Extended Release Niosomal Hydrogel for Ocular Targeting of Piroxicam: *In vitro* and *Ex vivo* Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Author BKAR designed the study, carried out all the experiments and literature search, performed data analysis and the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors OSA and FN managed the instrumental analytical experiments. Authors HAL, IMA and HAAA contributed to the laboratory work. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

This study aimed at the investigation of piroxicam-niosomal hydrogel for ocular targeting to prolong and enhance its local analgesic activity. Various formulations were prepared,

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characterized and evaluated *ex vivo* for their transocular permeation using excised cow cornea. The prepared niosomes had distinct spherical multi-lamellar shape and mean vesicle size between 1.25±0.81µm and 7.47±0.85µm. Relevant increase in drug EE% was obtained with increase of cholesterol content and surfactant's hydrophobicity. Drug retention in vesicles was significantly ($p<0.05$) higher at refrigerated condition than that at the room temperature. Prolonged drug release was achieved with high niosomal cholesterol content and the mechanism of drug release can be described as Fickian diffusion. The niosomal hydrogel showed 3.7 Permeability Improvement Ratio comparing to piroxicam aqueous suspension. The optimized niosomal gel showed prolonged drug release and enhanced piroxicam ocular bioavailability due to the formation of an amorphous drug form within the gel.

1. INTRODUCTION

The basic goal of a sustained drug therapy is to achieve a steady state blood or tissue level that is therapeutically effective and nontoxic for an extended period. The design of proper dosage regimen is an important element in accomplishing this goal [1]. Targeted drug delivery is an event where a drug carrier, complex/conjugate delivers the drug moieties exclusively to the preselected targeted cells in a specific manner [2]. A number of novel vesicular drug delivery systems have been developed that allow drug targeting and sustained or controlled release of drug [3].

Niosomes are either unilamellar or multilamellar vesicles which can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials. These micro-vesicles are formed on the admixture of a non-ionic surfactant and cholesterol with subsequent hydration in aqueous media [4].

Niosomes in topical ocular delivery are preferred over other vesicular systems because: (i) they are chemically stable as compared to liposomes; (ii) can entrap both lipophilic and hydrophilic drugs; (iii) they are biodegradable, biocompatible, non-immunogenic, and of low toxicity; (iv) exhibit flexibility in their structural characterization, e.g. in their composition, fluidity, and size; (vi) can improve the performance of the drug via better availability and controlled delivery at a particular site [5]. The penetration of drug molecules into the eye depends on the physicochemical properties of both the drug and vehicle. Vesicular systems provide prolonged duration of action at the corneal surface by preventing ocular metabolism in the lachrymal fluid (Fig. 1). Niosomes have been reported as a potential ocular vehicle for several medications for example cyclopentolate [6], acetazolamide [7], acyclovir [8] and gentamicin [9].

Mucoadhesive polymers such as hyaluronic acid and carpobol, have recently gained attention among pharmaceutical scientists as a means of improving ocular drug delivery. Niosomal based mucoadhesive dosage forms promote drugs precorneal retention time thus enhance drugs bioavailability and prolong their action [10].

Piroxicam is a non steroidal anti-inflammatory drug (NSAID) used in treatment of pain and inflammatory disorders, such as rheumatoid arthritis as well as for a range of ocular

inflammatory conditions [11]. Up to our knowledge there are no reports for using niosomal sustained release gel for piroxicam ocular administration, therefore the present study aimed at formulating piroxicam loaded niosomes that are incorporated into the mucoadhesive ocular gel, with optimizing and characterizing the designed formulation and then assessing their performance *in vitro* and *ex vivo.*
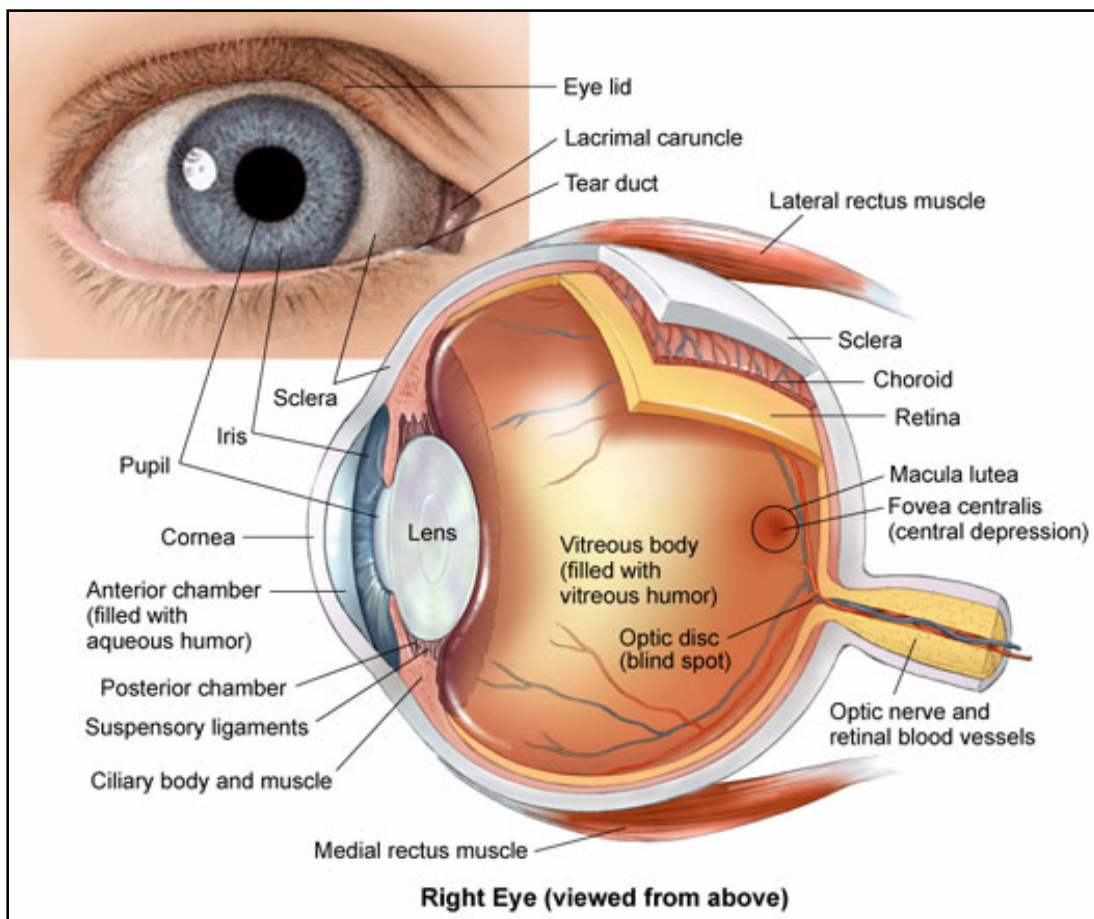

