wjpmr, 2017,3(9), 188-194

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.wjpmr.com

Research Article
ISSN 2455-3301
WJPMR

FORMULATION AND DEVELOPMENT OF ELASTIC VESICLE AS DRUG CARRIER FOR OPHTALMIC DRUG DELIVERY SYSTEM

Sheetal B. Tathe*[1], Pravin B. Suruse[2] and Umesh D. Shivhare[1]

[1]Sharad Pawar College of Pharmacy, Wanadongri, Hingna Raod, Nagpur-441108.
[2]Kamla Nehru College of Pharmacy, Butibori, Nagpur-441108.

***Corresponding Author: Sheetal B. Tathe**
Sharad Pawar College of Pharmacy, Wanadongri, Hingna Raod, Nagpur-441108.

Article Received on 09/08/2017 **Article Revised on 29/08/2017** **Article Accepted on 19/09/2017**

ABSTRACT

Conventional liquid ophthalmic formulations are most convenient from patient point of view, but these formulations were showed low bioavailability because of a constant lachrymal drainage in the eyes leads to increase dosing frequency. Moreover, the absorption of the drug drained through the nasolacrymal duct may results in undesirable side effects. To overcome these limitations different approaches has been applied such as preparation of ointments, gels, creams etc. These ophthalmic formulations also fails to show desired therapeutic responses because of their own disadvantages such as ointments makes blurred vision. So, two different systems were combined together as niosomes and *in situ* gel by incorporating niosomes in this gel formulation so that it is easy to administered and retain at the site for prolong period of time. The Ciprofloxacin HCL, a second generation fluoroquinolone derivative was used in eye infections needs frequent dosing in its solution form. Vesicular system reported prolonged and controlled action at corneal surface but it has again limitation of drainage along tear produced. In this study, first niosomes containing Ciprofloxacin HCL were prepared by applying 3^2 full factorial designs and evaluated for its vesicle size, percent entrapment, *in vitro* drug release kinetics and stability. Also, *in situ* gel formulation was prepared by dispersing the niosomes in solution of Carbopol 940 and Hydroxy Propyl Methyl Cellulose (HPMC) K4M. *In vitro* drug release kinetics from niosomal *in situ* gel formulation indicated that the minimum inhibitory concentration (MIC) of drug (4µg/ml) was achieved within 1-2 h (batch F1-F9).

KEYWORDS: Niosomes, *In-situ gel, ophthalmic* drug delivery system, Ciprofloxacin HCL, *F*actorial design.

INTRODUCTION

In the recent years considerable attention has been focused on the development of new drug delivery systems. The therapeutic efficacy and safety of drugs administered by conventional methods can be improved by more precise and temporal placement with in the body through a controlled drug delivery. Basically, there are three modes of drug delivery i.e. Targeted Delivery, Controlled Delivery and Modulated Delivery.[1]

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Numerous approaches have been attempted to increase the bioavailability and the duration of therapeutic action of various ocular drugs. The typical pulse entry type drug release behavior observed with ocular aqueous solutions (eye drops), suspensions and ointments can be replaced.[2]

Niosomal vesicular drug delivery system facilitate prolonged and controlled drug action at the corneal surface along with controlled ocular delivery through prevention of drug metabolism mediated by enzymes present at tear/corneal epithelial surface. However, disadvantage of precorneal and nasolacrimal drainage is more often associated with such niosomal system in the ophthalmic drug delivery. Therefore, several *in-situ* gel forming systems have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. Such delivery systems consist of phase transition polymers that are in liquid form at the time of instillation into the eye and thereafter shift to the gel phase once it is in the *cul-de-sac* of the eye due to variations in physiological parameters. But, the drug release rate from these systems was not found to be sustained release type. So, to overcome the disadvantages associated with individual systems (niosomes and *in-situ* gel) and the usual barriers of conventional therapies along with controlled and patient complying drug delivery to the site of action, present research is an attempt to combine these two systems together.[3-4]

Vesicles prepared from self-assembly of hydrated non-ionic surfactants molecules are called niosomes. Niosomes and liposomes are equally active in drug delivery potential and both increase drug efficacy as compared with that of free drug. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy. These types of vesicles were first reported in the cosmetic industries. Nonionic surfactants used in formulation of niosomes are polyglyceryl alkyl ether, glucosyl dialkyl ether, crown ether, polyoxyethylenealkyl ether, ester-linked surfactants, spans and tweens etc. Niosomes preparation is affected by processes variables, nature of surfactants, presence of membrane additives and nature of drug to be encapsulated.[1]

EXPERIMENTAL AND RESULTS

Materials

Ciprofloxacin HCl was obtained as a gift sample from Aarti Drugs Limited, Mumbai and all other chemicals used in the study were of Analytical grade.

