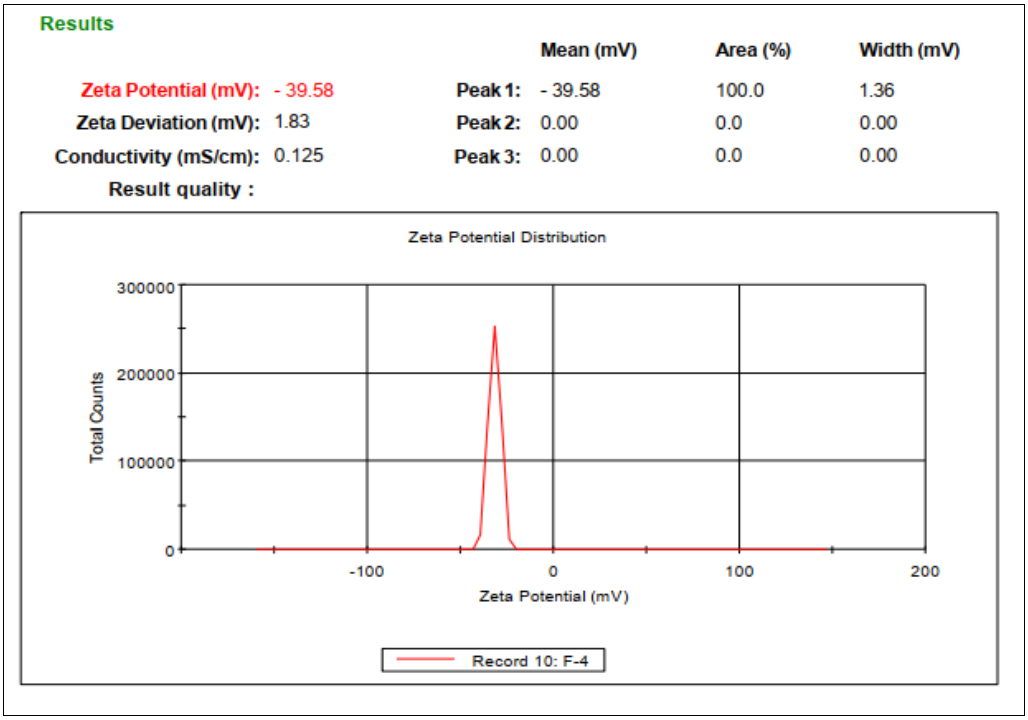

Figure 2: Graph of Zeta Potential (mV) optimized formulation F-4.

Table 3: Characterization of Optimized formulation of liposomes.

Formulation	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-4	165.58±0.45	73.32±0.58	-39.85±1.2

Characterization of gel based formulation of liposomes

Table 4: Characterization of gel based formulation of liposomes.

Gel formulation	Viscosity (cps)	pH	Drug content	Extrudability (g)	Spreadibility (g.cm/sec)
LG-1	2562±15	6.9±0.2	98.98±0.12	155±4	5.16±0.32
LG-2	2420±36	7.0±0.1	99.45±0.11	145±5	4.32±0.41
LG-3	2345±74	6.8±0.2	98.74±0.08	139±4	3.15±0.32

Table 5: *In vitro* drug release study of prepared optimized gel formulation LG-2.

S. No.	Time (hr)	% Cumulative Drug Release
1	0.5	16.65
2	1	35.56
3	2	45.65
4	4	55.89
5	6	68.78
6	8	73.32
7	10	82.23
8	12	98.78

CONCLUSION

Liposomes were prepared by rotator evaporation method and optimized on the basis of average vesicle size, % drug entrapment and polydispersity index. The optimized formulation was further incorporated with gel base (carbapol gel) and characterized for their viscosity, extrudability, spreadability and drug release study. In vitro drug release from liposomes was carried out using Franz diffusion cell method. Drug release from liposomes formulation was found in very sustained and controlled manner. It was concluded that prepared gel containing Econazole loaded liposomal formulation was optimized and successfully formulated in the form gel can be use for topical preparation for anti-Fungal effect.

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