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RESEARCH ARTICLE

DESIGN AND EVALUATION OF PRONIOSOMES AS DRUG CARRIER FOR OCULAR DELIVERY OF LEVOFLOXACIN

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ABSTRACT

The objective of present work is to develop proniosomal ocular gel of Levofloxacin and evaluation of their potential for sustained ocular delivery. The conventional liquid ophthalmic formulation is eliminated from the precorneal area immediately upon instillation and it is difficult to achieve good bioavailability of drug due to the tear production, non productive absorption, impermeability of corneal epithelium and transient residence time. As a result, frequent instillation of concentrated solutions is needed in order to achieve the desired therapeutic effects. The bioavailability can be enhanced by the use of vesicular systems such as Niosomes. But, Aqueous suspensions of Niosomes may exhibit aggregation, fusion, leaking of entrapped drug or hydrolysis of encapsulated drug, thus limiting the shelf life of the dispersion. The proniosomal approach minimises the above-mentioned problems and therefore there is ease of transfer, distribution, measuring and storage which makes proniosomes a versatile delivery system. Levofloxacin Proniosomal gel enhances the contact time and retention in the eye and provides sustained release action and better availability of drug.

Keywords: Levofloxacin, Proniosome, Gel, bioavailability

INTRODUCTION

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task. Non-ionic surfactant vesicles known as niosomes are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and dicetyl phosphate with subsequent hydration in aqueous media. Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via different routes specially Transdermal route. An attempt has been made to formulate Proniosomes of Levofloxacin for ocular drug delivery system. Proniosomes minimizes problems of niosomes physical stability such as aggregation, fusion and leaking and provide additional convenience in transportation, storage and dosing.[1-5]

MATERIAL AND METHODS

Material: Levofloxacin was obtained gift sample from Ranbaxy Laboratory Dewas, M.P India, lecithin was purchsed from Sigma aldrise, USA Spans were obtained from Zydus Cadila, Ahmadabad and Cholesterol was purchased from Rankem Laboratory, Mumbai. Other solvents were purchased from analytical grade in local firms.

Methods

PREFORMULATION STUDIES OF DRUG[6-9]

Identification Test: The different identification tests as per I.P. are done.

Physical appearance: The drug levofloxacin powder was examined for its organoleptic properties like color and odor.

Solubility study: The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent as water, methanol, ethanol, phosphate buffer, HCl, etc., in small test tubes and well solubilized by shaking.

Melting point determination:

Melting point of levofloxacin was determined by capillary method. A small quantity of powder was placed into a fusion tube. The tube was placed in the melting point determining apparatus (Tempo, Mumbai). The temperature of the apparatus was gradually increased automatically and the temperature at which powder started to melt and the temperature when all the powder gets melted were recorded[57].

Development of Calibration Curve for levofloxacin:

a) A stock solution of 1mg/ml of levofoxacin was prepared by dissolving 100 mg of drug in 100 ml of STF (pH 7.4) and sonicated for few seconds.

b) The stock solution was serially diluted to get solutions in the range of 2-10 µg/ml and λ max of the solution was found out.

c) The λ max of the solution was found out.

d) The absorbencies of the different diluted solutions were measured in a UV spectrophotometer.

Determination of partition coefficient

The partition behavior of drug was examined in n-Octanol: PBS (pH 7.4). It was determined by taking 5 mg of drug in separating funnel containing 10 ml portions of each n-Octanol and 10 ml of PBS (pH 7.4). The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically at 293 nm after appropriate dilution. The partition coefficient of the drug in phases was calculated by using formula:

$$\text{Partition Coefficient, } K = \frac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}}$$

Compatibility Studies

A Compatibility study was carried out in order to establish, that there would be no interaction between the drug and excipients used in the formulation. These studies were carried out by FT IR studies. The drug, individual polymer and 1:1 physical mixtures of drug and polymer (each 10 mg) were prepared and mixed with 400 mg of potassium bromide. About 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 10 tones pressure. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer. The IR spectra of physical mixtures were compared with those of pure drug and polymers to detect any appearance or disappearance of peaks. Similarly, IR spectrums of the physical mixture were scanned after 2 months and were matched with the original ones.

