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Transient and Steady State Characterization of Permeation Kinetics of Diclofenac through Ocular Tissue

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Mechanism of permeation kinetics of a model drug has been studied through sheep cornea. Hydroxypropyl methylcellulose matrix film containing triethanolamine as plasticizer and benzalkonium chloride (BZC) as preservative was prepared by solvent casting technique. In this study, semi-empirical models, such as Higuchi and Korsmeyer-Peppas models were utilized for describing drug permeation kinetics. Mean transient time, mean steady state time and mean permeation time evaluated using statistical moment analysis were decreased with the increase of triethanolamine concentration in presence of benzalkonium chloride. Results of scanning electron microscopy, Fourier transform infrared spectroscopy and X-ray diffractometry studies suggested the inhibition of the crystal growth of diclofenac in the film. Statistical moment analysis was employed for characterization of transient and steady state of kinetics of permeation. A correlation has also been established between the *ex vivo* permeation and *in vitro* dissolution of the formulations.

Keywords: Permeation kinetics, Transient state, Steady state, Statistical moment analysis.

INTRODUCTION

Investigational studies regarding passage of molecular mass across the biological membranes are of utmost significant with respect to array of perspectives related to the kinetics of drug transport. Various mathematical models and semi-empirical equation including first-order, Higuchi and Korsmeyer-Peppas models were tested for model fitting purpose [1-5]. First order kinetics of drug diffusion is concentration dependent and decrease exponentially with time. Higuchi model diffusion pattern from heterogeneous matrix system is directly proportional to the square root of time and involves tortuosity which leads to Fickian diffusion. Korsmeyer-Peppas model, a semi empirical equation relates exponential diffusion of drug to the elapsed time. Several parameters like drug type, polymorphic form, crystallinity, particle size and solubility in addition to the permeation enhancing component in the dosage form are capable of altering diffusion kinetic.

Drug transport through a biomembrane majorly is a two step phenomenon (*i.e.*, transient state and steady state) involving dissolution of drug molecule, followed by permeation of the dissolved species [6,7]. The flux of diffusing entities is a motion in response to a thermodynamic force arising from a concentration gradient. The entities on the move reach a steady drift speed, when the thermodynamic force is matched by the viscous drag [8]. Once the system reaches steady state it can be assumed that the diffusant concentration remains constant at all points on donor and receiver side of the barrier. Diffusional behaviour of chemical entities in liquid has been described in chemical engineering by using statistical moment analysis [9]. Statistical moment analysis is the non-compartmental approach for estimation of important pharmacokinetic parameters such as volume of distribution, mean residence time, clearance and bioavailability without the complicated non-linear regression processes used in compartmental analysis [10,11]. Pharmaceutical industry is giving prime importance to the non-compartmental approach owing to its ruggedness and versatility. Time lag factor [12] is the depiction of the transient state and deduced from finite time difference observed between the time at which the drug enters the biological barrier and the time at which the flow rate reaches a steady state.

In the past many investigations were carried out to characterize corneal permeability mechanism and predict drug delivery rates to the eye [13]. Several models have been examined to explain drug transport kinetics [14]. Absence of any specific tactic for evaluation and quantification of permeation behaviour warrants attention. Statistical moment analysis is a preferred tool for quantification of parameters in biological system. Numerous investigations were carried out in the past to characterize *in vitro* and *in vivo* correlation using statistical moment analysis [15,16]. After extensive literature survey it was found that majority of the experimental reports

utilized conventional model analysis rather than statistical moment analysis for the characterization of biomembrane permeation kinetics [17].

In the present study, conventional mathematical models along with statistical moment analysis were utilized to explain the mechanism of ocular permeation kinetics of diclofenac potassium film formulation fabricated with triethanolamine as plasticizer and benzalkonium chloride as preservative. Characterization was done by using analytical techniques [18] such as, X-ray diffractometry, Fourier transform infrared spectroscopy and scanning electron microscopy for understanding the crystal behaviour of the drug in the film formulations. The best fitted model was selected basing on the highest coefficient of determination (r^2) of the permeation data. Statistical moment analysis was applied to the permeation data and mean transient time (MTT), mean steady state time (MST) and mean permeation time (MPT) were determined statistically. Quantification of amount permeated in transient state (APT), amount permeated up to lag time (APL), amount permeated in the steady state (APS) and total drug permeated (TDP) were deduced. Pearson's product moment correlation has been attempted to establish Level "A" correlation between *in vitro* dissolution and *ex vivo* steady state permeation at each time point.

