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RESEARCH ARTICLE

Formulation and Evaluation of Liposomal Topical Gels of Linezolid

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ABSTRACT

The objective of the present study was to develop a Linezolid loaded liposomal gel for better anti-bacterial activity by sustaining the drug release and reduces adverse effects. Linezolid is a synthetic antibacterial agent of a new class of Oxazolidinones, which has more effective clinical utility in treatment of infectious diseases compared to Vancomycin, Methicillin. Linezolid liposomes were prepared by thin film hydration technique using soya lecithin, phospholipon 90 H, cholesterol and drug in different weight ratios. They were evaluated for particle size, entrapment efficiency and *in vitro* drug release. The liposomal dispersion which showed an entrapment of 84.3% and drug release of 61.93 % in 8 hrs was optimized. The optimized formulation was incorporated into gel using Carbopol 934, HPMC K4M and HPMC K15M. Optimized liposomal gel had the drug content of 95.36 and drug release of 49.84% in 8 hrs. Ex vivo studies were performed for the optimized liposomal gel. The flux (Jss) and Permeability coefficient (Kp) was found to be 1320.6 µg/cm^2/hr and 0.4402 cm/hr respectively. Stability studies indicated that optimized formulations were stable for a period of 3 months under refrigerated conditions. It was concluded that linezolid loaded liposomal gels were successfully formulated to increase the efficacy and sustain the drug release.

KEYWORDS

Liposomes, Liposomal Gel, Linezolid, Soya Lecithin, Phospholipon 90 H, Carbopol

INTRODUCTION

Topical drug delivery is an attractive route for local and systemic treatment. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatological diseases. Linezolid is a synthetic antibiotic, an oxazolidinone derivative that is active against infectious diseases caused mainly by aerobic gram positive bacteria, certain negative bacteria and anaerobic bacteria. Linezolid is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, headache, diarrhea and abdominal discomfort. Oral Linezolid cannot be taken in conjugation with a number of medications. In order to bypass these disadvantages, the liposomal gel formulations have been proposed as topical applications.

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Topically Linezolid is used in the treatment of skin and soft tissue diseases. To sustain the drug release for a prolonged period of time and minimize the side effects it can be formulated in the form of liposomes.

Liposomes have been widely used as drug carrier in topical treatment of diseases, especially in dermatology. They are capable of incorporate a variety of hydrophilic and hydrophobic drugs to enhance the accumulation

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of drug at the administration site and to reduce side effects.

However major limitation of using liposomes topically is the liquid nature of preparation. That can be overcome by their incorporation in vehicles such as carbopol 934, HPMC K4M and HPMC K15M.

The objective of the present study was to develop Linezolid loaded liposomal gels for better anti-bacterial activity by sustaining the drug release.

MATERIALS AND METHOD

Materials

Linezolid was obtained as a gift sample from Azakem Pvt. Ltd. Hyderabad. Soya lecithin was purchased from Himedia, phospholipon 90H was a gift sample from Lipoid from Germany and cholesterol, carbopol 934 P, HPMC K4M, HPMC K15M from SDFCL. All other chemicals and reagents were of analytical grade.

Preparation of Liposomes[6,7]

Aqueous liposomal dispersions were prepared by conventional thin film hydration method. Different weight ratios of soya lecithin, phosphotidylcholine, cholesterol and stearic acid were weighed and dissolved in chloroform and methanol mixture (2:1) in 250 ml round bottom flask. A thin film was formed on evaporating organic solvent under vacuum using rota evaporator at 45-60˚C. The dried lipid film was hydrated with 10 ml of phosphate buffer solution (pH 6.8) which containing drug. The dispersion was left undisturbed at room temperature for 2-3 hour to allow complete swelling of the lipid film and hence to obtain vesicular dispersion.

Preparation of Liposomal Gel[6,7,9,14]

Preparation of Gel

The gels of were prepared using hydroxy propyl methyl cellulose (K4M, K15M) and carbopol 934P in different ratios. Gels were prepared by adding gelling agents to the distilled water. Then, propylene glycol was added as a humectant and the mixture was neutralized by drop wise addition of triethanolamine. Mixing was continued until a transparent gel appeared. Then allowed the mixture overnight to swell.