Fig. 1. Anatomy of human eye

2. METHODS AND MATERIALS

2.1 Materials

Piroxicam (PRX), Span 20, Span 40, Span 60, and Span 80 were purchased from Sigma-Aldrich (USA).Cholesterol (CHOL), dihydrogen sodium orthophosphate, disodium hydrogen orthophosphate and phosphate buffer saline (PBS) tablets (pH 7.4) were obtained from Belami Fine Chemicals (Mumbai, India). Carbopol 943 was purchased from Wuxi Hexia Chemical Company (China). Glycerin and propylene glycol were gift from JULPHAR (RAK, UAE). All other chemicals were of analytical grade.

2.2 Formulation of PRX Niosomes

PRX loaded niosomes were prepared by thin-film hydration method [12]. Accurately weighed quantities of drug, non ionic surfactant Span, and CHOL were dissolved in chloroform/methanol system (2:1) in a round-bottom flask. The solvent was evaporated at 55-65°C under reduced pressure using a rotary evaporator (Heidolph, Germany). The thin films were hydrated in 5 ml of PBS pH 7.4 with gentle shaking and then left for 3h at room temperature. Table 1 shows the composition of the prepared formulas. Each formula contains 50mg of PRX. Formulations reproducibility was attained by preparing all the batches three times on different days. Samples were stored at 8°C for further analysis.

2.3 Morphological Characterization

The size, shape, and lamellar nature of the prepared vesicles were observed by optical microscopy (Kshitij Innovations, India) using a calibrated eyepiece micrometer, and photographs were taken at ×14 magnification with a digital camera (DigiEye, Indonesia).

2.4 Determination of PRX Entrapment in Vesicles

PRX niosomal formulations were centrifuged at 3500 ×g and 4°C for 30 min using a centrifuge (Hettich, Germany) to separate niosomes from non-entrapped drug. The niosomal dispersion was separated from the free drug which precipitated on the wall of Eppendorff tubes and diluted with 10mL methanol. The concentration of the entrapped drug was determined by UV spectrophotometer (NMB-6200, USA) at 334nm [13]. The percentage of drug entrapment in niosomes was calculated using Eq. 1:

$$\% \text{ drug entrapment} = (\text{total drug - drug in supernatant})/ \text{ total drug} \times 100 \qquad (1)$$

2.5 Drug Retention Studies

PRX niosomal formulations were sealed in tightly closed plastic containers and stored at refrigeration (2-8°C) temperature and room temperature (25 ±1°C). Samples from each batch at each temperature were withdrawn at definite time intervals and analyzed for the % drug entrapment to determine drug leakage rate from the vesicles.

2.6 FTIR Spectroscopy Analysis

Five milligrams of each sample were blended with 30 mg of KBr and compressed at 10 ton by a hydraulic press. The resulting disks were analyzed with FTIR-8400S (Shimadzu, Japan) spectrometer. The scanning range was (400-4000 cm^{-1}). The spectra were recorded for the pure drug, CHOL and formula S80-B.

2.7 *In vitro* Release Studies

PRX *in vitro* release was studied using a dialysis bags as donor compartment (Slide-A-Lyzer dialysis cassette, USA) fitted with a dialysis membrane, molecular weight is 10,000. The dialysis membrane was soaked into warm water for 30 sec and one end was sealed with a clip. Formulations or free PRX solution was pipette into the bag and the bag was sealed with another closure clip to prevent leakage. The dialysis bag was placed in the receptor compartment which contains 100 ml of PBS pH 7.4. The medium was stirred at 100 rpm and

temperature was maintained at 35± 2°C. Samples (3 ml) were withdrawn at pre-determined intervals and replaced with fresh buffer. The amount of drug released was assayed by UV spectrophotometer at 334 nm. Results were the mean values ±SD of three runs. Microsoft 2010 Excel sheet was used to determine drug concentration using linear regression analysis of PRX calibration curve (y=47.705x, $R^2 = 0.9989$).

2.8 Kinetics of Drug Release

To analyze the *in vitro* drug release, various kinetic models were used to describe the release data including:

1. Zero order kinetics where the rate of drug release rate is independent on its concentration [14]:

$$C = K_o \, t \tag{2}$$

Where, C is the initial drug concentration and K_o is zero-order rate constant.

2. First order kinetics where release rate is concentration dependent [15]:

$$lnCt = \ lnCo - \ kt \tag{3}$$

Where, Co is the initial drug concentration and K is first order constant.

3. Higuchi model which describes drug release from insoluble matrix as a square root of time [16]:

$$Q = K_H \, t^{1/2} \tag{4}$$

Where, Q is fraction of released drug and K_H is Higuchi release constant.