EXPERIMENTAL

Diclofenac potassium and hydroxypropyl methylcellulose (HPMC K15 M) were purchased from Tejani Life care, Cuttack, India and Himedia Laboratories Pvt. Ltd, India respectively. Benzalkonium chloride and triethanolamine were procured from Merck Ltd, Mumbai, India. All other chemicals were of analytical grades as required.

Preparation of film: Hydroxypropyl methylcellulose films were prepared by casting and solvent evaporation method [19-21]. Diclofenac potassium, triethanolamine and benzalkonium chloride (wherever required) were dissolved simultaneously in ethanol and incorporated in the swelled aqueous gel of HPMC for five formulations (F1, F2, F3, F4 and F5). The mass was poured over petridis and dried at 40 °C until constant weight. Triethanolamine and benzalkonium chloride were absent in F1 and all other formulations contain benzalkonium chloride (0.01 % dry wt/wt of polymer). F2, F3, F4 and F5 contain 10, 20 and 30 % triethanolamine as plasticizer respectively on dry wt/wt of polymer basis.

***in vitro* Dissolution:** USP type II apparatus (Electrolab, dissolution tester USP, TDT06L, India) was employed for the dissolution study. Precisely weighed strip of each film was cut and adhered to the glass slide with cyanoacrylate adhesive and placed inside bottom of the dissolution basket. The basket was filled with simulated tear fluid (pH 7.4). The dissolution was carried out at 34.0 ± 0.5 °C at 50 rpm for 360 min [13]. Samples were withdrawn through 0.45 μm membrane filter and analyzed spectrophotometrically at 276 nm as the mean of three determinations.

***ex vivo* Permeation:** Freshly excised sheep eyes were collected from local butcher shop within 1 h of its sacrifice. After cleaning the extraneous tissues, the cornea was harvested and permeation experiment was carried out at 34 °C using a vertical type diffusion cell. The dorsal side of the cornea faced the donor compartment vertically. Withdrawn samples were filtered through a 0.45 μm membrane filter and absorbance was recorded at 276 nm using UV-VIS spectrophotometer (JASCO V-630 spectrophotometer, Software: Spectra Manager).

Data analysis: The permeation data were fitted to various semi-empirical mathematical models including first-order, Higuchi and Korsmeyer-Peppas models. The best fitted model was selected based on the highest coefficient of determination (r^2) values. The lag time (t_d) was estimated from the x-intercept of the linear region of permeation profile using regression analysis. Transient and steady state parameters were elucidated from permeation profile according to Fick's law of diffusion. Following relations were employed for elucidation of mean transient time (MTT), mean steady state time (MST) and mean permeation time (MPT) using the first moment of amount of drug permeated *vs.* time curve.

$$\text{Mean transient time} = AUMC_{(0\text{-}2)}/AUC_{(0\text{-}2)} \quad (1)$$

$$\text{Mean steady state time} = AUMC_{(2\text{-}6)}/AUC_{(2\text{-}6)} \quad (2)$$

$$\text{Mean permeation time} = AUMC_{(0\text{-}6)}/AUC_{(0\text{-}6)} \quad (3)$$

where, AUMC is the area under the product of amount permeated and time (the first moment AUC) *versus* time curve. $AUMC_{(0\text{-}2)}$ was calculated from initial sampling time to sampling at 2 h and $AUMC_{(2\text{-}6)}$ was derived from 2 h to last sampling time employing MATLAB 7.0 software. $AUMC_{(0\text{-}6)}$ is the summation of $AUMC_{(0\text{-}2)}$ and $AUMC_{(2\text{-}6)}$.