Incorporation of Liposomes of Optimized Batch in to Gel

Liposomal formulation based on maximum entrapment efficiency and in vitro drug release profile was selected for the preparation of liposomal gel. The prepared gels were filled in glass vials and refrigerated at 4 to 8 °C.

Preliminary Studies

Drug-Polymer Interaction Studies[4,5,11]

The drug excipient compatibility studies were determined by Shimadzu 8400 S FTIR using KBR pellets of 0.1 mm. Samples of pure drug and physical mixtures of drug and excipients were scanned in the range between 400-4000 cm^{-1}.

Evaluation of Liposomes

Optimization of Formulation Variables[12]

A. Effect of Concentration of Cholesterol

Liposomal dispersions FL 1- FL 6 were prepared by the varying the cholesterol concentration. The dispersions were evaluated to optimize the cholesterol concentration.

B. Effect of Concentration of Lecithin

Liposomal dispersions FL 7- FL 13 were prepared by the varying the lecithin concentration. The dispersions were evaluated to optimize the lecithin concentration.

C. Effect of Concentration of Phospholipon 90 H

Liposomal dispersions FP 1- FP 6 were prepared by the varying the phospholipon 90 H concentration. The dispersions were evaluated to optimize the phospholipon 90 H concentration.

D. Effect of Sonication Time

The effect of sonication time on the physical characteristics of the formulated liposomes was examined for different sonication time intervals i.e 5, 10, 15 and 20.

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Table 1: Composition of liposomes

Formulation Code	Drug(mg)	Soya lecithin(mg)	Phospholipon 90H(mg)	Cholesterol(mg)	Stearic acid(mg)
FL 1	30	100	-	10	30
FL 2	30	100	-	20	30
FL 3	30	100	-	30	30
FL 4	30	100	-	40	30
FL 5	30	100	-	50	30
FL 6	30	100	-	60	30
FL 7	30	80	-	30	30
FL 8	30	90	-	30	30
FL 9	30	100	-	30	30
FL 10	30	200	-	30	30
FL 11	30	300	-	30	30
FL 12	30	400	-	30	30
FL 13	30	500	-	30	30
FP 1	30	-	80	30	30
FP 2	30	-	90	30	30
FP 3	30	-	100	30	30
FP 4	30	-	200	30	30
FP 5	30	-	300	30	30
FP 6	30	-	400	30	30
FP 7	30	-	500	30	30

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E. Vesicle Shape and Size Analysis of Liposomes

Size and shape of the vesicles were determined using optical microscopy and SEM (Hitachi S 3700N).

F. Particle Size Measurement

The average diameter of sonicated vesicles was determined by laser diffraction technique using Malvern mastersizer 2000.

G. Zeta Potential

The zeta potential of the liposomes was determined using Zetasizer.

H. Entrapment Efficiency (EE)[6,7,13]

The entrapment efficiency of liposomes were estimated by ultracentrifugation method where the liposomal dispersions were centrifuged at 14000 rpm for 30 minutes. The clear supernatant from the resulting solution was diluted appropriately using pH 6.8 phosphate buffer and analyzed for Linezolid spectrophotometrically. The percent of encapsulation efficiency (EE %) was calculated using the following equation:

$$EE\% = \frac{[\text{Total drug}] - [\text{diffused drug}]}{[\text{Total drug}]} \times 100$$

I. In Vitro Drug Release[6,9,10,11]

Studies were performed for all the formulations. The diffusion cell consisted of a hollow glass cylinder (length 14.6 cm and internal diameter 2.5 cm) made up of borosil glass. One end of the cylinder was covered with Himedia dialysis membrane (cut-off molecular weight: 12000-14000), which was previously soaked in warm water and placed on the receptor compartment. The temperature was maintained at 37°C.

Phosphate buffer pH 6.8 was placed in the receptor cell. Samples were withdrawn at specified time intervals and the medium was replaced with fresh phosphate buffer (pH 6.8). The samples were analyzed for drug using a UV-Vis spectrophotometer at 251 nm.

Characterization of Liposomal Topical Gel

Visual Appearance and pH[8,11,14]

The formulations were observed for the presence of any particular matter. The pH of liposomal topical gels were measured in triplicate using digital pH meter.

