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FORMULATION AND EVALUATION OF OCULAR NIOSOMAL *IN SITU* GELS OF LINEZOLID

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ABSTRACT: Niosomes, an emerging class of novel vesicular systems, are non-ionic surfactant vesicles which can entrap both hydrophilic and lipophilic drugs. Niosomes are incorporated into *in situ* gels for sustained release of drug and to prolong the residence time. The aim of present study is to formulate and evaluate ocular niosomal *in situ* gels of Linezolid. Linezolid is a potent synthetic oxazolidinone derivative active against a broad range of gram positive and gram-negative aerobic and anaerobic bacteria. Niosomes were prepared using various surfactants (span 20, span 40, span 60 and span 80) in different ratios using thin film hydration technique. They were evaluated for particle size, entrapment efficiency and *in vitro* drug release. Niosomes prepared using cholesterol and span 60 in the ratio 1:2 showed higher entrapment efficiency and better *in vitro* drug release. The optimized formulation was formulated as *in situ* gels using Carbopol 971P and HPMC K4M in different ratios and evaluated for gelling capacity, pH, viscosity, *in vitro* drug release, drug content, antimicrobial activity and ocular irritation test. The gels retained its antimicrobial efficacy and were proved to be safe and non-irritant on rabbit eyes. The niosomal *in situ* gel is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release.

INTRODUCTION: Niosomes are microscopic lamellar structures formed on hydration of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol, which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane [1]. Niosomes are biodegradable, biocompatible non-immunogenic, chemically stable and non-toxic in nature [2, 3].

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Niosomes enhance bioavailability by preventing the enzymatic degradation of the drug [4, 5].

In situ gels are polymeric solutions which upon instillation undergo phase transition in the ocular *cul-de-sac* to form viscoelastic gel thus increasing the precorneal residence time of the delivery system and enhancing the ocular bioavailability [6, 9]. In-situ gels exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter like ionic strength, pH or temperature [7, 8].

Linezolid is a synthetic antibiotic, an oxazolidinone derivative that is active against most gram-positive bacteria and is used to treat conjunctivitis and endophthalmitis [11, 12].

Incorporation of niosomes into *in situ* gels will improve the precorneal retention time, which in turn will increase the therapeutic efficacy of the drug. Niosomal *in situ* gel is used as an efficient vehicle to improve the patient compliance by reducing the frequency of administration and enhance ocular bioavailability of Linezolid.

MATERIALS AND METHODS:

Materials: Linezolid was a kind gift sample from Azakem Labs Pvt. Ltd, Hyderabad, India. Cholesterol, span 20, span 40, span 60, span 80, methanol, chloroform and carbopol 971P were procured from S.D Fine chemicals, Mumbai. HPMC K4M was procured from Colorcon Asia Pvt. Ltd and dialysis membrane from Hi media. All other reagents used were of analytical grade.

Preparation of niosomes: Linezolid niosomes were prepared using lipid film hydration technique using nonionic surfactants span 20, span 40, span 60 and span 80. Surfactant and cholesterol in different ratios were accurately weighed and dissolved in a mixture of chloroform and methanol (2:1v/v) as shown in Table 1. It was then vortexed in a cyclomixer at 60^0C for 30 minutes at a speed of 100 rpm and reduced pressure of 25 mm Hg to remove the solvent. The resulting film was hydrated with aqueous drug solution dissolved in phosphate buffer saline pH7.4 and vortexed for 30 minutes. The obtained colloidal dispersion was sonicated using Branson Q700 sonicator for 3 min at 150v. The niosomal suspension was left to mature overnight at 4°C [4].

TABLE 1: COMPOSITION OF NIOSOMES

Surfactant used	Formulation code	Cholesterol content (µmoles)	Surfactant (µ moles)	Cholesterol: Surfactant ratio
Span 60	N1	50	50	1:1
	N2	50	100	1:2
	N3	100	100	1:1
	N4	100	200	1:2
	N5	150	150	1:1
	N6	150	300	1:2
	N7	100	300	1:3
Span 40	N8	50	50	1:1
	N9	50	100	1:2
	N10	100	100	1:1
	N11	100	200	1:2
	N12	150	150	1:1
	N13	150	300	1:2
	N14	100	300	1:3
Span 60 + Span 40	N15	100	100 + 100	1:2
	N16	100	125 + 75	1:2
	N17	100	75 + 125	1:2
Span 80	N18	50	50	1:1
	N19	50	100	1:2
	N20	50	150	1:3
	N21	100	100	1:1
	N22	100	200	1:2
Span 20	N23	50	50	1:1
	N24	50	100	1:2
	N25	50	150	1:3
	N26	100	100	1:1
	N27	100	200	1:2

Preparation of pH triggered *in situ* gels of Niosomes: Niosomal formulation showing maximum entrapment efficiency and *in vitro* release profile was selected for the preparation of *in situ* gel.

