Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X

RESEARCH ARTICLE

Received on: 14-11-2014
Accepted on: 15-01-2015
Published on: 21-01-2015

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QR Code for Mobile users

Conflict of Interest: None Declared !

DOI: 10.15272/ajbps.v4i40.634

Development and evaluation of an ocular niosomal delivery system for some newly synthesized Beta blockers.

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Abstract

Topical beta-blocking agents are nowadays the drugs of choice as these have few ocular and systemic side effects. In the present study some selected beta blockers (PP-24, PP-34, PP-41, PP-48, PP-50), synthesized using 4-hydroxybenzaldehyde and isovanillin were encapsulated into niosomes possessing a high drug entrapment efficiency in order to be used as ophthalmic carriers for topical ocular treatment. Prepared niosomes were characterized for morphology, number of vesicles per cubic millimeter, entrapment efficiency and drug content. Incorporation of the drug into respective niosomal preparation was confirmed by DSC. TEM studies showed that vesicles were small in size, round in shape, bilamellar/unilamelar without any aggregation. No significant variation in particle size and drug content of prepared niosomes was observed, upon storage, over a period of 42 days at 4-8°C. Ex vivo permeability studies indicated that PP-24 formulation showed the maximum enhancement in corneal permeability (27.29%) as compared to the free drug (11.33%). Onset of action of all the developed formulations was 0.5 h which was comparable to timolol. Formulation PP-24 produced sustainable and better IOP lowering effect than formlation PP-34. This may be attributed to lower particle size and better entrapment efficiency of the PP-24 niosomal formulation.

Cite this article as:
Poonam Piplani, Pramod Kumar, Aditi Rohilla, Shaffali Singla, Indu Pal Kaur. Development and evaluation of an ocular niosomal delivery system for some newly synthesized Beta blockers. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (40); 2014 , 8-17.

INTRODUCTION

Ocular drug delivery has met with significant challenges posed by various ocular barriers that are inherent and unique to ocular anatomy and physiology making it an exigent task for drug delivery scientists. These barriers are specific depending upon the route of administration viz. topical, systemic and parenteral. Various preformulation and formulation factors need to be considered while designing an ophthalmic formulation (1).

β-Adrenergic blocking agents are the mainstay in glaucoma treatment. Glaucoma has been characterized by an increased intraocular pressure (2). The use of β-blockers in glaucoma began with the discovery of propranolol followed by betaxolol, levobunolol, carteolol, timolol and metipranolol (3,4). Although, all these drugs are applied topically, but they may enter the general circulation and reach concentrations high enough to elicit systemic effects, most serious of which are respiratory side effects. The simplest method to minimize the amount of drug in the systemic circulation following topical dosing is by keeping the eye closed after dosing and also by applying fingertip pressure to the inner corner of the eye to occlude the nasolacrimal drainage system. An alternative strategy is modifying the formulation of the drug.

Thus, drug delivery of ocular therapeutics is a tedious task as the treatment of ocular infections requires frequent topical drug administration. In order to overcome the problems of conventional ocular therapy such as short residence time, loss of drug through nasolacrimal drainage, impermeability of corneal epithelium and frequent instillation; newer ocular delivery systems are being explored by many researchers. Vesicular drug delivery systems used in ophthalmics broadly include liposomes and niosomes. Vesicles, consisting of one or more surfactant bilayers enclosing aqueous spaces, have been of particular interest because they offer several advantages over liposomes like that of chemical stability, lower cost and availability of materials as they require no special conditions such as low temperature or inert atmosphere for protection or storage (5,6).

A number of aryloxyaminopropanol derivatives were synthesized as antiglaucoma agents in our laboratory using 4-hydroxybenzaldehyde and isovanillin as the starting materials. The formyl group was allowed to introduce various substituents such as oxime and amino functions. The phenolic group was elaborated to the oxypropanolamine side chain normally present in β-adrenergic blocking agents. Tert-butylamino moiety and isopropylamino moiety were introduced in the oxyaminopropanol side chain. From the various synthesized compounds, five compounds PP-24, PP-34, PP-41, PP-48 and PP-50 (Fig. 1) were selected for the development of niosomal formulation on the basis of their IOP lowering effect and their physico-chemical properties. The purpose of the current study was to prepare encapsulated niosomes of beta-blockers possessing a high drug loading capacity in order to be used as ophthalmic carriers for topical ocular infection treatment.

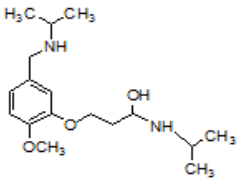
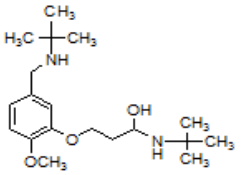
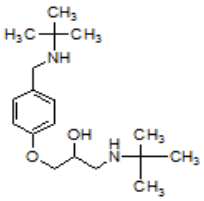
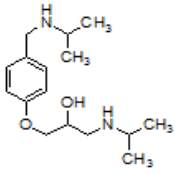
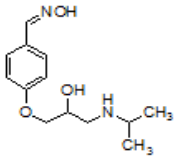
Compound code	Structure
PP-24	
PP-34	
PP-41	
PP-48	
PP-50	

Fig. 1: Structures of compounds synthesized.