Drug Content[8,9,11,14]

Drug content was estimated spectrometrically where 100 mg of formulation was taken and dissolved in methanol and filtered. The volume was made to 100 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 251 nm.

Viscosity[7,9,11]

Viscosity of the formulations were determined using Brookfield synchroelectric viscometer (DV Pro II) fitted with S-63 spindle at 5, 10, 50 and 100 rpm.

In Vitro Drug Release Studies

In vitro release studies were carried out using Franz diffusion cell and the temperature was adjusted to 37±0.5^{o}C. Samples were withdrawn at periodic intervals for 8 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible Spectrophotometer at 251 nm using phosphate buffer (pH 6.8) as blank.

Ex Vivo Drug Release Studies[11,13]

Ex vivo Studies were carried out using skin of albino rat. Tissue was inserted in the diffusion cell with permeation area of 3.8 cm^2. Temperature was adjusted to 37±0.5^{0}C. In situ gel was placed in the donor compartment. At predetermined time intervals, sample was withdrawn and replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed using UV-Visible Spectrophotometer at 251 nm using pH 6.8 phosphate buffer.

Accelerated Stability Studies[4,7,12]

The optimized liposomal dispersion which had higher entrapment efficiency was placed in vials

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and sealed with aluminium foil for a short term accelerated stability study at 25 ± 2ºC/ 60±5% RH and 5 ± 3ºC as per modified International Conference on Harmonization guidelines. Samples were analyzed at periodic time intervals for 3 months for pH and drug content.

RESULTS AND DISCUSSION

Preliminary Studies

Drug-polymer Interaction Studies

FTIR spectrum of pure drug and mixture of drug and polymers are shown in Fig. 1 and 2. From the spectral study, as shown in Table 2 and 3 it was observed that there was no significant change in the peaks of pure drug and drug polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.

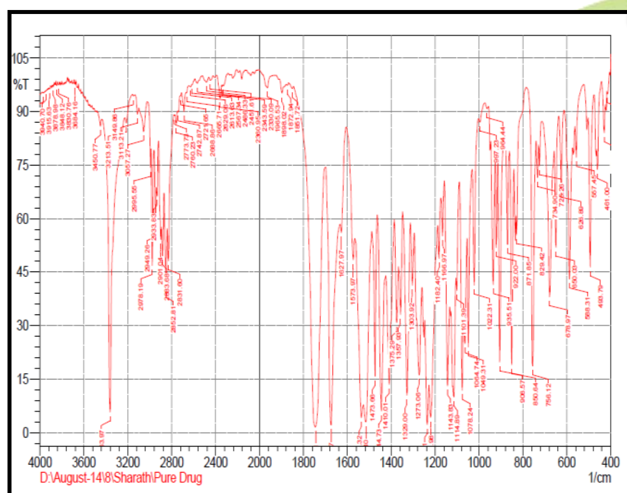

Figure 1: IR Spectrum of Drug

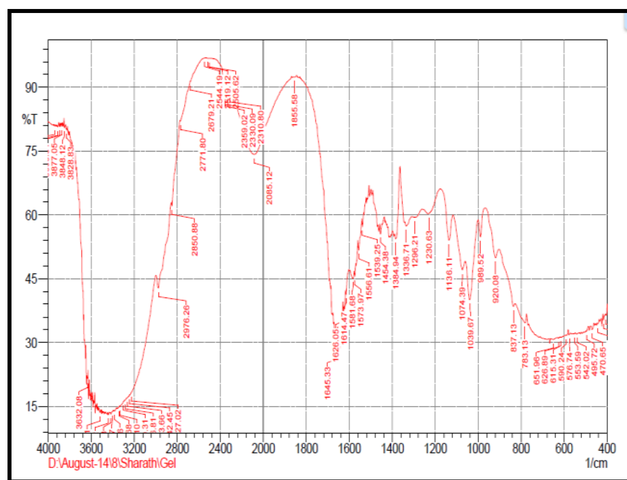

Figure 2: IR Spectrum of Drug and Excipients

Table 2: Characteristic IR peaks of Linezolid plain drug

Functional group	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
N-H Stretching	3100-3400	3213
C-H stretching	2820-3000	2995
C=O stretching	1800-1850	1851
N-H bending	1550-1640	1627

