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In Vitro Corneal Permeation of Diclofenac from Oil Drops

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In vitro transcorneal permeation of diclofenac from oil drops was studied using freshly excised goat cornea.The maximum apparent corneal permeability coefficient (Papp) was obtained with 0.2% (w/v) diclofenac drops in sesame oil followed by safflower oil, while formulation in castor oil provided minimal Papp. The addition of benzyl alcohol, a preservative, in oil drops, increased the Papp value of diclofenac. Partition experiments indicated increased partitioning of diclofenac in the aqueous phase in the presence of benzyl alcohol, and the same could be responsible for the benzyl alcohol-induced increase in Papp. The solubility of diclofenac was higher in castor, arachis, and sunflower oil. But drug permeation from 0.5–1.0% (w/v) diclofenac drops in castor oil or 0.5% (w/v) drops in arachis /sunflower oil was less than that observed with 0.2% (w/v) drops in sesame oil. Thus diclofenac 0.2% (w/v) drops in sesame oil containing 0.5 % (v/v) benzyl alcohol provides maximum Papp. The formulation increased corneal hydration indicating corneal damage. Since corneal hydration is less than 83% the damage appears to be reversible. The saturation solubility of diclofenac in sesame oil at 4℃ is 0.33% (w/v). Hence diclofenac 0.2% (w/v) solution in sesame oil will not precipitate at 4℃ and therefore the chances of crystallization of diclofenac from the formulation due to climatic change leading to physical instability appear to be remote.

Key words——diclofenac; sesame oil; benzyl alcohol; partition; Papp

INTRODUCTION

Steroids are used in the treatment of allergic ocular disorders, corneal burns, uveal tract inflammation, and other ocular inflammations but their use is limited by their tendency to increase intraocular pressure and to cause cataract upon chronic administration.[1] Steroids also exacerbate ocular infections and diminish corneal/stromal wound healing.[2] Nonsteroidal antiinflammatory drugs (NSAIDs) like indomethacin,[3] flurbiprofen,[4] ketorolac,[5] and diclofenac[6] have been found to be viable alternatives to steroids in treating ocular inflammation. Diclofenac is reported to be more effective than prednisolone sodium phosphate in inhibiting the breakdown of the blood-aqueous barrier.[6] Diclofenac and ketorolac have been found neither to attenuate the antiviral activity of cefdovir nor to facilitate adenoviral replication.[7,8] Diclofenac has been reported to be more potent than indomethacin and dexamethasone in inhibition of endothelial PGE2 synthesis induced by calcium ionophore A23187 or lipopolysaccharide (LPS) of *Salmonella typhimurium*.[9] Diclofenac is a phenyl acetic acid derivative NSAID.[10] It has poor aqueous solubility. Aqueous solution of diclofenac sodium (0.1% w/v) is commonly applied topically in the eye for the management of pain in corneal epithelial defects following surgery or accidental trauma, treatment of postoperative ocular inflammation, chronic noninfectious inflammation, and prevention of intraoperative miosis during cataract surgery and for symptomatic relief of seasonal allergic conjunctivitis.[11] A major impediment to the bioavailability of topically applied ophthalmic drugs is incomplete absorption due to nasolacrimal drainage. The time-honored approach to overcome this has been through prolonging the ocular contact time of the medication. Increased ocular contact time of the drug may be achieved by formulating the drug as oil solution. Earlier studies with pilocarpine,[12] tetracycline,[13] and ketorolac[14] revealed higher ocular availability of drugs from oily solutions.

This laboratory has recently reported the effects of formulation factors on corneal permeability of diclofenac from aqueous drops.[15] However, no such information is available on corneal permeation of

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diclofenac from oily solution. In the present study, the corneal permeation of diclofenac from oily solutions was investigated.

EXPERIMENTAL

Materials Diclofenac sodium (purity 98.58%) was obtained as a gift sample from Dabur Research Foundation (Ghaziabad, India). High-performance liquid chromatography (HPLC) grade acetonitrile, acetone, glacial acetic acid, triethylamine, and water were purchased from Qualigens Fine Chemicals (Mumbai, India). Refined food grade vegetable oils used in the study, arachis (Amrit Banaspati Co. Ltd., Punjab, India), mustard (National Diary Development Board, Gujarat, India), soybean (Adani Wilmar Limited, Gujarat, India), sesame (Shanker Udyog, Kanpur, India), kardi (safflower) (Marico Ltd., Mumbai, India), sunflower (Amrit Banaspati Co. Ltd., Punjab, India), olive (SOS Cuetara, Madrid, Spain) and castor oil (S.D. Fine Chem Ltd., Mumbai, India) were purchased from the local market. Benzyl alcohol was procured from Merck India Ltd., Mumbai. All other chemicals purchased were of analytical grade and were used as received. Fresh whole goat eye were obtained from a local butcher shop (Hisar, India).

