

THERMODYNAMIC PROPERTIES OF OCULAR PERMEATION OF DICLOFENAC: EFFECT OF TRIETHANOLAMINE

RAJARAM MOHAPATRA, SIBANANDA SENAPATI, CHINMAYA SAHOO, SUBRATA MALLICK*

Department of Pharmaceutics, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Khandagiri Square, Bhubaneswar, OR, India

*corresponding author: s_mallickin@yahoo.com; profsmalllick@gmail.com

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Abstract

The objective of the present study was to evaluate the effect of triethanolamine (TEA) as plasticizer on the thermodynamic properties of *ex-vivo* permeation of diclofenac potassium (DP) from hypromellose matrix films. The apparent permeability has been evaluated at different temperatures and utilized for estimation of activation energy (E_a), enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) of permeation, diffusion and partition. Permeation was found to be increasing with the increase of temperature and concentration of plasticizer. Improved permeation has been supported by the decreased value of activation energy. Positive ΔH value of all the films indicated increased permeation with the increase of temperature. The presence TEA in the formulation significantly lowered the ΔS value compared to the film without TEA. FTIR, DSC and SEM studies of the films revealed the inhibition of the crystal growth and transformation of crystalline nature of drug to amorphous state. The film (containing 30% TEA) was rehydrated *in vivo* upon lachrymal fluid uptake and progressed as a bioerodible swelled mass at the end of 5 h of application.

Rezumat

Obiectivul acestui studiu a fost evaluarea efectului trietanolinei (TEA), ca plastifiant, asupra proprietăților termodinamice ale permeației *ex-vivo* ale diclofenacului de potasiu (DP) din matricile de hipromeloză. Permeabilitatea aparentă a fost evaluată la diferite temperaturi și a fost utilizată pentru estimarea energiei de activare (E_a), a entalpiei (ΔH), a entropiei (ΔS) și a energiei libere (ΔG) de permeație, partiție și difuzie. Permeația s-a dovedit îmbunătățită prin scăderea valorii energiei de activare. Valorile pozitive ale entalpiei (ΔH) au indicat o creștere a permeației, odată cu creșterea temperaturii. Stratul filmat de TEA a scăzut semnificativ valoarea entropiei (ΔS). Studiile FTIR, DSC și SEM au relevat inhibiția creșterii cristalelor și transformarea acestora în stare amorfă. Filmul (cu un conținut de 30% TEA) a fost rehidratat *in vivo* pentru a favoriza absorbția fluidului lacrimal și a evoluat ca masă bioerodibilă după 5 h de la aplicare.

Keywords: hypromellose, ocular permeation, thermodynamic properties, activation energy, triethanolamine (TEA)

Introduction

Hypromellose (Hydroxypropyl methylcellulose) is an inert, viscoelastic biocompatible polymer widely used in ophthalmic and other topical pharmaceutical formulations [1]. The major problem associated with ophthalmic drug delivery system is the poor bioavailability of the topically applied drugs due to a rapid drainage from application site [2]. Properly designed ocular inserts can provide better therapeutic efficacy by improving the contact time and reducing the frequency of administration [3]. Transport of drug across the corneal barrier is mainly due to passive diffusion and the rate of mass transfer directly depends on its partition coefficient [4]. Transcorneal permeability can be calculated using the measured flux and the concentration gradient. Thus it can be summarized that the permeability is a function of diffusion coefficient of the drug in the barrier and partition coefficient of the drug into the

barrier [5]. Different factors which can influence permeability are the relative solubility in the membrane and surrounding medium, the chemical structure, physicochemical properties (*in situ* gelling) [6] and the molecular weight of the drug [7]. Several studies have been reported describing the change in permeability in response to variation in environmental temperature [7, 8]. Recent literature survey revealed that *in-situ* thermosensitive gel-forming systems have potential in increasing the ocular residence time of drug molecules for the therapeutic management of eye diseases [9]. Ocular absorption of tilisolol has been increased by utilizing thermosensitive *in situ* gel-forming solution [10]. In several reports the mechanism of corneal drug penetration has been described by determining the activation energy of permeation [4]. Diclofenac potassium is a commonly used non-steroidal anti-inflammatory drug usually meant for the treatment of pain and inflammation. It can also

be applied topically to the conjunctiva to reduce ocular inflammation caused by various diseases or to prevent postoperative inflammation. Diclofenac has limited ocular permeability at the physiological pH of the eye and several efforts have been undertaken earlier for permeation improvement [11]. The objective of the present study was to evaluate the *ex-vivo* permeation of diclofenac potassium from hypromellose matrix film formulations prepared by solvent casting method using sheep cornea. The use of an effective plasticizer imparts additive effect on the permeation of drug from the ocusert [12]. In zein-coated tablets triethanolamine (TEA) was used as an effective plasticizer [13]. Triethanolamine has been incorporated in the film as plasticizer [14] and its effect on transcorneal permeability has also been studied. Apparent permeability has been evaluated at different temperature such levels as 26, 30, 34 and 40°C and thermodynamic parameters such as enthalpy, entropy and free energy were estimated to describe the corneal penetration mechanism of diclofenac potassium. The extensive literature survey revealed that this type of research has not been undertaken earlier. FTIR (Fourier Transform Infrared), DSC (Differential Scanning Calorimetry) and SEM

(Scanning electron microscopy) studies were performed to characterize the formulated films.

Materials and Methods

Materials

Diclofenac potassium (DP) was obtained from Tejani Life Care, Cuttack, India. Hypromellose (HPMC K15 M) was received as a gift sample from Caplin Point Laboratories Ltd, India. Triethanolamine (TEA) was purchased from Merck Ltd, Mumbai, India. All other reagents were of analytical grade.