FORMULATION OF PRONIOSOMES[10-14]

Proniosomal gel was prepared by a coacervation-phase separation method. Precisely weighed amounts of surfactant, lecithin, cholesterol, poly ethylene oxalate and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (2.5 ml absolute ethanol) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) 1.6ml was added and warmed on a water bath till a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization.

Compositions of proniosomal gel formulations are given in Table 1.

Table 1: Composition of proniosomal formulations prepared

Proniosomal Code	Drug (mg)	Cholesterol (mg)	Lecithin (mg)	Span		PEO mg
				40	60	
PN1	10	20	50	10	--	10
PN2	10	20	20	20	--	10
PN3	10	20	20	30	--	10
PN4	10	20	20	--	10	10
PN5	10	20	20	--	20	10
PN6	10	20	20	--	30	10

EVALUATION OF PRONIOSOMES OF LEVOFLOXACIN

Optical microscopy and vesicle size determination[10]

A drop of niosomal dispersion prepared from proniosomes was spread on a glass slide and examined for the vesicle structure and presence of insoluble drug crystals under the light microscope with varied magnification power. Photomicrographs were taken for niosomes using a digital camera with 5X optical 200 m. The proniosomal gel (10 mg) was hydrated with PBS (10 ml) in a small test tube by manual shaking for 5 min and the resulting niosomes were observed under optical microscope at 100 X magnification. The average size of vesicles was measured using calibrated ocular and stage micrometer in the microscope.

Determination of Encapsulation Efficiency[11-12]

Percent encapsulation efficiency (EE) was determined by centrifugal method[56]. The proniosomal gel was converted to a niosomal dispersion, which was centrifuged (18000 rpm) for 40 min at 5°C in order to separate unentrapped drug. The supernatant was taken and diluted with PBS (pH7.4). The drug concentration in the resulting solution was assayed spectrophotometrically at 293 nm. The percentage of drug encapsulation was calculated by the following:

$$EE\ (\%) = [(Ct - Cf)/ Ct] \times 100,$$

Where Ct is the concentration of total drug and Cf is the concentration of unentrapped drug.

Scanning electron microscopy[12-13]

The niosomes formed from the hydration of proniosomal gel were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with gold/palladium using a vacuum evaporator and examined with the scanning electron microscope equipped with a digital camera at 25kV accelerating voltage.

In vitro release studies using dialysis cellophane membrane[12-14]

In vitro release studies on proniosomal gel were performed using Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor

compartment exposed to receptor compartment was 1.41cm^2. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was STF (stimulated tear fluid) pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1^{o}C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 293 nm.

Ocular Irritancy Test [15]

The optimized formulation was evaluated for in vivo performance in animal model (Albino rabbits). Three rabbits were used for this study. They were housed and maintained in the animal house at room temperature (27^{o}C) during the period of the study. They were fed with standard diet and water. The animals were placed in cages and the eyes were marked as test and control. The control group received no sample and the test eye received the formulation , and the eyes were observed for the ocular irritancy (includes the macroscopic observation of cornea, iris, and conjunctiva).

RESULT AND DISCUSSION

Identification

Physical Appearance: The drug (Levofloxacin) powder was examined for its organoleptic properties like colour and odour and it was observed that Levofloxacin was yellowish crytalline powder.

Solubility study: The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent as water, methanol, ethanol, ethyl acetate etc., in small test tube and well solubilized by shaking. Solubility study in different solvents at room temperature revealed that it is soluble in, ethanol, methanol, 0.1N NaOH, Glacial acetic acid etc and it is insoluble in distilled water (table. 2).

Table 2: Solubility studies of levofloxacin with different solvent

Sr. No.	Compound	Ratio	Solubility
1	Levofloxacin: Water	1:10	Slightly Soluble
2	Levofloxacin: Methanol	1:10	Completely soluble
3	Levofloxacin: Ethanol	1:10	Sparingly soluble
4	Levofloxacin: Dichloromethane	1:10	Slightly soluble
5	Levofloxacin: Glacial acetic acid	1:10	Completely soluble
6	Levofloxacin: 0.1N NaOH	1:10	Completely soluble
7	Lexvofloxacin : Ethyl acetate	1:10	Slightly soluble

Preparation of calibration curves

Levofloxacin solution was scanned in the U.V. range of 200-400 nm using Sistronic UV Visible spectrophotometer. The spectrophotometric method of analysis of Levofloxacin at λ_{max} 293 nm was found to be reproducible and highly sensitive. The standard curves of Levofloxacin was prepared in STF (pH 7.4), at λ_{max} 293 nm. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.99 was observed, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 2-20 µg/ml.