METHODS

Materials:

Non-ionic surfactant (NIS) Sorbitan i.e. Span 60, chloroform and solvent ether were obtained from S.D. Fine Chemicals Ltd (Mumbai, India) and cholesterol was obtained from Sigma Aldrich Chemie GmbH. All other reagents used in the study were of analytical grade.

Preparation of niosomes:

There are two methods by which niosomes were prepared: Ether injection method and Reverse phase evaporation method.

Ether injection method

Non-ionic surfactant (NIS) Sorbitan i.e. span 60 (S60) and cholesterol were taken in the ratio of 1:2 and dissolved in ether (10 ml). The compounds PP-24, PP-34 and PP-48 which were soluble in aqueous phase were dissolved in TDW (10 ml). The ether phase was slowly injected into the preheated aqueous phase (60 ^{0}C) which was stirred continuously on a magnetic stirrer, using an 18 gauge needle with the rate of addition maintained at approximately 0.25 ml/min. The developed vesicles were stored in hydration media viz. TDW. The compounds PP-41and PP-50 which were not soluble in aqueous phase were dissolved in organic phase (ether). Rest steps were same as explained above.

Reverse phase evaporation (REV) method

NIS span 60 (S60) and cholesterol in a ratio of 1:2 were dissolved in a mixture (12 ml) of ether and chloroform (2:1). The compounds PP-24, PP-34 and PP-48 which were soluble in aqueous phase were dissolved in TDW respectively. Aqueous phase was added such that the organic to aqueous phase ratio was 3:1. The mixture was then sonicated for 5 minutes using a probe sonicator. A stable emulsion so formed was dried in a rotary evaporator at 60^{0}C till a semi solid gel like structure was formed. Gel was then shaken vigorously on the vortex mixer and the resultant viscous dispersion was diluted suitably with TDW to make up the volume to 4 ml (volume of aqueous phase used initially).

Characterization of niosomal vesicles:

Morphology and structure of vesicles:

The morphological characters (viz. shape, uniformity and lamellarity) of prepared vesicles were monitored employing fluorescence microscopy (Nikon eclipsed i80; magnification 40X). Selected vesicles were observed under a fluorescence microscope. A thin layer of the diluted vesicular dispersion was spread over the glass slide and after covering it with a coverslip, were optically observed for structural attributes such as lamellarity, uniformity of size, shape and physical stability characteristics i.e. aggregation and/or irregularity.

Number of vesicles per cubic millimetre:

Vesicles were suitably diluted with water and the number of vesicles per cubic mm were counted by fluorescence microscopy using a hemocytometer (7). The vesicles in eighty small squares were counted and numbers of vesicles/mm^3 were calculated by using the formula given below.

Total no. of vesicles per cubic mm =

$$\frac{\text{Total no. of vesicles counted x Dilution Factor x 400}}{\text{Total number of squares counted}}$$

Entrapment efficiency of the vesicles:

The entrapment of the drug by the vesicles was determined using dialysis method and ultracentrifugation technique. Percentage entrapment was calculated as given below, using IPA to disrupt the vesicles.

Percentage entrapment:

$$\text{Percentage entrapment} = \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \times 100$$

Drug content:

IPA (Isopropyl alcohol) was chosen as a suitable solvent for disrupting the prepared vesicles. 1 ml of the aqueous dispersion was suitably diluted with IPA and the absorbance was recorded at respective λmax.

Transmission electron microscopy (TEM):

Vesicles were further evaluated using TEM for lamellarity, uniformity of size, shape and physical stability characteristics i.e. aggregation and/or irregularity at an accelerated voltage of 100 kV. A drop of the sample was placed on a carbon-coated copper grid to leave a thin film on the grid. The excess of the solution was drained off with a filter paper. The grid was air dried thoroughly and samples were viewed under TEM. Selected vesicles (at magnification 20000 X and 200000 X) were further evaluated for the aforesaid structural attributes.

Vesicle size and size distribution:

The size and distribution of vesicles was determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetamaster, ZEM 5002, Malvern, U.K.).

pH of the formulations:

pH of the formulations were measured using Cyberscan, Eutech pH 510, at 25^{0}C.

Differential scanning calorimetry:

Samples of pure compounds PP-24, PP-34, PP-41, PP-48 and PP-50 and all excepients (cholesterol and span 60) were scanned using differential scanning calorimeter (DSC) and thermograms so generated were analyzed for any significant shift in the peak, disappearance of a peak, or appearance of any new peak, with respect to niosomal formulations. Samples were weighed in an aluminium pan and heated from 25-30^{0}C at a rate of 10 ^{0}C /min.