Amount permeated in transient state (APT), amount permeated upto t_d (APL), amount permeated in the steady state (APS) and total drug permeated upto 6 h (TDP) were evaluated from the amount permeation *vs.* time profile to understand the effect of triethanolamine on permeation threshold of the films. Level "A" correlation between *in vitro* dissolution and *ex vivo* steady state permeation at each time point has been attempted using Pearson's product moment correlation employing following relations.

$$r_p = \left(1/n\sum xy - \overline{xy}\right)/S_xS_y$$

where, r_p = Pearson's product moment correlation co-efficient, n = Number of pairs of sample, x = Amount dissolution, y = Amount permeation, $S_x = \sqrt{1/n\sum x^2 - \overline{x}^2}$, $S_y = \sqrt{1/n\sum y^2 - \overline{y}^2}$.

Instrumental analysis of film: Scanning electron microscopy (JSM- 6390, JEOL, Tokyo, Japan) was employed for the detailed study of surface morphology of the films. The dried samples were mounted on metal stab for gold coating under applied vacuum. The photomicrographs were taken at different magnifications employing electron imaging at an accelerated voltage of 10 kV. FT-IR spectra for the pure drug, polymer and films were obtained with a JASCO spectrometer (Japan) employing the KBr disc technique. The XRD pattern of pure diclofenac and the films were recorded using X-ray diffractometer (Model: Philips analytical X-ray with PC-APD, Diffraction Software). The voltage and current were 40 kv and 15 mA, respectively. Anode material Cu, K_α (radiation 1.5406 Å) was used as a source of X-rays. Measurements were undertaken at a scan speed of 1 °/min for the scanning angle ranging from 10° to 60° (2θ).

RESULTS AND DISCUSSION

Drug dissolution and kinetics of permeation: *in vitro* Dissolution of diclofenac potassium has been improved with the increased amount of triethanolamine and the presence of benzalkonium chloride in the film formulation (Fig. 1). F5 film (30 % triethanolamine) exhibited significantly improved dissolution compared to other films (20, 10 and 0 % triethanolamine). Fig. 2 depicts the amount of drug permeated from formulated films at 34 °C as a function of time. An increasing trend of amount permeation was observed with increasing triethanolamine content in presence of benzalkonium chloride. Amount permeation of different formulations was found in the following order as: F1 < F2 < F3 < F4 < F5. For sensible preference of mechanism of diffusion kinetics, the permeation data was examined for the applicability of several mathematical models. Goodness of fit test of permeation data with first order, Higuchi and Korsmeyer-Peppas model was examined. The calculated values of $t_{d,}$ time required for 50 % permeation ($t_{50\,\%}$), rate constant (k) and r^2 values corresponding to each model were depicted in Table-1. The projected t_d and $t_{50\,\%}$ were in the decreasing trend with the increased value of permeation rate constant as triethanolamine increased in the formulation, confirming its positive influence on permeation.

Steady state permeation parameters of the film formulations are reported in Table-2. It was evident that the value of the lag time has been tagged on to a paradigm with respect to plasticizer concentration. Gradual reduction in t_d was observed as the plasticizer concentration increased. Triethanolamine, a hydrophilic plasticizer decreased the lag time of permeation from 0.49 to 0.20 h when its concentration was varied from 0 to 30 %. Fundamental relationship of Fick's first law used in the pharmaceutical diffusion process was applied for the estimation of $t_{50\,\%}$, steady state rate constant (k_{ss}) and permeation flux (J). Parameters indicating improved permeation ($t_{50\,\%}$ values 7.66 to 4.73 h; k_{ss} values 8.09 to 11.44 μg h^{-1} and J values 273.02 to 387.86 μg cm^{-2} h^{-1}) were also resulted by the increased content of triethanolamine in the film formulation. Permeation parameters MTT, MST and MPT were estimated by the statistical moment analysis and depicted in Table-3. Increase in triethanolamine from 0 to 30 % lowered the MTT from 1.77 to 1.49 h. Analogous reduction pattern in MST (4.56 to 4.39 h) and MPT (4.49 to 4.14 h) was followed with the increase in triethanolamine. A comparative illustration of amount permeated in transient state (APT), amount permeated upto t_d (APL), amount permeated in the steady state (APS) and total drug permeated upto 6 h (TDP) were depicted in Table-3. This result supports the positive involvement of plasticizer in the progress of drug permeation. The correlation coefficient and regression equation of *in vitro* dissolution and steady state *ex vivo* permeation (Fig. 3) have been tabulated in Table-4. The Pearson's product moment correlation co-efficient for F1, F2, F3, F4 and F5 was found to be 0.993, 0.991, 0.992, 0.974 and 0.963, respectively.

