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Brief/Technical Note

Effect of pH and Formulation Variables on *In Vitro* Transcorneal Permeability of Flurbiprofen: A Technical Note

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INTRODUCTION

Most of the topically applied drugs have to enter the eye through the corneal route and therefore epithelial and stromal layer of the cornea act as barrier to transcorneal permeability of both hydrophilic and lipophilic drugs respectively. Paracellular and transcellular pathways are the main two routes for corneal drug transport. Lipophilic drugs cross the cornea through transcellular pathway while the hydrophilic drugs cross through paracellular pathway (1,2). It has been reported based on studies involving different series of compounds that the fraction absorbed through corneal route varies from less than 1% for hydrophilic drugs to 7% for lipophilic drugs (1–3). Corneal epithelium and stroma exert varying degree of resistance to penetration depending on the nature of the drug (4). A corneal permeability coefficient of the order of 0.1×10^{-5} to 4.0×10^{-5} cm/s can be considered a measure of efficient corneal drug permeation (5). Corneal permeability increases when the corneal integrity is compromised by the usage of high concentration of certain formulation excipients like, preservatives and chelating agents (5).

Rabbit cornea or eye is most commonly used for *in vitro* and *in vivo* studies on ocular drug delivery. An investigation on the mammalian corneal epithelium in different species including human, rabbit and pig revealed morphological similarity of corneal cell layers (6). Nevertheless dissimilarities do exist between these species in terms of response of the eye to irritation and trauma (7). Rabbit eyes were found to be more sensitive to irritant than other species. Reer and his coworkers have suggested that rabbit cornea is not always the model of choice and the great morphological uniformity of mammalian cornea allow a different model like goat cornea to be chosen for *in vitro* permeation studies (8). Goat corneal membrane has been used for *in vitro* transcorneal permeability studies of ketrolac tromethamine from aqueous drops, (9) oil based drops and ointments (10). Effect of preservatives on *in vitro* transcorneal permeability of ibuprofen and flurbiprofen across goat cornea has also been reported (11). Taking the above reports in view, goat cornea was chosen for *in vitro* permeation studies. A practical advantage of goat cornea is its easy availability so that larger number of experiments could be performed in a shorter time period.

The present study was aimed to investigate the effect of various formulation variables in ophthalmic formulation on the *in vitro* transcorneal permeability of flurbiprofen in excised goat cornea using modified Franz diffusion cell. Effect of pH of the buffer, pH of the unbuffered solution and formulation excipients like, preservatives and chelating agents on the transcorneal permeability of the drug was investigated. To support transcorneal permeability data, solubility studied were carried out in buffered and unbuffered systems. Also investigated was the transcorneal permeability of flurbiprofen from various 5% *w/v* gels made of viscosity enhancing polymers and from various vegetable oil vehicles like, olive oil, linseed oil and sesame oil. All the animal experiment protocols were approved by Institutional Animal Ethics Committee, BITS, Pilani, India.

MATERIALS AND METHODS

Materials

Pure flurbiprofen was obtained as gift sample from Optho Remedies Pvt. Ltd., Allahabad, India. All the other polymers, chemicals and reagents used were of pharmaceutical or analytical grade and were used as received. Freshly excised whole eyeballs of goats were obtained from butcher's shop (M/s Dawood Butcher House, Pilani, India) within an hour of slaughtering of the animal in cold (4°C) normal saline. A commercially available ophthalmic drop of flurbiprofen (OCUFLUR of FDC Ltd., Aurangabad, India) was selected from the local market on random basis.

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OCUFLUR contained flurbiprofen sodium USP—0.03% *w/v*, phenyl mercuric nitrate I.P.—0.001% *w/v* and water for injection IP—q.s.