Preparation of Calibration Curve of Levofloxacin in STF (PBS pH 7.4)

The calibration curve was plotted between the concentration and absorbance. The calibration curve of 2-20µg/ml was carried out. The slope and intercept of the calibration curve were 0.051 and 0.008 respectively. The correlation coefficient 'r^2' values were calculated as 0.999 as shown in table 3 and figure 1.

Table 3: Standard Curve of Levofloxacin in Phosphate Buffer Solution (pH 7.4) at 293 nm

S.No.	Drug Conc. (µg/ml)	Absorbance	Statistical Parameters
1.	2	0.102	Correlation coefficient- **r** = 0.999 Slope **m** = 0.051 Intercept **c** = 0.008 Equation of Line- y = 0.051 X + 0.008
2.	4	0.210	
3.	6	0.321	
4.	8	0.421	
5.	10	0.522	
6.	12	0.629	
7.	14	0.721	
8.	16	0.832	
9.	18	0.932	
10.	20	1.02	

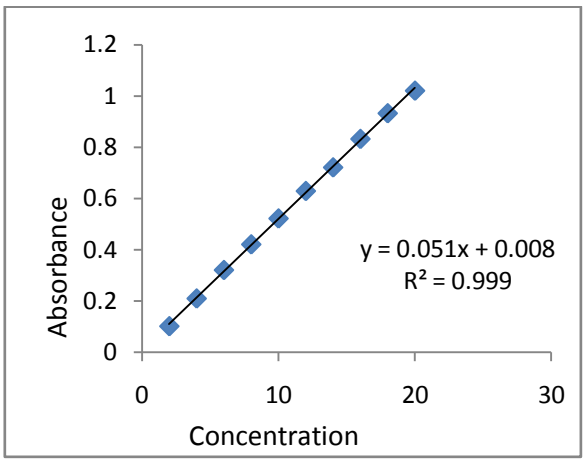

Fig 1: Calibration curve of Levofloxacin in phosphate buffer at λ_{max} 293

Melting point determination: Melting point of Levofloxacin was found at 218 ºC

Partition coefficient: Partition coefficient studies are carried out to find out extent of drug transfer in the aqueous and the other non aqueous layer. This phenomenon usually is done to obtain the drug concentration in the either layer. Partition coefficient value of Levofloxacin also revealed its hydrophobic nature which is listed a as shown in table 4.

Table 4: Partition coefficient values of Levofloxacin

S. No.	Solvent system	Partition Coefficient
1.	n-Octanol/Distilled water	0.666
2.	n-Octanol/PBS (pH 7.4)	0.459

Fourier-Transform Infra Red spectroscopy (FTIR)

The IR spectrum of drug substance was authenticated using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. Various peaks of the drug are shown in figure 2 and shown in table 5 with its band frequencies.