Preparation of ocular film

Ocular films were prepared by casting and solvent evaporation method [14-16]. HPMC was subjected to swelling in cold water for a period of 15-20 h and stirred continuously to get homogeneous jelly like liquid. DP and TEA (0, 10, 20, and 30 % wt/wt of polymer on dry basis) were co-dissolved in ethanol and added to the polymeric dispersion under stirring. Stirring was continued until a clear mixture was obtained. The clear mixture was transferred to a Petri dish and dried at 45°C until constant weight. Formulation of diclofenac ocular film containing triethanolamine as plasticizer has been summarised in Table I.

Table I

Formulation and physical properties of diclofenac potassium ocular films

Film code	DP (mg)	HPMC K15 (mg)	Triethanolamine (% w/v of HPMC)	Solvent system	Thickness (mean $\pm$ SD; n = 6) (μ m)	Folding endurance	Moisture uptake (%) at 75% RH (mean $\pm$ SD; n = 3)	Assay (%) (mean $\pm$ SD; n = 3)
Dpt ₀	376	1133	0	Ethanol-water	245 $\pm$ 8	26 $\pm$ 2 (mean $\pm$ SD; n = 3)	13.57 $\pm$ 1.48	28.16 $\pm$ 1.76
Dpt ₁₀	376	1133	10	Ethanol-water	244 $\pm$ 4	> 200	10.23 $\pm$ 0.68	25.39 $\pm$ 1.25
Dpt ₂₀	376	1133	20	Ethanol-water	246 $\pm$ 7	> 200	9.38 $\pm$ 2.12	24.92 $\pm$ 0.58
Dpt ₃₀	376	1133	30	Ethanol-water	242 $\pm$ 6	> 200	8.97 $\pm$ 1.72	26.61 $\pm$ 1.54

Evaluation of films

Film containing around 10 mg equivalent of drug was precisely weighed, dissolved in 100 mL of simulated tear fluid (phosphate buffer, pH 7.4) and subjected to sonication (Ultrasonic Cleaner: Trans-O-Sonic, Mumbai, India) for 2 h for complete drug solubilisation. Drug content of the film was determined by analysing the filtered (0.45 μ membrane filter) samples using UV-Vis spectrophotometer (JASCO V-630 spectrophotometer, Software: Spectra Manager) at 276 nm.

Film thickness was determined using digital micrometer screw gauge (Mitutoyo, Japan) at six different positions. Folding endurance of the films was carried out by applying recurring folds to a piece of the film at the same position till broken or

folded up to 200 times without breaking. For moisture uptake determination, precisely weighed films kept in desiccators at ambient temperature for 24 hours were taken out and placed in Humidity Cabinet at 75 % RH (Ikon Instruments, India) until a constant weight of the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight and mean of three determinations was recorded for each film.

The swelling index of the randomly selected film samples (1 cm $\times$ 1 cm approx.) was determined by placing them in Petri dishes containing 40 mL of simulated tear fluid (pH 7.4). Excess liquid on the surface was wiped out and the weight gain by the films was recorded at regular intervals. The swollen

films were dried at 60°C overnight and subjected to desiccation afterwards for another 24 h. The weight of the dried films was recorded and percent swelling [14] and percent matrix erosion were calculated using the following formulas. The determinations were made in triplicate and mean data was used for calculations.

$$\text{Percent swelling} = [(W_{\text{Hyd}} - W_i)/W_{\text{Hyd}}] \times 100,$$

$$\text{Percent matrix erosion} = [(W_i - W_{\text{Sw}})/W_i] \times 100,$$

where W_{Hyd} = Weight of hydrated film; W_i = Initial weight of film; W_{Sw} = Weight of swollen film.

FT-IR, DSC and scanning electron microscopy

Spectra were recorded by a FT-IR spectrophotometer (Jasco FT-IR 4100 type A) equipped with a standard light source and a detector using Spectra Manager software. Analysis of pure drug, films and polymer was carried out by KBr disc technique. All the powdered samples were dried prior to analysis for removal of traces of residual moisture. Each spectrum was scanned at a speed of 2 mm/sec at a resolution of 4 cm^{-1} and spectral window ranging from 4000 to 400 cm^{-1} . The thermal behaviour of the pure DP and films was characterized by differential scanning calorimetry (Universal V4.2E TA Instruments). Samples (about 2 - 3 mg) were weighed into an aluminium pan and scanning was carried out at a rate of 10°C/min at temperatures between 30°C and 330°C, using a nitrogen gas purge at 50 mL/min. The surface topography of the pure drug and films was observed using a scanning electron microscope (JSM- 6390, JEOL, Tokyo, Japan). The dried samples were mounted on metal grids for vacuum gold coating before observation in microscope. The photomicrographs were taken at different magnifications employing electron imaging at 10 kV.

Ex-vivo ocular permeation studies

Fresh sheep eyes were collected from slaughter house within 1 h of its sacrifice. The cornea with attached 5 - 6 mm wide scleral ring was excised from the whole eye. After applying a properly cut piece of film on the centre, the dissected cornea was mounted on one side of diffusion tube with clamps. The corneal epithelium was placed facing the donor compartment vertically with an effective area of 1.43 cm^2 and the diffusion media (simulated tear fluid of pH 7.4) was placed in the receptor compartment. The diffusion was continued for 6 h at 26, 30, 34 and 40°C under constant stirring. Samples (10 mL) were withdrawn at regular time intervals from the sampling port and filtered through a 0.45 μm membrane filter. A stock solution of diclofenac potassium 100 mL was prepared in simulated tear fluid (1000 $\mu\text{g/mL}$). Working standard solutions of 5, 10, 15, 20, 25, 30, 35, 40 and 50 $\mu\text{g/mL}$ were made after appropriate

dilution of the stock solution. Absorbance data of the withdrawn samples were recorded at 276 nm (λ_{max}) using UV-Vis spectrophotometer (JASCO V-630 spectrophotometer, Software: Spectra Manager). Same amount of fresh buffer solution was replaced to the donor compartment to maintain a constant volume.