Table 3: Characteristic IR peaks of Linezolid and excipients

Functional group	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
N-H Stretching	3100-3400	3242
C-H stretching	2820-3000	2850
C=O stretching	1800-1850	1855
N-H bending	1550-1640	1626

Evaluation of Liposomes

Table 4: Effect of sonication time

Sonication time (min)	Vesicle size (µm)	Characteristics of vesicles
0	>15	Large vesicles
10	10	Irregular circular vesicles
15	6-7	Oval to circular vesicles

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20	5-6	Evenly sized circular vesicles
25	3-4	Ruptured vesicles

From the Table 4, it can be concluded that spherical liposome vesicles were not observed after 20 min sonication, suggesting that >20 min exposure to sonication may damage the vesicles. Sonicating the formulation for 20 minutes produced vesicles with a uniform spherical structure.

Vesicle Shape and Size of Liposomes

SEM images, microscopic evaluation showed that most of the vesicles were spherical in shape as shown in Fig. 3 and 4.

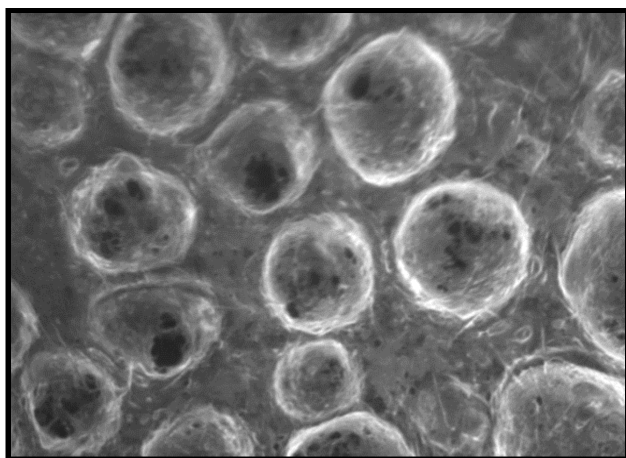

Figure 3: Scanning Electron Microscopic Images of Linezolid liposomes

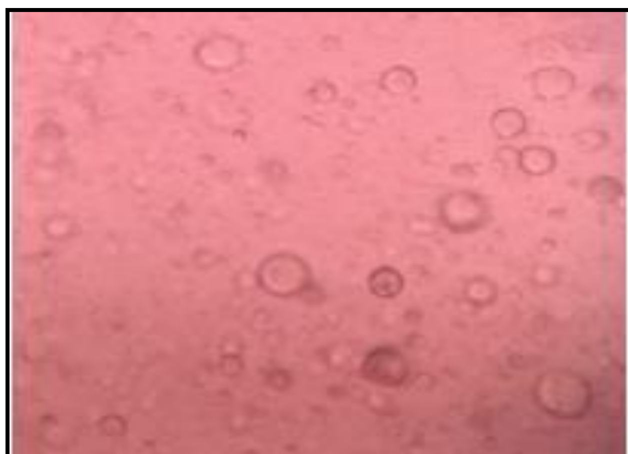

Figure 4: Photomicrograph of Linezolid loaded liposomes

From Fig 5(a) it was observed that most of the vesicles were spherical in shape. The diameter (nm) of leptosomes was found to be in the range of 100 to 1000 nm. The average size of liposomes were found to be 151.1 nm

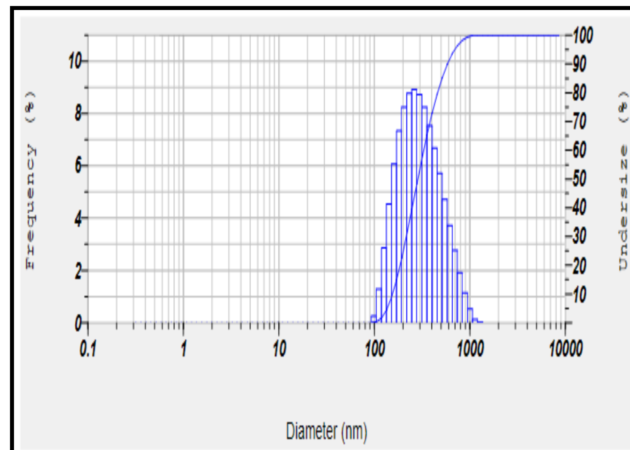

Figure 5(a): Particle size analysis of linezolid loaded liposomes

Zeta Potential

The zeta potential of the liposomes were determined using Zetasizer and the value of the liposomes was found to be -41.5 mV which indicates that liposomes were stable.