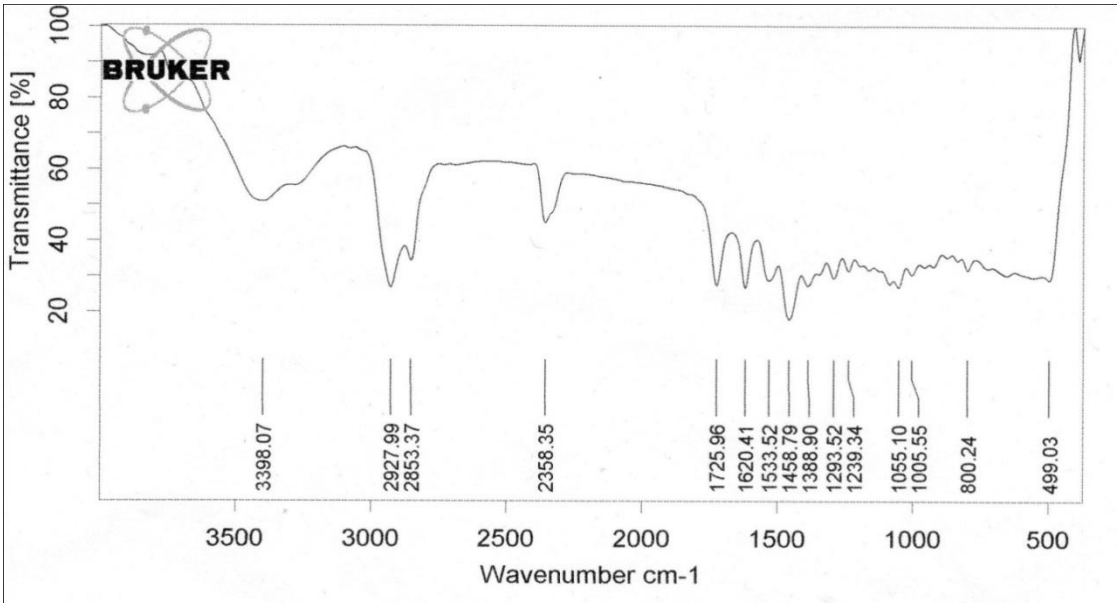

Fig 2: FT-IR of Levofloxacin

Table 5: Important band frequencies in IR spectrum of Levofloxacin

S. No.	Named Group	Reported Band frequency (cm^{-1})	Band frequency obtained (cm^{-1})
1.	C-O(cyclic ether)	1200	1055
2.	COOH group	1680	1620
3	C=O	1700	1725
4.	C-N	1200	1293
5.	N-H(piprazine)	3200	3398

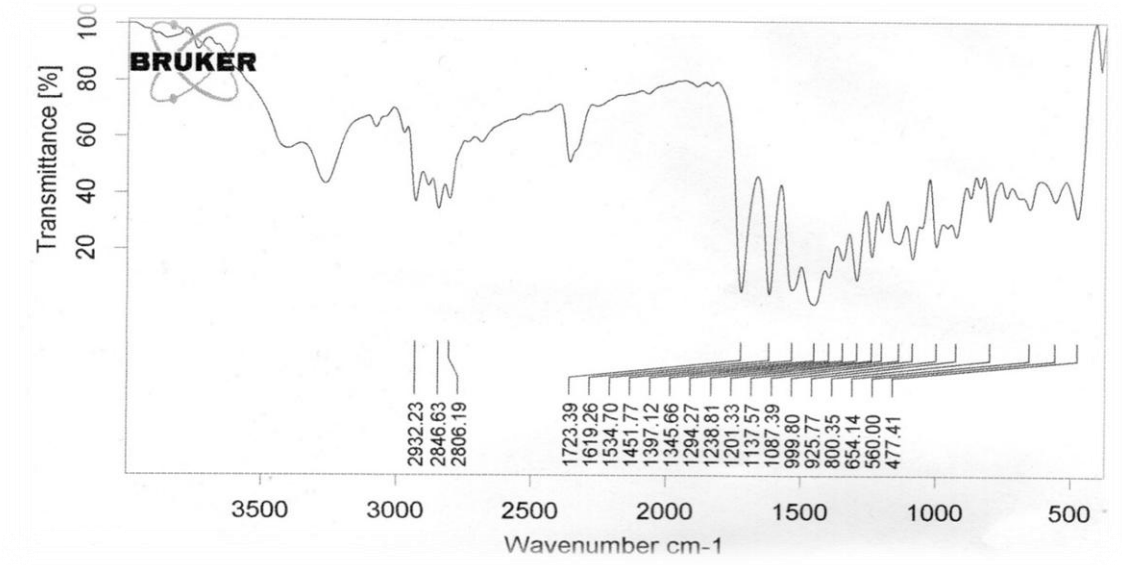

Fig 3: FT-IR of drug (Levofloxacin) and surfactant (span 60)

FT-IR Spectra of Levofloxacin,Span physical mixture of drug : carrier and F4 formulation were recorded. The Levofloxacin present in the formulation F4 was confirmed by FT-IR spectra.The characteristic peaks due to pure Levofloxacin shows IR absorption at 1055cm^{-1}(C-O stretching),1620cm^{-1}COOH carboxylic group,1725cm^{-1}(C=O stretching), 1293cm^{-1}(C-N stretching),3398cm^{-1}{N-H(piprazine)bending}. All these peaks have appeared in pure levofloxacin, physical mixture and formulation indicating no chemical interaction between levofloxacin and carrier. It also confirmed that the stability of drug during formulation.

