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Formulation and Evaluation of a Sustained Release Dosage Form

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ABSTRACT

Most ocular diseases are treated with topical application of eye drops. After instillation of an eye drop, typically less than 5% of the applied drug penetrates the cornea and reaches intraocular tissues, while a major fraction of the instilled dose is absorbed and enters the systemic circulation. As ocular efficiency of topically applied drugs is influenced by the corneal contact time, most common method of improving ocular availability of drugs is to increase pre-corneal residence time by using vesicular system and hydrogel. One such approach to improve bioavailability of drug is the use of in-situ gelling system, which gets converted from sol-to-gel as a result of change in pH. By using Carbopol940 – a pH sensitive gelling agent, an attempt was made to develop niosomal *in-situ* gelling systems of Norfloxacin, a fluoroquinolone antibiotic; useful in the treatment of bacterial conjunctivitis, to increase the ocular residence time of drug. HPMC K15M was incorporated as viscosity enhancing agent. The promising formulations M1, M2, M3 and M4 were evaluated for pH, drug content, *in vitro* gelation, *in vitro* drug release, *in vivo* drug release, viscosity and stability. Percent drug content of 82.5%, 86.7%, 85.3% and 90.8%; viscosity of 320cP, 405cP, 440cP and 500cP at 100 rotation per minute (RPM), cumulative percent release of 37.9%, 47.7%, 48.2% and 49.7%, was observed for formulation M1, M2, M3 and M4 respectively. The developed formulations were therapeutically efficacious, stable, non-irritant and provided sustained release of the drug for 12 hours.

Introduction

Ophthalmic drug delivery is one of the most interesting challenges faced by pharmaceutical scientists. Successful delivery of drugs into the eye is extremely complicated because the eye is protected by a series of complex defense mechanisms, which make it difficult to achieve an effective concentration of the drug within the target area of the eye [1]. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy [2]. A significant challenge to the formulator is to bypass the protective barriers of the eye without causing permanent tissue damage.

Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result in poor bioavailability of drug in the ocular cavity. The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration [3]. The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems such as vesicles and particulates, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing pre-corneal drug loss which can be achieved by amphiphilic vesicles [4].

Niosomes are non-ionic surfactant vesicles obtained by hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs [5]. Niosomes are promising vehicle for drug delivery and being non-ionic; they are less toxic and improve the therapeutic index of drug by restricting their action to target cells.

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolonged period of time [6]. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of a gel system that are instilled as drops into the eye and undergo a sol to gel transition in the cul-de-sac. The present work is intended to formulate and evaluate niosomal in-situ hydrogel of Norfloxacin with increased lipophilicity as well as reduced particle size that will enhance corneal permeation of the drug. Carbopol940 and HPMC K15M were used as gelling agent and viscosity enhancing agents, respectively. The sol to gel conversion of formulation will allow ease of administration and improved residence time of drug in the eye.

Materials And Methods:

Norfloxacin was obtained as a gift sample from Cipla Ltd., Vikhroli. HPMC K15M was also obtained as a gift sample from Colorcon. Spans, cholesterol, carbopol940 and other excipients were purchased from Molychem and dialysis membrane was purchased from Hi-Media.

Preparation of Niosomes by thin film hydration method:

Accurately weighed quantity of Norfloxacin was taken in a beaker and dissolved in chloroform and methanol in 2:1 ratio. Then required quantity of surfactant and cholesterol were dissolved in above drug solution. The solution was transferred to round bottom flask (RBF) which was attached to a rotary vacuum evaporator (Rotavap) as shown (fig. 1). The organic

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solvent mixture was allowed to evaporate under very low vacuum. Temperature was maintained at 60°C and solution stirred at 100 RPM for the entire duration of the process till a thin film was formed on the wall of RBF (approximately 1 h). After complete evaporation of the organic solvent, vacuum was switched off. Phosphate buffer saline (10ml) was added into the RBF and allowed to rotate for half an hour for hydration of the film to form niosomes [7]. (Table 1)