The *in situ* gelling systems of linezolid niosomes were prepared utilizing the phase transition properties of hydroxy propyl methyl cellulose (K4M) and carbopol 971P in different ratios.

They were prepared by adding HPMC K4M to the suspension and carbopol was then added and allowed to hydrate overnight. The gel was made isotonic using sodium chloride (0.9%). Benzalkonium chloride was added as a

preservative. The prepared gels were filled in glass vials and refrigerated at 4 to 8^{o}C.

PRELIMINARY STUDIES:

Drug-polymer interaction studies: The drug excipient compatibility was 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 NIOSOMES:

Vesicle shape and size analysis of niosomes: Size and shape of the vesicles was determined using optical microscopy and SEM (Hitachi S 3700N).

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

Entrapment Efficiency (EE): The entrapment efficiency of niosomes was estimated by ultracentrifugation method where the niosomal dispersions were centrifuged at 14000 rpm for 90 minutes. The clear supernatant from the resulting solution was diluted appropriately using pH 7.4 phosphate buffer and analyzed for Linezolid spectrophotometrically. The percent of encapsulation efficiency (EE %) was calculated using the following equation:

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

***In vitro* drug release:** 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. The diffusion cell was placed in a 500 ml beaker that served as the receptor cell. The temperature was maintained at 37^{o}C.

Simulated tear fluid (100 ml) pH 7.4 was placed in the receptor cell. Samples were withdrawn at specified time intervals and the medium was compensated with fresh simulated tear fluid (pH 7.4). The samples were analyzed for drug using a UV-Vis spectrophotometer at 251 nm.

Characterization of Niosomal *in situ* gel:

Visual Appearance and pH: The formulations were observed for the presence of any particular matter. The pH of niosomal *in situ* gels was measured in triplicate using digital pH meter.

***In vitro* gelation study:** Gelling strength of formulations was evaluated by placing a drop of polymeric solution in vials containing 2 ml of freshly prepared simulated tear fluid, equilibrated at 37^{o}C. The gel formed and time taken for gelation was assessed visually.

Drug content: Drug content of niosomal *in situ* gel was determined by adding n-propanol to the formulation for lysis of the vesicles. 0.1 ml of niosomal *in situ* gel was then diluted to 100 ml with STF of pH 7.4. Drug content was estimated spectrophotometrically at 251 nm.

***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 24 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible Spectrophotometer at 251 nm using simulated tear fluid as blank.

Viscosity: Viscosity of the formulations was determined using Brookfield synchroelectric viscometer (DV Pro II) fitted with S-63 spindle at 5, 10, 20, 50 and 100 rpm.

Antimicrobial activity: Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems. This was determined in the agar diffusion medium employing Cup plate technique. Sterile solution of marketed Ciprofloxacin eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile nutrient agar previously seeded with Staphylococcus aureus organisms.

The gels were allowed to diffuse for two hours and then the plates were incubated for 24 hrs at 37^{o}C.

The zone of inhibition (ZOI) was compared with that of the standard.

Isotonicity studies: The optimized formulation was subjected to isotonicity test by adding few drops of blood and observing it under microscope and comparing it with standard marketed ophthalmic formulation (ciprofloxacin eye drops). The shape of blood cell (bulging or shrinkage) was compared with standard marketed ophthalmic formulation.

Ocular irritancy studies (Draize Test): Ocular irritation studies were performed on three albino rabbits weighing 1.5-2 kg after obtaining the Ethical committee clearance with SVCP/IAEC/ 2013/001. According to the Draize test, the amount of substance applied to the eye i.e. 100µl was placed into the lower cul-de-sac with observation of the various criteria made at a required time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. One eye is treated as test eye and the contra lateral eye serves as control.

The rabbits were observed periodically for redness, swelling, watering of the eye.

Accelerated stability studies: The optimized niosomal dispersion which had higher entrapment efficiency was placed in vials and sealed with aluminium foil for a short term accelerated stability study at 25º±2º C/ 60±5% RH and 4º±2º C as per modified International Conference on Harmonization guidelines. Samples were analyzed every 30 days for appearance, pH, gelling studies 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 3 and 4** 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.