Equipment

A scanning spectrofluorimeter (Jasco, Tokyo, Japan, model FP-777) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220–750 nm and 10 mm quartz cells was used for fluorescence intensity measurement of samples obtained from *in vitro* transcorneal permeability experiments. For samples at low drug concentration, Jasco model liquid chromatograph equipped with two-pump gradient system (PU-1580), Rheodyne injector (7725i) fitted with a 20.l loop, UV detector (UV-1575) and BORWIN-I software was used.

In vitro transcorneal permeation experiments were carried out in modified Franz diffusion cell fabricated in-house (12). The water jacketed receiver chamber had an internal volume of 10 ml and was fitted with a side arm for sampling of receiver fluid and circulating warm water bath to maintain 37±1°C. The donor chamber with an internal area of 0.47 cm^2 (for permeation across the cornea) was clamped on top of the receiver chamber with corneal membrane sandwiched between the two.

Analytical Method

Two analytical methods were employed for analysis of fluribiprofen obtained from transcorneal permeability and solubility determination studies. A spectroflourimetric method using 1:1 mixture of methanol and 0.1 N H_2SO_4 at the λ_{ex} and λ_{em} of 250 nm and 314 nm respectively was employed for analysis in case of solubility determination and permeability study samples that did not show potential interference from vehicles or other chemical agents employed (13). The limit of quantitation (LOQ) was obtained as 3.32 ng/ml for the drug using this method. For the analysis of drug samples obtained from transcorneal permeability studies that showed potential interference from vehicles or other chemical agents employed, a liquid chromatographic method was used involving RP-C18 column in 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (0.1 M, pH 5.6) at a flow rate of 0.75 ml/min with UV detection at 248 nm (14). The chromatographic column used was a reverse phase 4.6× 125 mm LiChroCART® Purospher® endcapped C18 LC column (E. Merck, Darmstadt, Germany) with 5 μm particles. The LOQ for the method was found to be 50 ng/ml.

In vitro Permeability Study

Following procedure was employed for studying the *in vitro* transcorneal permeability of flurbiprofen across goat cornea.

(a) Corneal preparation: Freshly excised whole eyeballs of goat were transported from butcher's shop to laboratory in cold (4°C) saline within 1 h of slaughtering. The eyeballs were taken only from 6–7 months old goats so as to avoid obtaining corneas with pigmentation or other corneal abnormalities. The corneas were carefully dissected along with 2–4 mm of surrounding scleral tissue from the eyeball and washed with cold saline to remove any adhering pigments. The washed cornea were preserved in freshly prepared balance base buffer (pH 7.4) with % *w/v* composition of NaCl—0.57, $NaHCO_3$—0.361, KCl—0.04, K_2HPO_4—0.023, $MgSO_4$—0.007 and $CaCl_2$—0.08 in glass distilled water containing additionally aliquot amount of adenosine and bubbled with O_2 to keep the cornea in viable state (12).

(b) Permeation experiment: Fresh cornea obtained by the above procedure was mounted on the modified Franz diffusion apparatus by sandwiching the scleral tissues between the clamped donor and the receiver chamber. Care was taken to maintain the convex surface shape of the cornea by suitable design of the clamp, receiver and donor chamber edge and also to ensure that the epithelial surface of the cornea is towards the donor side. Balance base buffer (composition same as given in previous section) was filled in receiver chamber after expelling all the air bubbles by inverting the diffusion cell and then allowing the bubbles to travel through the sampling port. The receiver fluid was maintained at 37±1°C with the help of circulating warm water and kept under stirring using a teflon coated magnetic bead. An aliquot (1 ml) of test sample was placed on the epithelial surface of the cornea in the donor chamber and covered with glass slip using silicone grease to prevent evaporation. The permeation was continued for 120 min At predetermined time points of 15, 30, 45, 60, 90, and 120 min, a 250 μl sample was withdrawn through the sampling port, suitably diluted and analyzed by spectroflourimetric or liquid chromatographic method discussed earlier.