Table 1: Composition of phosphate buffer saline (PBS) PH7.4

Ingredient	Quantity
Disodium Hydrogen Phosphate	2.38g
Potassium Dihydrogen Phosphate	0.19g
Sodium Chloride	8.0g
Distilled Water	1000ml

Niosomes with different Cholesterol:Surfactant molar ratio:

Initially, a test batch was prepared to check the ease of formation of niosomes. Then, various batches were prepared with different Cholesterol: Surfactant molar ratios as given (Table 2, 3, 4 and 5).

Table 2: Niosomes With Varying Cholesterol: Surfactant Molar Ratio (SPAN20)

Sr. No.	Cholesterol:Span20	Drug (mg)	Cholesterol (mg)	Span 20 (mg)
F1	1:1	30	193	173
F2	1:2	30	127	228
F3	1:3	30	96	259
F4	1:4	30	77	276

Table 3: Niosomes with varying cholesterol: surfactant molar ratio (SPAN40)

Sr. No.	Cholesterol:Span40	Drug (mg)	Cholesterol (mg)	Span 40 (mg)
F5	1:1	30	193	201
F6	1:2	30	127	265
F7	1:3	30	96	302
F8	1:4	30	77	322

Table 4: Niosomes with varying cholesterol: surfactant molar ratio (span60)

Sr. No.	Cholesterol:Span60	Drug (mg)	Cholesterol (mg)	Span 60 (mg)
F9	1:1	30	193	215
F10	1:2	30	127	284
F11	1:3	30	96	322
F12	1:4	30	77	344

Table 5: Niosomes with varying cholesterol:surfactant molar ratio (SPAN80)

Sr. No.	Cholesterol:Span80	Drug (mg)	Cholesterol (mg)	Span 80 (mg)
F13	1:1	30	193	214
F14	1:2	30	127	283
F15	1:3	30	96	321
F16	1:4	30	77	343

Evaluation of Niosomes:

The prepared niosmes were evaluated for drug entrapment efficiency by centrifugation method. The shape and surface morphology were observed by scanning electron microscopy. Particle size distribution and zeta potential were assessed by particle size analyzer and zeta sizer, respectively.

Preparation of niosomal in-situ gel:

On the basis of drug entrapment efficiency and morphology, the batch which gave maximum entrapment and good surface morphology was selected for preparation of in-situ gel. The appropriate quantity of Carbopol940 and HPMC K15M were sprinkled over niosomal dispersion under constant stirring with a glass rod, taking care to avoid formation of lumps and allowed to hydrate. Other ingredients were incorporated and triethanolamine was added in sufficient quantity to adjust the pH (Table 6).

Table 6: Composition of niosomal in-situ gel of norfloxacin

S.No.	Ingredients	M1	M2	M3	M4	Role
1	Niosomal dispersion eq. % w/v	0.3	0.3	0.3	0.3	Vehicle
2		0.4	0.5	0.4	0.5	Gelling agent
3		0.4	0.4	0.5	0.5	
4	Carbopol940 % w/v	0.01	0.01	0.01	0.01	Viscosity enhancing agent
5	HPMC K15M % w/v	q.s.	q.s	q.s.	q.s.	Preservative
	Benzalkonium chloride % v/v					pH adjusting agent
	Triethanolamine					

Evaluation of niosomal in-situ gel:

The prepared niosomal in-situ gel was evaluated for various physicochemical properties such as visual appearance, clarity, color and gelling capacity. The viscosity of the formulation before and after gelation was assessed by using Brookfield viscometer with small sample adapter.

Drug content analysis:

Drug content of niosomal in-situ gel was determined by taking in-situ gel containing a known amount of drug, dissolving the contents in absolute ethanol and diluting with STF pH7.4. Then the absorbance was measured using STF as blank by UV spectrophotometer at 272nm to calculate the percentage drug content.