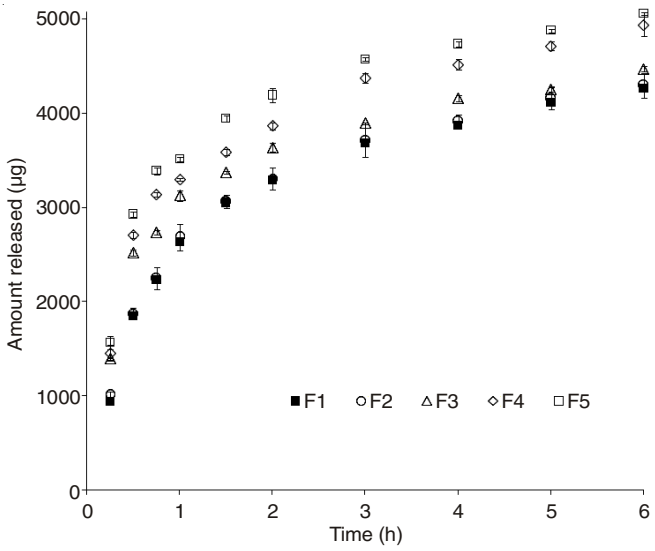

Fig. 1. *in vitro* Dissolution profiles of diclofenac potassium film formulation

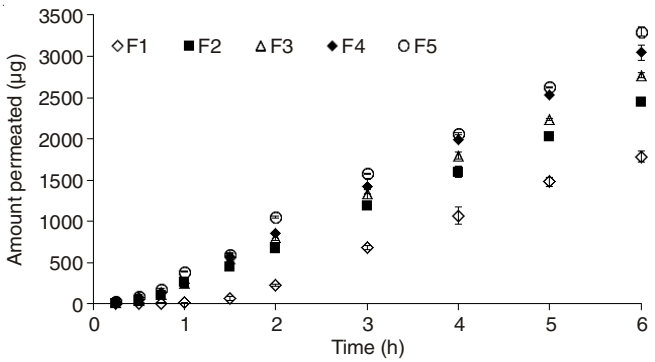

Fig. 2. *ex vivo* Permeation profiles of diclofenac potassium film formulation across cornea at 34 °C

TABLE-2
STEADY STATE PERMEATION PARAMETERS OF DIFFERENT FORMULATIONS

Film code	t_d (h)	$t_{50\,\%}$ (h)	k_{ss} (μg h^{-1})	J (μg cm^{-2} h^{-1})	r^2
F1	0.49	7.66	8.09	273.02	0.994
F2	0.43	6.01	8.48	307.48	0.983
F3	0.29	5.62	9.80	337.19	0.992
F4	0.26	5.19	10.58	384.86	0.986
F5	0.20	4.73	11.44	387.86	0.994

TABLE-1
GOODNESS OF FIT TEST OF PERMEATION DATA WITH DIFFERENT KINETIC MODELS

Film code	First order				Higuchi				Korsmeyer-Peppas		
	t_d (h)	$t_{50\,\%}$ (h)	k (h^{-1})	r^2	t_d (h)	$t_{50\,\%}$ (h)	$k^{\%rel.-1/2}$	r^2	$t_{50\,\%}$ (h)	n	r^2
F1	0.018	9.66	0.079	0.955	0.95	3.32	21.35	0.916	5.67	2.93	0.981
F2	0.013	6.25	0.121	0.980	0.54	2.69	25.18	0.961	4.66	1.39	0.954
F3	0.010	5.83	0.132	0.975	0.52	2.61	26.41	0.955	3.26	1.69	0.967
F4	0.007	5.19	0.151	0.964	0.51	2.37	30.56	0.950	2.88	1.53	0.990
F5	0.005	4.53	0.176	0.968	0.49	2.21	33.63	0.958	2.15	1.55	0.978

