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FORMULATION, DEVELOPMENT AND EVALUATION OF *ELASTIC LIPOSOMES* OF ERYTHROMYCIN

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Abstract

Erythromycin is an antibiotic used for the treatment of a number of bacterial infections. This includes respiratory tract infections, skin infections, Chlamydia infections, pelvic inflammatory disease, and syphilis. Topical erythromycin is used for the treatment of inflammatory acne vulgaris that occurs due to activity against propioni bacterium acne. It is slightly soluble in water and freely soluble in methanol. Erythromycin base are examples of topical drugs with poor dermal localization due to lipophilicity. Elastic liposomes could be suitable carriers for these drugs with a potential impact on their dissolution. Total Six formulations were prepared using varying amount of Soya-phosphatidylcholine, Span 80 and drug and evaluated for Vesicle size and Entrapment efficiency. Formulation F4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation F4 Sleeted as optimized formulation for further evaluation.

The optimized batch of elastic liposomes was further incorporated into gel base and evaluated for pH, Spreadability, Measurement of viscosity, Drug content and *In-vitro* diffusion study. The value of vesicle size, and entrapment efficiency. The vesicle size of all elastic liposomes varied between 145.45±0.24 and 225.45±0.21nm where as entrapment efficiency was found between 65.85±0.45 to 73.32±0.12%. Erythromycin loaded elastic liposomes were successfully prepared with an excellent loading efficiency of about 73.32±0.12%. The Erythromycin loaded elastic liposomes exhibited adequate size, stability and flexibility characteristics. Furthermore, elastic liposomes extended the Erythromycin release time, achieving sustained release for almost 10 h.

Key Words: *Elastic liposomes,* Formulation, Evaluation, Erythromycin

Introduction

Novel drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimization of drug related side effects due to controlled therapeutic blood levels instead of oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered

Various drug delivery systems have been developed and some of them under development with an aim to minimize drug degradation or loss, to prevent harmful side effects and to improve drug bioavailability and also to favour and facilitate the accumulation of the drug in the required bio- zone (site). There are no. Of novel carries which have been established and documented to be useful for controlled and targeted drug delivery. It is important to critically evaluate different terms used under the different broad categories of novel drug delivery system.

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*., 2004).

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 (Kumar *et al*., 2010).

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).

Elastic liposomes (EL) are some of the most versatile deformable vesicular carriers that comprise physiologically biocompatible lipids and surfactants for the delivery of numerous challenging molecules and have marked advantages over other colloidal systems. They have been investigated for a wide range of applications in pharmaceutical technology through topical, transdermal, nasal, and oral routes for efficient and effective drug delivery. Increased drug encapsulation efficiency, enhanced drug permeation and penetration into or across the skin, and ultradeformability have led to widespread interest in Elastic liposomes to modulate drug release, permeation, and drug action more efficiently than conventional drug- release vehicles.

Erythromycin is an antibiotic used for the treatment of a number of bacterial infections. This includes respiratory tract infections, skin infections, Chlamydia infections, pelvic inflammatory disease, and syphilis. Topical erythromycin is used for the treatment of inflammatory acne vulgaris that occurs due to activity against propioni bacterium acne. It is slightly soluble in water and freely soluble in methanol. Erythromycin base are examples of topical drugs with poor dermal localization due to lipophilicity. Elastic liposomes could be suitable carriers for these drugs with a potential impact on their dissolution.

Material and Methods

Materials

Erythromycin obtained as gift sample from Bioplus life science pvt ltd, Bangalore, phospholipids, purchased from **Himedia Laboratory, Mumbai.** Ethanol, purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from **Himedia Laboratory, Mumbai.** All other ingredients used were of analytical grade.

Methods

Preparation of Erythromycin loaded elastic liposomes

Elastic liposomes were prepared by rotator evaporation method given by Touitou *et al.,* (2000) with slight modification in which drug was dissolved in methanol to give a concentration of 1.0% w/v of drug solution. The accurately weighed amounts of phospholipids and surfactant were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in minimum quantity of ethanol (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 2% w/v of drug solution in distilled water v/v, followed by rotating the flask containing mixture of drug by rotation at speed of 60 rev/min for 1 hr. After complete hydration of film, the prepared formulation of elastic liposomes was subjected to sonication at 4ºC 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 (Hussain *et al.,* 2016).

Preparation of Gel Base

Carbopol 934 (1-3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. 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. Gel was also prepared with plain drug by adding 10 mg of drug and dispersed

properly by following same procedure given above. The same procedure was used to formulate liposome containing gel, Elastic liposomes preparation corresponding to 0.75% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base (Sharma *et al.,* 2012).