In-vitro drug release study:

The Franz diffusion cell was used to study the drug release of in-situ gel (fig. 2). Optimized batches were used for drug release study. The buffer salts were dissolved in distilled water as per the quantity given (Table 7). Dialysis membrane was soaked overnight in simulated tear fluid. Sample was applied on membrane and then membrane was placed between donor and receiver compartment of the cell. Aliquots of 1ml were withdrawn at 0,1,2,…, 12hrs and absorbance read at 272nm [8].

Table 7: Composition of STF

Ingredient	Quantity
Sodium Chloride	0.678g
Sodium Bicarbonate	0.218g
Potassium Chloride	0.138g
Calcium Chloride Dihydrate	0.0084g
Distilled Water	100ml

Antimicrobial activity of niosomal in-situ gel:

Antimicrobial study was carried out to check antibacterial efficiency of Norfloxacin in-situ gel. The test organisms used were Escherichia coli and Staphylococcus aureus. Growth media used was Nutrient Agar. The cup plate method was used to carry out antimicrobial study. The method is based on the principle of diffusion of drug from vertical cup through solidified agar layer in petri plate to an extent such that growth of the added microorganisms is prevented entirely in zone around the cup containing a solution of test substance. After incubation, plates were observed for zone of inhibition and observations were recorded [9].

Stability studies:

The formulations M3 and M4 showed higher % drug release and were kept for accelerated stability study at 25°±2°C/ 60±5% relative humidity (RH) and 4°±2°C for 3 months. The formulations were analyzed every 15 days for appearance, pH, gelling studies and % drug content [10].

Results And Discussion:

In this research work, ophthalmic niosomal in-situ gel of Norfloxacin was prepared to sustain the release of drug and

increase the drug residence time in the eye. From evaluation of formulation, following conclusions can be drawn:

The results for % entrapment efficiency of all the prepared batches are shown (fig. 3, 4, 5 and 6). Of all the above batches, F10 showed maximum drug entrapment efficiency in the order of Span60>Span40>Span80>Span20. Increase or decrease in surfactant concentration showed no linear relationship with entrapment efficiency. The decreased entrapment efficiency in Span20 and Span80 is due to the presence of unsaturated alkyl chain. Span40 and Span60 showed higher entrapment efficiency than the other surfactants due to high phase transition temperature. The only drawback of Span60 is rapid leakage of drug from the vesicles. The formulations with decreased cholesterol content (F4, F8, F12 and F16) had poor entrapment efficiency. The incorporation of cholesterol into bi-layer composition of niosomes induces membrane stabilizing activity. Cholesterol has the ability to cement the leaking space in the bi-layer membranes. Hence, incorporation of cholesterol into bi-layer increases entrapment efficiency.

On the basis of results of entrapment efficiency, F10 batch was subjected to SEM study. Most of the vesicles were found to be spherical in shape and within the range of size required for ophthalmic formulation (fig. 7).

From the graph, average particle size was found to be 1.5µm, which is acceptable size range for ophthalmic formulation (fig. 8). The negative value of zeta potential i.e. -40.7mV indicates that the formulation is stable (fig. 9).

Clarity of all the formulations was found to be satisfactory. The pH of the formulations was found to be satisfactory and was in the range of 6.2-6.8. The formulations were liquid at room temperature and at the pH formulated (Table 8).

Table 8: Results of color, clarity and pH

Formulations	Color	Clarity	pH
M1	Yellowish white	Clear	6.2
M2	Yellowish white	Clear	6.1
M3	Yellowish white	Clear	6.4
M4	Yellowish white	Clear	6.8

Gelling capacity of M4 was found to be most among all the formulations (Table 9) and the drug content was found to be in acceptable range for all the formulations (Table 10).

