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Development and Characterization of Elastic Liposomes of Metronidazole for the Treatment of Bacterial Infection

Priyam Chaurasiya*, Ritesh Agarwal, Kavita R. Loksh

Oriental College of Pharmacy, Raisen Road, Patel Nagar, Bhopal, Madhya Pradesh 462022

Abstract

Objective: The objective of present study is to develop and evaluate the elastic liposomes of metronidazole so as to provide the sustained release and improve its bioavailability.

Methods: Elastic liposomes were prepared by rotary evaporation method using Span 80 and Span 60 as a surfactants. The prepared elastic liposomes were evaluated for entrapment efficiency, vesicle size, *In vitro* drug release.

Results: The drug release profiles from different elastic liposomes-in-vehicle formulations were in agreement with the physicochemical properties of the formulations. The formulation prepared showed an average vesicle size 185.4nm. The amount of drug entrapped into the elastic liposomes formulations was determined. The entrapment efficiency was found to be 73.45±0.78 %. A good amount of drug was entrapped in the liposome formulations prepared. Based on different parameters formulations of batch TG2 was found to be the best formulations. Stability study was performed on the selected formulation TG2. When the regression coefficient values of were compared, it was observed that 'r' values of first order was maximum i.e. 0.993 hence indicating drug release from formulations was found to follow Korsmeyer Peppas model release kinetics

Conclusion: These results indicate that elastic liposome can function as probable drug delivery systems to enhance transdermal permeation of metronidazole for treating the topical infections.

Keywords: Metronidazole, Elastic liposomes, Topical administration, Skin infection

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*Address for Correspondence:

Priyam Chaurasiya, Oriental College of Pharmacy, Raisen Road, Patel Nagar, Bhopal, Madhya Pradesh 462022

INTRODUCTION

There are different forms of drug delivery systems have been tried in the past years so that the drug can be applied and delivered to the targeted area that has been affected. In recent times, innovative liposome vesicles are used and the performance of the drugs can be also improved. In the process, better use of extracts from plants is affected [1]. At present scenario, the most recent development in vesicle design for transcutaneous bioactive delivery is the use of elastic liposomes, which differ from conventional liposomes due to their characteristic fluid membrane with high elasticity [2].

The use of lipid vesicles in a delivery system for skin treatment has attracted increasing attention in recent years, but it remains controversial. Most relevant reports cite the localization effect of liposomes with transport processes reported in a few cases, depending on the formulation. In recent years, there has been an increasing interest in the development of novel vesicular approaches for effective transcutaneous immunization. Vesicular carriers *i.e.* elastic liposomes, conventional liposomes, Niosomes etc., elicit immune response by different mechanisms. [3, 4]. Some lipids directly lower the skin permeability barrier, which resides primarily in the stratum corneum. The most recent development in vesicle design for transcutaneous bioactive delivery is the use of elastic liposomes, which differ from conventional liposomes and Niosomes by their characteristic fluid membrane with high elasticity. In elastic liposomes, Elasticity is stress controlled, owing to the composition dependence of the membrane bending energy [5]. Elastic liposomes passage through the normally confining pores is then governed by the basic principles of elastomechanics.

Elastic liposomes are biocompatible bilayer vesicular systems that can deliver numerous drugs for therapeutic, biochemical, and cosmetic purposes. Elastic liposomes can accommodate challenges faced by traditional drug delivery vehicles due to their improved physicochemical and pharmacokinetic properties [6]. Elastic liposomes consist of phospholipids, surfactants such as edge activators (EA), and an inner aqueous compartment enclosed within a lipid bilayer capable of encapsulating hydrophilic (in an aqueous chamber) and lipophilic (in a lipid bilayer) molecules. Moreover, materials such as charged lipids (cationic and anionic), polyethylene glycol (PEG; PEGylation), ethanol, cyclodextrin complexes, and gels have been employed [7].

Metronidazole is an antibiotic that can be used to eliminate both anaerobic and aerobic bacteria. The gel is highly active against gram-positive and -negative bacteria and is the treatment of choice for anaerobic infections [8]. Metronidazole is classified therapeutically as an antibacterial, antiprotozoal drug which is used in the treatment of trichomoniasis also. A review concluded that metronidazole as a cream, gel, lotion or intravenous solution is useful in the treatment of malodorous pressure ulcers [9]. Thus the present study were to refine the formulation of elastic liposomes containing metronidazole for the treatment of skin disorder that will release the drug over a prolonged period of time i.e. sustained release, enhanced anti-fungal activity.