Preparation of Diclofenac (Acid) Diclofenac sodium was dissolved in distilled water and diclofenac-free acid was precipitated by acidifying the solution with 0.1N HCl to pH 2.0. The precipitate so obtained was collected by filtration and washed repeatedly with distilled water until it was free from chloride ions. The product was characterized by spectroscopy.

Solubility of Diclofenac in Oil An excess amount of diclofenac was added to oils to prepare a saturated solution at 50°C. The solution of diclofenac in oils was then cooled and left overnight at 4°C. The solution was subsequently centrifuged at 4°C at 5000 rpm (Remi Equipments Ltd., Mumbai, India). One milliliter of the clear supernatant was dissolved in 25 ml of acetone and analyzed for diclofenac content by reverse-phase HPLC.

Preparation of Test Formulations Diclofenac ophthalmic solutions in different oily vehicles: Required amounts of diclofenac (acid) were dissolved in oily vehicles to give diclofenac (0.2%, w/v) solution in arachis, castor, safflower, mustard, olive, sesame, sunflower, and soybean oil. Similarly diclofenac (0.5 % w/v) solutions in arachis, sunflower, or castor oil, and diclofenac (1.0 %, w/v) solution in castor oil were also made.

Diclofenac ophthalmic solutions containing preservative: Diclofenac 0.2% (w/v) solutions in all the oils, 0.5% (w/v) solutions in arachis, sunflower, or soybean oil, and 1.0% (w/v) solution in castor oil were made as above. Benzyl alcohol (0.5%, v/v) was added as a preservative in each formulation.

Permeation Studies Whole goat eyes were transported from the local butcher shop to the laboratory in cold (4°C) normal saline within 1h of slaughter. The corneas were carefully excised along with 2 to 4 mm of surrounding scleral tissue and washed with cold normal saline until the washing was free from protein. Isolated corneas were mounted by sandwiching the surrounding scleral tissue between clamped donor and receptor compartments of an all-glass modified Franz diffusion cell[16] in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.95 cm^2. The receptor compartment was filled with 11 ml of freshly prepared bicarbonate ringer (pH 7.4). One milliliter of ophthalmic solution was placed on the cornea and the opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained at 35°C with constant stirring, using a Teflon-coated magnetic stir bead. One milliliter sample was withdrawn from the receptor compartment at various time intervals up to 120 min and was analyzed for diclofenac content using reverse-phase HPLC. Each withdrawn sample was replaced with an equal volume of bicarbonate ringer. At the end of the experiment, each cornea (freed from sclera) was weighed, soaked in 1 ml methanol, dried overnight at 90°C, and reweighed. From the difference in weights corneal hydration was calculated. The study was designed with paired corneas, *i.e.*, one cornea of an animal received formulation without benzyl alcohol while the contralateral cornea received formulation with benzyl alcohol.

Calculation of Apparent Permeability Coefficient The apparent permeability coefficient was calculated using the following equation:

$$Papp = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{(A \cdot Co \cdot 60)} \quad (1)$$

where $\Delta Q/\Delta t$ ($\mu g/min$) is the flux across the corneal tissue, A is the area of diffusion (cm^2), Co ($\mu g/cm^3$) is the initial concentration of drug in donor

compartment, and 60 is taken as the factor to convert minutes into seconds. The flux across the cornea was obtained from the slope of the regression line obtained from the linear part of the curve between the amount permeated (Q) *vs* time (t) plot.