Data analysis

In usual practice where the permeant concentration (C_f) does not show significant alterations throughout the permeation process, permeability coefficients (P) through barrier are derived from the slope of a linear regression line of amount permeated in the receptor compartment versus t as:

$$W = PAC_f \times t, \quad (1)$$

where $C_f = W/V$; V = film volume; S = surface area of the film.

As C_f , the drug concentration of the film at any given point of time, t (min) of permeation, changed appreciably in the present study, the permeability coefficient, P (cm/sec) has been estimated from the slope of $\text{Log } C_f$ versus t as [5]:

$$\text{Log } C_f = \text{Log } C_o - P \cdot A t / 2.303 V_f, \quad (2)$$

where, C_o is the initial film concentration at zero time, A (cm^2) is the surface area of the film, V_f (mL) is the volume of film. Eq. (2) holds good for calculation of P in the case of significant changes in C_f during the permeation study with progressive time. Fick's law was employed for estimation of the lag time (t_l) which was estimated from the x -intercept of linear regression line obtained from permeation profile of cumulative drug release in receiver compartment [1, 17]. Using the lag time (t_l) the diffusion coefficient (D) was calculated using following the relation:

$$D = h^2/6t_l, \quad (3)$$

where h is the thickness of the sheep cornea (0.05 cm)

The partition co-efficient (K) was estimated by the following relation:

$$K = Ph/D, \quad (4)$$

Activation energy (E_a) for permeation, diffusion and partition were estimated from the slope of linear regression line of Arrhenius equation:

$$k = A \cdot \exp (-E_a/RT), \quad (5)$$

where k is the rate coefficient, A is the frequency factor, E_a is the activation energy, R is the universal gas constant and T is the temperature (in Kelvin). Arrhenius plot of $\text{Ln } P$, $\text{Ln } D$ and $\text{Ln } K$ versus $1/T$ was constructed and E_a was calculated from the slope. Other significant thermodynamic parameters like ΔH (enthalpy of activation), ΔS (entropy of activation), and ΔG (free energy of

activation) were calculated using following the relations:

$$E_a = \Delta H + RT, \quad (6)$$

$$\Delta G = \Delta H - T \Delta S, \quad (7)$$

$$\Delta G = -RT \ln(X), \quad (8)$$

In Eq. (8) X represents P, D and K.

Results and Discussion

Evaluated parameters of films

All the evaluated parameters of the diclofenac films have been summarised in Table I. Films obtained were found uniform in drug content (24.92 ± 0.58 to 28.16 ± 1.76 %) and also in thickness (242 ± 6 to 246 ± 7 μm). The presence of triethanolamine as plasticizer (Dpt₁₀, Dpt₂₀ and Dpt₃₀) increased the folding endurance to more than 200 compared to its absence in the film Dpt₀ (26 ± 2). The presence of plasticizer in 10, 20 and 30 % in the films Dpt₁₀, Dpt₂₀ and Dpt₃₀ respectively made the films sufficiently tough, flexible and not fragile compared to Dpt₀. Presence of moisture and its uptake by the ocular film are important to maintain its flexibility, consistency and integrity without being completely dried and brittle which are related to patient compliance. Moisture uptake of the films at 75% RH has been decreased (13.57 ± 1.48 to 8.97 ± 1.72 %) with the increased content of triethanolamine. Swelling and matrix erosion studies were important due to the presence of hydrogel forming polymer (HPMC) in the film formulations. Swelling mechanism, drug release kinetics and ocular retention of the film could be understood well from these studies. The swelling profile of the films (Figure 1) indicated that it increased percentually with time but the swelling capacity was gradually increased with the increased content of plasticizer (Dpt₁₀ < Dpt₂₀ < Dpt₃₀). The absence of plasticizer has shown a lower level of swelling compared to

the other films. The plasticizer played a role in increasing the flexibility by increasing the inter-molecular separation of the polymer molecules and allowed a higher rate of water access causing enhanced swelling. Film formulations have shown significant matrix erosion after a period of 150 min of swelling and the films were fragmented afterwards losing their wholeness. It was difficult to handle and recover the films from the aqueous simulated tear fluid after 150 min. The film without plasticizer (Dpt₀) has been eroded to the maximum extent (64.23 ± 3.65 %) and incorporation of TEA decreased the percentual erosion (Figure 1) with a lowest value of 26.70 ± 4.26 % (Dpt₃₀). Percentual matrix erosion of the films was found in the order: Dpt₃₀ < Dpt₂₀ < Dpt₁₀ < Dpt₀. Literature reports also revealed that the tendency of erosion of HPMC matrix increased as the drug solubility decreased [18].

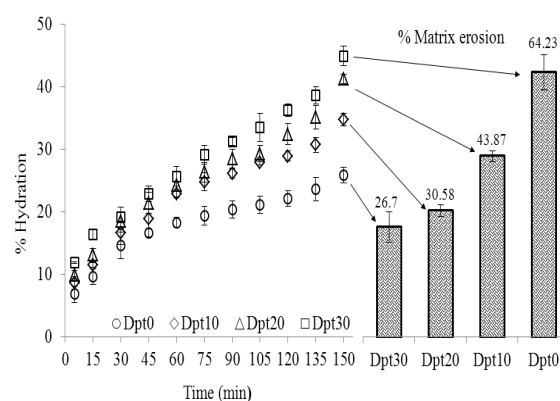


Figure 1.

Dynamic swelling and erosion studies of the film formulations (mean $\pm$ SD; n = 3).

FT-IR study

The Fourier transform IR spectra of pure drug, polymer and film formulations have been exhibited in Figure 2.