4. Hixson-Crowell cube root equation which describes the release from systems where there is a change in surface area and diameter of particles [17]:

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}C_t \tag{5}$$

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in system and K_{HC} is Hixson-Crowell rate constant.

5. Korsmeyer–Peppas model was used to find out the mechanism of drug release from the niosomal formulations [18]:

$$Mt / M\infty = K \, t^n \tag{6}$$

Where, Mt / M∞ is fraction of drug released at time t, k is the rate constant and n is the release exponent. The n value is used to characterize drug release mechanisms from niosomes.

2.9 Preparation of PRX Niosomal Gel

Carbopol 943 gel bases were prepared at various concentrations (0.5%, 1%, and 1.5%) by scattering a definite amount of the polymer onto warm distilled water with continuous stirring. The dispersions were left for complete hydration. A required amount of formula S80-B was incorporated into the dispersions and the pH was neutralized to 5.9±0.1 with 0.1N NaOH solution. The final weight was adjusted with distilled water up to 1g. The gel bases were sonicated for 30 minutes at 25°C and kept overnight to remove air bubbles [19].

2.10 *Ex vivo* Drug Transcorneal Permeation

2.10.1 Corneal preparation

Freshly excised whole eyeballs of cow were transported from local butcher's shop (Dubai, UAE) to the laboratory in cold (4°C±2) phosphate saline solution within 1 h after slaughtering. The eyeballs were taken only from 6–8 months old cows in order to avoid obtaining corneas with pigmentation or other corneal abnormalities. The method of cornea dissection was the same as described by Malhotra and Majumdar [20]. The corneas were carefully dissected along with 2–4 mm of surrounding sclera tissue from the eyeball and washed with cold saline to remove any adhering pigments. The washed cornea were preserved in freshly prepared PBS (pH 7.4) and stored at -20°C till the time of the study.

2.10.2 Permeation experiment

The cornea obtained by the above procedure was mounted on Franz diffusion cell (Copley, UK) by sandwiching the sclera tissues between the clamped donor and the receiver chamber. Care was taken to ensure that the epithelial surface of the cornea is towards the donor side. The receiver chamber was filled with PBS pH 7.4. One gram of test sample (PRX niosomal hydrogels or PRX niosomal dispersion or PRX aqueous suspension 0.1% w/w) was placed on the epithelial surface of the cornea and covered with the plastic cover to prevent samples evaporation. The receiver fluid was maintained at 35±1°C and 50 rpm. At predetermined time intervals samples were withdrawn through the sampling port and analyzed for the drug content by UV spectrophotometer at 334 nm.

2.10.3 *Ex vivo* data analysis

Mean cumulative amount permeated was calculated at the end of 8h based on triplicate experiments for each sample. The apparent corneal permeability coefficient (P_{app}) in centimeters per second was determined according to Eq. 7 [21].

$$P_{app} = Flux/ (60. Co) \qquad (7)$$

Where, flux (mg/cm^2·min) was obtained as ratio of slope [determined based on linear regression analysis of the plot between cumulative amount permeated vs. time (in minutes)] and the exposed corneal surface area of 1.767cm^2. Co is the initial concentration of drug in the donor cell and 60 represents the conversion factor for minutes to seconds. Permeability improvement ratio was calculated as the ratio of P_{app} of the test sample to the P_{app} of the control (PRX aqueous suspension) [22].

2.11 Differential Scanning Calorimeter Analysis (DSC)

The DSC analysis was carried out with DSC-60 (Shimadzu, Japan). Samples of PRX, CHOL, carbopol 943, blank gel and eye cornea before and after permeation study with S80-B niosomal gel) were placed in an aluminum pan and heated at a rate of 10°C/min to 300°C. The instrument was calibrated with indium and an empty pan was used as reference. Dry nitrogen was used as a carrier gas with a flow rate of 25 ml/min.

2.12 Data Analysis and Statistics

Data are expressed as mean ± SD. Statistical analysis was performed by Students' T-test using GraphPad Prism (version3.0). Significance was defined at p values <0.05.

3. RESULTS AND DISCUSSION

PRX loaded niosomes were prepared by the thin-film hydration method using different proportions of surfactant: cholesterol. In this method, surfactants/lipids are casted as layers of film from their organic solution under reduced pressure and dispersed in an aqueous medium. Upon hydration, the lipids swell and peel off from the wall of the round bottom flask. The mechanical energy required for the swelling of the lipids and dispersion of casted lipid film is imparted by manual agitation.

3.1 Morphology and Size Analysis of Niosomes

The size, shape, and lamellar nature of the prepared vesicle formulations were observed by optical microscopy. The prepared niosomes had distinct spherical shape as shown in Fig. 2. This result was expected since thin-film hydration method usually forms typical multi-lamellar spherical niosomal vesicles [23]. The mean vesicle size was between 1.25±0.81μm and 7.47±0.85μm. Cholesterol content and HLB value of the surfactant were the main factors affecting the niosomal vesicle size. The increase in cholesterol content as well as surfactant hydrophobic chains of the used surfactants resulted in accompanied increase of niosomal size. The ranking of hydrophobicity of surfactants is as follows: Span 80> Span 60> Span 40 > span 20 and maximum vesicular size was observed with Span80 (HLB value is 4.3), as shown in Table 1.

3.2 Niosomal Entrapment Efficacy

The formulation technique was optimized for hydration medium volume and hydration time to get maximum drug entrapment using formula S80-B. Maximum EE% was obtained with 5 ml of PBS pH 7.4 and 3h duration, as shown in Table 2.