EVALUATION OF PREPARED PRONIOSOMES OF LEVOFLOXACIN

Optical Microscopy and Vesicle Size Determination

The photomicrographs of hydrated PN1 and PN3 proniosomal formulations, composed of Span 40 and cholesterol in 1:1 and 2:1 ratios, are shown in Figure 4 and Figure 5 respectively. The photographs reveal that the niosomes are unilamellar vesicles having spherical shape and no aggregation or agglomeration is observed. Apparently, PN1 niosomal formulation gives vesicles of larger sizes.

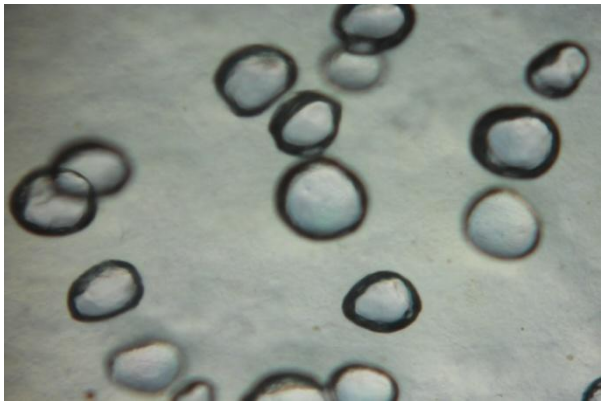

Fig 4. Photomicrograph of hydrated PN1 (proniosomal formulation)

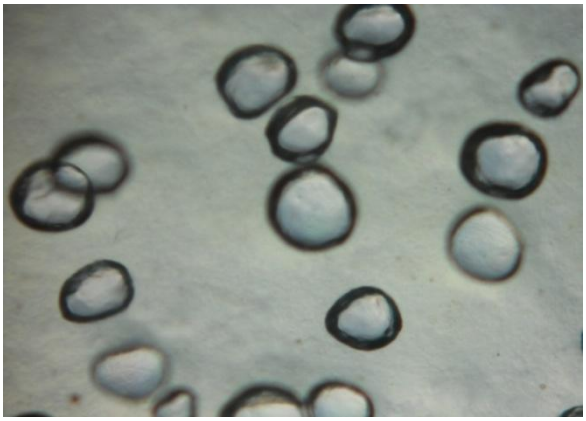

Fig 5. Photomicrograph of hydrated PN2 (proniosomal formulation)

% Encapsulation Efficiency of levofloxacin proniosomes

Proniosomes prepared with non-ionic surfactants of alkyl ester Span (sorbitan esters) 40 and 60 were utilized to determine the encapsulation of associated levofloxacin. As shown in Table-8, encapsulation efficiency of proniosomes formed from span 40 exhibits a very high value and the entrapment efficiency was found to be highest with the formulation PN3. These Span surfactants give the least leaky proniosomes as have the highest phase transition temperature. This result was consistent with the entrapment efficiency of levofloxacin in proniosomes incorporated with Span 40. Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Span can form the micelles on hydration in the presence of cholesterol .The encapsulation of levofloxacin can be entrapped into proniosomes composed of span60 however, the encapsulation efficiency was relatively low as compared to those composed of Span40 (Table -6).

Soya lecithin was selected over egg lecithin because the former gives vesicles of larger size, possibly due to

differences in the intrinsic composition of soya and egg derived lecithin. Preparations with a white semi-solid appearance were obtained with span and cholesterol. Incorporation of lecithin results in a gel-like appearance. The types of alcohol affect the size of niosomal vesicles as well; ethanol gave the largest size vesicles. The larger size with ethanol is due to the slower phase separation because of its greater solubility in water.