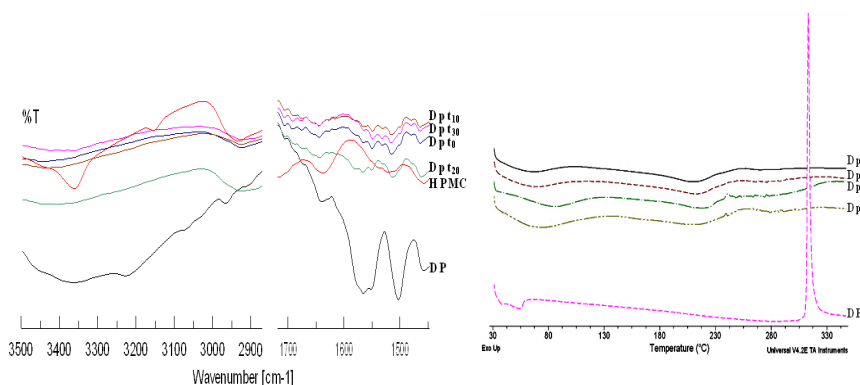


Figure 2.

FTIR spectra and DSC thermogram of pure drug, polymer and film formulations

The IR spectrum of pure drug showed N-H stretch at 3364 cm^{-1} and C-H stretch at 2965 cm^{-1} . The broad band at 3224 cm^{-1} could be represented as the $\nu(\text{NH} \cdots \text{O})$ mode, due to intramolecular hydrogen bonding [19] and a sharp band at 1566 cm^{-1} due to the carboxylate carbonyl group. The IR spectrum of the HPMC portrayed the characteristic peaks of 3361 cm^{-1} (OH stretching) and 2932 cm^{-1} (C-H stretching). In the films the foremost spectral attribute was a strong asymmetric packet gathered around 3409 cm^{-1} to 3439 cm^{-1} , emerging from the overlay of the stretching vibrations of N-H group of DP and O-H stretching of HPMC. Disappearance of peak at 3224 cm^{-1} in all the films might be the indication of intermolecular hydrogen bonding between drug and HPMC. Intensity of the characteristic peaks corresponding to diclofenac potassium in the films were reduced, might be due to the inhibition of the crystal growth of the drug. Broadening and reduction in peak intensity might have occurred due to transformation of crystalline nature of drug to amorphous state [20].

Thermal property of the films

Differential Scanning Calorimetry of pure drug (DP) and films was analyzed in order to investigate the effect of polymeric fusion of drug on its thermal profile (Figure 3). Pure drug presented a complex exotherm of decomposition at 313.2°C . This characteristics exothermic thermal profile of DP was previously reported by Fini *et al.* [21]. In addition, films revealed no endothermic or exothermal peaks corresponding to DP in that thermal range suggesting molecular incorporation of DP in the HPMC matrix and major modification in drug crystallinity. HPMC in the films portrayed a broad endotherm at $60 - 90^\circ\text{C}$, addressed to loss of labile water [14]. In all the films (Dpt₀, Dpt₁₀, Dpt₂₀ and Dpt₃₀) tiny step-like patterns in the range of 210 to 220°C was the manifestation of glass transition of DP.

Film morphology

To establish the role of TEA as plasticizer in the DP loaded HPMC matrix films towards the crystal habit modification, the appropriate exploration of the nature of obtained patch surface is a matter of high importance. SEM micrographs (Figure 3) of pure drug revealed distinct interconnected crystals with and without twinning (Figure 3a, 3b). Homogeneous and smooth surfaces of DP loaded HPMC films indicated interpenetration and appreciable solubilisation of DP in the polymeric network which confirmed lowering of crystalline intensity. HPMC network in the matrix acted as the major crystal growth inhibitor and a habit modifier [22]. Crystals grown in the presence of TEA were smaller in comparison to crystals grown in its absence in the films. Literature also supported the fact that incorporation of TEA as plasticizer in the films produced relatively uniform surfaces. The SEM of Dpt₀ exposed the occurrence of plate shaped agglomerated crystals dispersed in the polymeric matrix (Figure 3c, 3d) while in Dpt₁₀ phase separation between polymer and drug was reduced to discrete crystal form (Figure 3e, 3f). Dpt₂₀ (Figure 3g, 3h) and Dpt₃₀ (Figure 3i, 3j) indicated less prominent manifestation of crystal geometry. Phenomenon such as fusion prone state of drug crystallites and interfacial adhesion between drug and polymer phases was indicated by appearance of relatively smaller crystal in Dpt₂₀. Increasing concentration of TEA induced the reduction of crystallite size. Effective wetting of drug particle by the polymer in Dpt₃₀ exhibited considerably decreased crystallite size to the maximal extent. The fused appearance of drug and polymer in Dpt₃₀ might be the result of association between individual crystallites and polar surface of the polymer fibres.

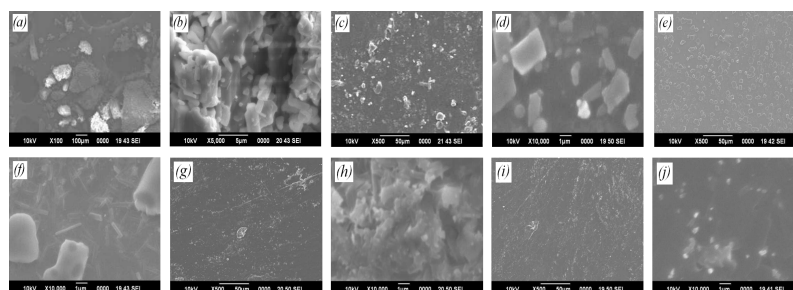


Figure 3.