Preformulation studies

Organoleptic evaluation

The Organoleptic characteristics of Ciprofloxacin HCl were carried out and it was showed faintly yellowish to light crystalline powder.

Melting point

The melting point of Ciprofloxacin HCl was determined by capillary method and was found to be 309-312 ^{0}C which complies with the melting point reported in BP.

Solubility

Ciprofloxacin HCl was found to be freely soluble in methanol, propanol and sparingly soluble in water.

UV visible spectroscopy

The solution of Ciprofloxacin HCl in 0.1 N HCl was scanned from 400 to 200 nm. An absorbance maximum (λ_{max}) was found to be 276 nm which compiles with the λ_{max} reported in BP.[1]

Estimation of MIC of Ciprofloxacin HCl

Different concentrations of Ciprofloxacin HCl (1-10 µg/ml) were prepared and introduced into the series of nutrient broth tubes and inoculated with standard test organism *Staphylococcus aureus*, to find out the MIC of Ciprofloxacin HCl. MIC was estimated in terms of the lowest concentration of Ciprofloxacin HCl that prevents growth of a particular pathogen. The lowest concentration of drug resulting in no growth of microorganisms indicated by no turbidity after incubation for 24 h was considered as MIC of Ciprofloxacin HCl for the above said organism.[2]

Table 1: Minimum inhibitory concentration of Ciprofloxacin HCL.

S. No.	Volume of culture medium (ml)	Volume of test solution (ml)	Turbidity (after 24 h)
1	9.5	0.5	+
2	9.0	1.0	+
3	8.5	1.5	–
4	8.0	2.0	–
5	7.5	2.5	–
6	7.0	3.0	–
7	6.5	3.5	–
8	6.0	4.0	–
9	5.5	4.5	–
10	5.0	5.0	–
11	10.0 (control inoculated)	0	+
12	10.0 (no control inoculated)	0	-

Formulation and development niosomes

The method for the preparation of niosomes was selected by considering the criteria of highest entrapment efficiency and the release of drug. For this niosomes were prepared by ether injection method, ethanol injection method and thin film hydration method.[2]

In ether injection method, the weighed quantity of drug was added to the ether solution of span 60 and cholesterol (1:1). This solution was then injected drop by drop through 20 G needle in warmed solution of the phosphate buffer pH 7.4 with continuous stirring until complete evaporation of ether and allowed to mature overnight. Similarly, ethanol injection method was carried out to prepare niosomes by using ethanol as a solvent instead of ether.[2]

Niosomes by thin film hydration method were prepared by using vacuum rotary evaporator. In this technique weighed quantity of Ciprofloxacin HCI was added to the solution of span 60 and cholesterol (1:1) dissolved in solvent system of chloroform: methanol (2:1). Prepared solution was transferred to round bottom flask maintained at 60 ± 2^{0}C with 10 Pa vacuum and attached to rotary vacuum evaporator. Rotation of round bottom flask was continued till complete removal of solvent leaving behind thin film of residue in the flask. The dried film was then hydrated using phosphate buffer pH 7.4 (60^{0}C) with continuous shaking of flask. The flask was then bath sonicated and kept for maturation overnight.[2]

Preparation of Ciprofloxacin HCl loaded niosomes

A 3^2 full factorial design were used to study the effect of independent variables on quality attributes of niosomes. Independent variables have been selected on basis of highest entrapment and desired *in vitro* drug release.

In present study, thin film hydration method was used to prepare niosomes containing Ciprofloxacin HCl as it

gives better entrapment supported by percent entrapment efficiency study. For optimization of span 60 and cholesterol ratio, 3^2 full factorial design was used and niosomal batches (N1-N9) were prepared as per runs obtained in design using Design Expert 7.0 software. The independent variables selected were amount of span 60 (X_1) and amount of cholesterol (X_2).[2]

Table 2: Factorial design layout.