Table 9: Results of gelling capacity

Formulations	Gelling capacity
M1	+
M2	+
M3	++
M4	+++

Table 10: Results of % drug content

Formulations	% Drug content
M1	82.5
M2	86.7
M3	85.3
M4	90.8

The results for the viscosity measurements of the formulations before and after gelation are shown (Table 11 and 12). The rheological study of the formulations indicates that the viscosity decreases upon increasing the shear rate due to the pseudo-plastic behavior of the formulations.

Table 11: Data of viscosity measurements before gelation

Angular velocity (rpm)	Viscosity (Cp)			
	M1	M2	M3	M4
10	550	825	1120	1380
20	380	640	790	860
50	260	420	440	485
100	190	240	260	280

The % CR at 12th h of all the formulations is given (Table 13). When compared with all the formulations, M4 showed slower but maximum drug release due to high gelling capacity. For the drug to be released in the medium, it has to pass through the vesicle wall and then through the hydrogel matrix. Initially, the un-entrapped drug was released from the in-situ gel which can serve as the initial loading dose. Thereafter, the niosomes can release drug in a sustained manner making the formulation suitable for once a day dosing.

Table 12: Data of viscosity measurements after gelation

Angular velocity (rpm)	Viscosity (Cp)			
	M1	M2	M3	M4
10	1320	1405	2600	2020
20	710	1110	1320	1545
50	460	680	735	890
100	320	405	440	500

Table 13: values of % cumulative release

Formulation	% CR
M1	37.9
M2	47.7
M3	48.2
M4	49.7

Table 14: Result of antimicrobial study of niosomal in-situ gel and marketed eye drop

Formulation	Microorganism	Zone of inhibition (mm)
Niosomal in-situ gel (10µg/ml)	Staphylococcus aureus	24
	Escherichia coli	25
Marketed eye drop (10µg/ml)	Staphylococcus aureus	25
	Escherichia coli	30

Table 15: Results of ph for formulations kept for stability study

Time in days	pH			
	M3		M4	
	4±2^{o}C	25±2^{o}C	4±2^{o}C	25±2^{o}C
0	6.4	6.4	6.8	6.8
15	6.49	6.52	6.84	6.9
30	6.58	6.68	6.9	7.05
45	6.62	6.79	7.02	7.21
60	6.68	6.9	7.12	7.25
90	6.75	7.35	7.18	7.3

Antimicrobial activity of formulation was done (Table 14). Zone of inhibition for niosomal in-situ gel and marketed eye drop (standard) was measured. It was found to be comparable to the standard (fig. 10 and 11).

Table 16: Results of gelling capacity for formulations kept for stability study

Time in days	Gelling capacity			
	M3		M4	
	4±2^{o}C	25±2^{o}C	4±2^{o}C	25±2^{o}C
0	++	++	+++	+++
15	++	++	+++	+++
30	++	++	+++	+++
45	+	++	++	+++
60	+	+	++	+++
90	+	+	++	++

The formulations M3 and M4 were kept for stability study as per ICH guidelines. Niosomes are unstable at higher temperatures. Therefore, the stability study was carried out only at two temperatures; at 4±2^{o}C and at 25±2^{o}C. The formulations were evaluated for appearance, pH, gelling capacity and % drug content (Table 15, 16 and 17). The stability study indicates that the % drug content of formulations stored at 4±2^{o}C was more than at 25±2^{o}C (fig. 12 and 13). Thus, increase in temperature

and storage period decreases the % drug content of the formulation.

Table 17: Results of % drug content for formulations kept for stability study

Time in days	% Drug content			
	M3		M4	
	4±2°C	25±2°C	4±2°C	25±2°C
0	85.3	85.3	90.8	90.8
15	82.7	79.9	87.9	87.4
30	78.6	71.7	86.3	78.3
45	75.5	68.1	82.7	74.6
60	74.1	56.2	79.2	66.6
90	69.2	47.9	76.1	58.6

Present work resulted in the formulation of a stable Niosomal in-situ gel that undergoes sol to gel transformation at physiological pH. Further studies are required on animal models to correlate the results of in-vitro studies.

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