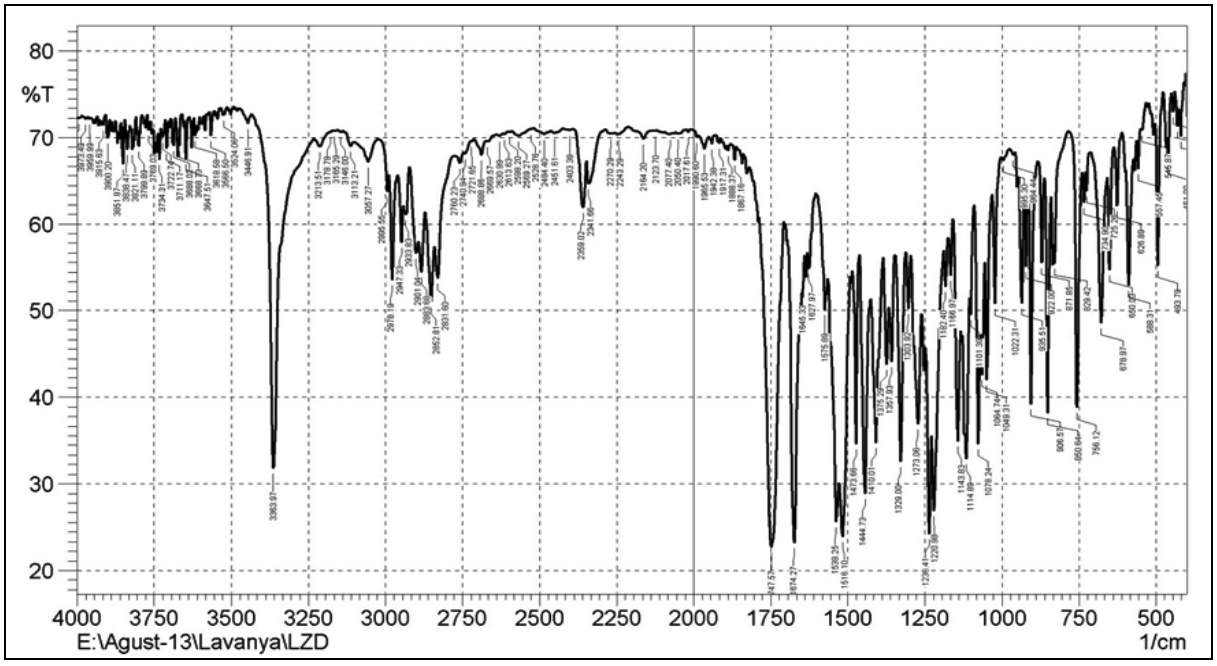

FIG. 1: IR SPECTRUM OF DRUG

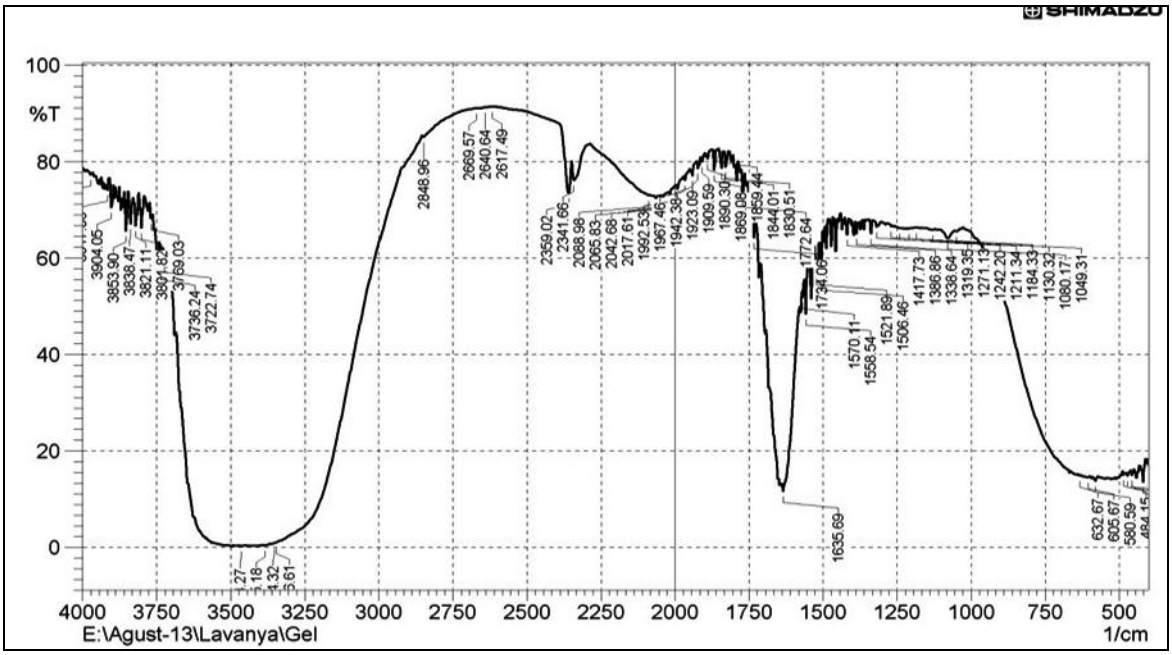

FIG. 2: IR SPECTRUM OF DRUG AND EXCIPIENTS (SPAN 60 + CARBOPOL + HPMC K4M

TABLE 3: CHARACTERISTIC IR PEAKS OF LINEZOLID PLAIN DRUG

Functional group	Reported frequencies (cm^{-1})	Observed frequency (cm^{-1})
N-H stretching	3100-3400	3363
C-H stretching	2820-3000	2816
C=O stretching	1760-1820	1335
N-H bending	1550-1640	1452

TABLE 4: CHARACTERISTIC IR PEAKS OF LINEZOLID AND EXCIPIENTS

Functional group	Reported frequencies (cm^{-1})	Observed frequency (cm^{-1})
N-H stretching	3100-3400	3334
C-H stretching	2820-3000	2848
C=O stretching	1760-1820	1815
N-H bending	1550-1640	1558

Evaluation of Niosomes:

Vesicle shape and size of niosomes: SEM images microscopic evaluation showed that most of the vesicles were spherical in shape as shown in **Fig. 3 and 4**. Niosomes prepared using span 60 were large in size compared to other niosomal formulations. This