Formulation development of Erythromycin loaded elastic liposomes

Table 1: Optimization of Erythromycin loaded elastic liposomes

Formulation code	Soya PC (% w/v)	Span 80 (% w/v)	Drug (mg)	Ethanol (ml)
F1	4	2	50	5
F2	5	3	50	5
F3	6	4	50	5
F4	7	5	50	5
F5	8	6	50	5
F6	9	7	50	5

Characterization of elastic liposomes

Microscopic observation of prepared elastic liposomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared elastic liposomes formulation.

Vesicle size

Microscopic analysis was performed to determine the average size of prepared elastic Liposomes. 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 (Maurya *et al.,* 2010).

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

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

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the elastic liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Utreja *et al.,* 2011).

Entrapment efficiency

Erythromycin entrapped within the elastic liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the elastic liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai) at 18000 rpm at a temperature of 4°C for 45 minutes, where upon the pellets of liposomes and the supernatant containing free drug were obtained. The elastic liposomes pellets were washed again with phosphate buffer to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with phosphate buffer solution by measuring absorbance at 282 nm using Labindia 3000+ spectrophotometer (Nava *et al.,* 2011).

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

Characterization of Elastic Liposomes Containing Gel

Measurement of viscosity

Viscosity measurements of prepared topical liposomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm (Kamra *et al.,* 2017).

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 (Jenita *et al.,* 2012).

Drug content

Accurately weighed equivalent to 100 mg of topical liposome gel was taken in 10 ml volumetric flask, add 5 ml of methanol and sonicate it for 10 min and after sonication volume was made upto 10 ml with methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 0.1mL 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} 282 nm. Drug content of topical liposome based gel is shown in table no 7.7 (Mohamed *et al.,* 2013).

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 6cm upon adding 20g of weight was noted, good spreadibility show lesser time to spread.

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

***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 282nm of drug (Mitkari *et al.,* 2010).

Results and Discussion

Total Six formulations were prepared using varying amount of Soya-phosphatidylcholine, Span 80 and drug and evaluated for Vesicle size and Entrapment efficiency. Formulation F4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation F4 Sleeted as optimized formulation for further evaluation.

The optimized batch of elastic liposomes was further incorporated into gel base and evaluated for pH, Spreadability, Measurement of viscosity, Drug content and *In-vitro* diffusion study. The value of vesicle size, and entrapment efficiency. The vesicle size of all elastic liposomes varied between 145.45±0.24 and 225.45±0.21nm where as entrapment efficiency was found between 65.85±0.45 to 73.32±0.12%.

Results showed that in formulation F4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation F4 Sleeted as optimized formulation for further evaluation.

Drug content is most important in elastic liposomes formulation and the data found are satisfactory. It was found to be 98.45±0.15 to 99.65±0.32% which shows the good capacity of formulation to hold the drug. The maximum drug content was found in formulation ELG-2 (99.65±0.32).

In transdermal drug delivery system pH plays an important role, the result of elastic liposomes formulation shows that all the formulations are suitable for skin delivery. The pH value of the prepared elastic liposomes gels was found to be in limits of 6.58±0.02 - 7.01±0.01. The pH of optimized formulation ELG-2 was found 7.01±0.01.

A modified apparatus was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of 11.45±0.78 – 13.25±0.95gms. cm. /sec. The gels should have optimum spreadability because very high and very low spreadability values indicate that the application of the gel to the site is difficult. The spreadability of optimized formulation ELG-2 was found to be 13.25±0.95.

The Viscosity of optimized formulation was found to be 32375±12cps.

The dissolution models were applied to determine the kinetics of optimized formulation ELG-2 and the data obtained the value of R^2 for Higuchi model was found to be higher than that of zero, first order model and korsermeyer peppas model. So, the ELG-2 Optimized formulation confirmed to show sustained Higuchi order release. In case of korsermeyer peppas the value of n found to be 0.891.

Table 2: Evaluations of elastic liposomes for vesicle size and entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
F1	185.25±0.15	65.85±0.45
F2	178.85±0.25	68.89±0.36
F3	163.32±0.36	67.74±0.25
F4	145.45±0.24	73.32±0.12
F5	187.78±0.35	70.15±0.25
F6	225.45±0.21	69.95±0.36