Zeta potential:

Zeta potential of the vesicular dispersions was measured using Malvern's zetasizer. The measurements were done at 25^{0}C and the electric field strength was around 23.2 V/cm. The zetasizer measures the zeta potential based on the Smoluchowski equation.

$\zeta = UE\ \eta/\varepsilon$

Where ζ is zeta potential, UE is electrophoretic mobility, η is viscosity of the medium and ε is dielectric constant.

Stability of formulation:

The formulations were kept in 15 ml sealed glass ampoules and stored under different conditions (refrigerator i.e. 4-8 ºC, ambient room temperature and at 45 ± 2 ºC). Aliquout samples from the formulations were withdrawn at definite time intervals and analyzed spectrophotometrically at the respective λmax. of the selected compounds for the extent of entrapment.

***Ex vivo*/Corneal permeability studies:**

GBR (Glutathione Bicarbonate Ringer) was used as the diffusion medium considering that it acts as a simulating tear fluid (STF). This solution is reported to preserve the integrity of the excised cornea for upto 6 h.

Solutions were stored in a refrigerator and used within 3 weeks of preparation. The final solution was equilibrated at 35 ± 0.5^{0}C, aerated for 5 minutes before use and the pH of the solution was adjusted between 7.2 - 7.4 by passing CO2 (CaCO3 was taken in Buchner flask and concentrated hydrochloric acid was added drop wise using a dropping funnel).

Preparation of cornea:

The whole eye bulbus (obtained from the local slaughter house) was enucleated from its socket immediately after slaughtering the pigs. For preparation, the bulbus was placed with the corneal surface facing upwards so as to avoid contamination of the epithelial surface or physical trauma to the tissue. Starting at a distance of approximately 5 mm from the corneal rim the sclera was incised until the vitreous body was reached. The cornea was excised circularly at that distance. The whole anterior part of the eye was lifted, lens and iris still attached to the corneal part. This was important in order to avoid corneal wrinkling. The iris/cilliary body and lens were carefully removed using forceps taking care that the cornea remained well shaped. The latter was washed in the ringer solution before being mounted onto the apparatus. It was ensured that the cornea remains in its place by lightly coating the rim of the donor compartment with an adhesive and then the two compartments were fixed together using hooks (on the assembly) and rubber bands.

Method:

For the in vitro corneal permeability studies, membrane diffusion technique was used. Studies were conducted within a jacketed cell, maintained at a constant temperature (37 ± 0.2^{0}C), under mixing conditions using a magnetic stirrer. The cell used was a two-limbed reservoir; on one limb of which cornea was mounted and the other limb was used as the sampling port (volume = 20 ml). The preparation (0.2 ml) to be studied was placed on the cornea. The cornea was mounted within half an hour of sacrifice of the animals. Diffusion medium used was freshly prepared GBR solution equilibrated at 37 ± 0.2^{0}C, pH 7.4. Aliquots of the medium were withdrawn after a fixed time interval from the sampling port and were replaced with equal quantity of fresh GBR to maintain a constant volume. Sink conditions were maintained throughout the study. Samples were analyzed spectrophotometrically at the respective λmax of the compounds.

The apparent corneal permeability coefficient (Papp) of different formulations was determined according to the following equation

$$P_{app} = \frac{\Delta Q}{\Delta t.60.A.C_O}(cms^{-1})$$

where $\frac{\Delta Q}{\Delta t}$ is the steady state slope of the linear portion of the plots of the amount of drug in the receiving chamber (Q) vs. time (t). A is the exposed corneal surface area (1.327 cm2), the initial concentration of drug in the donor cell and 60 represents the conversion of minutes to seconds.

Pharmacodynamic evaluation (Intraocular pressure lowering effect):

Animals:

Adult male rabbits weighing 1.5-2.0 kg were used for the studies and the permission for conducting these experiments was obtained from Institutional Animal Ethical Committee. The rabbits were provided with food and water in a temperature controlled room. All rabbits used in these experiments were normotensive and were housed in proper conditions. They were exposed to the normal (ambient) light and dark cycles. Intraocular pressure (IOP) was measured using a Reichert non-contact hand-held pneumatonometer. All IOP measurements were carried by the same operator, using the same tonometer. IOP was measured three times at each time interval and the means were taken. The animals used, were accustomed to the experimental procedure. The only restrain was the hand of the investigator lightly on the back and shoulder of the rabbit.

Method:

Formulations were instilled topically into the upper quadrant of the eye and the eye was manually blinked three times, one eye received 40 µl of the formulation, and the contralateral eye served as the control. The IOP was measured immediately prior to giving the drug, and at fixed time intervals. Each formulation was tested on a group of at least five healthy male rabbits. Each animal was given a washout period of three days after every treatment.

Change in IOP (ΔIOP) is expressed as follows

ΔIOP= IOPdosed eye- IOPcontrol

ΔIOP is reported as the mean ± S.E.M.