can be attributed to the fact that vesicles with high drug entrapment are large and the mean size of niosomes increases proportionally with decrease in HLB value of surfactants [10].

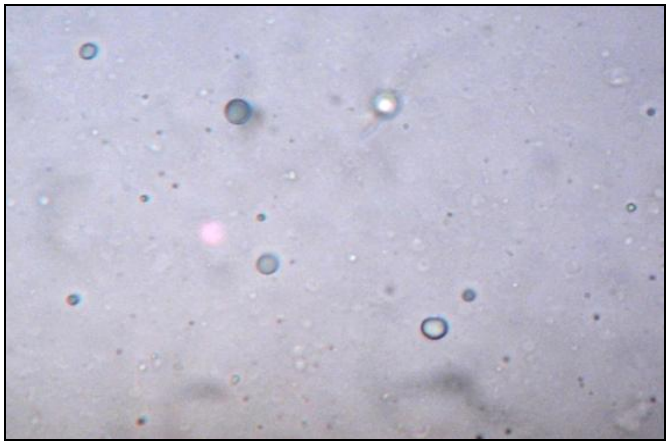

FIG. 3: PHOTOMICROGRAPHS OF LINEZOLID LOADED NIOSOMES

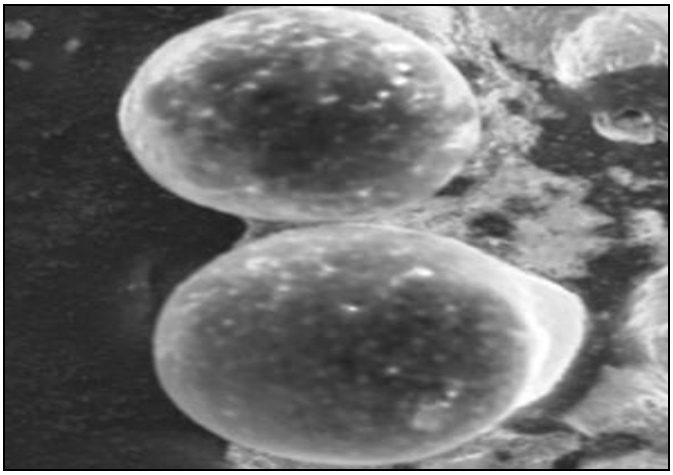

FIG. 4: SCANNING ELECTRON MICROSCOPIC IMAGE OF LINEZOLID NIOSOMES

Entrapment efficiency: Percentage entrapment efficiency of Linezolid in niosomes was found to be in the range of 40 – 85% as shown in **Fig. 5**. The entrapment efficiency was found to be higher for the formulation N4 (84.35%), prepared using span 60. The order of entrapment efficiency is span 60 > span 40> span 80 > span 20. The order of entrapment efficiency increased as the lipophilicity of the surfactant increased (HLB value decreased). Span 80 has the lowest HLB value but it has an unsaturated alkyl chain in its structure leading to lower entrapment efficiency [10].