Scanning electron micrographs of (a) diclofenac pure drug crystals 100x; (b) 5000x; and film formulations: (c) Dpt₀ 500x; (d) 10000x; (e) Dpt₁₀ 500x; (f) 10000x; (g) Dpt₂₀ 500x; (h) 10000x; (i) Dpt₃₀ 500x; and (j) 10000x

Permeation profile, lag time and activation energy

Standard calibration curve in simulated tear fluid (pH 7.4) was plotted and found linear in the concentration range of $5 - 50\text{ }\mu\text{g/mL}$. The cumulative

amount of drug in receiver compartment (Q) was estimated by employing the vertical diffusion apparatus and the permeation profiles were exhibited in Figure 4a - 4d. A gradual increase in Q

was found with the rise in temperature. The increase in TEA content in the film also showed improvement in permeation at respective particular

temperature. While comparing the formulations, Q was found to be in following order at a particular temperature: $Dpt_0 < Dpt_{10} < Dpt_{20} < Dpt_{30}$.

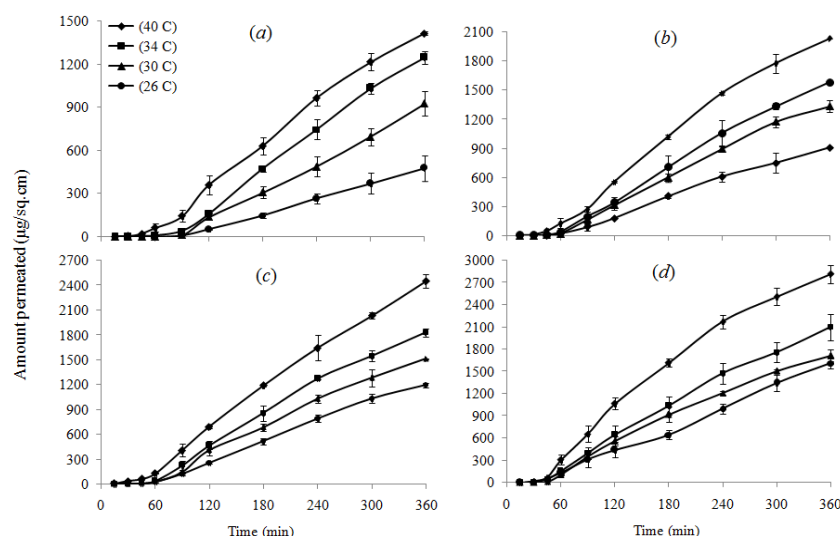


Figure 4.

Permeation profiles of diclofenac potassium from film formulations across cornea (a) Dpt₀; (b) Dpt₁₀; (c) Dpt₂₀; and (e) Dpt₃₀ at 26, 30, 34 and 40°C (mean $\pm$ SD; n = 3)

The diffusional properties of a drug have relevance in consideration of processes such as the dissolution and transport of the drug through corneal tissue. The estimated diffusional parameters such as P, K and D have been presented in Table II. Temperature and plasticizer factors (T_f and P_f respectively) have been mentioned in the Table II to get a comparative view. The permeation coefficient of Dpt₀ escalated up to 3.42 fold from 1.10082E - 09 cm/sec at 26°C

to 3.76965E - 09 cm/sec at 30°C. Temperature increase of 26 to 30°C, 30 to 34°C and 34 to 40°C yielded 1.71, 2.85 and 3.42 fold improvements in P value respectively. This increase in P was due to 1.73 fold increase in D value from 8.00367E-06 to 1.39075E-05 cm²/sec and 1.97 fold increase in K value from 6.87698E-06 to 1.35526E-05 as P is a function of D and K.

Table II

Effect of triethanolamine on permeation of diclofenac potassium (P ($\times 10^{-9}$) cm/sec; K ($\times 10^{-6}$) Unit less; D ($\times 10^{-6}$) cm²/sec)

X	t (°C)	Dpt ₀	T _f	P _f	Dpt ₁₀	T _f	P _f	Dpt ₂₀	T _f	P _f	Dpt ₃₀	T _f	P _f
P	26	1.10 $\pm$ 0.14	1.00	1.00	2.20 $\pm$ 0.02	1.00	2.00	3.06 $\pm$ 0.14	1.00	2.77	4.25 $\pm$ 0.34	1.00	3.86
	30	1.88 $\pm$ 0.20	1.71	1.00	3.54 $\pm$ 0.12	1.60	1.88	4.10 $\pm$ 0.27	1.34	2.18	4.72 $\pm$ 0.37	1.10	2.50
	34	3.13 $\pm$ 0.15	2.85	1.00	4.32 $\pm$ 0.23	1.96	1.37	5.33 $\pm$ 0.35	1.74	1.69	5.93 $\pm$ 0.46	1.39	1.89
	40	3.77 $\pm$ 0.26	3.42	1.00	6.31 $\pm$ 0.07	2.87	1.67	7.99 $\pm$ 0.36	2.61	2.11	10.89 $\pm$ 1.06	2.56	2.88
K	26	6.87 $\pm$ 0.79	1.00	1.00	7.67 $\pm$ 0.07	1.00	1.11	10.10 $\pm$ 0.48	1.00	1.46	10.42 $\pm$ 1.03	1.00	1.51
	30	9.35 $\pm$ 1.02	1.36	1.00	9.90 $\pm$ 0.35	1.29	1.05	10.66 $\pm$ 0.70	1.05	1.14	10.70 $\pm$ 0.85	1.02	1.14
	34	11.27 $\pm$ 0.56	1.64	1.00	11.31 $\pm$ 0.62	1.47	1.00	12.41 $\pm$ 0.95	1.22	1.10	12.42 $\pm$ 1.01	1.19	1.10
	40	13.55 $\pm$ 0.94	1.97	1.00	14.45 $\pm$ 0.17	1.88	1.06	17.31 $\pm$ 0.79	1.71	1.27	19.76 $\pm$ 1.93	1.89	1.45
D	26	8.00 $\pm$ 0.90	1.00	1.00	14.34 $\pm$ 0.13	1.00	1.79	15.13 $\pm$ 0.72	1.00	1.89	20.40 $\pm$ 2.06	1.00	2.54
	30	10.06 $\pm$ 1.10	1.25	1.00	17.87 $\pm$ 0.64	1.24	1.77	19.24 $\pm$ 1.27	1.27	1.91	22.03 $\pm$ 1.76	1.07	2.18
	34	11.00 $\pm$ 0.55	1.37	1.00	19.09 $\pm$ 1.05	1.33	1.73	21.45 $\pm$ 1.41	1.41	1.95	23.85 $\pm$ 2.32	1.16	2.16
	40	13.90 $\pm$ 0.97	1.73	1.00	21.84 $\pm$ 0.26	1.52	1.57	23.06 $\pm$ 1.06	1.52	1.65	27.55 $\pm$ 2.69	1.35	1.98