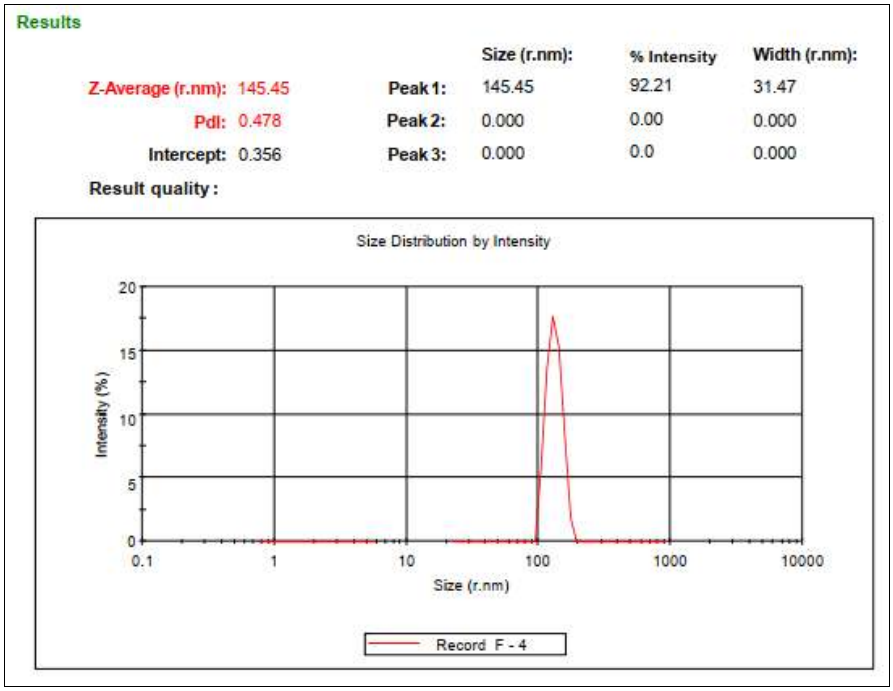

Figure 1: Vesicle Size of Optimized elastic liposomes formulation F4

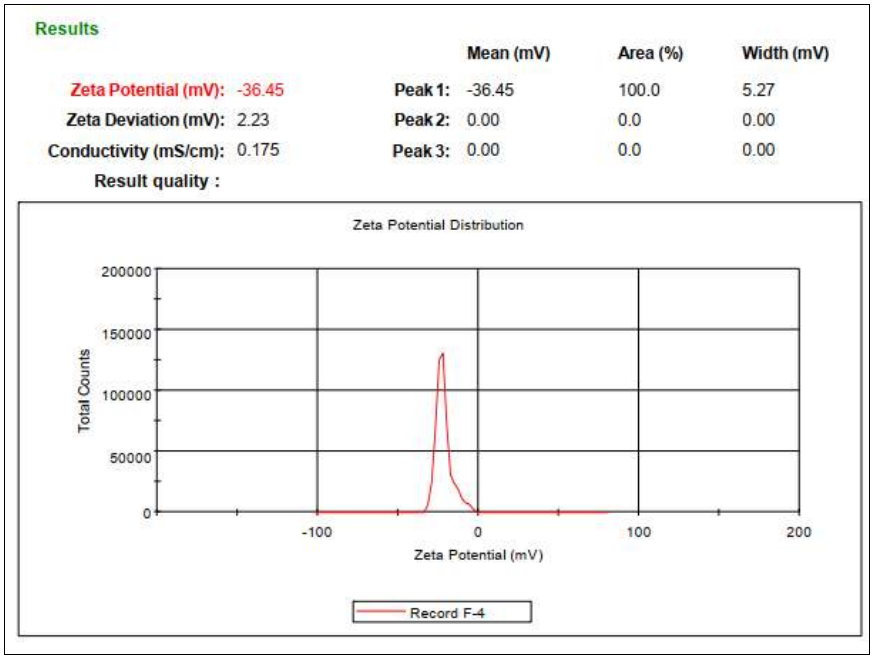

Figure 2: Zeta potential of Optimized elastic liposomes formulation F4

Table 3: Vesicle size and entrapment efficiency of optimized formulation

Formulation Code	Vesicle Size (nm)	Entrapment Efficiency (%)	Zeta potential (mV)
F4	145.45±0.24	73.32±0.12	- 36.45

Table 4: Results of elastic liposomes gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
ELG1	98.45±0.15	6.58±0.02	14.65±1.05	2645±15
ELG2	99.65±0.32	7.01±0.01	13.25±0.95	2375±12
ELG3	98.74±0.24	6.81±0.02	11.45±0.78	2210±14

Table 5: *In-vitro* drug release data for ELG2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.70711	-0.301	16.65	1.221	83.35	1.921
1	1	0	32.25	1.509	67.75	1.831
2	1.41421	0.301	45.65	1.659	54.35	1.735
4	2	0.602	59.98	1.778	40.02	1.602
6	2.44949	0.778	67.45	1.829	32.55	1.513
8	2.82843	0.903	75.65	1.879	24.35	1.386
10	3.16228	1	98.41	1.993	1.59	0.201

*Average of three reading

Table 6: Regression analysis data of elastic liposomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
ELG2	0.941	0.747	0.967	0.891

Conclusion

Erythromycin loaded elastic liposomes were successfully prepared with an excellent loading efficiency of about 73.32±0.12%. The Erythromycin loaded elastic liposomes exhibited adequate size, stability and flexibility characteristics. Furthermore, elastic liposomes extended the Erythromycin release time, achieving sustained release for almost 10 h.

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