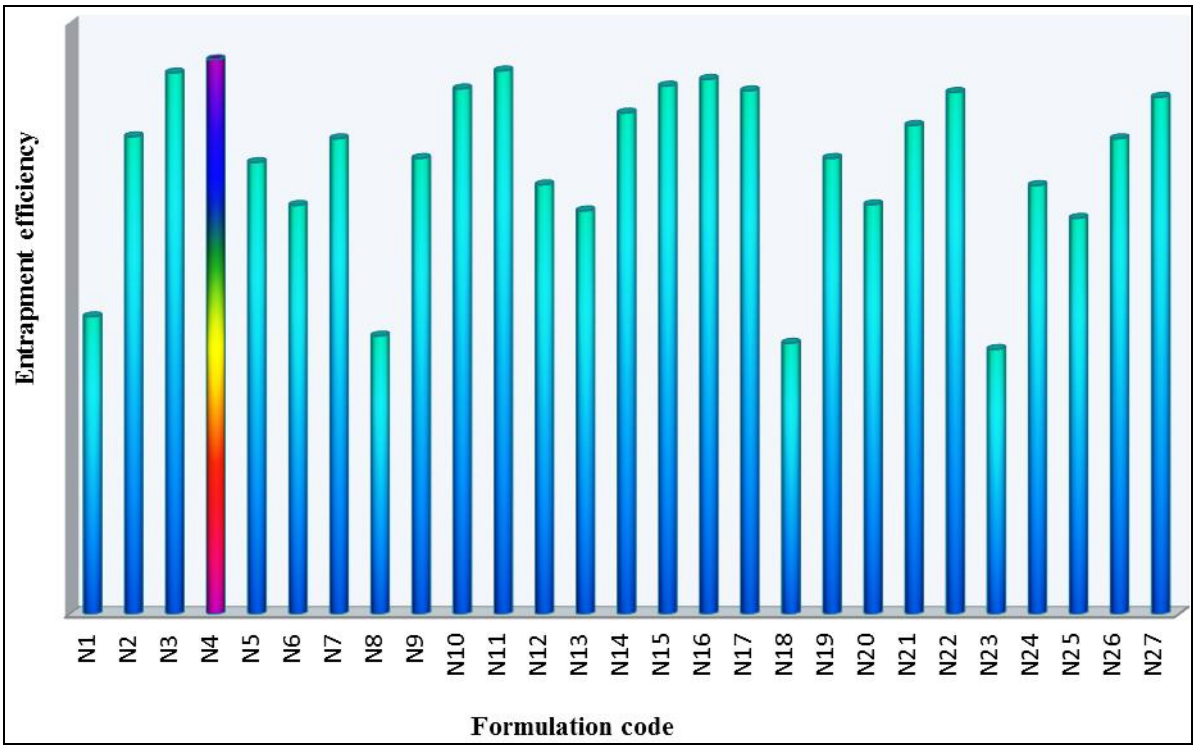

FIG 5: ENTRAPMENT EFFICIENCY OF NIOSOMES

***In vitro* drug release:** The cumulative percentage of drug release from various niosomal formulations are shown in **Fig. 6-10**. The experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. Formulation N4 showed higher drug release than other formulations. Hence, it was chosen to be formulated as niosomal *in situ* gel.