MATERIALS AND METHODS

Materials

Metronidazole 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.

Preparation of metronidazole loaded elastic liposomes

Elastic liposomes were prepared by rotator evaporation method given by Touitou *et al.,* (2000 & 2011) 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 ethanol in 7% 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 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 [10, 11].

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 10 ml 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 [12].

Table 1: Optimization of lipid: surfactant concentration

Formulation code	Soya PC (% w/v)	Span 80 (% w/v)	Drug (mg)	Ethanol (ml)
F1	4	2	4	5
F2	5	3	6	5
F3	6	4	8	5
F4	7	5	10	5
F5	8	6	12	5
F6	9	7	14	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 [13]. The average diameter was calculated using the flowing formula:

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

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–Smoluchowski 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.

Entrapment efficiency

Metronidazole 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 Equipment, 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 278 nm using Labindia 3000+ spectrophotometer.

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

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.

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.

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} 278 nm [14].

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 6 cm upon adding 20g of weight was noted, good spreadibility show lesser time to spread [15].

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

In vitro drug diffusion study

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane was 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 3.14 sq.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 was phosphate buffer pH 7.4. The receptor compartment was 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 [16, 17]. During each sampling interval, samples were withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn were analyzed spectrophotometrically at wavelength 278 nm of drug.

Release kinetics study

To study the release kinetics of drug from the elastic liposomes, the data obtained from the *in vitro* release study were analysed using various kinetic models to describe the mechanism of drug release from the hydrogels [18].

In order to investigate the mode of release from the polymeric nanoparticles loaded in hydrogel, the release data were analyzed with the following mathematical models.

$$Qt = K_0\, t \text{ (Zero Order Kinetics)}$$

$$\text{Log } (Q_t / Q_0) = - K_1\, t / 2.303 \text{ (First order Kinetics)}$$

$$Qt = K_{KP}\, t^n \text{ (Korsmeyer and Peppas equation)}$$

$$Qt = K_H\, t^{1/2} \text{ (Higuchi's equation)}$$

Where, Qt is the percent of drug released at time't', K_0, K_1, K_{HC}, K_{KP} and K_H are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

Stability test

The behavior of the liposome to retain the drug was studied by storing the liposome at 4 to 8 °C (refrigerator RF) for a period of 4 weeks. The liposomal preparations were kept in sealed vials [16].

RESULTS AND DISCUSSION

Evaluation of vesicle size and entrapment efficiency

Table 1 includes the value of vesicle size, and entrapment efficiency. The vesicle size of all elastic liposomes varied between 178.89 and 245.65 nm whereas entrapment efficiency was found between 61.15 to 75.65%.

Table 1: Evaluations of elastic liposomes for vesicle size and entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
F1	200.0±5.45	68.52±0.85
F2	210.3±8.32	65.58±0.65
F3	225.5±5.41	70.32±0.35
F4	208.5±9.62	69.95±0.74
F5	185.5±7.45	73.45±0.78
F6	210.9±9.12	70.65±0.41

Results showed that in formulation F2 which contain smallest vesicle size and increase in entrapment efficiency, Formulation F2 was selected as optimized formulation for further evaluation.

Table 2: Vesicle size and entrapment efficiency of optimized formulation

Formulation Code	Vesicle Size (nm)	Entrapment Efficiency (%)	Zeta potential (mV)
F2	185.4	73.45±0.78	- 25.4

In the present study, 5% 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. The formulation prepared showed an average vesicle size 185.4nm. The amount of drug entrapped into the elastic liposomes formulations was determined. The entrapment efficiency was found to be 73.45±0.78 %. A good amount of drug was entrapped in the liposome formulations prepared. Zeta potential measurement study supported by the above hypothesis, as zeta potential tends to be more negative.

Evaluation of elastic liposomes gel

Drug content is most important in elastic liposomes formulation and the data found are satisfactory. It was found to be 96.58±0.45 to 98.98±0.15 % which shows the good capacity of formulation to hold the drug. The maximum drug content was found in formulation TG-2 (98.98±0.15).