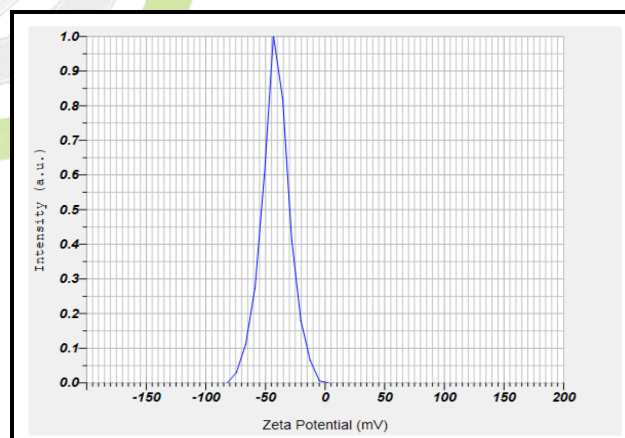

Figure 5(b): Zeta potential of linezolid loaded liposomes

Entrapment Efficiency

Percentage entrapment efficiency of Linezolid in liposomes was found to be in the range of 63 – 84% as shown in Fig. 6. The entrapment efficiency was found to be higher for the formulation FL 10 (84.30%), prepared using lecithin.

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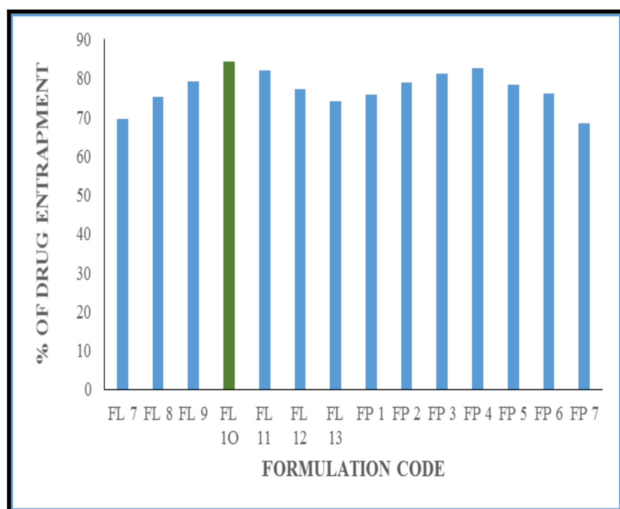

Figure 6: The entrapment efficiency of the liposomes prepared using Lecithin, Phospholipon 90 H

In Vitro Drug Release

The cumulative percentage of drug release from various liposomal formulations is shown in Fig. 7-9. The experimental studies showed that the rate of drug release depends on the concentrations of the contents of the formulations. Formulation FL 10 showed higher drug release than other formulations. Hence, it was chosen to be formulated as liposomal topical gel.

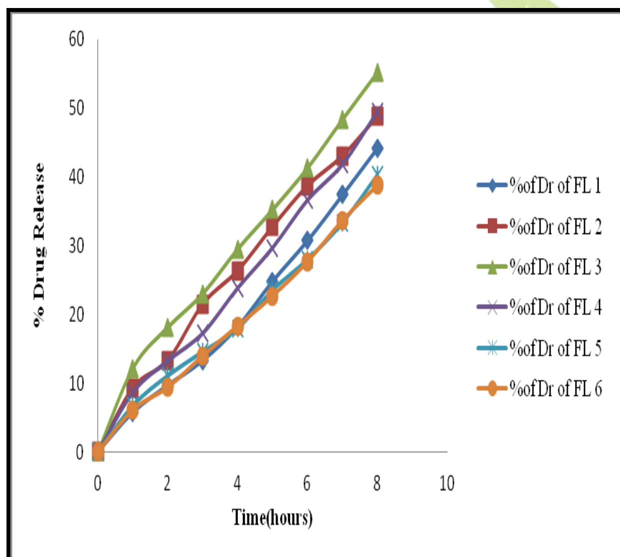

Figure 7*: In vitro* percentage drug release of liposomes prepared by different cholesterol concentrations