T_f and P_f are the temperature factor and the plasticizer factor by which permeation parameter (P, K and D) has been multiplied by that of 26°C and Dpt₀ respectively (mean $\pm$ SD, n = 3).

For other formulations (Dpt₁₀, Dpt₂₀ and Dpt₃₀) similar type of incremental behaviour with temperature was detected. In case of Dpt₁₀, Dpt₂₀ and Dpt₃₀ the P value increased up to 2.87, 2.61 and

2.56 folds from 2.20E-09 to 6.31E-09, 3.06E-09 to 7.99E-09 and 4.25E-09 to 1.09E-08 cm/sec respectively with temperature elevation. Incremental changes of 1.88, 1.71 and 1.89 fold in

K value and 1.52, 1.52 and 1.35 fold in D value were established for D_{pt10} , D_{pt20} and D_{pt30} respectively, which contributed for improvement of permeation.

Significant improvement of permeation has been observed with the increased contribution of plasticizer in the formulated films (Table II). The permeation coefficient escalated up to 3.86 fold from $1.10E-09$ to $4.25E-09$ cm/sec through the increase of 0 - 30% TEA at 26°C. Plasticizer variation like 0 to 10, 10 to 20 and 20 to 30 % yielded 2.00, 2.77 and 3.86 fold increase in P value respectively. This increase in P was attributed to 2.54 fold improvement in D value ($8.00E-06$ to $2.04E-05$ cm²/sec) and 1.51 fold improvement in K value ($6.88E-06$ to $1.04E-05$) with rise in TEA (0 to 30 %). Similar trends were also detected at 30, 34 and 40°C. P value increased up to 2.50, 1.89 and 2.88 fold with increasing concentration of TEA. Enhancement of 1.14, 1.10 and 1.45 fold in K value, and 2.18, 2.16 and 1.98 fold in D value have been exhibited at 30, 34 and 40°C respectively.

The permeation process can be divided into its transient and steady state steps. The transient or the dynamic components can be represented by the “time lag parameter” obtained from finite time difference observed between the time at which the drug enters the corneal tissue and the time at which the flow rate reaches a steady state of permeation. It is evident from the Table III that the values of the lag time (t_l) followed a pattern with respect to temperature and plasticizer concentration. Formulated films showed gradually reduced t_l when the temperature increased from 26 to 40°C. D_{pt0} showed reduced t_l from 52.06 to 29.96 min whereas, D_{pt30} exhibited 20.42 to 15.12 min when temperature increased from 26 to 40°C. Triethanolamine is a hydrophilic substance containing hydroxyl and amino functions and exhibits high plasticizing effects [23]. Presence of TEA in the film made the transient state shorter. When the plasticizer concentration was taken into account, the greater difference of t_l was observed as 31.64 min at 26°C ($52.06 - 20.42^\circ\text{C}$), rather than 14.84 min at 40°C ($29.96 - 15.12^\circ\text{C}$).

Table III

Lag time of permeation and activation energy of permeation, diffusion and partition coefficient as a function of temperature (mean $\pm$ SD, n = 3)

Film Code	Lag time (min)				Activation Energy (kJ/mol)		
	26°C	30°C	34°C	40°C	P (E_{ap})	D (E_{ad})	K (E_{ak})
D_{pt0}	52.06 ± 3.22	41.40 ± 2.02	29.98 ± 2.73	29.96 ± 1.07	69.13 ± 2.97	36.94 ± 2.08	32.19 ± 2.33
D_{pt10}	29.05 ± 2.32	23.32 ± 1.97	21.83 ± 2.96	19.08 ± 1.09	56.33 ± 1.44	33.36 ± 3.16	22.97 ± 2.22
D_{pt20}	27.54 ± 2.53	21.66 ± 2.01	19.42 ± 1.09	18.07 ± 1.34	53.10 ± 3.12	30.53 ± 1.43	22.56 ± 2.48
D_{pt30}	20.42 ± 1.76	18.91 ± 1.02	17.46 ± 2.07	15.12 ± 1.83	52.87 ± 1.98	30.36 ± 1.56	22.51 ± 1.91

The simultaneous determination of activation energy of permeation, partition and diffusion coefficient (E_{ap} , E_{ak} and E_{ad}) with and without the presence of plasticizer, enthalpy, entropy and free energy gives an idea regarding thermodynamic

behaviour [24-26] of permeation of the film. Arrhenius plot of permeation, diffusion and partition has been depicted in the Figure 5a, 5b and 5c respectively and activation energy was estimated from the slope of linear regression line.