(c) Preparation of test samples: To study the effect of pH of phosphate buffer (0.01 M) on the transcorneal permeability of flurbiprofen, a 750 μg/ml solution of the drug was prepared in pH 5.4, 6.4, 7.4 and 8.4 system (15). Similar drug concentration solutions (0.01 M) were prepared in unbuffered pH (4.5, 5.5, 6.5, 7.5 and 8.5) using 0.01 N HCl and 0.01 N NaOH. In all the pH dependent experiments ionic strength was maintained at 0.2 with NaCl. An aqueous solution of the drug in triple distilled water with benzyl alcohol (0.5% *v/v*) served as in-house control in all these studies and the marketed formulation (OCUFLUR) of flurbiprofen was used as external control.

Effect of formulation excipients (isotonicity agent, preservatives and chelating agents) like, sodium chloride (NaCl; 0.9% *w/v*), thiomersal (THM; 0.004% *w/v*), benzalkonium chloride (BAC; 0.01% *w/v*), chlorbutanol (CB; 0.5% *w/v*), phenyl mercuric nitrate (PMN; 0.002% *w/v*), ethylenediamine tetraacetic acid (EDTA; 0.01% *w/v*), methyl hydroxy benzoate (MHB; 0.04% *w/v*) and propyl hydroxy benzoate (PHB; 0.02% *w/v*) in phosphate buffer (pH 7.4) on the transcorneal permeability was also investigated. Composition of these treatments are presented in Table I.

Various viscosity enhancing polymeric gel type formulations were prepared for permeability studies containing 5% *w/v* carboxy methyl cellulose (CMC), 5% *w/v* methyl

Table I. Composition of Various Treatment Vehicles

Treatment Name	Components[a]					
	Flurbiprofen (mg)	Additive	Polymer	Benzyl alcohol (ml)	Phosphate Buffer (pH 7.4)	Vegetable oil
Aqueous solution	75.0	–	–	0.5	–	–
NaCl (0.9% *w/v*)	75.0	0.9 g NaCl	–	–	q.s. to 100 ml	–
BAC (0.01% *w/v*)	75.0	0.01 g BAC	–	–	q.s. to 100 ml	–
THM (0.004% *w/v*)	75.0	0.004 g THM	–	–	q.s. to 100 ml	–
CB (0.5% *w/v*)	75.0	0.5 g CB	–	–	q.s. to 100 ml	–
PMN (0.002% *w/v*)	75.0	0.002 g PMN	–	–	q.s. to 100 ml	–
EDTA (0.01% *w/v*)	75.0	0.01 g EDTA	–	–	q.s. to 100 ml	–
MHB (0.04% *w/v*)	75.0	0.04 g MHB	–	–	q.s. to 100 ml	–
PHB (0.02% *w/v*)	75.0	0.02 g PHB	–	–	q.s. to 100 ml	–
CMC-5% *w/v*	75.0	–	5.0 g CMC	0.5	q.s. to 100 ml	–
MC-5% *w/v*	75.0	–	5.0 g MC	0.5	q.s. to 100 ml	–
NaCMC-5% *w/v*	75.0	–	5.0 g NaCMC	0.5	q.s. to 100 ml	–
PCB-5% *w/v*	75.0	–	5.0 g PCB	0.5	q.s. to 100 ml	–
Linseed oil	75.0	–	–	0.5	–	Linseed oil q.s. to 100 ml
Olive oil	75.0	–	–	0.5	–	Olive oil q.s. to 100 ml
Sesame oil	75.0	–	–	0.5	–	Sesame oil q.s. to 100 ml

NaCl Sodium chloride, *THM* thiomersal, *BAC* benzalkonium chloride, *CB* chlorbutanol, *PMN* phenyl mercuric nitrate, *EDTA* ethylenediamine tetraacetic acid, *MHB* methyl hydroxy benzoate, *PHB* propyl hydroxy benzoate, *CMC* carboxy methyl cellulose, *MC* methylcellulose, *NaCMC* sodium carboxymethyl cellulose, *PCB* polycarbophil 934 NF, *q.s.* quantity sufficient

[a] In final volume of 100 ml

cellulose (MC), 5% *w/v* sodium carboxy methyl cellulose (NaCMC) and 5% *w/v* polycarbophil (PCB). CMC and PCB were neutralized using 0.1 M NaOH before use. Various vegetable oils like, olive oil, linseed oil and sesame oil were selected as vehicle for dissolving the drug and studied for permeation. Composition of these treatments are also given in Table I.