Factors (independent variables)	Levels used			Responses (dependent variables)
	-1	0	+1	
A = amount of span 60 (mg)	30	45	60	R_1 = % entrapment efficiency
B = amount of cholesterol (mg)	30	40	50	R_2 = *in vitro* drug release

Table 3: Formulation of niosomal batches as per 3^2 full factorial designs.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
Span 60 (X1) (mg)	30 (-1)	30 (-1)	30 (-1)	45 (0)	45 (0)	45 (0)	60 (1)	60 (1)	60 (1)
Cholesterol (X2) (mg)	30 (-1)	40 (0)	50 (1)	30 (-1)	40 (0)	50 (1)	30 (-1)	40 (0)	50 (1)
Ciprofloxacin HCL (mg)	30	30	30	30	30	30	30	30	30

Characterisation of niosomes

Microscopic examination

The niosomal suspension was placed on a glass slide covered with cover slip and examined for the structure and lamellarity under motic plus 2.0 microscope at magnification powers of (10X and 45X) and photomicrographs were recorded.

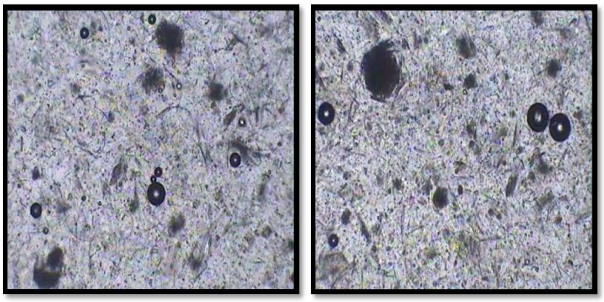

Fig 1: Photomicrograph of noisome at 10X magnification power.

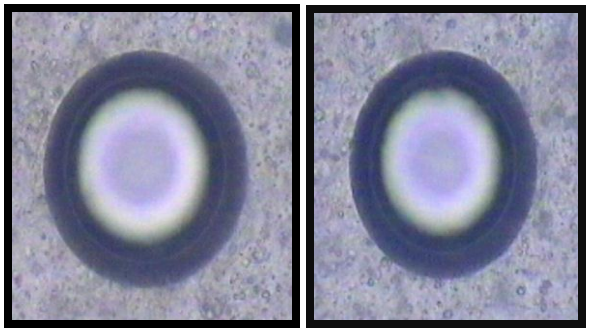

Fig 2: Photomicrograph of noisome at 45X magnification power.

Vesicle size

The mean particle size diameter and size distribution (polydispersity index, PI) was determined by Malvern Zetasizer nano. This instrument was based upon photon correlation spectroscopy (PCS) technique. The monochromatic coherent He-Ne laser with a fixed wavelength of 633 nm was used as the light source. The statistical intensity fluctuations in light scattered from particles were measured. These fluctuations were due to Random Brownian motion of the particles. Each sample was run 3 times and analysis was carried out at 25 ^{0}C with an angle of detection 173°.[2]

Entrapment efficiency

An Entrapment efficiency of Ciprofloxacin HCl niosomes were determined by centrifugation method. For removal of unentrapped drug, prepared niosomal suspension was centrifuged at 10000 rpm for 60 min and supernatant was decanted. Niosomes settled at bottom of centrifugation tube were re-suspended in freshly prepared phosphate buffer solution, centrifuged and same process was repeated until last detection of Ciprofloxacin HCl in the supernatant. Unentrapped drug content was estimated by analyzing supernatant decanted every time spectrophotometrically using UV spectrophotometer (Shimadzu, UV-1800, Thane). The amount of drug entrapped was determined by subtracting the unentrapped drug content from total drug added. The percent entrapment efficiency was determined by formula given below.[2]

$$\% \text{ Drug entrapped} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

Table 4: Characterization of noisome.