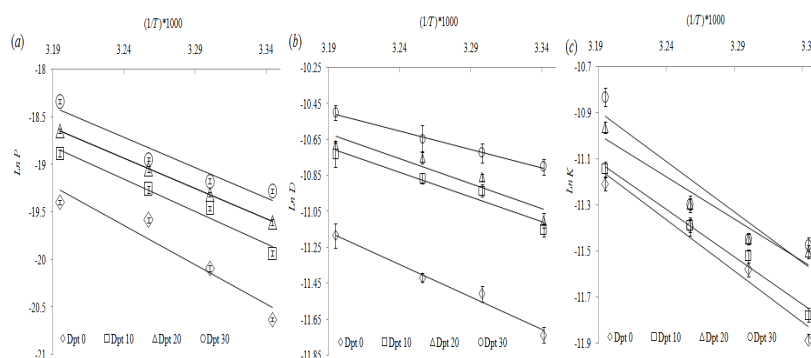


Figure 5.

Arrhenius plots for the (a) permeation coefficient (P), (b) diffusion coefficient (D) and (c) partition coefficient (K) in the ocular tissue against the inverse of temperature of receiver compartment (mean $\pm$ SD; n = 3)

The observed value of E_{ap} of all the films was found to be comprising of E_{ak} and E_{ad} (Table III). E_{ap} of D_{pt0} ($69.13 \text{ kJ mol}^{-1}$) consisted of 36.94 kJ

mol^{-1} of E_{ak} and $32.19 \text{ kJ mol}^{-1}$ of E_{ad} . Activation energy of permeation reduced to 56.33, 53.10 and $52.87 \text{ kJ mol}^{-1}$ with 10, 20 and 30 % of increase in

plasticizer respectively. A parallel decrease in the activation energy for partition ($33.36 \text{ kJ mol}^{-1}$) and diffusion ($22.97 \text{ kJ mol}^{-1}$) has been noticed in Dpt₁₀. This decreasing pattern has also been viewed in case of Dpt₂₀ (30.53 and $22.56 \text{ kJ mol}^{-1}$) and Dpt₃₀ (30.36 and $22.51 \text{ kJ mol}^{-1}$), due to an increase in TEA concentration. This gradual reduction in E_a with the increase in TEA suggested a positive involvement of TEA in the permeation process.

Enthalpy, entropy and free energy of activation

Enthalpy, entropy and free energy of activation of diffusion, partition and permeation of DP from films through sheep cornea were estimated using the above thermodynamic relations. The free energy barrier for permeation is composed of enthalpic and entropic components. Enthalpy is the favoured idiom for changes in system energy which clears the conception of thermodynamic potential of the

system. The positive ΔH values for P, D and K as mentioned in the Table IV, V and VI suggested that the system was endothermic and temperature dependent. Increased concentration of TEA boosted the permeation, diffusion and partition by decreasing ΔH at all the temperatures. ΔH of permeation, diffusion and partition has been decreased from 66.64 to 50.39 , 29.70 to 20.03 and 34.46 to $27.87 \text{ kJ mol}^{-1}$ respectively at 26°C .

Entropy (ΔS) is a comparative measure of the numbers of microstates substance can exist in a thermodynamic system. The value of entropy depends on number of microstates. Here we considered permeated and non-permeated drug molecules as two microstates. The presence of TEA in the formulation (Dpt₁₀, Dpt₁₀ and Dpt₁₀) significantly decreased ΔS value compared to Dpt₀ (Table IV, V and VI).

Table IV

Effect of triethanolamine on activation enthalpy, entropy, and free energy of permeability (P) (mean $\pm$ SD, n = 3)

t (°C)	$\Delta H (\text{kJ mol}^{-1})$				$\Delta S (\text{J mol}^{-1} \text{K}^{-1})$				$\Delta G (\text{kJ mol}^{-1})$			
	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀
26	66.64 ± 5.66	53.84 ± 2.44	50.61 ± 2.27	50.39 ± 1.98	51.37 ± 4.44	14.34 ± 1.24	6.26 ± 0.58	6.32 ± 0.33	51.28 ± 5.43	49.56 ± 2.23	48.74 ± 2.19	48.50 ± 4.13
30	66.60 ± 7.06	53.81 ± 2.95	50.57 ± 2.78	50.35 ± 3.54	52.79 ± 3.86	15.80 ± 1.86	6.36 ± 0.34	6.30 ± 0.71	50.61 ± 4.57	49.22 ± 3.70	48.65 ± 2.67	48.36 ± 3.91
34	66.57 ± 3.66	53.78 ± 3.49	50.54 ± 3.28	50.32 ± 5.35	54.05 ± 2.97	15.03 ± 1.22	6.24 ± 0.54	6.40 ± 0.44	50.00 ± 2.75	49.16 ± 3.19	48.62 ± 3.16	48.36 ± 4.79
40	66.52 ± 1.66	53.73 ± 2.41	50.49 ± 2.77	50.27 ± 2.01	51.27 ± 1.28	14.67 ± 1.56	6.29 ± 0.61	6.21 ± 0.37	50.48 ± 1.26	49.14 ± 2.21	48.52 ± 2.66	47.72 ± 1.89

Table V

Effect of triethanolamine on activation enthalpy, entropy, and free energy of diffusivity (D) (mean $\pm$ SD, n = 3)

t (°C)	$\Delta H (\text{kJ mol}^{-1})$				$\Delta S (\text{J mol}^{-1} \text{K}^{-1})$				$\Delta G (\text{kJ mol}^{-1})$			
	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀
26	29.70 ± 2.90	20.49 ± 1.82	20.07 ± 1.91	20.03 ± 1.39	1.75 ± 0.21	-24.21 ± 1.21	-25.15 ± 1.25	-23.89 ± 2.32	29.18 ± 3.03	27.72 ± 1.39	27.60 ± 1.33	27.17 ± 1.10
30	29.67 ± 1.78	20.45 ± 1.92	20.04 ± 2.01	20.00 ± 1.72	2.24 ± 0.13	-23.39 ± 1.05	-24.15 ± 1.08	-23.16 ± 0.95	28.99 ± 1.73	27.54 ± 3.03	27.36 ± 2.23	27.10 ± 1.87
34	29.63 ± 3.05	20.42 ± 1.53	20.00 ± 1.11	19.96 ± 0.93	3.54 ± 0.36	-23.83 ± 1.78	-24.21 ± 1.94	-23.47 ± 1.34	28.54 ± 3.21	27.74 ± 1.81	27.44 ± 1.50	27.17 ± 1.23
40	29.58 ± 0.98	20.37 ± 0.81	19.95 ± 1.19	19.91 ± 1.65	-1.53 ± 0.03	-24.15 ± 0.98	-25.02 ± 1.33	-23.68 ± 0.71	29.10 ± 0.72	27.93 ± 1.11	27.79 ± 2.66	27.32 ± 0.76