TABLE-3
KINETIC PARAMETERS OF PERMEATION DATA AFTER STATISTICAL MOMENT ANALYSIS

Film code	MTT (h)	MST (h)	MPT (h)	APT (µg)	APL (µg)	APS (µg)	TDP (µg)
F1	1.77 ± 0.02	4.56 ± 0.01	4.49 ± 0.02	228 ± 5.86	2.12 ± 0.12	1555 ± 13.58	1783 ± 26
F2	1.58 ± 0.03	4.42 ± 0.04	4.20 ± 0.03	661 ± 11.53	18.12 ± 0.47	1783 ± 21.53	2444 ± 28
F3	1.54 ± 0.02	4.41 ± 0.03	4.19 ± 0.03	808 ± 17.71	20.11 ± 0.26	1960 ± 19.36	2768 ± 68
F4	1.51 ± 0.03	4.40 ± 0.03	4.18 ± 0.02	849 ± 2.16	29.32 ± 1.47	2197 ± 13.82	3046 ± 47
F5	1.49 ± 0.02	4.39 ± 0.06	4.14 ± 0.01	1050 ± 6.24	69.70 ± 4.50	2251 ± 56.24	3301 ± 43

MTT = Mean transient time; MST = Mean steady state time; MPT = Mean permeation time; APT = Amount permeated in transient state; APL = Amount permeated upto T_{lag}; APS = Amount permeated in the steady state; TDP = Total drug permeated upto 6 h

TABLE-4
RESULTS OF CORRELATION OF *in vitro* DISSOLUTION AND STEADY STATE *ex vivo* PERMEATION AT THE SAME TIME POINT

Film code	r^2	Regression equation	r_p^*
F1	0.986	y = 1.606x-5120	0.993
F2	0.981	y = 1.771x-5279	0.991
F3	0.949	y = 2.103x-7434	0.992
F4	0.983	y = 2.333x-7733	0.974
F5	0.928	y = 2.583x-9995	0.963

*Pearson's product moment correlation coefficient.

Characterization of film: Photomicrograph of pure diclofenac potassium contains distinct compact drug crystals in micron scale (Fig. 4). All the films were microporous and of uniform surface morphology. Keen observation demonstrated that film without plasticizer and preservatives (F1) presented a relatively irregular, rigid, packed and porous surface with embedded minute drug crystals. Films with plasticizer and preservatives exhibited a smoother and continuous surface (F2,

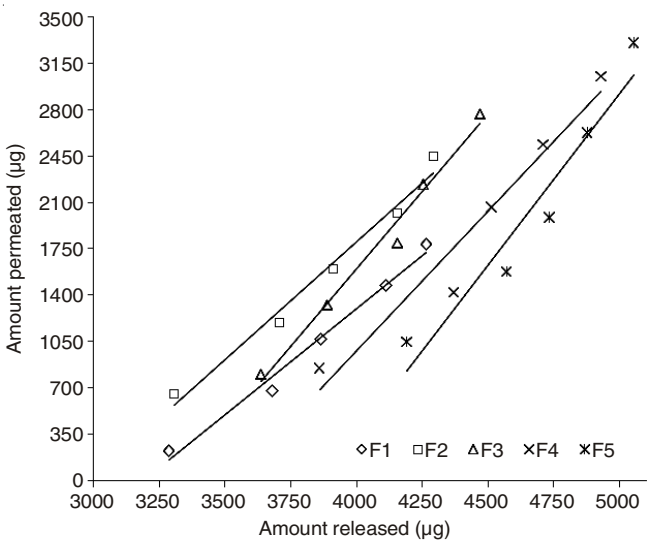

Fig. 3. Correlation of *in vitro* dissolution and *ex vivo* permeation (steady state) at the same time point