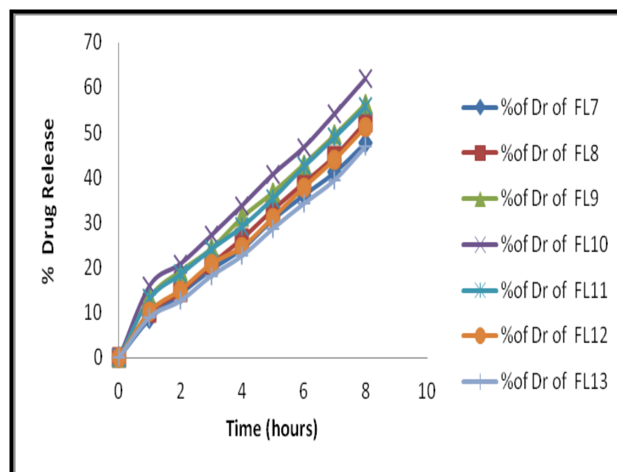

Figure 8: *In vitro* percentage drug release of liposomes prepared by different lecithin concentrations

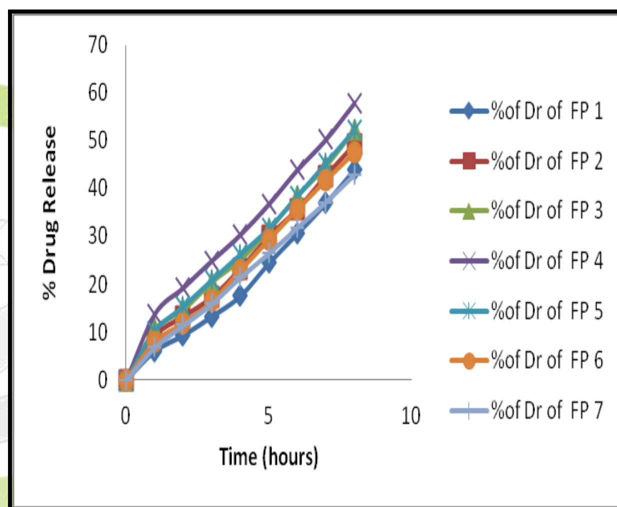

Figure 9: *In vitro* percentage drug release data of liposomes prepared by varying phospholipon 90H concentrations

Evaluation of Liposomal Topical Gel

Visual Appearance

All formulations were opaque, white in colour, odourless, semi-solid in nature and had smooth appearance.

pH

pH of all formulations were in the range between 5.9 to 6.6. The optimized formulation (LG 3) pH was found to be 6.5.

Drug Content

The solutions were analyzed for drug content spectrophotometrically at 251nm. All the

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formulations exhibited fairly uniform drug content. This ensures intended delivery of drug to the site after administration of the gel formulation. Results revealed that drug content of all developed formulations were in the range of 94to 97% as shown in Table 5.

Table 5: % of Drug content

S.No	Formulation Code	% Of Drug Content
1	LG 1	96.52±4.76
2	LG 2	95.75±4.58
3	LG 3	95.36±5.26
4	LG 4	94.14±4.84
5	LG 5	95.78±4.68
6	LG 6	95.62±5.23
7	LG 7	94.52±5.43
8	LG 8	95.49±5.37
9	LG 9	95.96±4.76
10	LG 10	94.78±4.72
11	LG 11	94.46±5.28
12	LG 12	94.18±4.58

Viscosity

The viscosity of the all gel formulations ranged from 1309-7943cps shown in Fig. 10. The viscosity of the formulations decreased on increasing the shear rate.