Statistical analysis:

The raw data obtained from in-vitro studies were analyzed by applying correction factor for volume and drug losses during sampling using the following equation

$$Ac = Au + \left(\frac{V_s}{V_t}\right)\sum_{i=1}^{n-1} Ai$$

For the nth sample:

Vs = Volume of the sample withdrawn Ac = Corrected absorbance

Vt = Total volume of receptor medium Ai = Uncorrected absorbance

Au = Uncorrected absorbance of the nth sample

All results are expressed as the mean ± S.D. The results were analyzed for statistical significance by one way ANOVA test followed by Tukey's test or student's pair t-test wherever applicable.

RESULTS

Solubility studies:

Solubility studies of the selected compounds were performed in various solvents as per I.P 2007. This study was done to select a suitable medium which would give perfect sink condition during ex-vivo permeation study. Compound PP-24 was freely soluble in TDW and GBR, while the compounds PP-34 and PP-48 were soluble in TDW and GBR. Three compounds PP-24, PP-34 and PP-48 were also soluble in IPA. Compounds PP-41 and PP-50 were freely soluble in 50 % ethanol, 50 % ethanol in GBR and IPA but insoluble in TDW.

Standard plot:

UV- spectroscopic method is reported in literature for the analysis of beta blockers (8). Keeping in mind the ease and low cost, UV- spectroscopic method was selected as a method of analysis for the selected compounds to calculate their extinction coefficient ( ). Standard plot of the compounds PP-24, PP-34, and PP-48 were prepared in three solvents viz. triple distilled water (TDW), isopropyl alcohol (IPA) and glutathione bicarbonate ringer (GBR) solution respectively. While the standard plot of the compounds PP-41 and PP-50 were prepared in 50% ethanol, 50% ethanol in GBR and isopropyl alcohol (IPA) respectively.

For compounds PP-41 and PP-50, 50% ethanol was used as the medium for entrapment studies. In order to provide sink condition in release studies, 50% ethanol in GBR was used as the medium for compounds PP-41 and PP-50. TDW was used as hydration media while IPA was used for disrupting the vesicles (Table 1).

Preparation of niosomes:

Two methods were selected to prepare these vesicular systems: ether injection method and reverse phase evaporation method. But the ether injection method proved to be more suitable because the number of vesicles per cubic mm were more with this method (Table 2).

Compound code	Solvent	λ_{max}	$E^{1\%}_{1cm}$	Linearity
PP-24	TDW	230	720 ± 0.02	0.998
	GBR	230	720 ± 0.02	0.999
	IPA	231	750±0.01	0.999
PP-34	TDW	230	940±0.02	0.998
	GBR	230	930±0.02	0.999
	IPA	231	990±0.03	0.998
PP-48	TDW	225	1230±0.05	0.999
	GBR	225	1300±0.07	0.999
	IPA	227	1000±0.06	0.999
PP-41	50% ethanol	225	930±0.02	0.998
	50% ethanol in GBR	225	920±0.02	0.999
	IPA	225	1590±0.07	0.999
PP-50	50% ethanol	265	1840±0.08	0.998
	50% ethanol in GBR	265	1830±0.06	0.999

Table 1: Data for Standard plot.

Ether injection method		Reverse phase evaporation method	
Needle gauge:	18	Sonication (Amplitude):	98
Speed of injection:	0.25 (ml/min.)	Time of sonication:	5 (min.)
Stirring:	100 (rpm)	Temperature for thin film formation:	60 (ºC)
Temperature:	60 (^{0}C)	Speed of rotation:	90-100 (rpm)
Number of vesicles per cubic mm (n=3):	158.8± 0.03 (No .of vesicles/mm3x102)	Time for hydration:	15-20 (mins)
		Time for swelling:	Overnight
		Number of vesicles per cubic mm (n=3):	128.8±0.04(No. of vesicles/mm 3x102)

Table 2: Various process parameters for the preparation of vesicles and the final selected values are enlisted.

Number of vesicles per cubic mm:

Niosomes prepared from different methods were diluted twenty-five times with TDW and the number of niosomes per cubic mm was counted by optical microscopy employing a haemocytometer.

Turbidity studies:

Using UV spectrophotometer, % transmittance of various formulations was determined keeping the ratio of cholesterol: span60 constant. Lower the transmittance higher the number of vesicles. Amongst all the formulations, PP-24 showed least %

transmittance (58.34%), thereby, indicating highest number of vesicles.

Entrapment efficiency of the vesicles:

The entrapment studies were carried out after separating the non-vesicular free drug. For studying drug separation, ultracentrifugation technique was used for PP-24, PP-34 and PP-48 as these drugs are freely soluble in TDW. The formulation was rotated at a speed of 35,000 rpm for 2 h at 4°C in an ultracentrifuge. This resulted in the formation of a pellet containing the drug entrapped in the vesicles. These were dissolved in IPA in order to release the drug entrapped in the vesicles and with subsequent dilution the amount of entrapped drug was determined by UV spectrophotometry.