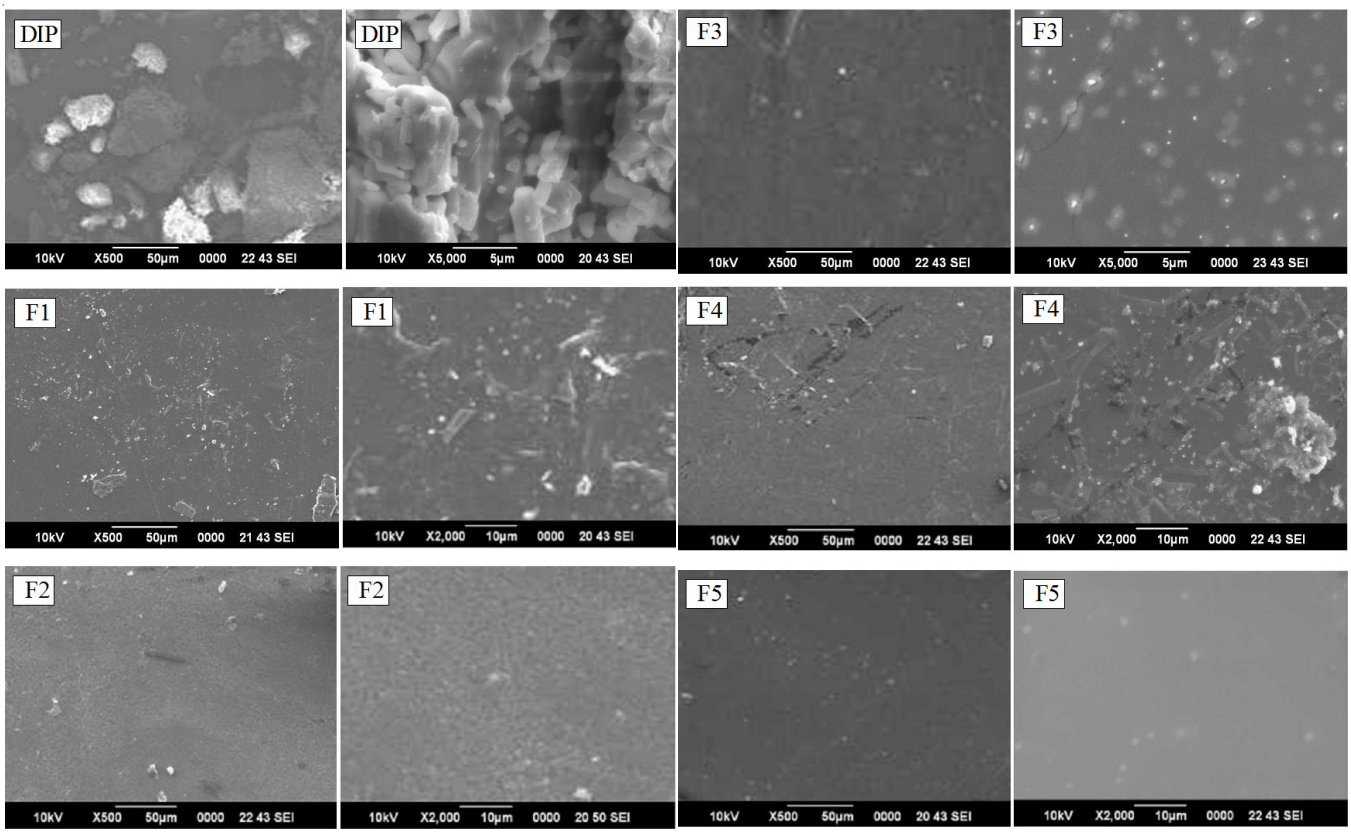

Fig. 4. Scanning electron micrographs of diclofenac potassium pure drug crystals (diclofenac potassium) 500×, 5000×; and film formulations in HPMC matrix: (F1) 500×, 2000×; (F2) 500×, 2000×; (F3) 500×, 5000×; (F4) 500×, 2000×; and (F5) 500×, 2000×

F3, F4 and F5). Crystals grown in the film in presence of both triethanolamine and benzalkonium chloride (F3, F4 and F5) were found smaller than the crystals grown without triethanolamine (F1 and F2).