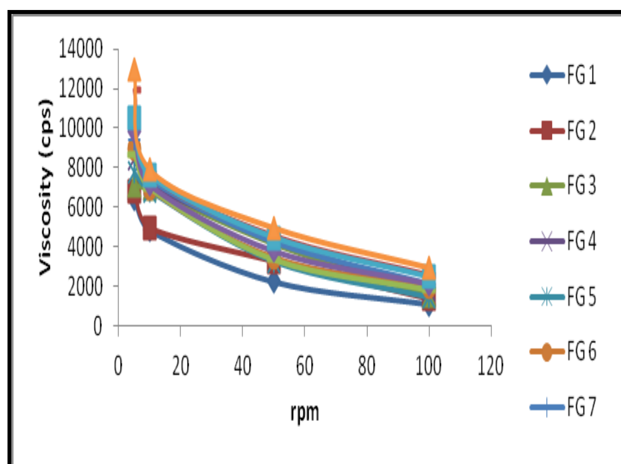

Figure 10: Viscosity of the liposomal gel formulations at different shear rates

In Vitro Release

The results of *in vitro* release after incorporation of liposomes in hydrogels are shown in Fig. 11, 12, 13 and 14. The cumulative percentage drug release for 8 hrs was highest for formulation LG 3 formulated using 2% carbopol 934.

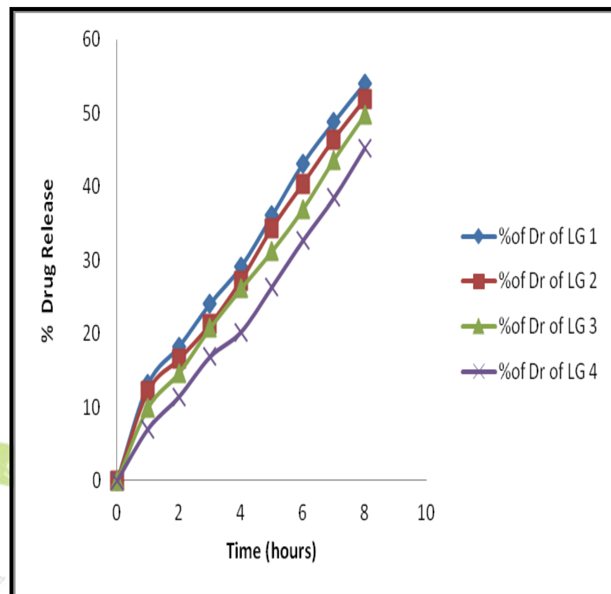

Figure 11: Cumulative percentage drug release of Linezolid from liposomal gels by using carbopol 934

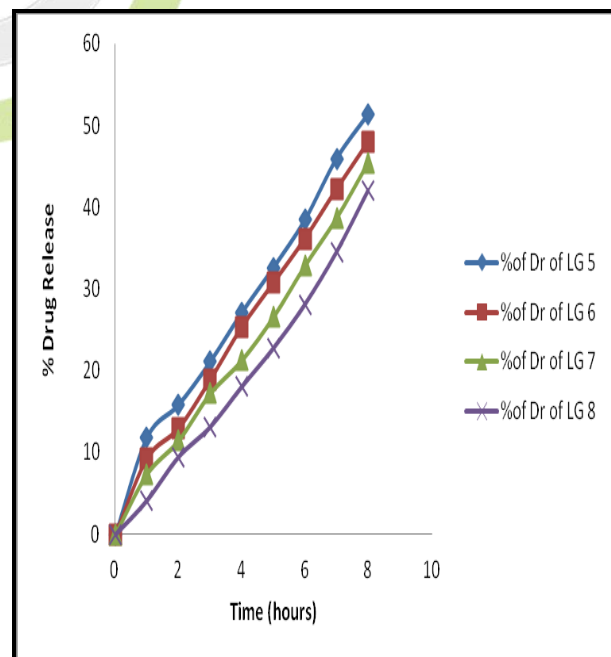

Figure 12: Cumulative percentage drug release of Linezolid from liposomal gels by using HPMC K4M

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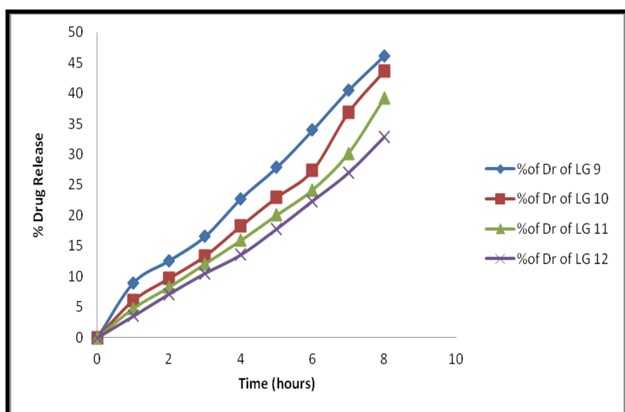