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 6.98±0.12 - 7.02±0.15. The pH of optimized formulation TG-2 was found 7.02±0.15.

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 9.56±1.32– 13.75±1.32gms. 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 TG-2 was found to be 11.65±1.74.

The best method for the selection of spindle was trial and error starting from T91 spindle. Spindles in increasing number were used depending on the % torque and error. The goal is to obtain a viscometer dial or display (% torque) reading between 10 & 100, the relative error of measurement improves as the reading approaches 100. Spindle T 95 was found to be suitable and was used for the measurement of viscosity of all the gels. The Helipath T- Bar spindles were rotated up and down in the sample giving variable viscosities at a number of points programmed over the time. Five readings taken over a period of 60 seconds were averaged to obtain viscosity. The viscosity of optimized formulation was found to be 3570±23cps. The outcomes of the result are tabulated in table 3.

Table 3: Results of elastic liposomes gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
TG1	96.58±0.45	6.98±0.12	13.75±1.32	3025±18
TG 2	98.98±0.15	7.02±0.15	11.65±1.74	3570±23
TG 3	97.56±0.25	6.87±0.08	9.56±1.32	4123±12

In order to determine the release model, the *in vitro* drug release data were analyzed according to zero order, first order, and diffusion controlled mechanism according to Higuchi model. The results revealed that the release of metronidazole from the optimized formulae obeyed the Higuchi model of matrix diffusion with highest R^2 values, as shown in table 4. To confirm the release mechanism, the data were fitted to the Korsmeyer-Peppas equation to describe the drug release mechanism from matrix. The drug release kinetic study revealed that drug release followed Korsmeyer-Peppas model in the formulations indicates that drug is diffused through the

membrane. The Korsmeyer Peppas graphs were plotted and the release rate constant, k, and the slope n, determined. When the regression coefficient values of were compared, it was observed that 'r' values of first order was maximum i.e. 0.993 hence indicating drug release from formulations was found to follow Korsmeyer Peppas model release kinetics. Therefore it can be inferred that the drug may have followed case II transport mechanism.

Table 4: *In-vitro* drug release data for TG2

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	15.65	1.195	84.35	1.926
1	1	0	22.36	1.349	77.64	1.890
2	1.41421	0.301	39.98	1.602	60.02	1.778
4	2	0.602	55.78	1.746	44.22	1.646
6	2.44949	0.778	69.98	1.845	30.02	1.477
8	2.82843	0.903	78.85	1.897	21.15	1.325
10	3.16228	1	98.36	1.993	1.64	0.215

*Average of three reading

Table 5: 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
LG2	0.973	0.796	0.991	0.993

Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 28 ± 0.5ºC for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the elastic liposomes gel.

Ethanol used in the preparation of elastic liposome is a well-known penetration enhancer and increase penetration of metronidazole through skin was suggested of a synergistic mechanism between ethanol vesicles and skin lipid. Elastic liposome are colloidal lipid nanocarriers composed of unique components with characteristic ultradeformability and elasticity to squeeze across microlamellar spaces (which are 1/10th the vesicle diameter) among keratinocytes to pass intact across the skin layer and increase skin hydration to improve transepidermal water loss (TEWL) [19]. Presently, the enhanced permeability of elastic liposome is generally significant enough due to synergistic effects of elastic liposome acting as a carrier and penetrant. However, results from recent studies have suggested that the former mechanism is more prominent than the latter [20]. Impressively, elastic liposome vesicles can penetrate the skin without disintegration [21]. Numerous researchers have realized the potential applications of elastic liposome as compared to other vesicular systems, wherein enhanced permeation, improved bioavailability, increased solubility, higher encapsulation, reduced side effects, and improved pharmacokinetic profiles have been reported with certain limitations. However, the success of elastic liposome based formulations is only possible if pharmaceutical industries take up the development as well as guarantee widespread use of vesicular elastic liposome systems.

CONCLUSION

Elastic liposomes are especially optimized particles or vesicles which can provide a novel solution for transport related problems. The elastic liposomes were prepared by thin rotator evaporation method successfully. The elastic liposomes provided sustain drug release, which is one of the desired characteristics for local skin infection. The selected elastic liposomes formulations of batch TG2 showed good stability profile without any much declination in their properties. Thus study concludes that metronidazole can be formulated in the liposomal carrier which finds its best way for the topical administration for skin infection.

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