Relevant increase in EE% was obtained with the increase of cholesterol niosomal content (Table 1). Hence, the incorporation of cholesterol into the composition of niosomes results in membrane stability and drug leakage reduction thus increases entrapment efficiency and vesicular size [24]. In general surfactants with alkyl chain length from C12-C18 had been widely utilized for preparation of noisomes as drug delivery systems, including oral, transdermal and ocular delivery. Our results indicated that Span 80 showed the superior EE% in comparison to other sorbitan surfactants used in this study. This may be attributed to the higher alkyl chain length in its structure (Fig. 3). Supporting to the previous opinion, a larger alkyl chain lowers the HLB value of a surfactant thus be more compatible with the hydrophobic drugs such as PRX [25]. The maximum entrapment efficiency (78.9%) was obtained with formula Span80-B.

Table 1. Composition and characteristics of PRX loaded niosomal formulations

Formula code	Surfactant used	Span: CHOL Molar ratio (µM)	Mean vesicle* size ±SD (µm)	% EE (Zero Time)	% EE (after 6weeks)	
					Room Temp.	Refrigerated
S20-A	Span 20	300 : 200	1.25±0.81	23.6	21.5	23.1
S20-B	Span 20	250 : 250	2.75±0.54	24.1	22.1	23.2
S20-C	Span 20	200 : 200	1.82±0.25	18.9	18.5	19.2
S40-A	Span 40	300 : 200	2.53±0.51	36.1	32.1	35.8
S40-B	Span 40	250 : 250	3.94±0.65	38.6	35.7	36.5
S40-C	Span 40	200 : 200	2.25±1.25	30.1	27.1	28.8
S60-A	Span 60	300 : 200	2.99±0.30	50.9	45.4	50.8
S60-B	Span 60	250 : 250	4.71±0.91	52.4	45.3	51.9
S60-C	Span 60	200 : 200	3.92±0.75	40.8	31.2	38.7
S80-A	Span 80	300 : 200	6.57±0.72	74.5	71.1	73.7
S80-B	Span 80	250 : 250	7.47±0.85	78.9	72.1	78.7
S80-C	Span 80	200 : 200	4.47±0.25	66.6	54.2	66.9

Results are given as mean ±SD (n=3)

3.3 Drug Retention in Vesicles

The results from drug leakage study for all formulas indicated that the percent drug retention in vesicles was significantly ($p<0.05$) higher at refrigerated condition than that at the room temperature (Table 1), since high temperature melts and fluidize the vesicular lipid contents of niosomes resulting in more drug leakage [26]. Formula S80-B maintained the highest drug retention at both storage conditions.

3.4 *In vitro* Drug Release

Fig. 4 shows the release profile of PRX from the prepared niosomal formulations. From all release profiles, it is obvious that formulas with high surfactant content (S20-A, S40-A, S60-A and S80-A) showed the maximum rate and extent of drug release followed by formulas (S20-C, S40-C, S60-C and S80-C), while formulas of high cholesterol content (S20-B, S40-B, S60-B and S80-B) showed prolonged drug release profile. This order may be due to the retention of PRX, poor water soluble drug, in the lipid layers of the vesicles. As well as, more cholesterol content increased rigidity of the lipid layers which result in prolonged drug release. Student t-test reveals that the difference is insignificant ($p<0.05$) among these formulas and because of highest EE% of S80-B in comparison to others, therefore S80-B was chosen for the preparation of the niosomal hydrogel, in order to prepare effective sustained release ocular PRX formulation.

Table 2. Optimization of process variables using formula S80-B

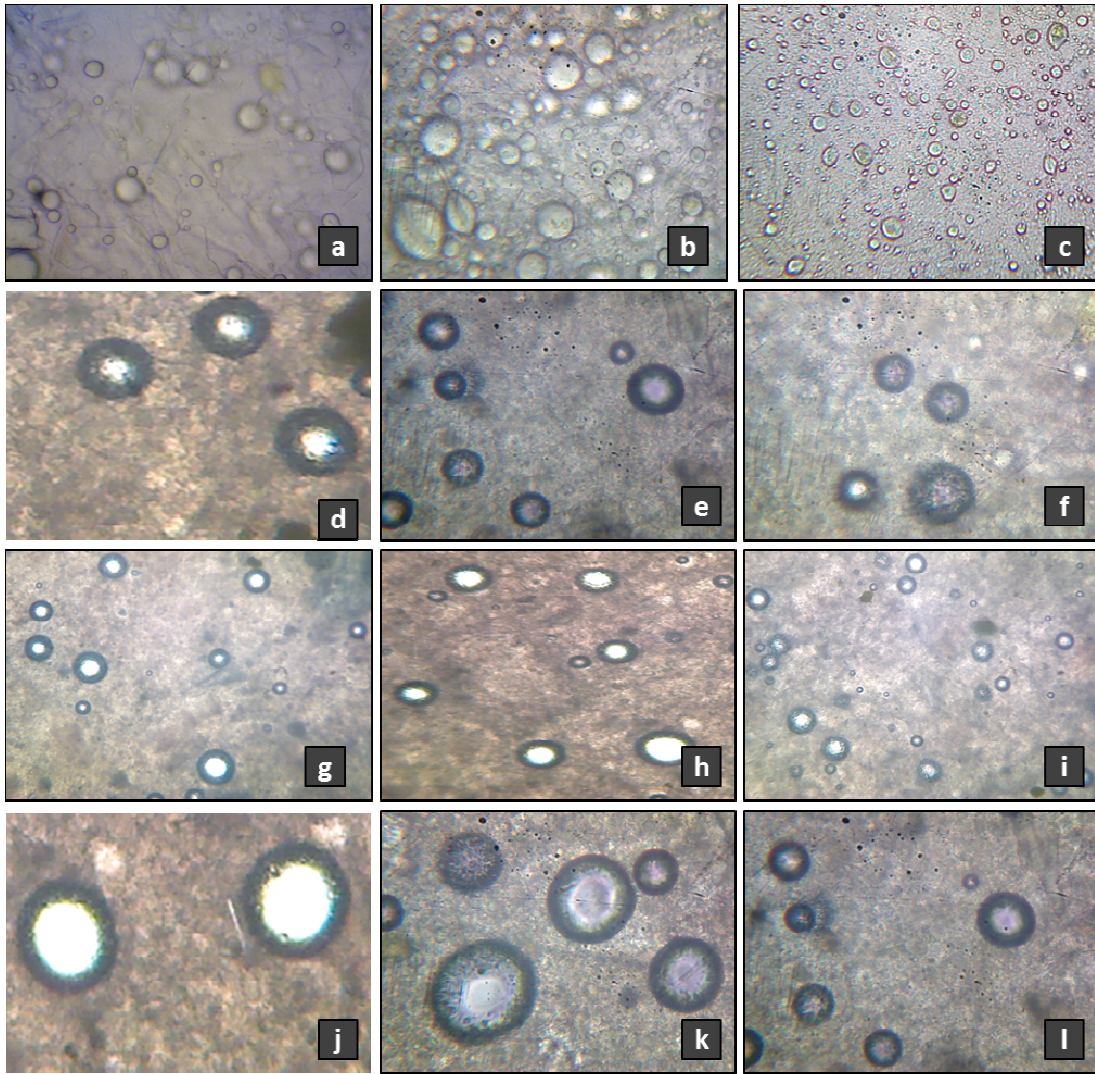| Time (h)	Volume (mL)	EE (%)
3	2	56.7
3	3	67.3
3	5	78.9
4	5	77.8
6	5	62.1