The entrapment of PP-41 and PP-50 was determined by dialysis membrane method using 50% ethanol as the release medium, as these drugs were not soluble in TDW.

The niosomal formulations of the compounds PP-24, PP-34 and PP-48 showed a significantly high entrapment i.e. 83.78%, 72.4% and 75.25% respectively which is more in comparison to the niosomal formulations of the compounds PP-41 (54.34%) and PP-50 (53.3%). This may be explained because of more hydrophilic nature of the compounds PP-24, PP-34 and PP-48. Also the log P value of compound PP-24 (1.200) was found to the lowest.

Drug content:

Actual amount added for all practical purposes was 5.0 mg/ml and the drug content of all the formulations was not found to be significantly different from the added amount.

Vesicle size and size distribution:

Particle size is an important parameter in in-process control and particularly in quality assurance, because the physical stability of vesicular dispersions depends on particle size and particle size distribution. Particle size of the ophthalmic preparation should be less than 10 μm in order to avoid irritation to the eyes (9). Average particle size of the developed vesicular system (Table 3), was well within this range and hence is appropriate for ocular use.

Zeta potential:

In dispersion, the Brownian motion results in frequent collision between the particles. Such interactions are mainly responsible for the stability of the dispersion system. When attraction predominates, the particles adhere after collision and tend to aggregate. When repulsion predominates, the particles rebound after collision and remain individually dispersed. Zeta potential is defined as the difference in potential between the surface of the tightly bound layer and electroneutral region of solution (10). A high zeta potential value signifies greater interparticle distance or in other words repulsion between vesicles and hence a good stability of the system. Zeta potential of the formulations PP-24, PP-34, PP-41, PP-48 and PP-50 was measured employing Malvern's zetasizer at 25°C. Observed zeta-potential of the respective formulations is shown in table 3.

Formulation code	% Transmittance	% Entrapment (n=3)	Drug content (mg/ml) (n=3)	Particle size (nm)	Zeta potential (mV)	DSC
						Peak °C
PP-24	58.34	83.78 ± 0.13	4.82 ± 0.03	461.4	-35.5	133.18
PP-34	67.5	72.41 ± 0.03	4.95 ± 0.01	542.7	-38.6	133.06
PP-41	81.35	54.18 ± 0.23	4.93 ± 0.01	355.5	-40	132.49
PP-48	64.67	75.21 ± 0.16	4.97 ± 0.03	202.9	-36.5	133.35
PP-50	79.35	53.29 ± 0.193	4.93 ± 0.01	323.5	-36.7	131.98

Table 3: Various observed parameters of formulations.

Formulations	Onset time (h)	Peak effect time (h)	Effective time period (h)	Duration of action (h)	% lowering of IOP at peak effect	Timolol activity of Formulation	Timolol activity of free drug
Timolol	0.5	1.5-2.0	0.5-5	4.5	16.1	1	1
PP- 24	0.5	1.5-3.0	0.5-6	5.5	15.8	0.98	0.69
PP- 34	0.5	1.0-2.0	0.5-5.5	5	12.3	0.76	0.91
PP- 41	0.5	1.5-2	0.5-5.5	5	10.5	0.65	0.64
PP- 48	0.5	1.0-1.5	0.5-5.5	5	12.5	0.78	0.69
PP- 50	0.5	1	0.5-4.5	4	8.77	0.54	0.56

Table 4: Activity parameters of the various niosomal formulations.

Formulation

The negative value of zeta-potential obtained for all the formulations indicates a low scope of coalescence and high stability of the vesicular system.

pH Measurement:

The ideal pH for maximum comfort when an ophthalmic preparation is instilled in the eye is indicated to be of the order of 7.2 ± 2 (11).

The pH value of all the formulations was found close to the tear fluid (7.19, 7.01, 7.21, 7.13, 7.36 for PP-24, PP-34, PP-41, PP-48, PP-50 respectively), so the developed formulations would not cause any irritation to the eye after its application and hence were used as such and not buffered.

Vesicle shape and type:

All the formulations were examined microscopically (optical microscope, Nikon eclipsed i90; magnification 40X). Optical inspection indicated that the vesicles were small in size, round in shape, bilamellar/unilamelar and no aggregation or irregularities were observed in the system. The particles were uniformly distributed as shown (Fig. 2).

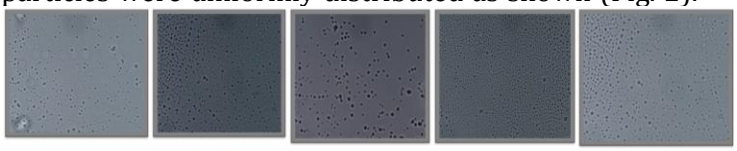


Fig. 2. Optical photomicrographs (40X) of formulations PP-24, PP-34, PP-41, PP-48 and PP- 50 respectively.