Mean cumulative amount permeated was calculated at the end of 120 min based on triplicate experiments for each treatment group. The apparent corneal permeability coefficient (P_{app}) in centimeters per second for each treatment group was determined according to the Eq. 1 given below (4,16).

$$P_{app} = \text{Flux}/(60.C_o) \quad (1)$$

Where, flux ($\mu g/cm^2 \cdot min$) 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 0.47 cm^2. The linear portion of the curve was identified using linear regression and goodness of fit of the permeation data obtained between 15 to 120 min. Also in the equation, C_o 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 (aqueous solution).

(d) Determination of corneal hydration: At the end of the experiment, each cornea was freed from adjoining sclera, weighed and soaked overnight in 2 ml of methanol. The soaked corneal membrane on the subsequent day was dried to constant weight at 90°C and reweighed. From the difference of the two weights corneal hydration percentage was calculated.

Solubility Studies

To support reasoning of the permeation data, the effect of various buffers, pH of the buffer and unbuffered system on the solubility of flurbiprofen was also evaluated. Different buffers at 0.01 M concentration were prepared as per earlier reported method (15). To study the effect of pH of phosphate buffer on the solubility of flurbiprofen, solubility of the drug was determined at 37±1°C in pH 5.6, 6.6, 7.0, 7.6 and 8.0 with ionic strength adjusted to 0.2 using sodium chloride. Effect of pH of unbuffered solution (0.01 M) on solubility of flurbiprofen was studied at pH 2.2, 3.2, 4.0, 5.7, 6.5, 7.0, 7.5, 8.2, 9.1, 10.4 and 12.2. The pH was adjusted using varying proportion of 0.01 N NaOH and 0.01 N HCl and the ionic strength was adjusted to 0.2 with sodium chloride. Solubility of the drug in triple distilled water (pH 6.65) served as control. Solubility was also determined in benzyl alcohol and the three vegetable oils (olive oil, linseed oil and sesame oil) selected for the studies.

Statistical Analysis

All values presented in this study are average of triplicate experiments for the same time points. Least

square regression equations and the correlation coefficients were calculated using Microsoft Office 2003 Excel package. Differences in transcorneal permeability profile of flurbiprofen under different conditions were tested statistically using one-way analysis of variance and Tukey's multiple range tests at different level of significance (17).

RESULTS AND DISCUSSIONS

Flurbiprofen was found to have a poor solubility of 61.5±3.5 μg/ml in triple distilled water (pH 6.65) at 37±1°C but very high solubility of 141.9±12.0 mg/ml in benzyl alcohol. To overcome the solubility issue in aqueous phase, 0.5% *v/v* of benzyl alcohol was used in aqueous solution and other vehicles (like unbuffered solutions and viscosity enhancing polymers) where the solubility of the drug was lower.

Cumulative amount permeated (in 120 min), calculated transcorneal flux, apparent permeability coefficient (P_{app}) and permeability improvement ratio as well as the percentage corneal hydration under different treatment conditions are presented in Table II. Corneal permeability of flurbiprofen from aqueous solution was very low with only 33.2±2.7 μg of the drug permeating with calculated flux of only 0.58± 0.05 μg/cm^2.min and P_{app} of 1.30 (±0.11)×10^{-5} cm/s. This aqueous solution with only 0.5% *v/v* of benzyl alcohol was used as in-house control for all further comparison. In comparison to aqueous solution in case of market formulation, the permeability was considerably enhanced with permeability improvement ratio of 2.9 (Table II). The increase in permeability in case of market formulation can be attributed to the presence of formulation additives like PMN in appreciable concentration in the marketed formulation. Earlier studies involving PMN has established its ability to enhance transcorneal permeability of drugs by interacting with corneal membrane sulphydryl groups (18). Our studies have shown similar results with drug permeability increasing three times in the presence of PMN 0.002% *w/v*.