Fig. 2. Microscopic images of (a) S20-A; (b) S20-B; (c) S20-C; (d) S40-A; (e) S40-B; (f) S40-C; (g) S60-A (h) S60-B; (i) S60-C; (j) S80-A; (k) S80-B; (l) S80-C. (H&E x40)
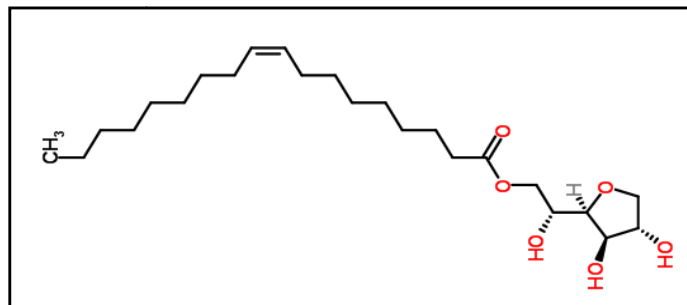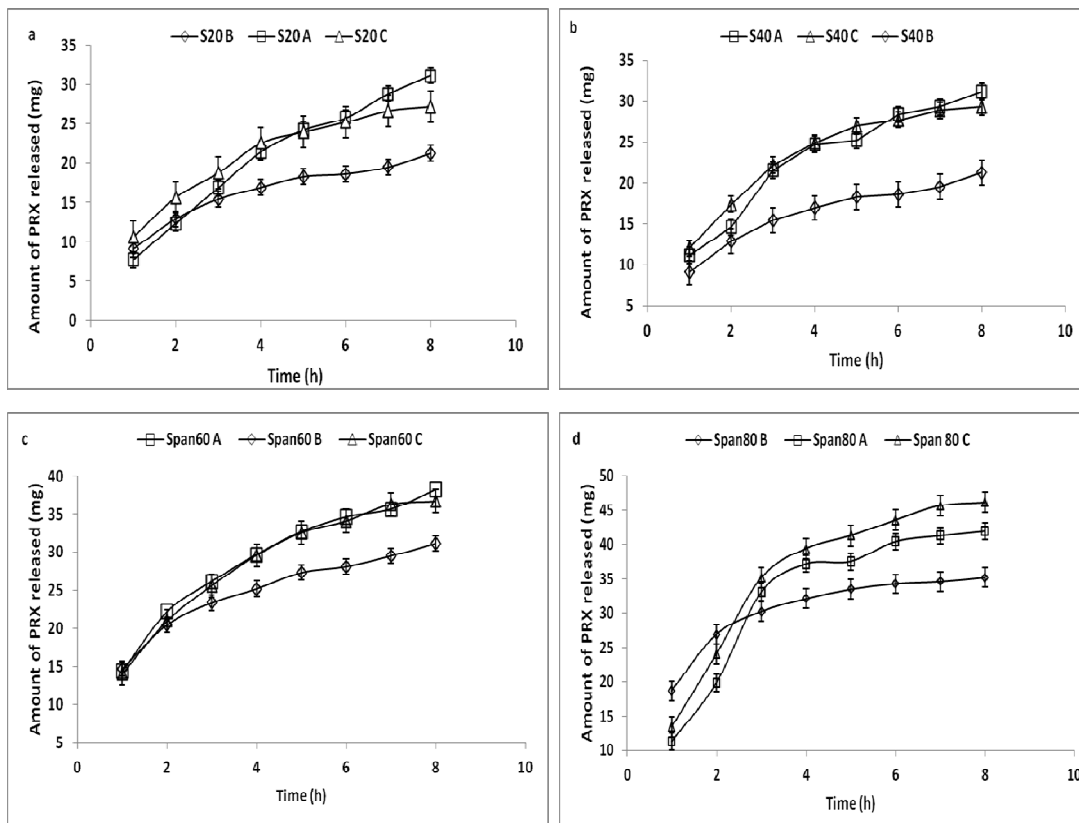

Fig. 3. Chemical structure of sorbitan monooleate (Span 80)

Fig. 4. *In vitro* release profile of niosomal formulations contain: a) Span20; b) Span40; c) Span60; and d) Span80, (mean ±SD, n=3)

3.5 Kinetics of Drug Release

Regression analysis of the obtained results showed that, Higuchi model gave the highest value of R^2 with a significant difference ($p<0.05$) compared to other kinetic models, as shown in Table 3. Therefore, Higuchi model was found to be the most appropriate model to describe the release kinetics of PRX from the niosomes preparations examined in the present study. Higuchi model describes the release of drugs from an insoluble matrix where the cumulative amount of the permeated drug per unit area is proportional to the square root of time. Since n value of all niosomal formulas, except S80-A, is ≤0.5 therefore the mechanism of PRX release from the niosomes can be described as Fickian diffusion which occurs by the usual molecular diffusion of the drug due to a chemical potential gradient. For S80-A, n value is 0.738, therefore drug release from this formula can be described as anomalous (non-Fickian) diffusion that includes disentanglement and erosion of the niosomal matrix [18].