A comparative overlay of the FTIR spectra of the pure drug, polymer and film formulations were made to establish any spectral shifts (Fig. 5). Spectra of pure diclofenac rendered characteristics band at 3364 and 2965 cm^{-1} due to C-H and N-H stretch respectively. Shallow band found around 3224 cm^{-1} was attributed to intra-molecular hydrogen bonding [22]. OH and C-H stretching vibration in polymer spectra resulted sharp characteristics peaks at 3361 and 2932 cm^{-1}, respectively. As far as films were concerned, characteristics peaks corresponding to pure drug overlapped to form strong asymmetric packet gathered around 3439 to 3409 cm^{-1}, emerging from the overlay of the stretching vibrations of N-H group of pure drug and O-H stretching of polymer.

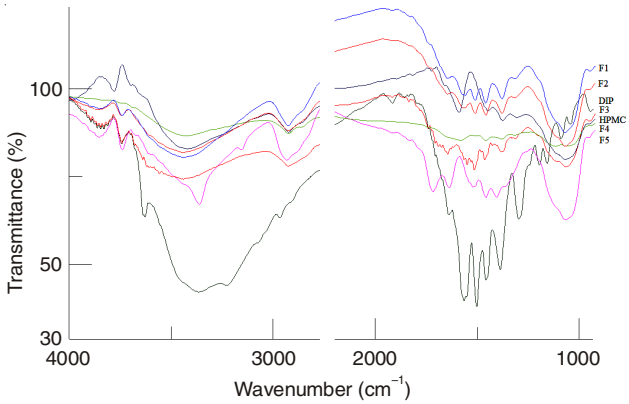

Fig. 5. FT-IR spectra of pure drug (diclofenac potassium), polymer (HPMC) and film formulations

The XRD spectra of the diclofenac potassium and drug loaded polymeric films were shown in Fig. 6. Characteristic peaks at 13.25, 20.40, 21.07, 25.24, 26.17, 30.15 and 26.82 2θ values in the XRD pattern of pure diclofenac potassium were observed. A notable increase in the full width at half maximum (FWHM) of all the films with respect to diclofenac potassium was noticed from the defractogram. Reduced intensity signals of F1 (without triethanolamine and benzalkonium chloride) were attributable to the partial amorphization of the drug under the influence of HPMC only. Prominent characteristic peaks corresponding to diclofenac potassium were absent in the diffractogram of other film formulations (F2, F3, F4 and F5).

Permeation of drug through a biomembrane conventionally can be characterized by transient state and steady state. Lag time is the transient component of permeation process, achieved from fixed time differentiation monitored between the time of corneal drug entry and the time at which the permeation rate reaches a steady state. Steady state permeation parameters such as t_d, $t_{50\ \%}$, J and k_{ss} were influenced by increased loading of triethanolamine (plasticizer) which acted as the permeation booster and improved permeation by breaking the physiological barriers of the corneal tissue. Films with lower plasticizer concentration possessed higher retention values as a sign of permeation retardation. Yasueda *et al.* [23]