Batch code	Vesicle size range (µm)	Entrapment efficiency (%)*
N1	0.35 -2.69	53.60 ± 0.05
N2	0.35 – 2.08	55.78 ± 0.01
N3	0.35 – 2.72	54.81 ± 0.01
N4	0.35 – 2.39	61.01 ± 0.6
N5	0.35 – 2.08	66.23 ± 0.06
N6	0.35 – 2.11	63.82 ± 0.03
N7	0.35 – 2.34	53.57 ± 0.02
N8	0.35 – 3.07	60.23± 0.04
N9	0.35 – 3.21	58.79 ± 0.1

*indicates average ± standard deviation (n=3)

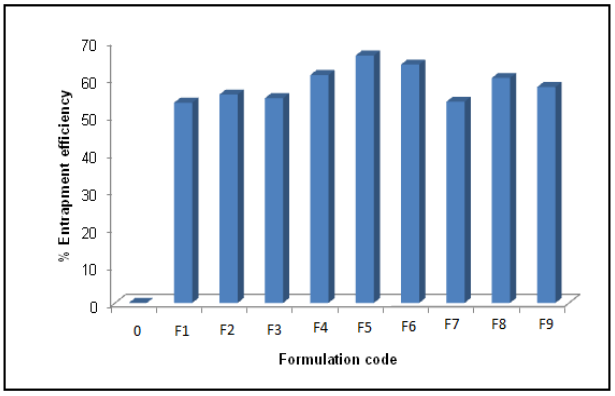

Figure 3: % Entrapment efficiency for formulation N1-N9.

***In vitro* release study**

The *in vitro* diffusion study was performed in a modified Franz diffusion cell of capacity 15 ml using cellophane membrane. The cellophane membrane was activated by keeping it in the mixture of phosphate buffer (pH 7.4) and ethanol for overnight. A section of membrane was cut, measured and placed on the receiver compartment. The donor compartment was filled with niosomal formulation. A 15 ml of aliquot of 4:6 (v/v) Ethanol: phosphate buffer (pH 7.4) was used as receptor medium. The receptor medium was maintained at 37 ^{0}C and stirred by a magnetic bar at 300 rpm. Aliquots of 0.5 ml of the receptor medium were withdrawn and immediately replaced by equal volume of fresh receptor solution at appropriate interval of 1 h up to 10 h. The 0.5 ml sample is diluted to 10 ml receptor medium and observed spectrophotometrically at λ_{max} 275nm.[2]

Treatment of drug release data with different kinetic equations

Different mathematical models were applied for describing the kinetics of the drug release process.

Table 5: Kinetic treatment of drug release data of various formulations F1-F9.

Formulation Code	Zero order model	First order model	Higuchi's model	Korsmeyer- Peppa's model
	R^2			
F1	0.9794	0.8514	0.8127	0.9935
F2	0.9527	0.7993	0.9444	0.9761
F3	0.9422	0.7844	0.9311	0.9755
F4	0.9657	0.8037	0.8981	0.9802
F5	0.9284	0.7679	0.9130	0.9697
F6	0.9070	0.7578	0.9633	0.9669
F7	0.9732	0.8198	0.7673	0.9871
F8	0.9351	0.8006	0.9712	0.9814
F9	0.9540	0.8176	0.9464	0.9878

The results of *in vitro* drug release profile obtained for all the ophthalmic formulations were plotted in modes of data treatment as Cumulative percent drug released versus time (zero-order kinetic model), Log cumulative percent drug remaining vs. time (First-order kinetic model), Cumulative percent drug released vs. square root of time (Higuchi's model) and Log cumulative percent drug released vs. log time (Korsmeyer-Peppa's equation).[5]

Optimization

The runs or formulations based on 3^2 full factorial designs were evaluated for the response variables. The response values were subjected to multiple regression analysis to find out the relationship between the factors and the response values. The response values subjected for this analysis were entrapment Efficiency (%) and *in vitro* cumulative drug release (%).[6]

The Entrapment Efficiency and *in vitro* cumulative drug release were chosen to analyze the relationship of effect of concentration of Span 60 and effect of concentration of Cholesterol.

Stability study

The optimized niosomal formulation OF10 were sealed in 30 ml clear glass vials and kept for stability study at refrigeration temperature (4 ± 2^{0}C), room temperature (25 ± 2^{0}C) and hot condition (45 ± 2^{0}C). After 7, 15, 30 and 45(d) the entrapment efficiency of each sample were determined and compared to the freshly prepared niosomes.[7]

Table 6: Stability study analysis.