Differential scanning calorimetry:

Successful formulation of a stable and an effective dosage form depends on the careful selection of the excipients. The latter can facilitate administration, promote a consistent release and bioavailability of the drug and protect it from degradation. Samples of pure compounds PP-24, PP-34, PP-41, PP-48 and PP-50 and all excipients (cholesterol and span 60) were scanned using differential scanning calorimeter (DSC) and thermograms so generated were analyzed for any significant shift in the peak, disappearance of a peak, or appearance of any new peak , with respect to niosomal formulations. The niosomal formulations of the compounds PP-24, PP-34, PP-41, PP-48 and PP-50 gave peaks at 133.18 ^{0C}, 133.06 ^{0C}, 132.49 ^{0C}, 133.35 ^{0C} and 131.98 ^{0C} respectively and no peaks were observed corresponding to the selected compounds, indicating the entrapment of the compounds within the niosome.

The compound PP-24 showed a sharp endothermic peak at 191.77 ^{0C} indicating the melting of the oxalate. In case of compounds PP-34, PP-41, PP-48 and PP-50 the peaks were observed at 257.80 ^{0C}, 102.27 ^{0C}, 185.58 ^{0C} and 141.98 ^{0C} indicating the melting of the respective compounds.

Transmission electron microscopy:

The niosomal formulations were observed for their morphology under transmission electron microscope. The TEM photographs of the niosomal formulations of the respective compounds showed that most of the vesicles were nearly spherical in shape and were present as individual entities rather than agglomerates confirming their stability against any kind of coagulation as are obvious from microscopy photographs. (Fig. 3)

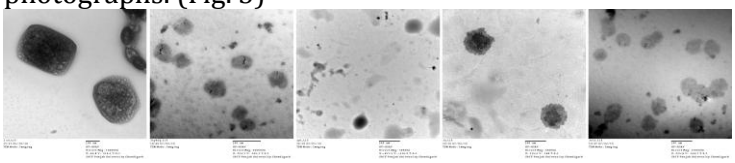


Fig. 3. TEM of niosomal formulation PP- 24, PP- 34, PP- 41, PP- 48 and PP- 50 respectively.

Ex-vivo/ Corneal permeability studies:

For the developed niosomal formulations, the linear permeability plot with correlation coefficient (r2) were obtained in the range of 0.966-0.964, 0.970-0.965, 0.972-0.982, 0.976-0.971 and 0.989-0.954 for PP-24, PP-34, PP-41, PP-48 and PP-50 respectively. All the formulations were behaving as a non-fickian diffusion system. The total dose permeated, % permeation, steady state flux and the apparent permeability coefficient (Papp) of all the free drugs and the niosomal formulations were calculated. A one way ANOVA followed by Tukey test was applied on the data. Statistical analysis of the data indicates that the niosomal formulations of PP-24, PP-34 and PP-48 were significantly better than the niosomal formulations of PP-41 and PP-50 at all time intervals. All three formulations PP-24, PP-34 and PP-48 showed an improvement in intrinsic apparent permeation coefficient (Papp).

An increased ocular bioavailability of water-soluble compounds PP-24, PP-34 and PP-48 entrapped in niosomes, may be due to the fact that surfactants which are the chief constituent of niosomes, act as penetration enhancers as they can remove the mucus layer and break junctional complexes (12-14). No significant difference was found between formulations PP-24 and PP-34 in terms of total amount permeated; however, they were significantly better than the formulation PP-48 in terms of cumulative amount permeated (µg).

The percentage amount permeated is highest in case of formulation PP-24 (27.29%) and lowest in case of formulation PP-50 (10.04%). The result of the formulation PP-24 (27.29%) was almost similar to the formulation PP-34 (26.39%) and their values were significantly higher than the values of formulation PP-41 and PP-50. The apparent permeability coefficient of niosomal formulation PP-24 and PP-34 was not significantly different. The formulation PP-24 showed maximum steady state flux (0.622 ± 0.04). On

comparison of various free drug solutions and their respective niosomal formulations in terms of total amount permeated, % permeation, steady state flux, it was found that all the niosomal formulations showed greater total amount permeated, % permeation, steady state flux with respect to their respective free drug solutions. The apparent permeability coefficient (Papp) of free drug solutions of PP-24 and PP-34 was greater than their respective niosomal formulations. Fig. 4 shows percentage permeated at various time intervals of all the formulations and 0.5% solution of respective compounds through porcine cornea.