Table VI

Effect of triethanolamine on activation enthalpy, entropy, and free energy of partition (K) (mean $\pm$ SD, n = 3)

t (°C)	$\Delta H (\text{kJ mol}^{-1})$				$\Delta S (\text{J mol}^{-1} \text{K}^{-1})$				$\Delta G (\text{kJ mol}^{-1})$			
	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀	Dpt ₀	Dpt ₁₀	Dpt ₂₀	Dpt ₃₀
26	34.46 ± 3.47	30.87 ± 2.69	28.05 ± 2.54	27.87 ± 1.84	16.39 ± 2.01	5.32 ± 0.29	-2.81 ± 0.15	-3.32 ± 0.21	29.55 ± 3.04	29.28 ± 1.61	28.90 ± 1.58	28.90 ± 1.23
30	34.42 ± 1.89	30.84 ± 1.54	28.02 ± 1.40	27.84 ± 0.92	17.32 ± 0.95	5.97 ± 0.29	-2.76 ± 0.13	-3.29 ± 0.12	29.18 ± 1.60	29.12 ± 1.45	28.84 ± 1.44	28.80 ± 1.76
34	34.39 ± 2.29	30.81 ± 2.15	27.99 ± 1.67	27.80 ± 1.54	17.29 ± 2.16	5.64 ± 0.39	-2.76 ± 0.16	-3.35 ± 0.27	29.18 ± 3.14	29.07 ± 2.03	28.83 ± 1.72	28.80 ± 1.93
40	34.34 ± 0.68	30.76 ± 1.07	27.94 ± 1.81	27.75 ± 1.65	16.51 ± 1.33	5.60 ± 0.19	-1.90 ± 0.12	-1.39 ± 0.09	29.17 ± 0.58	29.00 ± 1.01	28.53 ± 1.85	28.20 ± 1.21

This decreased value of ΔS referred transformation between microstates i.e. non-permeated to permeated

state and also potentiated the impulsive permeation by making the free energy change of the transformation

process more effective. In this study there was no evidence of major changes in ΔS found with escalating temperature, whereas, variation in plasticizer brought about eye catching difference in ΔS value at particular temperature. ΔS changed 51.37 to 6.32, 1.75 to -23.89 and 16.39 to -3.32 J mol⁻¹ K⁻¹ in permeation, diffusion and partition respectively at 26°C when TEA varied from 0 to 30 %. The free energy (ΔG) is the *in-house* energy of the thermodynamic system which is usable whereas, entropy is the negative part of that energy which is unusable. A positive ΔG indicates that the net effort done is getting sucked for completion of reaction and the system is not impulsive. Here it has been observed that the presence of TEA in the films (Dpt₁₀, Dpt₂₀ and Dpt₃₀) decreased the ΔG value when compared with absence of that (Dpt₀). ΔG decreased 51.28 to 48.50, 29.18 to 27.17 and 29.55 to 28.90 kJ mol⁻¹ in permeation, diffusion and partition respectively at 26°C when plasticizer varied from 0 to 30 %. At other temperatures, similar behaviour for ΔH , ΔS and ΔG established the presence of plasticizer on permeation, diffusion and partition process.

Increases in both temperature and TEA content showed a promising enhancement in permeability. Fang *et al.* described the non-interference of TEA with the structural integration or functional basis of the corneal barrier for betterment of permeation. The increase in the permeation flux might be due to correlation of basicity of positively charged amine group in TEA with carboxyl group of diclofenac to form ion pair at permeation site as well as in the formulated films. Diclofenac potassium being a salt, a dominant hydrophilicity is expected from it. This nature of DP was attenuated by counter ion TEA with the formation of ion pair. The resultant complex compensated the prevailed hydrophilicity with overriding hydrophobicity, sufficient enough to ensure permeation across corneal tissue. This concept has been strongly supported by the increase in the coefficients for permeation, diffusion and partition with TEA loading. The reduction in the activation energy and lag time was also a significant marker in this aspect.

Conclusions

Increases in temperature (26 through 40°C) brought about gradually increased transcorneal permeation of DP and gradually reduced t_l from all HPMC matrix film formulations. K and D have also been improved and contributed for the improvement of permeation as P is a function of D and K. Presence of TEA as plasticizer in the film led to formation of ion pair with DP and resulted in higher permeability. The highest permeability data was obtained for the formulation containing the highest

TEA concentration (Dpt₃₀) at 40°C. The gradual reduction in activation energy (E_{ap} , E_{ak} and E_{ad}) with the increase in TEA suggests a positive involvement of TEA in the permeation process. The activation energy of permeation was found as the sum of activation energy of partition and diffusion and the lowest activation energy in Dpt₃₀ referred to the highest permeation amongst all formulations. The presence of TEA significantly decreased ΔS value compared to its absence (Dpt₀) which referred transformation between microstates i.e. non-permeated to permeated state. Here it has been observed that the presence of TEA in the films (Dpt₁₀, Dpt₂₀ and Dpt₃₀) decreased the ΔG value when compared with the absence of it (Dpt₀). Analytical studies revealed intermolecular hydrogen bonding between drug and HPMC and transformation of crystalline drug to its amorphous state.

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