3.6 FTIR Spectroscopy Study

After formulation, CHOL and PRX substances will be in a liquid crystalline state in which interaction between them is possible thus may affect drug release from the dosage form. In FTIR spectrum of cholesterol the broad absorption peak at 3377 cm^{-1} corresponds to

stretching OH vibration. The asymmetric and symmetric C-H stretching peaks appeared centered at 2953 cm^{-1}. Other characteristic C-H bending peak appeared at 1460 cm^{-1} (Fig. 5a).

While in FTIR spectrum of piroxicam the absorption peak appeared at 3338 cm^{-1} was attributed to N-H stretching of amide group. Other characteristic bands appeared at 1629 cm^{-1} and 1529 cm^{-1} were assigned to stretching of amide carbonyl and stretching of the second amide band respectively. Peaks at 1435 cm^{-1} and 1352 cm^{-1} assigned for stretching of asymmetric and symmetric methyl groups, respectively. A peak at 1149 cm^{-1} is for stretching of –SO2-N- group and 775 cm^{-1}as stretching of ortho-di substituted phenyl (Fig. 5b).

The FTIR of formula S80-B showed all the peaks of piroxicam and cholesterol, however, -O-H peak of cholesterol were merged with –N-H stretching peaks of piroxicam and showed distinct two peaks appeared at 3378 cm^{-1} and 3340 cm^{-1}, not significantly different wave number than their corresponding parent IR spectral value (Fig. 5c). Appearance of the other characteristic absorption band of piroxicam and cholesterol indicates that there were no interaction in between piroxicam and cholesterol. Thus, behavior of niosomes as depot that released the drug in a prolonged way is mainly due to entrapment of PRX inside niosomal lipid content [27].

3.7 *Ex vivo* PRX Transcorneal Permeation

Transcorneal permeability studies revealed that PRX ocular permeability was affected by two factors namely; drug formulation design and carpobol gel concentration, as shown in (Fig. 6). PRX corneal permeability was enhanced significantly by incorporation of PRX niosomal vesicles into the hydrogel base comparing to its niosomal dispersion and aqueous suspension. Cumulative amount permeated (in 480min), calculated transcorneal flux, apparent permeability coefficient (P_{app}), as well as permeability improvement ratio under different treatment conditions are presented in Table 4. Corneal permeability of PRX from aqueous suspension was the lowest with only 197.17±3.7µg of the drug permeating, calculated flux of0.122 ±0.02µg/cm^2.min and P_{app} of 1.0 (±0.11) × 10^{-3}cm/s. The aqueous suspension contains 0.1% w/w of PRX was used as control for further comparison. Though, the cumulative amount permeated from formula S80-B niosomal suspension was 316.17 ± 1.8µg, the flux was 0.386±0.01µg/cm^2 and P_{app} of 3.2±0.23× 10^{-3}cm/s; this means that niosomal formulation showed around 3.1-folds higher flux than drug aqueous suspension. In previous studies, it was reported that nisomes improve the bioavailability of poorly soluble drug though their adsorption to the corneal surface and transfer their membrane-associated drug directly to the corneal epithelial cell membranes, thereby facilitate drug transport across the cornea [28,29]. However, niosomal hydrogels showed higher permeability improvement ratio in comparison to the control (Table 4). This can be explained on the fact that, the mucoadhesive properties of carbopol hydrogel base ensure an intimate contact between niosmes and corneal membrane and create a reservoir effect which prolongs the retention of the niosomal formulation at the site of administration and consequently enhance drug permeation [30]. Insignificant difference ($p<0.5$) was obtained from the niosomal gels 0.5% and 1% in their flux improvement effect while the least effect showed by the niosomal gel 1.5%. This decrease in drug permeation can be attributed to the gel viscosity. Niosomal gel prepared with 1.5% carpobolwas stiff therefore; this high resistance to drug diffusion was due to the increase in the gel viscosity [31]. Furthermore, carbopol gel prepared with 0.5% w/w was of low consistency where as carbopol gel 1% w/w was found to be of good consistency and acceptable feel. Hence 1% carbopol gel was considered as the candidate base for the formulation of PRX niosomal gel for ocular delivery.

Table 3. *In vitro* release kinetic parameters of PRX-loaded niosomal formulations