The vehicle pH under both buffered and unbuffered condition was also found to increase the transcorneal permeability of flurbiprofen. In case of phosphate buffer, increasing the pH of the vehicle from 5.4 to 8.4 resulted in statistically significant decrease in the corneal permeability and thus the transcorneal flux, P_{app} and permeability improvement ratio for flurbiprofen (Table II) when compared with that of aqueous solution. The P_{app} varied from 4.35 (±0.14)×10^{-5} cm/s at pH 8.4 to 8.00 (±0.06)× 10^{-5} cm/s at pH 5.4 with the corresponding permeability improvement ratio obtained as 3.3 and 6.2 respectively. A similar phenomenon was observed with unbuffered solutions of varying pH with permeability of the drug decreasing significantly with increase in the pH of the unbuffered vehicle. The permeability improvement ratio varied from 3.0 at pH 8.5 to 5.5 at pH 4.5 (Table II). Similar results have been reported earlier for ibuprofen and flurbiprofen from unbuffered solutions (11).

The permeability results inversely correlated with solubility data (Figs. 1 and 2). Flurbiprofen is a weakly acidic drug with 4.27 as its pK$_a$ value (19). It is expected that the drug will be ionized to a greater extent at pH above 4.27 with the degree of ionization increasing with increase in pH. This increase in ionization of the drug contributes to increase its solubility in aqueous vehicle and a proportional decrease in unionized fraction thereby resulting in decreased permeability. The difference in the permeability profile of the drug from buffered and unbuffered media could be attributed to the difference in the ionization capacity of hydrochloride and sodium hydroxide ions used for adjusting the pH in case of unbuffered solutions when compared to mono basic sodium mono phosphate and dibasic sodium mono phosphate ions (used for preparation of phosphate buffer of varying pH in buffered systems). Another reason for pH dependent permeability of the drug (reduced permeation at higher pH) could be because of the fact that corneal membrane has an isoelectric pH of 3.2 and at pH above this, cornea attain negative charge (20). Flurbiprofen being acidic drug would be anionic at higher pH thereby resulting in decreased permeation at higher pH due to enhanced repulsive forces between the corneal surface and the drug.

Studies on the effect of formulation additives on the transcorneal permeability revealed that compounds like BAC, THM, CB, PMN, EDTA, MHB and PHB (which are commonly used as formulation additives in ophthalmic formulations) increased the extent of flurbiprofen transcorneal permeation at pH 7.4 in phosphate buffer by statistically significant amount (Table II). Flurbiprofen permeability has been reported to be increased in the presence of above additives from 0.5% *w/v* drops of pH 6.4 in normal saline (11). A statistically significant ($p<0.05$) increase was also observed in case of formulation prepared using 0.9% *w/v* NaCl when compared to aqueous solution. Organomercurials like THM and PMN as mentioned earlier interact with the membrane sulphydryl groups thereby altering the membrane permeability and transport systems (18,21). In the present study, THM (0.004% *w/v*) and PMN (0.002% *w/v*) increased the permeability improvement ratio to 5.7 and 3.0 respectively (Table II). In our studies, BAC at 0.01% *w/v* concentration was found to enhance the corneal permeability of the drug by 6.1 times. Electrophysiological studies using BAC has been reported to cause irreversible damage to the corneal cell layer followed with neovascularisation (22). Compound CB at 0.5% *w/v* concentration was found to enhance the corneal permeability of flurbiprofen to the maximum extent with P_{app} value and permeability improvement ratio of 8.72 (±0.08)×10^{-5} cm/s and 6.7 respectively. CB is known to reduce oxygen utilization in the cornea and thereby resulting in loosened epithelial cell adhesion and thus, increased permeation of drugs through cornea (23). It has also been shown to contribute in enhanced corneal drug permeability by producing granularity and small vacuole in epithelial cells (24). EDTA a known calcium chelating agent is reported to act on epithelial cell junctions by interfering with calcium ions and altering membrane intercellular integrity and causing increased intercellular permeability (25,26). EDTA (0.01% *w/v*), also increased the corneal permeability of flurbiprofen by a factor of 6.5. Commonly employed preservatives in ophthalmic formulations, MHB (0.04% *w/v*) and PHB (0.02% *w/v*), were also found to cause statistically signif- icantly increase ($p<0.05$) in the transcorneal permeability of flurbiprofen (Table II).