HPLC Analysis Assay of diclofenac in samples was carried out by injecting 20 μl of the solution spiked with ketorolac tromethamine as internal standard into a chromatographic system equipped with 600 pump controllers (Waters), 2487 dual λ absorbance detector (Waters), and 7725i Rheodyne injector. The resolution of diclofenac was achieved using acetonitrile : water : acetic acid : triethylamine (60 : 38.25 : 1.65 : 0.10 vol/vol) at a flow rate of 1 ml/min as the mobile phase in an isocratic run through a Spherisorb (Waters) C 18, 5 μ (250×4.6 mm *i.d.*) column. The eluent was monitored for diclofenac at 276 nm. The retention time and the lowest limit of quantification of diclofenac were 5.6 min and 0.4 μg/ml, respectively.

Determination of Partition Coefficient Ten milliliters each of the test formulation and phosphate buffer (Sorenson's phosphate buffer, pH 7.4) were shaken in a reciprocating shaker bath (Narang Scientific Works, Delhi, India) for 2 h at 35℃. Samples from each phase were withdrawn and analyzed for diclofenac content using HPLC.

Statistical Analysis Statistical calculations were done with one-way analysis of variance (ANOVA) followed by Dunnett's test. The paired t-test was used for studies with paired corneas. A P value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 shows the solubility of diclofenac in different oils and its partition characteristics. Solubility was measured at 4℃. Diclofenac was found to have maximum solubility (% w/v) in castor oil (1.63) followed by arachis (0.72) and sunflower oil (0.55). In the rest of the oils like safflower, soybean, sesame, olive, and mustard oil, the solubility was between 0.25 to 0.38 %. The partition coefficient of diclofenac between oil and phosphate buffer (pH 7.4) was also found to be maximum with castor oil, followed by arachis oil, while the minimum partition coefficient was observed with olive oil.

Table 2 and Fig. 1 present the permeation characteristics of diclofenac from 0.2% (w/v) oil solutions. The results reveal the maximum apparent permeability coefficient (Papp) of diclofenac with drops formulated in sesame oil, and minimal Papp from formulation in castor oil. Permeation of drug from castor oil drops had a lag phase of 60 min. The apparent corneal permeability of diclofenac from drops formulated in soybean, sunflower, mustard, and olive oil was similar. On comparing the Papp of diclofenac from olive oil with others, it was observed that significantly ($p<0.05$) higher permeability of diclofenac was provided by sesame and safflower oils, while significantly ($p<0.05$) less permeability was provided by drops formulated in arachis and castor oils. The lesser corneal permeability of diclofenac from castor oil- and arachis oil-based drops could be attributed to the higher partitioning of diclofenac in castor and arachis oils. Similarly, higher permeability of the drug from sesame and safflower oil drops could be due to the lower partition coefficient of drug between the oil and aqueous phase. The normal cornea has a hydration level of 75–80%.[17] In an earlier study[18] it was reported that a 83–92% hydration level, *i.e.*, 3–7 percentage units or more above the normal value, denotes damage of the epithelium and/or endothelium. The maximal corneal hydration level attainable without producing irreversible damage to the tissue is 83%. Sesame, soybean, safflower, mustard, castor, and arachis oil drops of diclofenac showed corneal hydration of around 81%, indicating slight corneal damage (Tables 1 and 2). Earlier studies with ketorolac[16] also reported less permeation of drug from castor oil-based drops and higher permeation from sesame oil formulation.

Benzyl alcohol, a commonly used preservative, was added to oil formulations at 0.5% (v/v) concentra-

Table 1. Solubility and Partition Characteristics of Diclofenac

Oil	Solubility (%, w/v)	Partition coefficient*	
		Without BA	With BA
Arachis	0.720±0.09	6.44±0.47	2.91±0.13
Castor	1.633±0.06	11.88±2.06	3.36±0.34
Mustard	0.252±0.04	2.27±0.18	1.95±0.13
Olive	0.291±0.03	1.60±0.12	1.33±0.07
Safflower	0.383±0.04	1.86±0.13	1.54±0.19
Sesame	0.325±0.03	2.10±0.16	1.71±0.10
Soybean	0.327±0.03	3.55±0.27	2.93±0.31
Sunflower	0.549±0.03	3.12±0.13	2.46±0.21

* Partition coefficient between oil and phosphate buffer (pH 7.4), Values are mean±SD ($n=3$). BA, benzyl alcohol.