Sampling intervals (d)	Cold	Room Temperature	Hot
OF10_ fresh	64.39 ± 0.13	64.39 ± 0.41	64.39 ± 0.22
OF10_7	63.84 ± 0.24	63.62 ± 0.32	61.13 ± 0.09
OF10_15	63.27 ± 0.56	63.03 ± 0.25	59.61 ± 0.27
OF10_30	62.78 ± 0.10	62.40 ± 0.33	53.22 ± 0.46
OF10_45	62.30 ± 0.18	61.83 ± 0.19	48.37 ± 0.52

Preparation of Ciprofloxacin HCL *in-situ* gel formulation

The prepared optimized niosomal batches OF10 were further processed to form *in-situ* gel formulation. For this combination of two polymers (Carbopol 940 and HPMC K4M) was selected to overcome the drawbacks associated with single polymer (Carbopol 934). These drawbacks are associated with higher Carbopol concentration and include formation of stiff gel after instillation in the eye and increased risk of stimulation in the eye tissue due to acidic nature of Carbopol. To overcome these drawbacks, the total content of single polymer must be reduced without compromising the gelling properties. This can be achieved with use of combination of two polymers in the formulation.[2]

Polymer solution was prepared by soaking the weighed quantity of Carbopol 940 and HPMC K4M in phosphate buffer pH 6.2 for 24 h. The gelling capacity was determined by placing a drop of the polymer solution in a vial containing 2 ml of freshly prepared simulated tear fluid (STF) equilibrated at 37^0 C.[2]

Table 7: Selection of Carbopol 940 and HPMC K4M ratio for *in-situ* gel formulation.

Batch	Concentration (% w/v)		Gelling capacity*	Viscosity (cP at 20 rpm)
	HPMC K4M 940	Carbopol		
1	0.5	0.2	—	889
2	0.6	0.2	—	1013
3	0.5	0.3	+	1151
4	0.6	0.3	++	1221
5	0.5	0.4	++	1292
6	0.6	0.4	+++	1321

*Where — no gelation, + gelation after few minutes, ++ immediate gelation retained for few hours, +++ immediate gelation retained for prolonged period

Thereafter, visual assessment of the gel formation was done and time required for gelation and dissolution of gel formed was noted.[8] The viscosity of solution was measured using Brookfield viscometer (DV-II+ Pro Viscometer, Bangalore) in small sample adaptor at 20 rpm and 25 ± 2^0C.

After removal of unentrapped drug, optimized niosomal batch (OF10) were dispersed in optimized Carbopol 940 and HPMC K4M polymer solution to form *in-situ* niosomal gel formulation. Furthermore, benzalkonium chloride as preservative and sodium chloride as to maintain isotonicity with tear fluid. Amount of sodium chloride to be added was calculated by using sodium chloride equivalent method.[2]

Evaluation of niosomal Ciproloxacin HCL *in- situ* gel

pH determination of the gel formulation

The pH of the gel formulation containing Ciprofloxacin HCl was measured using digital pH meter. The gel formulations were diluted in ratio 1:25 using distilled water. Standard buffer solution of pH 4, 7 and 10 were used for calibration of pH meter. The gel formulation was tested in triplicate to obtain mean pH value. The diluted gel was in contact with pH electrode for 10 minutes to allow the pH values to stabilize. The electrode was thoroughly washed between each sample.[8]

Viscosity determination of gel formulation

The viscosity of the gel formulations were determined by Brookfield viscometer using spindle no. 7. The gel sample was taken in a beaker and the dial reading was noted at 100 rpm for 60 s and at temperature of 30^0C.[9]

Drug content uniformity of the gel drug content

The drug content was determined by dissolving the 0.5 ml of in-situ gel containing dispersed niosomes in methanol to break the niosomal structure and release the entrapped drug. After suitable dilution with phosphate buffer pH 7.4, samples were analyzed spectrophotometrically using UV-visible spectrophotometer (Shimadzu, UV-1800, Thane) at wavelength of 275 nm and drug content was determined.[10]

Drug content uniformity in niosomal gel formulation (OF5) was determined by UV spectroscopy at λ_{max} 287 nm and was found to be 97.56 ± 0.73%. This ensures uniform distribution of drugs in gel formulation.[10]