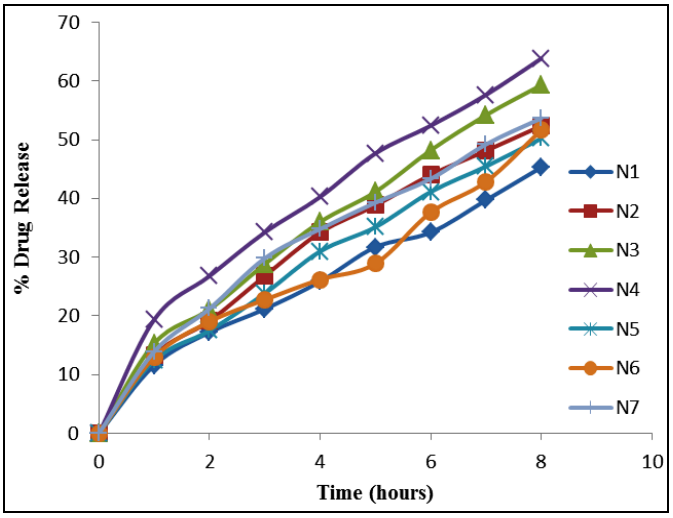

FIG. 6: *IN VITRO* RELEASE PROFILE OF NIOSOMES FORMULATED USING SPAN 60

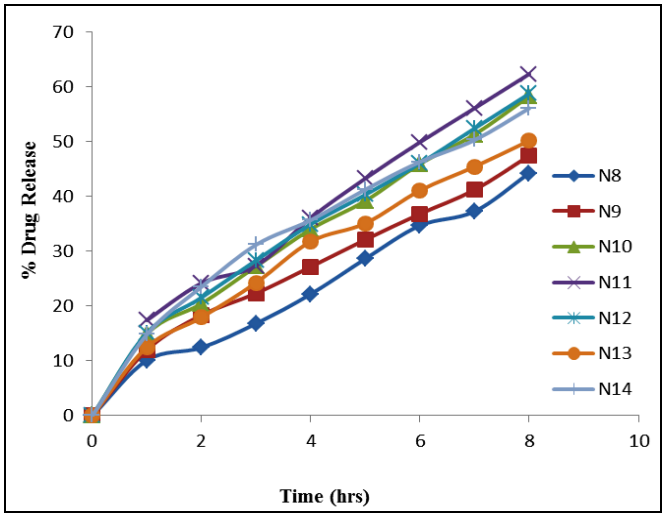

FIG. 7: *IN VITRO* RELEASE PROFILE OF NIOSOMES FORMULATED USING SPAN 40

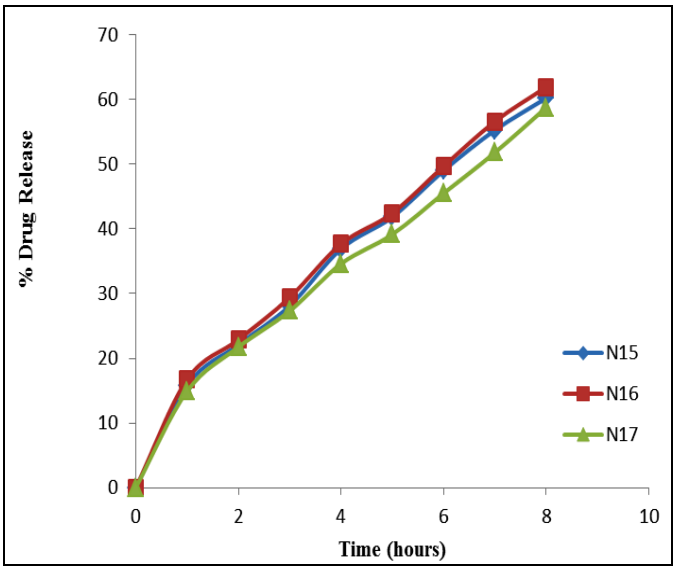

FIG. 8: *IN VITRO* RELEASE PROFILE OF NIOSOMES FORMULATED USING SPAN 60 AND SPAN 40

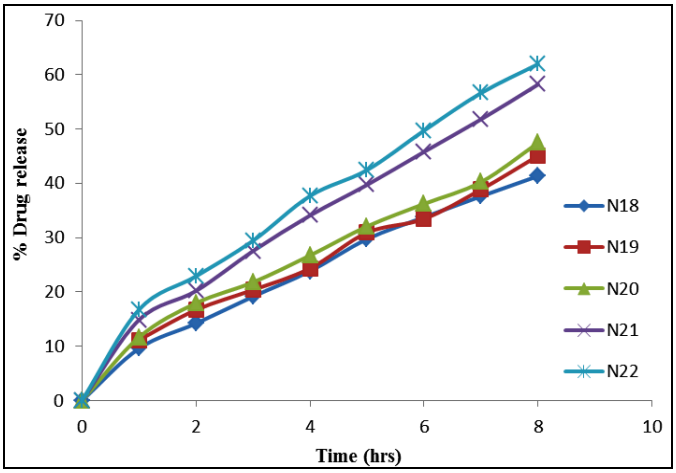

FIG. 9: *IN VITRO* RELEASE PROFILE OF NIOSOMES FORMULATED USING SPAN 80

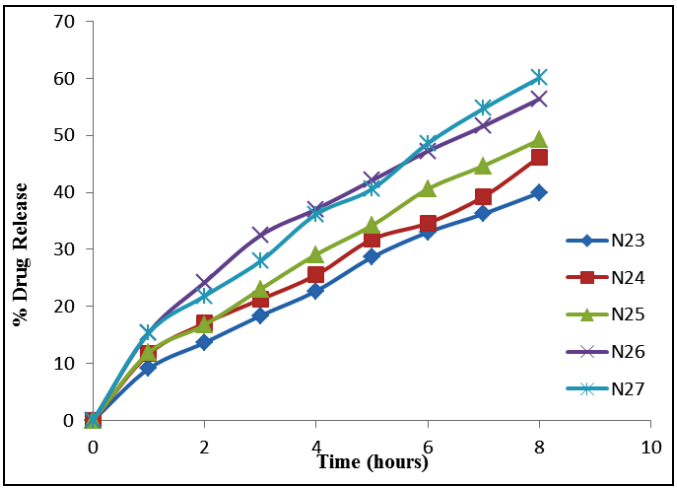

FIG. 10: *IN VITRO* RELEASE PROFILE OF NIOSOMES FORMULATED USING SPAN 20

Evaluation of Niosomal *in situ* gel:

Gelation studies: *In vitro* gelation studies revealed that the formulations G2, G3, G4, G5, G6 and G7 formed gels immediately which remained for extended period of time while G1 exhibited immediate gelation which remains for 2 – 3 hours as shown in **Table 5**.

Drug content: The solutions were analyzed for drug content spectrophotometrically at 251nm. All the 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 93 to 98% as shown in **Table 5**.

TABLE 5: EVALUATION OF NIOSOMAL *IN SITU* GELS

Formulation code	State of the gel	Appearance	pH	Drug content	Gelation capacity
G1	Liquid	Translucent	6.0	98.01	++
G2	Liquid	Translucent	6.0	97.66	++
G3	Liquid	Translucent	6.0	96.08	+++
G4	Liquid	Translucent	6.0	98.25	+++
G5	Liquid	Translucent	6.0	97.05	+++
G6	Liquid	Translucent	6.0	95.99	+++
G7	Liquid	Translucent	5.9	94.34	+++
G8	Liquid	Translucent	5.9	93.67	++++

- : No gelation; +: Gels slowly and dissolves; ++: Gelation immediate and remains for; +++: Gelation immediate and remains for extended period of time

Viscosity: The viscosity of the all gel formulations before and after gelation ranged from 141-1200 cps and 300-4000 cps as shown in **Fig. 11 and 12**. The viscosity of the formulations decreased on increasing the shear rate.

***In vitro* release:** The results of *in vitro* release after incorporation of niosomes in hydrogels are shown in **Fig. 13**. The cumulative percentage drug release for 24 hrs was highest for G2.