Table 2. Corneal Permeation of Diclofenac from 0.2% (w/v) Oil Drops

Oil	Papp* (cm/sec×10^7)		Rel Papp		Corneal hydration* (%)	
	Without BA	With BA	Without BA	With BA	Without BA	With BA
Arachis	1.85±0.10†	2.94±0.22[a]	0.69	0.86	81.05±0.54	81.71±0.31
Castor	1.27±0.21†	2.70±0.15[a]	0.47	0.79	80.88±0.35	81.56±0.84
Mustard	2.55±0.24	3.42±0.31[a]	0.95	1.01	80.95±0.57	81.46±0.39
Olive	2.66±0.22	3.38±0.54	1	1	80.14±0.76	81.22±0.41
Safflower	5.76±0.35†	7.31±0.70†	2.17	2.16	80.86±0.75	81.86±0.15
Sesame	9.18±0.86†	13.95±1.47†[a]	3.45	4.12	81.44±0.47	82.10±0.54
Soybean	2.44±0.15	3.27±0.75	0.91	0.96	81.12±0.46	81.75±0.32
Sunflower	2.56±0.44	3.29±0.91	0.96	0.97	80.54±0.57	81.20±0.67

* Values are mean±SD (n=3). † Statistically significant ($p<0.05$) compared with olive oil drop as determined by 1-way ANOVA followed by Dunnett's test.
[a] Statistically significant ($p<0.05$) compared with drops without benzyl alcohol, as determined by the paired t-test. BA, benzyl alcohol.

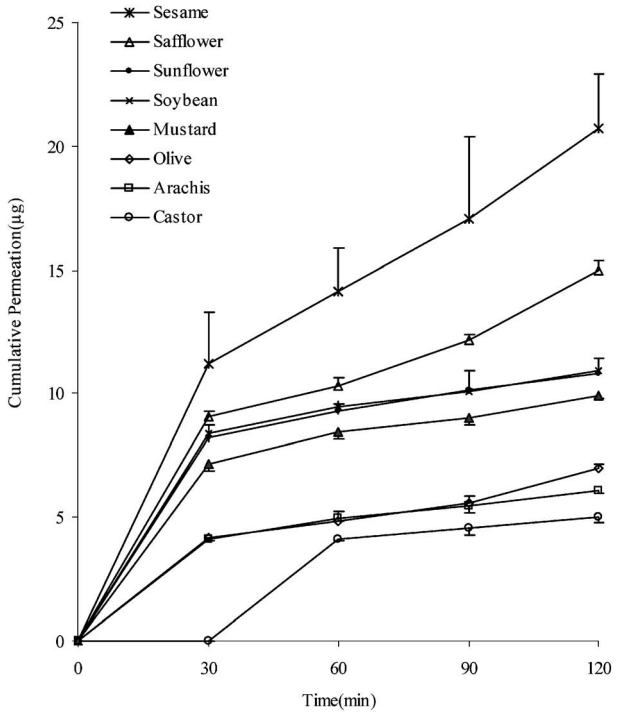

Fig. 1. Cumulative Permeation Profile of Diclofenac from Diclofenac (0.2%, w/v) Oil Drops

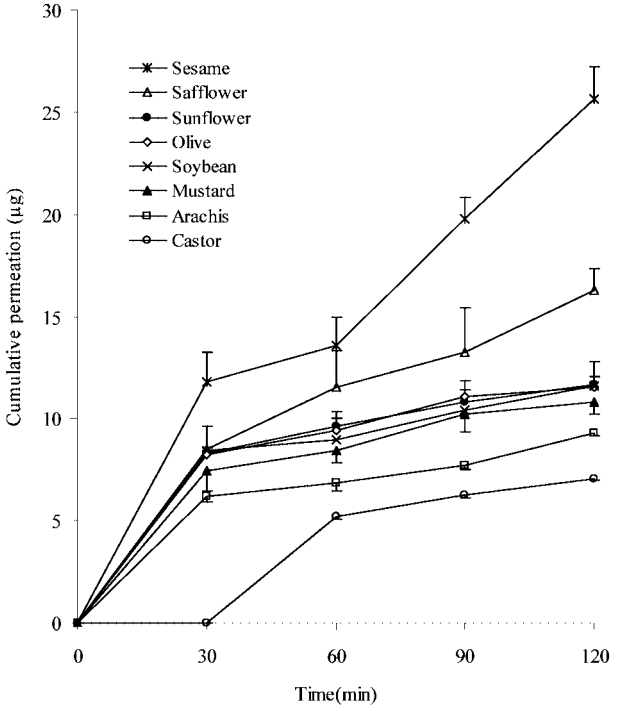

Fig. 2. Cumulative Permeation Profile of Diclofenac from Diclofenac (0.2%, w/v) Oil Drops Containing Benzyl Alcohol