| Formula code	Zero order		First order		Higuchi		Hixon-crowell		Korsmeyer-peppas		
	R^2	K_0 $(mg. h^{-1})$	R^2	K_1 (h^{-1})	R^2	K_H $(h^{-1/2})$	R^2	K_{HC} $(h^{-1/3})$	R^2	n value	K_{KP}
S20A	0.97	3.28	0.88	0.183	0.99	12.95	0.92	0.157	0.99	0.497	7.77
S20B	0.91	1.53	0.83	0.102	0.97	6.17	0.86	0.084	0.98	0.407	9.46
S20C	0.91	2.28	0.83	0.121	0.97	9.19	0.86	0.106	0.99	0.492	10.89
S40A	0.92	2.78	0.85	0.136	0.96	11.14	0.87	0.123	0.96	0.504	11.00
S40B	0.91	1.5	0.83	0.102	0.97	6.17	0.86	0.084	0.98	0.447	9.46
S40C	0.87	2.34	0.79	0.113	0.94	9.52	0.82	0.102	0.98	0.481	12.42
S60A	0.92	3.12	0.83	0.120	0.98	12.51	0.87	0.118	0.98	0.486	15.00
S60B	0.91	2.10	0.84	0.091	0.97	8.43	0.87	0.086	0.98	0.436	15.30
S60C	0.91	3.13	0.83	0.124	0.97	12.60	0.86	0.120	0.99	0.502	14.44
S80A	0.79	4.09	0.70	0.160	0.98	16.85	0.73	0.155	0.94	0.738	12.18
S80B	0.76	1.99	0.70	0.072	0.99	8.25	0.72	0.072	0.94	0.503	19.95
S80C	0.83	4.33	0.72	0.149	0.92	17.74	0.76	0.151	0.95	0.472	14.58
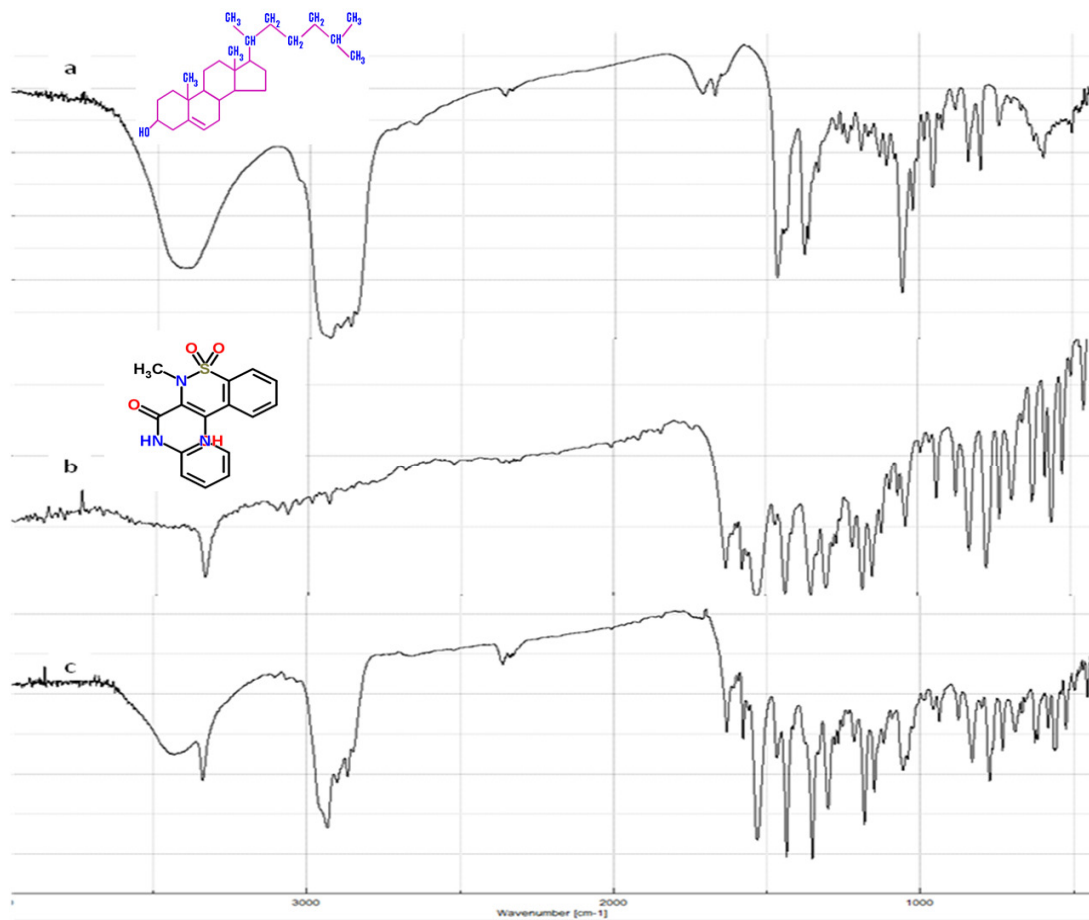
Fig. 5. FTIR spectra of a) CHOL; b) PRX; and c) Formula S80-B

3.8 DSC Analysis

The DSC thermogram of piroxicam demonstrating a sharp characteristic endothermic peak at 205°C near its melting point (198-200°C), (Fig. 7a). This indicates that piroxicam used was in pure crystalline state. The DSC thermogram of carbopol showed two endothermic peaks at 72 and 242°C and an exothermic one at 285°C indicating the decomposition of carbopol (Fig. 7b). Cholesterol shows a sharp characteristic endothermic peak around its melting point 149 (m.p. 147 to 150°C), (Fig. 7c).