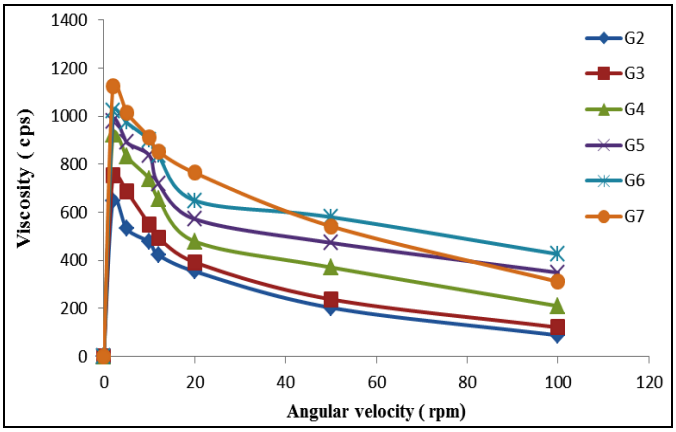

FIG. 11: VISCOSITY OF NIOSOMAL *IN SITU* GELS BEFORE GELATION

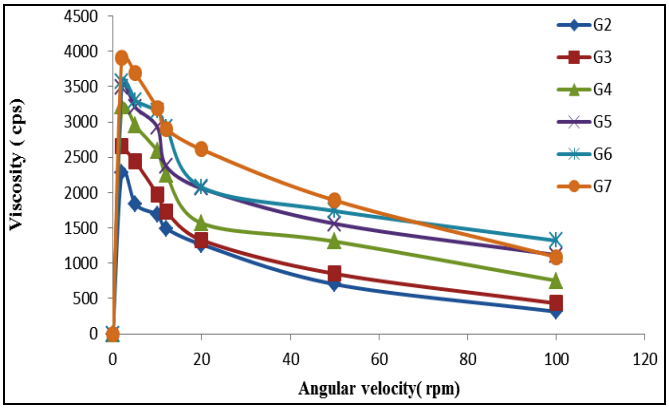

FIG. 12: VISCOSITY OF NIOSOMAL *IN SITU* GELS AFTER GELATION

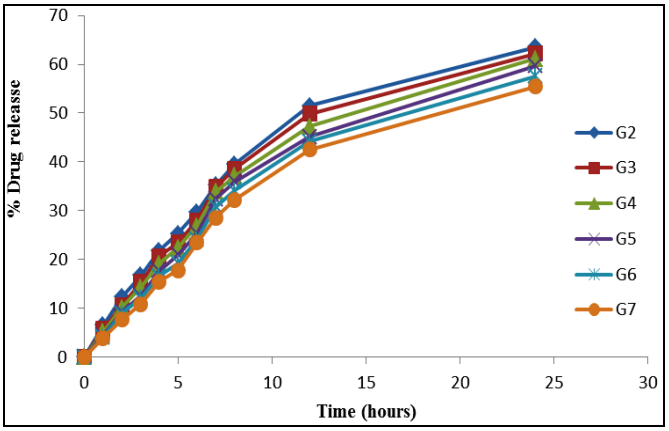

FIG. 13: CUMULATIVE PERCENTAGE DRUG RELEASE FROM NIOSOMAL *IN SITU* GELS

Isotonicity studies: The formulation G2 exhibited no change in the shape of blood cells (bulging or shrinkage), which reveals the isotonic nature of the formulation.

Antimicrobial activity: The optimized in situ gelling formulations showed antimicrobial activity when tested microbiologically by the Cup- Plate technique. The results as shown in **Fig. 14** indicate that linezolid retained its antimicrobial efficacy when formulated as an *in situ* gelling system.

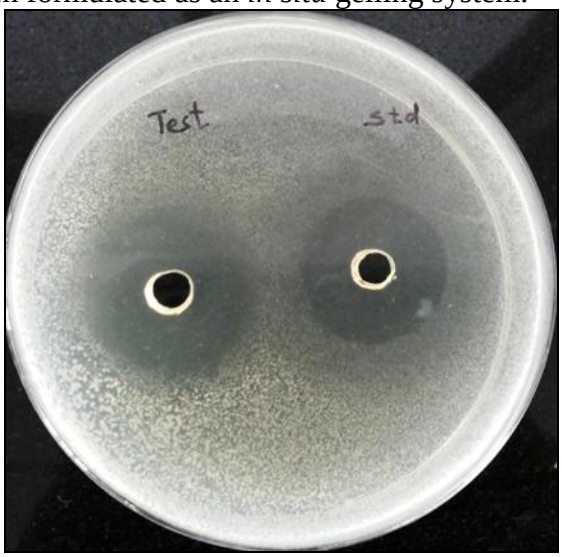

Fig. 14: Zone of inhibition of Linezolid

Ocular irritation studies: The results of the ocular irritation studies indicate that formulations have no average score (Zero) according to Draize scale [13]. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible.

Stability studies: The stability studies of niosomal *in situ* 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**. Niosomal *in situ* formulations retained good stability throughout the study.

TABLE 6: STABILITY DATA OF FORMULATION G2

Storage conditions	Drug content			
	Initial	1 month	2 months	3 months
5°C±2°C	97.66%	97.32%	96.97%	96.25%
25°C±2°C/60±5% RH	97.66%	97.01	96.51%	95.59%

CONCLUSION: From the study, it can be concluded that the niosomal *in situ* gel is a viable alternative to conventional eye drops as it enhances bioavailability by prolonging the contact time of the drug with the cornea and its ability to release the drug in a sustained manner. It also results in better patient compliance by reducing the frequency of administration.

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