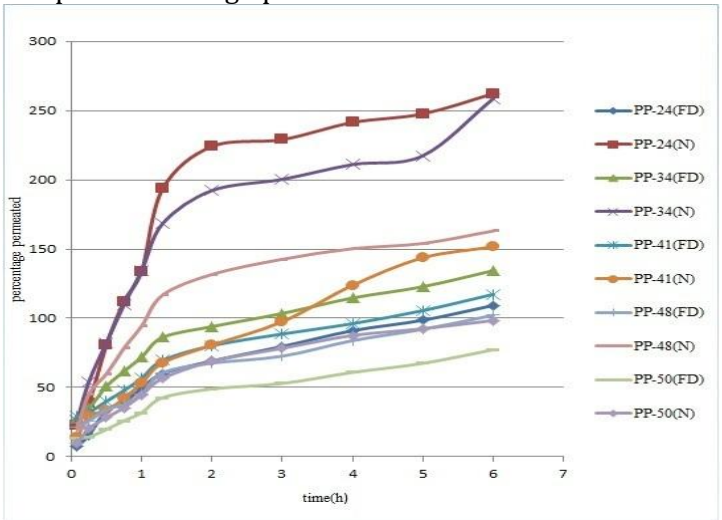


Fig. 4: Comparison of % permeation vs. time (min) data of niosomal formulations (0.5% solution) of PP-24, PP-34, PP-41, PP-48 and PP-50.

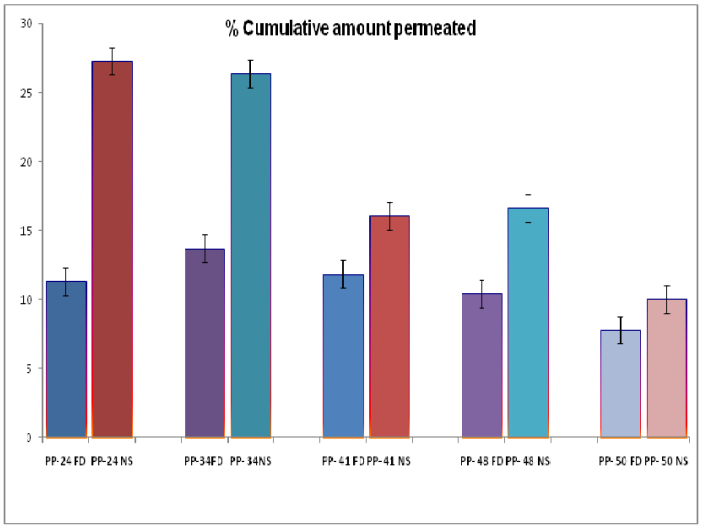


Fig. 5: Comparison of % cumulative amount permeated of the free drug solutions and their niosomal formulations. (NS) = Niosomal formulation, (FD) = Free drug

From the Fig. 5, it's clear that the niosomal formulation PP-24 gave the highest rate and extent of drug permeation followed by the formulation PP-34, PP-48, PP-41 and PP-50. This can be explained in terms of highest percentage entrapment of PP-24 compound in the vesicles. Because of more hydrophilic nature of compounds PP-24, PP-34 and PP-48 they cannot cross the biological membrane. Therefore, the permeation of the respective free drug solutions were less as compared to the free drug solutions of compounds PP-41 and PP-50 which were more lipophilic.

Pharmacodynamic evaluation (Intraocular pressure lowering effect):

The pharmacodynamics effects (IOP lowering effects) of the niosomal formulations of the compounds PP-24, PP-34, PP-41, PP-48 and PP-50 were evaluated in male albino rabbits, using Reichert tonometer. The change in IOP upon instillation of these formulations into the rabbit eye was observed. The formulations have been compared in terms of their activity parameters (onset time, peak effect time, effective time period, duration of action, % lowering of IOP at peak effect). One way ANOVA followed by Tukey's test was carried out for all the formulations to compare each formulation with Glucomol® (0.5% solution of Timolol maleate, Allergan) taken as control, and among themselves, at different time intervals. Table 4 shows the effect of all the formulations on lowering of intraocular pressure. Comparison of activity of free drug and their respective niosomal formulation taking % IOP lowering effect of timolol as unit activity is shown in Table 4. On comparison of the compounds in terms of their activity parameters, it was observed that onset of action of all the formulations was 0.5 h.

In comparison to timolol, it was observed that all the formulations had comparable onset of action. The percentage lowering of IOP at peak effect of formulation PP-24 (15.8%) was comparable to timolol (16.1%). On comparing the formulation PP-24 which has isopropylamino substituted chain at meta-position provided a sustained and better IOP lowering effect than formulation PP-34 which has tert-butylamino substituent at the same position. This may be attributed to lower particle size and better entrapment efficiency of the PP-24 niosomal formulation. Latter may be attributed to lower molecular weight of PP-24. Compounds having the same substituents at C-1 and C-3 of the aromatic ring i.e. either tert-butylamino or isopropylamino (PP-24 and PP-34) showed good IOP lowering effect .The reduction in IOP was comparable to that achieved with timolol (Timolol-16.1%; PP-24-15.8%; PP-34-12.3%; and PP-48- 12.5%) (Table 4). Smaller particle size especially of PP-48 niosomal formulation may be responsible for its better effect in comparison to PP-34 niosomal formulation. Furthermore, PP-34 and PP-48 niosomal formulation showed a 0.5 h increase in duration of action with respect to their free compounds. However niosomal formulation of PP-41 and PP-50 did not show

enhancement in their IOP lowering effect even after their entrapment into niosome (Fig. 6).