The DSC thermogram of eye cornea after transocular permeation of the niosomal gel demonstrates a shifting of the broad endothermic peak of carbopol from 72°C to 107°C and completes disappearance of the characteristic peak of piroxicam at 205°C, as shown in (Fig. 7d). This can be explained on the fact that piroxicam lost its crystalline form with the formation of an amorphous solid solution within carbopol resulting in enhancement of PRX ocular bioavailability.
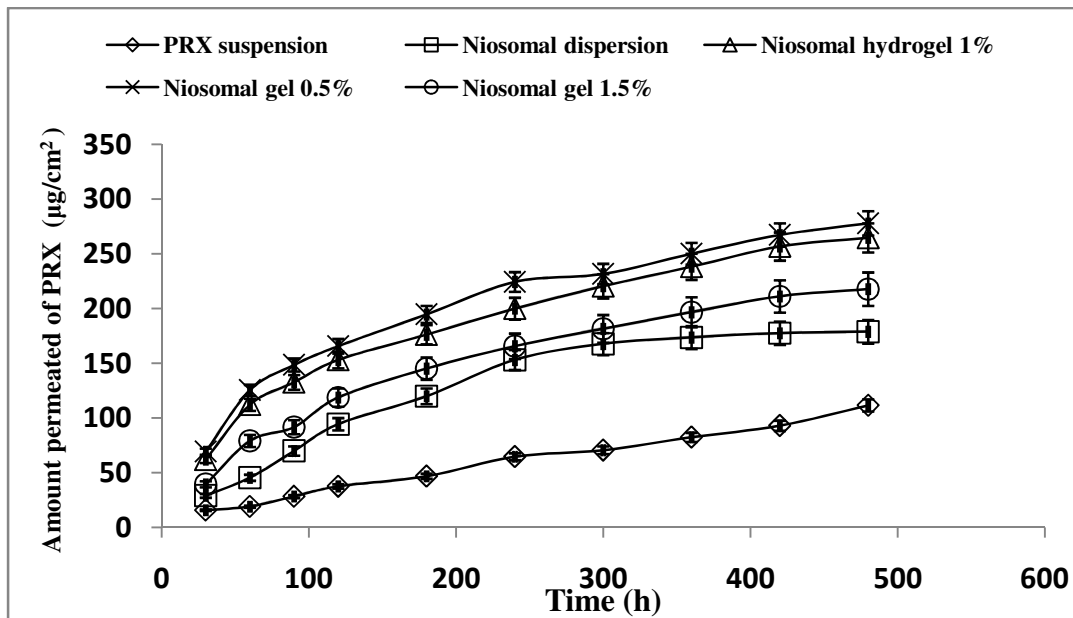
Fig. 6. Transcorneal permeartion profile of PRX from niosomal hydrogels and niosomal dispersion in comparison to niosomal suspension, (mean ±SD, n=3)
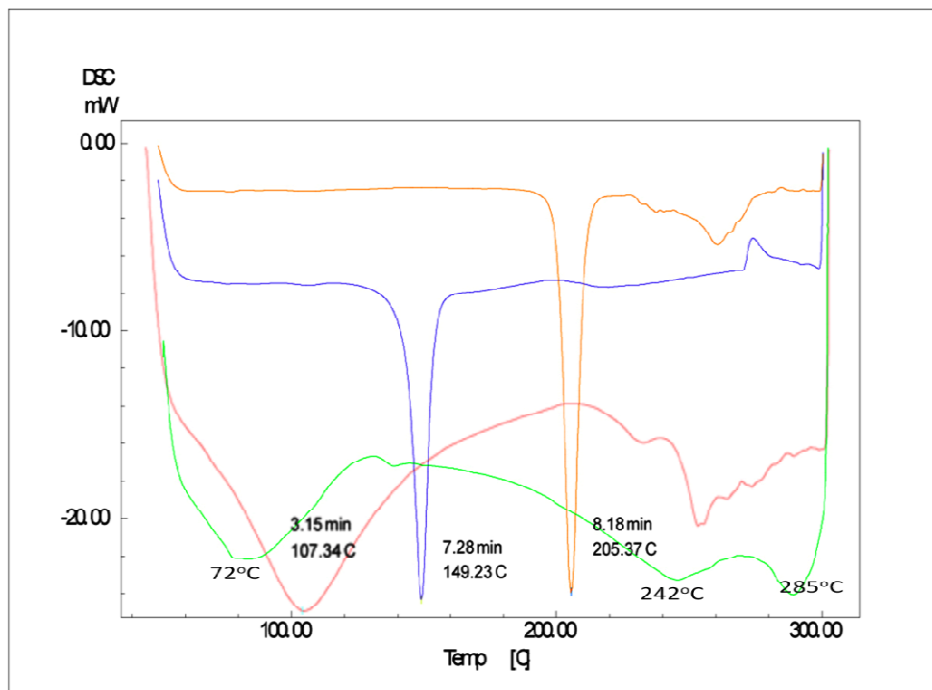

Fig. 7. DSC thermograms of a) PRX; b) carbopol; c) CHOL; and d) the eye cornea after transocular permeation of the niosomal gel. Color code: (a) orange, (b) green, (c) purple, and (d) pink

Table 4. Transcorneal Permeability Parameters of PRX across Cow Corneal Membrane under Different Treatment Conditions

Formulations	Amount permeated[a] (μg in 480 min)	Flux[a] (μg/cm^2·min)	P_{app}[a] x 10^{-3} (cm/s)	Permeability improvement ratio
PRX aqueous suspension	197.17±3.7	0.122±0.02	1.0±0.11	----
PRX-niosomal dispersion	316.17±1.8	0.386±0.01	3.2±0.23	3.1
PRX- niosomal hydrogel (0.5% w/w)	489.22±2.3	0.454±0.09	3.8±0.08	3.7*
PRX- niosomal hydrogel (1% w/w)	467.83±1.9	0.453±0.08	3.8*±0.10	3.7*
PRX- niosomal hydrogel (1.5% w/w)	303.48±5.1	0.391±0.11	3.3±0.013	3.2

a Data presented are mean ± SD, n=3
* Statistically significant difference at p<0.05 from the aqueous suspension

4. CONCLUSION

PRX niosomal formulas were prepared successfully by lipid film hydration technique using cholesterol and sorbitan surfactants. The highest entrapment efficiency and optimum controlled drug release was obtained with S80-Bformula which consists of Span 80 and high content of cholesterol. Incorporation of the niosomes inside carbopol gel resulted in further prolonged drug release and significant enhancement of PRX ocular bioavailability in comparison to the control. In conclusion, S80-Bniosomal gel can be considered as a novel drug carrier aimed at an ocular targeting of PRX for its sustained analgesic and anti-inflammatory effect. However future in vivo pharmacological studies are required to support our results.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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