A normal intact goat cornea has been reported to have 76 to 80% hydration level (27). An increased hydration level

Table II. Various Transcorneal Permeability Parameters of Flurbiprofen Across Goat Corneal Membrane Under Different Treatment Conditions

Treatment	Amount permeated[a] (μg in 120 min)	Flux[a] (μg/cm^2·min)	P_{app} (±SD)[a] cm/s (×10^5)	Permeability improvement ratio	Corneal hydration[a] (%)
Aqueous Drop	33.2±2.7	0.58±0.05	1.30 (±0.11)	1.0	79.8±0.3
Market preparation	98.1±5.4	1.70±0.10	3.78 (±0.21)*	2.9	78.3±0.5
Effect of pH of phosphate buffer (0.01 M and ionic strength of 0.2)					
pH 5.4	201.5±1.4	3.60±0.03	8.00 (±0.06) **	6.2	77.9±0.3
pH 6.4	136.9±3.2	2.46±0.06	5.46 (±0.13) **	4.2	77.7±0.4
pH 7.4	122.4±6.6	2.26±0.12	5.02 (±0.27) **	3.9	76.4±0.3
pH 8.4	108.5±3.4	1.96±0.06	4.35 (±0.14) *	3.3	78.6±0.5
Effect of pH of unbuffered solution (0.01 M and ionic strength of 0.2)					
pH 4.5	179.9±0.9	3.20±0.02	7.11 (±0.04)**	5.5	78.9±0.6
pH 5.5	145.0±2.1	2.59±0.04	5.75 (±0.08)**	4.4	80.1±0.4
pH 6.5	126.5±5.4	2.27±0.10	5.05 (±0.22)**	3.9	78.2±0.2
pH 7.5	116.5±6.0	2.13±0.11	4.73 (±0.24)**	3.6	77.9±0.8
pH 8.5	97.7±4.2	1.76±0.08	3.92 (±0.17)*	3.0	78.3±0.5
Effect of different formulation excipients at pH 7.4					
NaCl (0.9% *w/v*)	81.6±4.7	1.41±0.08	3.14 (±0.18)*	2.4	79.3±0.6
BAC (0.01% *w/v*)	211.2±3.1	3.57±0.05	7.93 (±0.12)***	6.1	84.6±0.7
THM (0.004% *w/v*)	189.6±3.5	3.32±0.06	7.37 (±0.14)**	5.7	83.9±0.4
CB (0.5% *w/v*)	235.3±2.2	3.92±0.04	8.72 (±0.08)***	6.7	85.3±0.3
PMN (0.002% *w/v*)	105.6±2.1	1.78±0.04	3.96 (±0.09)*	3.0	78.6±0.1
EDTA (0.01% *w/v*)	225.4±2.1	3.81±0.04	8.46 (±0.10)***	6.5	85.7±0.6
MHB (0.04% *w/v*)	74.8±1.8	1.29±0.03	2.86 (±0.07)*	2.2	78.6±0.5
PHB (0.02% *w/v*)	80.1±2.2	1.26±0.04	2.81 (±0.08)*	2.2	79.2±0.2
Effect of different viscosity enhancing polymers					
CMC-5% *w/v*	110.5±7.1	2.15±0.14	4.77 (±0.31)**	3.7	78.6±0.3
MC-5% *w/v*	145.4±4.7	2.32±0.08	5.16 (±0.17)**	4.0	76.9±0.4
NaCMC-5% *w/v*	101.3±5.3	1.98±0.10	4.41 (±0.23)*	3.4	78.5±0.2
PCB-5% *w/v*	151.4±3.3	2.73±0.07	6.06 (±0.16)**	4.7	79.6±0.4
Effect of different vegetable oil vehicle					
Linseed oil	123.5±3.1	2.07±0.05	4.60 (±0.12)*	3.5	78.6±0.7
Olive oil	102.8±2.7	1.86±0.05	4.14 (±0.11)**	3.2	80.2±0.5
Sesame oil	97.3±2.3	1.78±0.04	3.96 (±0.09)*	3.0	77.6±0.7