Eye irritancy test

Three healthy rats were selected to evaluate the ocular irritancy effects of the optimized niosomal formulations. The animals were housed at a temperature and relative humidity of 20 ± 3°C and 50–60%, respectively, with 12-h light and 12-h dark cycle. For feeding, conventional laboratory diets were used with an unrestricted supply of drinking water. A niosomal formulation in which 2-3 drop was instilled into the conjunctival sac of left eye of each animal (initially to one animal) and the untreated eye served as a control. Each of the animals was observed visually with a slit lamp for the severity of ocular reactions such as corneal ulceration, iritis, conjunctival redness, and conjunctival edema at various intervals of 1, 24 and 48 h. The animal experiment was conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations.[11-15]

DISCUSSION

UV scanning and standard calibration curve of Ciprofloxacin HCl

From the standard calibration curve in 0.1 N HCl it was found that drug obeys Beer-Lamberts law in concentration range of 5-50µg/ml (figure2). The value of correlation co-efficient indicates the linear correlation between concentration and absorbance.

Estimation of MIC of Ciprofloxacin HCl

MIC of Ciprofloxacin HCl was found to be 4 µg/ml for *Staphylococcus aureus*, where no growth of microorganisms has been observed as indicated by no turbidity method. Estimation of MIC will be helpful in confirming the time required for release of sufficient amount of drug necessary for achievement of MIC to exemplify the therapeutic action.

Formulation and characterization of niosomal dispersion

Vesicle size

Vesicle size of niosomes (N1-N9) has been observed in between 0.35 to 3.21 µm which was in accordance with previously reported size for avoiding irritation to the eye.

Entrapment efficiency

The entrapment efficiency is the most important parameter from the pharmaceutical view point in the niosomal formulations. The use of cholesterol up to certain extent leads to an increase in the entrapment efficiency of niosomes. The improvements in the drug entrapment with the desired cholesterol content and the major reduction in drug entrapment when the cholesterol content was further increased may be due to two conflicting factors:

- With the desired quantity of cholesterol, the bilayer hydrophobicity and the stability increased and permeability decreased which may lead to the efficiently trapping of hydrophobic drug into the bilayers as the vesicles formed.
- In contrast, the higher amounts of cholesterol may compete with the drug for packing space within the bilayer, hence excluding the drug as the amphiphiles assemble into the vesicles.

The span 60 was found to have significantly higher entrapment efficiency. This could be due to the surfactant chemical structure. The span 60 having the highest phase transition temperature provided the highest entrapment of the drug and vice versa.

Stability study

Stability study indicates that the formulations are more stable at 4^0C and minimum leakage.

Drug release kinetics

The mechanism and kinetics of drug release are dependent on the composition of niosomes and the viscosity of the gelling polymer.

The *in vitro* drug permeation data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi and Korsmeyer models to ascertain the mechanism of drug release.

When the regression coefficient 'R_2' value of zero order and first order plots were compared, it was observed that the 'R_2' values of zero order were in the range of 0.96 to 0.97 drug release from all the formulations were found to follow zero order kinetics.

Characterization of niosomal in-*situ gel* formulation

pH and appearance

Addition of Carbopol leads into a slight decrease in pH of buffer system due to acidic functional groups present in Carbopol structure. The observed pH of *in-situ* gel formulation was within range of 6.69-6.72. Niosomal *in-situ* gel was appeared turbid when observed visually. This might be due to the dispersion of niosomes in polymer solution.

Drug content

Drug content of *in-situ* gel formulation was found to 97.89 ± 0.63 %. Additionally, response surface plot (Figure 10 b) clearly indicates profound effect of cholesterol in determining the drug content of *in-situ* gel.

Viscosity

Viscosity of optimized gel batch was found 1269 cps. It has been observed that cholesterol played a major role in deciding the viscosity of *in-situ* gel. Highest viscosity of gel leads to retarded drug release up to considerable extent. Intermediate viscosity of formulations has shown maximum retardation of drug release than for release. However, Carbopol and HPMC as polymer system have contributed majorly towards building viscosity of formulation formulations with highest viscosity indication other factors (membrane integrity) were also responsible for retarded drug.