Figure 13: Cumulative percentage drug release of Linezolid from liposomal gels by using HPMC K15 M

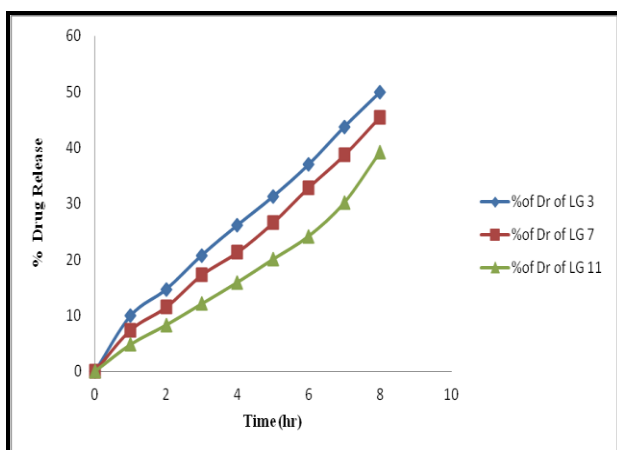

Figure 14: Comparison of cumulative percentage drug release of Linezolid from liposomal gels prepared using Carbopol, HPMC K4M and HPMC K15

Ex Vivo Studies

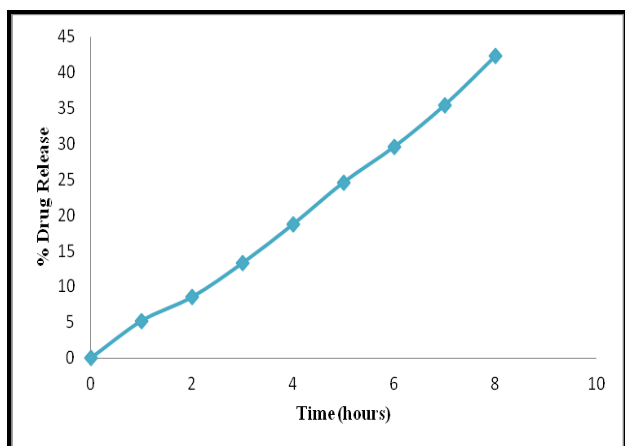

Figure 15: Ex vivo percentage drug release of Linezolid from liposomal gel prepared using 2% carbopol 934

Formulation code	Jss ($\mu g/cm^2/h$)	Kp (cm/h)
LG 3	1320.658	0.440219

Ex vivo drug permeation study was performed for optimized formulation (LG 3), the flux (Jss) and permeability coefficient (Kp) was found to be 1320.658 $\mu g/cm^2/h$ and 0.440219 cm/h respectively as shown in Fig 15.

Stability Studies

The stability studies of liposomal gel was performed at 5°C±2°C and 25°C±2°C/ 60±5% RH for 3 months. The formulations were examined visually for precipitation. The drug content, pH and gelling capacity were determined for every 30 days for 3 months. It was observed that there was no change in the physical appearance of the formulation. The drug content was analyzed and there was marginal difference between the formulations kept at different temperatures as shown in Table 6. Liposomal topical gel formulations retained good stability throughout the study.

Table 6: Stability data of formulation LG 3

Storage conditions	pH			
	Initial	1 month	2 month	3 month
5 ± 3°C	6.5	6.51	6.53	6.61
25 ± 2°C/ 60 ± 5% RH	6.5	6.54	6.59	6.3

Storage conditions	Drug content			
	Initial	1 month	2 month	3 month
5 ± 3°C	95.36%	95.14%	94.66%	94.45%
25 ± 2°C/ 60 ± 5% RH	95.36%	94.06%	93.82%	93.36%

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CONCLUSION

Linezolid loaded liposomal gels were successfully formulated. It can be concluded that the liposomal topical gels prolongs the contact time of the drug with the skin and releases the drug in a sustained manner.

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