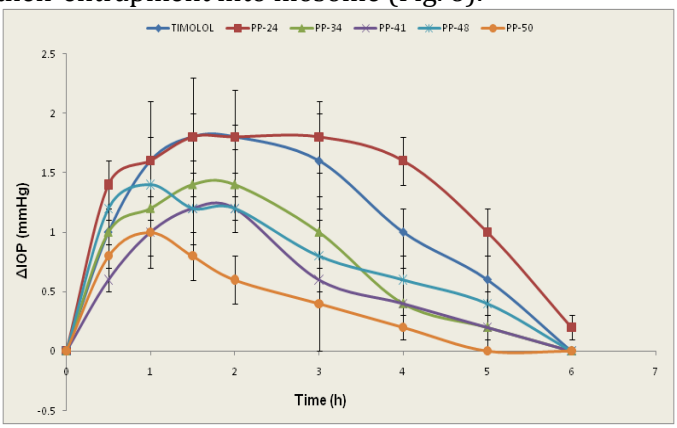


Fig. 6: Comparison of IOP lowering effect of niosomal formulations of PP-24, PP-34, PP-41, PP-48, PP-50 and timolol as standard.

This can be explained in terms of a poor entrapment (~50%) of these compounds within niosome, and almost 50% of the compound was still in free form. Further a poor water solubility of these compounds when present as free agents would limit their ocular bioavailability (a drug needs to be presented in a soluble form to elicit a physiological effect in the eye). Same may be responsible for their poor entrapment. Further studies are required to work out/optimize system to enhance the entrapment of these agents such that an improved pharmacodynamics may be observed.

Stability studies:

Drug retention in niosomes ultimately governs the shelf life of the niosomal formulations during the storage period. Drug leakage behavior of the developed niosomal formulations were evaluated, at different time intervals, at ambient condition (room temperature 30 ± 2 ^{0C}), under refrigeration (4-8 ^{0}C) and at accelerated conditions of 45 ± 2 ^{0C} and 75% relative humidity (RH).

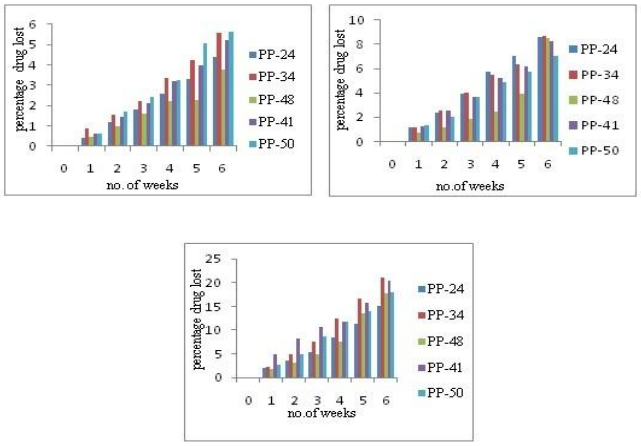


Fig. 7: Comparison of % drug loss from niosomal formulations vs temperatures 4 ± 2ºC(a), 30 ± 2ºC (b), 45 ± 2ºC (c).

The results in Fig. 7 indicated that the niosomes were more stable when stored under refrigeration; while at a higher temperature the rate of drug loss was very high. The increase in the leakage of drug from the niosome at elevated temperature may be related to the degradation of lipids in the vesicle bilayers resulting in defects in membrane packaging, making them leaky. Hydrolysis of lipids and degradation of drug at elevated temperature conditions may be other reasons responsible for greater loss of drug. This marked leakage at higher temperatures demands the storage conditions somewhat lower than the normal room temperature. Therefore, refrigerated conditions seem to be the best storage conditions for the prepared system.

CONCLUSION

Niosomes of aryloxypropanolamine derivatives synthesized at U.I.P.S were prepared by the ether injection method and the average particle size of the formulations was in the nano-range and well appropriate for ocular use. The water soluble compounds showed a significantly high entrapment probably because of the hydrophilic cavity of the niosome. The negative value of zeta potential obtained for all the formulations indicated low scope of coalescence and high stability of the vesicular system. Optical inspection indicated that the particles were uniformly distributed with small size, spherical shape and no aggregation or irregularities. Niosomes were stable when stored at a temperature of 4-8 ^{0C}. DSC thermogram of the developed formulations showed the absence of any peaks corresponding to the pure compounds indicating the successful entrapment of the respective compounds into the niosome. Ex vivo corneal permeability data establishes the usefulness of incorporating these compounds into niosome; compound PP-24 showed the maximum enhancement in corneal permeability from 11.33% to 27.29% i.e. 2.4 fold increase after its entrapment in niosome with respect to its free drug solution. Onset of action of all the developed formulations was 0.5 h which was comparable to timolol.

Formulation PP-24 produced sustained and better IOP lowering effect than formulation of PP-34 this may be attributed to lower particle size and better entrapment efficiency of the PP-24 niosomal formulation.

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