[a] Data presented are mean of three experiments per group with SD
*Statistically significant difference at $p<0.05$ from the aqueous solution
**Statistically significant difference at $p<0.005$ from the aqueous solution
***Statistically significant difference at $p<0.001$ from the aqueous solution

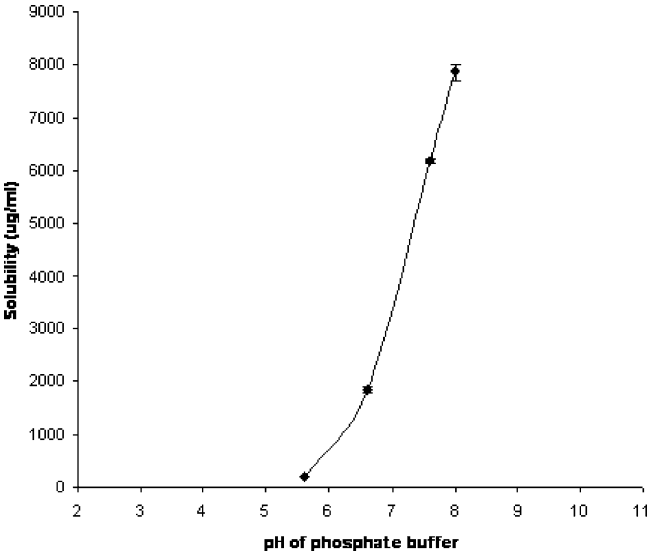

Fig. 1. Solubility profile of flurbiprofen in phosphate buffer of varying pH

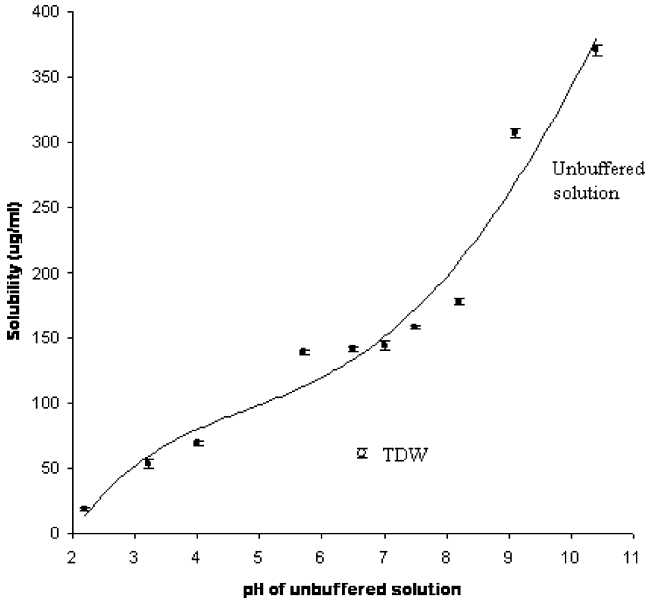

Fig. 2. Solubility of flurbiprofen in unbuffered solutions and triple distilled water (TDW)

of 83 to 92% is usually seen in case of corneal damage (4). Our experiments on freshly excised untreated goat cornea showed a hydration level of 78.9±1.6%. Of all the treatments studied only in case of vehicle containing BAC, THM, CB or EDTA, the post treatment corneal hydration was found to be higher than 80% (Table II). These results further confirm the earlier reports of adverse effect of these agents on the corneal cell structure and its integrity (21–26).