Table 6: physicochemical characterization of proniosome batches of Levofloxacin

S.No.	Proniosomal code	Encapsulation efficiency
1.	PF1	95.67
2.	PF2	96.71
3.	PF3	97.12
4.	PF4	78.62
5.	PF5	81.45
6.	PF6	85.51

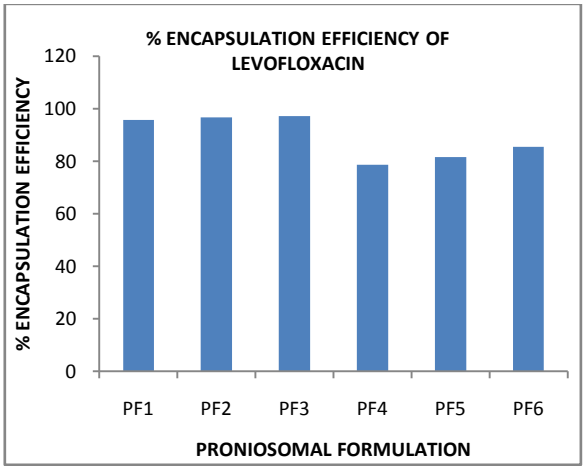

Fig 6: % of encapsulation efficiency of levofloxacin proniosomes

Scanning electron Microscopy:

Shape and surface characteristic of proniosome were examined by Scanning Electron Microscopy analysis. Scanning electron microscopy shows the porous surface of the pure levofloxacin particles (figure 7), this makes them effective carrier and provides more surface area for the coating of the surfactant mixture. Surface morphology illustrates the smooth surface of proniosome formulation. Preparing proniosomes on levofloxacin was formulate in different surfactant like span 20 and span 40 also being include lecithin but it was necessary that the solution be incorporated in very small amounts and complete drying be ensured before further additions are made.

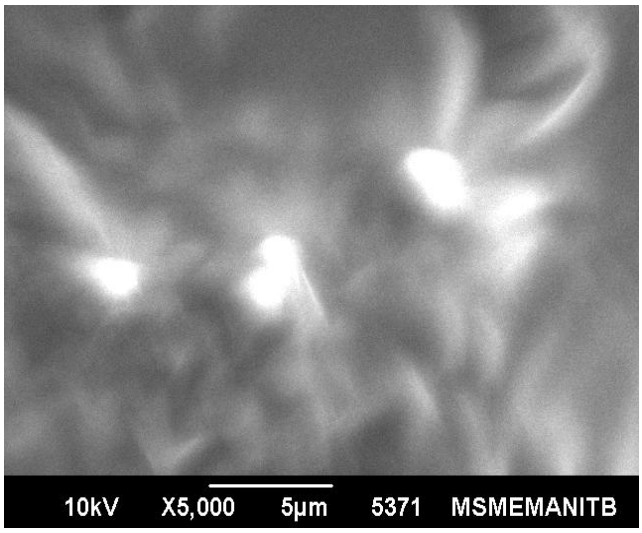

Fig 7: SEM of Levofloxacin Proniosomes:

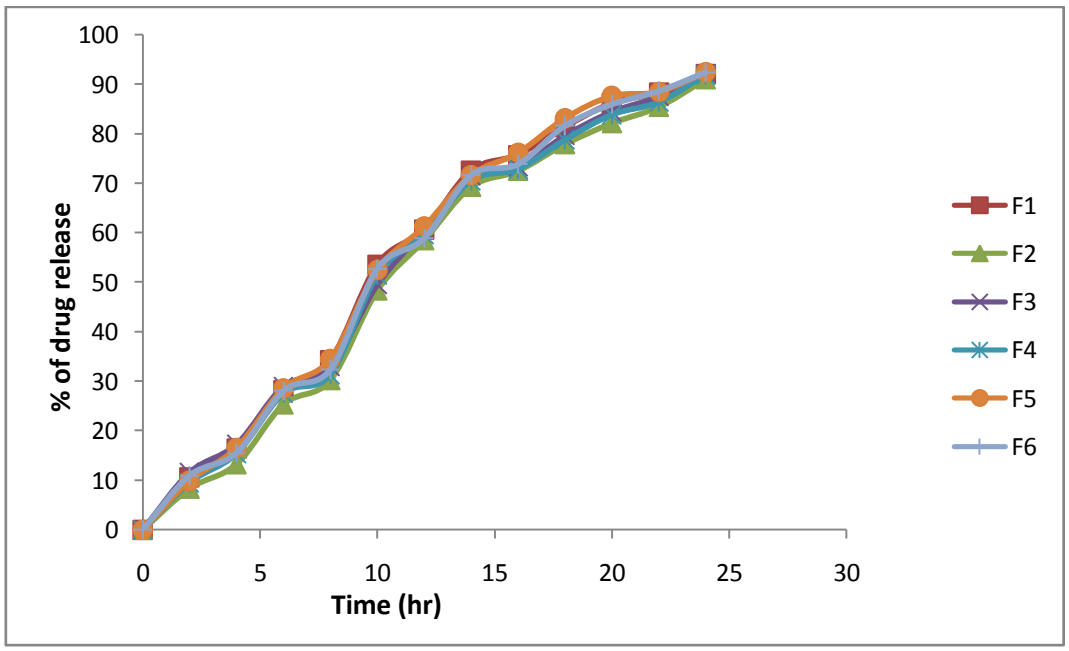

Fig 8: *In-vitro* release of all formulation

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In Vitro release studies of levofloxacin proniosomes

The release study was conducted for all the six formulations as shown in the figure 8. All of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes,this found to prevent the leakage of drug from the proniosomal formulation.The slower release of drug from the multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment .The above specified three best formulations PN3,PN5, and PN6 were found to give a cumulative release of 92.092%,92.437%,and92.438% respectively over a period of 24 hrs

***In-vivo* Ocular Irritancy test**

Ocular Irritancy Test of Levofloxacin Proniosome

The potential ocular irritancy test of proniosomal formulations PN1, PN2 and PN3 were evaluated by observing them for any redness, inflammation, or increased tear production, upon application to the eyes of albino rabbits. There is no irritancy effect on PN3. The miner redness was observed on PN1 and PN2.

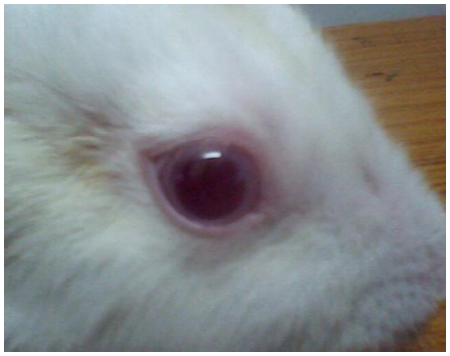

Fig 9. PN 1 Left Eye

Fig 10: PN 10 Left Eye

Fig 11: PN 3 Left Eye

CONCLUSION

Proniosomes of Levofloxacin were prepared by coacervation-phase separation method. The drug encapsulation efficiency was studied for all the six formulations represented in table 8. The entrapment efficiency was found to be highest with the formulation PF3 (97.12%), which may have an optimum surfactant ratio to provide a high entrapment of levofloxacin.

The release study was conducted for all the six formulations as shown in the Figure 9. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 90% release within a period of 24 hours. The formulation which have optimum ratio PN3 was found to sustain the drug release than other formulations. Among all formulations PN3 was selected as best formulation because of its highest entrapment efficiency and consistent release profile of levofloxacin.

Vesicles with smaller diameter are believed to better permeate through the skin as smaller vesicles tend to fuse readily. Shape and surface characteristic of proniosome were examined by Scanning Electronic Microscopy analysis. Surface morphology showed the smooth surface of optimised proniosomal formulation Figure.8. On conclusion,this novel drug delivery system ,Proniosome as compared to noisome suspension, represent a significant improvement by eliminating physical stability problems, such as aggregation or fusion of vesicle and leaking of entrapped drug during long term storage.

Proniosome derived niosomes are superior in their convenience if storage, transport and dosing as compared to niosomes prepared by conventional methods.

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