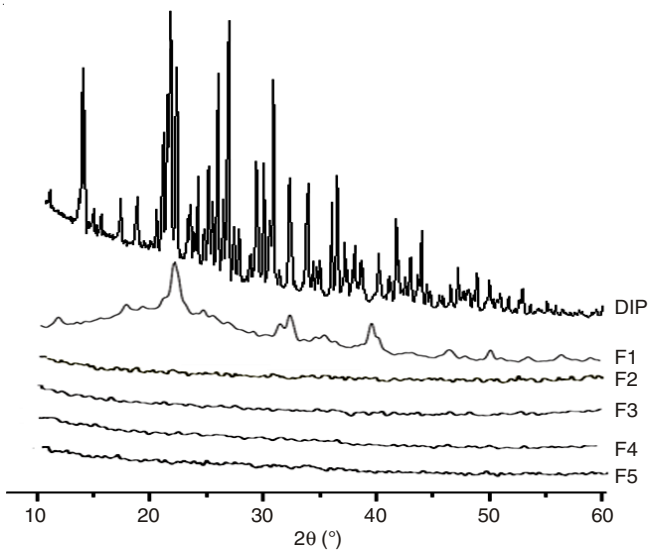

Fig. 6. XRD pattern of pure drug (diclofenac potassium) and film formulations

recently reported that the corneal permeability of dexamethasone and lomefloxacin has been increased in de-epithelialized cornea with consequent decrease in lag time as compared to epithelialized cornea (physiological barriers). In the present study statistical moment analysis has been applied to enumerate model independent factors like MTT, MST and MPT. Decreased value of MTT, MST and MPT due to increased concentration of triethanolamine in presence of benzalkonium chloride signified improved permeation. Possible formation of ion pair between hydroxyl and amino functions containing triethanolamine and weakly acidic drug diclofenac potassium elevated the permeation flux [24] thus reduced values of MTT, MST and MPT.

Literature revealed that though the permeation follows Korsmeyer-Peppas model, its rate is not only limited to drug diffusion but also to the factors like polymeric chain relaxation [25] affected by plasticizer and surfactant effect of preservative. The inherent nature of triethanolamine to decrease the intermolecular forces throughout the polymer chains brought motion within the polymer hence attributed to its flexibility [26]. Triethanolamine as plasticizer, played important role in polymeric chain relaxation and flexibility and permitted easy ingress of drug molecule [27]. The permeation process was also facilitated in presence of surfactant activity of benzalkonium chloride.

In the present study correlation coefficient (0.928-0.986) of *in vitro* dissolution and steady state *ex vivo* permeation of the films indicated a good level "A" correlationship between them. Moreover, in the Pearson's product moment correlation analysis, the critical value at 5 % significance level ($r_{crit} = 0.878$ for n = 5) means that there is only 5 % chance of getting a result of 0.878 or greater if there is no correlation between the variables. Therefore the study indicates the likely existence of a corelationship between the *ex vivo* permeation and *in vitro* dissolution of the formulations. The good correlationship indicated batch to batch consistency in the *ex vivo* performance of the films by the use of such *in vitro* values.

SEM micrographs of formulated films revealed homogeneous mixing of dug crystal in the polymer matrix of HPMC.

Here, HPMC played a key role as drug crystal growth inhibitor and a habit modifier [28]. Broadening and reduction in FT-IR peak intensity of diclofenac might be the result of inhibition of the crystal growth of the diclofenac and probable amorphization [29]. Characteristic peaks at 13.25, 20.40, 21.07, 25.24, 26.17, 30.15 and 26.82 2θ values in the XRD pattern of pure diclofenac potassium clearly revealed the crystalline nature of the drug. The increase of the full width at half maximum (FWHM) of all the films with respect to diclofenac potassium supported molecular dispersion of pure drug and polymer. Reduced intensity signals of F1 (without triethanolamine and benzalkonium chloride) were attributable to the partial amorphization of the drug under the influence of HPMC only. The absence of prominent peaks in the diffractogram of other film formulations (F2, F3, F4 and F5) confirmed the existence of the drug in microcrystalline form in the polymer matrix. It may be inferred from the study that a major portion of the drug incorporated existed as solid-solid solution in the polymer matrix whereas, the small remaining fraction has been crystallized in the microcrystalline form in the polymeric matrices.

Conclusion

First order, Higuchi and Korsmeyer-Peppas models have been used for describing ocular permeation kinetics of diclofenac potassium film formulation through sheep cornea. Mean transient time, mean steady state time and mean permeation time were evaluated using statistical moment analysis for characterization of transient and steady state of permeation more appropriately compared to conventional mathematical models avoiding model related error. Drug permeation through ocular tissue has been improved with the increase of triethanolamine concentration in presence of benzalkonium chloride. Analytical techniques such as SEM, FTIR and XRD studies revealed the inhibition of the crystal growth of diclofenac in the film formulation and improved permeation. Linear correlation between the *ex vivo* permeation and *in vitro* dissolution of the formulations has been established.

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