Transcorneal permeability studies involving selected formulations prepared using various mucoadhesive polymers revealed that corneal permeability of flurbiprofen was increased with the use of these polymers to different extent (Table II). Maximum increase in permeability was seen in case of 5% *w/v* PCB with a flux of 2.73±0.07 μg/cm^2.min and cumulative permeation of 151.4±3.3 μg and the least increase was seen in case of 5% *w/v* NaCMC with cumulative permeation of 101.3±5.3 μg and a flux of 1.98±0.10 μg/cm^2. min. The P_{app} was found to be 6.06 (±0.16)×10^{-5} cm/s and 4.41 (±0.23)×10^{-5} cm/s in case of 5%*w/v* PCB and NaCMC respectively. 5% *w/v* MC and CMC showed intermediate enhancement in permeability. The increase in corneal permeation in case of polymeric gels can be attributed to the increased intimate adherence of the formulation to the corneal epithelium thereby facilitating partitioning and permeation of the drug from the vehicle to the corneal membrane.

Studies with selected vegetable oil based vehicles showed that the permeability increased in all cases (Table II). A maximum of 3.5 times increase in permeability was seen in case of linseed oil. The solubility data obtained showed that flurbiprofen has maximum solubility in sesame oil (23.86± 0.57 mg/ml) followed by olive oil (21.63±0.40 mg/ml) and linseed oil (14.71±0.32 mg/ml). It is expected that oil/water partition coefficient for flurbiprofen will also follow the same order thereby proportionately decreasing partitioning of the drug to cornea from oil where solubility is comparatively higher. The results also reinforced this idea, wherein maximum permeability was seen in case of linseed oil (123.5±3.1 μg in 120 min with a flux of 2.0714 μg/cm^2 min) followed with olive oil (102.8±2.7 μg in 120 min with a flux of 1.8635 μg/cm^2·min) and sesame oil (97.3±2.3 μg in 120 min with a flux of 1.7841 μg/ cm^2.min).

SUMMARY AND CONCLUSIONS

Corneal permeability of flurbiprofen from aqueous solution was found to be very low whereas in case of market formulation (OCUFLUR) the permeability was considerably enhanced due to the presence of formulation additives like, PMN. In case of buffered and unbuffered vehicles of varying pH the permeability decreased with increase in pH of the vehicle. Studies on the effect of formulation additives on the transcorneal permeability revealed that compounds like BAC, THM, CB, PMN, EDTA, MHB and PHB which are commonly used as formulation additives in ophthalmic formulations increased the transcorneal permeation of flurbiprofen by statistically significant amount. However in case of BAC, THM, CB and EDTA the corneal hydration value varied between 83 to 86% suggesting adverse effect of these agents on the corneal cell structure and its integrity thereby increasing the drug permeability.

Transcorneal permeability was found to increase with gels prepared using various viscosity enhancing polymers and drops prepared using vegetable oils. Since the donor chamber is not stirred, there exists a possibility of development of concentration gradient in the donor phase in case of viscous preparations. That could be limitation of transcorneal permeability determined using modified Franz diffusion cell. On the basis of the present data, it can be concluded that flurbiprofen ophthalmic drops buffered at neutral pH will favor higher transcorneal permeability. Preservatives like PMN, MHB and PHB will also favor better transcorneal permeation of the drug without significant corneal damage.

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