Eye irritancy Test

It was observed that over the study period (48 h) none of the tested formulae showed any signs of redness, inflammation or increased tear production. Thus it could concluded that the non-ionic surfactants namely span 60, Cholesterol used in the niosomal formulations as well as the other excipients were non-irritant to the eye.

CONCLUSION

The UV Visible spectroscopic method was developed for the analysis of Ciprofloxacin HCl. The formulation of Ciprofloxacin HCl loaded niosomes were prepared by film hydration method as it was found to be well-suited and sound approach at laboratory scale to obtain the stable multilamellar vesicles. Niosomes were found to be spherical multilamellar vesicles in the size range of 0.35-3.07 µm. Variables such as amount of surfactant and the amount of cholesterol were found to have a profound effect on the percentage entrapment efficiency

and vesicle size. The niosomes were found to be more stable at refrigerator temperature than the room temperature. The formulations were optimized at pH slightly less than 7. This study provides the evidence that the niosomal vesicles are valuable as the ophthalmic delivery carrier to enhance the penetration and deposition of the Ciprofloxacin HCl.

ACKNOWLEDGEMENT

The authors are grateful to Dept. of Pharmaceutics, Sharad Pawar College of Pharmacy, Nagpur for providing necessary facilities to carry out the research work.

REFERENCES

1. Patel R, Patel K, Patel J, Patel H. Opthalmic drug delivery system; Der Pharmacia letter, 2010; 2; 100-15.
2. Gaikwad V, Gujar V, Rao P, Chaudari P. Formulation and evaluation of niosomal *in-situ* gel for Ophthalmic use. Am J PharmTech Res, 2012; 2: 2249-3387.
3. Nefise S, Mozafari M. Noisomes as nanocarrier system: M.R. Mozafari (ed), Nonmaterial Nanosystem for Biomedical Application, 2007; 67-81.
4. Budai M, Chapela P. Budai L, Wales M, Petricovies I, Zimmer A, Grof P, Szilasi M. Liposomal Oxytetracycline and Doxycycline: studies on enhancement of encapsulation efficiency. Drug Disco Ther, 2009; 3: 13-17.
5. P. Arunothayanun, I. F. Uchegbu, D. Q. M. Craig, J. A. Turton, A. T. Florence, *in vitro*: *in vivo* characterization of polyhedral niosomes, Int J Pharm, 1999; 183: 57–61.
6. Kaur I , Rana C, Singh M , Bhushan S , Singh H ,Kakkar S. Development and evaluation of Fluconazole. J Ocular Pharmacol Ther, 2012.
7. Manjunatha N, Naidu P, Sutrave V, Patel K, Samanta M. Preparation and evaluation of liposomes of an antiviral drug. Indian J Novel Drug delivery, 2009; 1: 25-31.
8. Kulkarni V, Shankar M, Rao B, Kumar P, Sandeep N. Formulation and evaluation of Latanoprost ophthalmic gels. J Glonal Pharma Tech, 2009; 183: 57–61.
9. Bartoletti A, Morand K, Barratt G, Brandely M, Chast F. Liposomal Amphotericin B eye drop to treat fungal keratitis. Int J Pharm, 2007; 150-53.
10. Hajdu M, Budai L, Budai M, Noszal b, Klebovich I, Istvan Antal, Beni S, Grof P. Gel and liposomes in optimized ocular drug delivery; Int J Pharm, 2007; 34-40.
11. Gevaria H. Formulation and evaluation of Betaxalol for ophthalmic drug Delivery system. In Handbook of Experimental Pharmacology, sears, MC (ed) spring verlag, 2013; 69: 116.
12. Morand K, Bartoletti A, Barratt G, Chast F. Formulation of Amphotericin-B eye drops to treat fungal keratiis. Int J pharm, 2007; 433: 150-53.
13. Prabhu P, Kumar N, Koland M, Dhondge G. Preparation and evaluation of nanovesicles of Brimonidine tartrate as an ocular drug delivery system.
14. Pandey VP, Deivasigamani K. Preparation and characterization of Ofloxacin nonionic surfactant vesicles for ophthalmic use. J Pharm Res, 2009; 2: 1330-1334.
15. Mudgil M, Gupta N, Nagpal M, Pawar P. Nanotechnology: A new approaches for ocular drug delivery system. Int J Pharm Sci., 2012; 4: 202.