tion. The addition of benzyl alcohol to oil drops resulted in increased permeation of diclofenac from all the formulations compared with the formulations without the preservative. However, a significant increase ($p<0.05$) in Papp of diclofenac on the addition of benzyl alcohol was observed only with arachis, castor, mustard, and sesame oil drops, while the sesame oil formulation showed the maximum increase. Partition experiments indicated higher partitioning of drug from the oil to aqueous phase in the presence of benzyl alcohol. There was increased corneal hydration associated with the use of benzyl alcohol, indicating slight corneal damage. Permeation of drug from castor oil drops containing benzyl alcohol had a lag phase of 60 min (Tables 1, 2 and Fig. 2).

Table 3 and Figs. 3 and 4 show the effects of drug concentration on the corneal permeation of diclofenac. Increased diclofenac concentration in arachis, castor, and sunflower oils from 0.2% to 0.5 % resulted in a significant increase in Papp but the increase in permeation was not consistent with the in-

Table 3. Effects of Drug Concentration on Corneal Permeation of Diclofenac from Oil Drops

Oil	Drug conc. (%, w/v)	Papp* (cm/sec×10^7)		Rel Papp		Corneal hydration* (%)	
		Without BA	With BA	Without BA	With BA	Without BA	With BA
Arachis	0.2	1.85±0.10	2.94±0.22[a]	1	1	81.05±0.54	81.71±0.31
	0.5	2.13±0.11[†]	2.88±0.11[a]	1.15	0.97	82.06±0.27	83.05±0.54
Castor	0.2	1.27±0.21	2.70±0.15[a]	1	1	80.88±0.35	81.56±0.84
	0.5	1.83±0.07[†]	2.80±0.10[a]	1.44	1.03	82.38±0.41	83.46±0.32
	1.0	1.63±0.12	2.46±0.13[a]	1.28	0.91	83.05±0.44	84.14±0.36
Sunflower	0.2	2.56±0.44	3.29±0.91	1	1	80.54±0.57	81.20±0.67
	0.5	3.33±0.16[†]	3.74±0.06	1.30	1.13	82.17±0.28	83.08±0.44

* Values are mean±SD (n=3). † Statistically significant ($p<0.05$) compared with diclofenac 0.2% (w/v) drops, as determined by 1-way ANOVA followed by Dunnett's test. [a] Statistically significant ($p<0.05$) compared with drops without benzyl alcohol, as determined by the paired t-test. BA, benzyl alcohol.

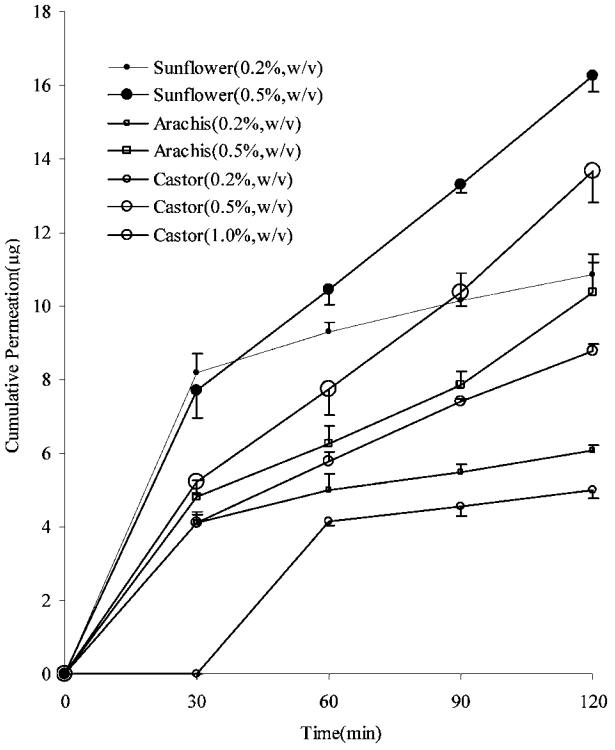

Fig. 3. Effects of Concentration on *in vitro* Transcorneal Permeation of Diclofenac from Oil Drops

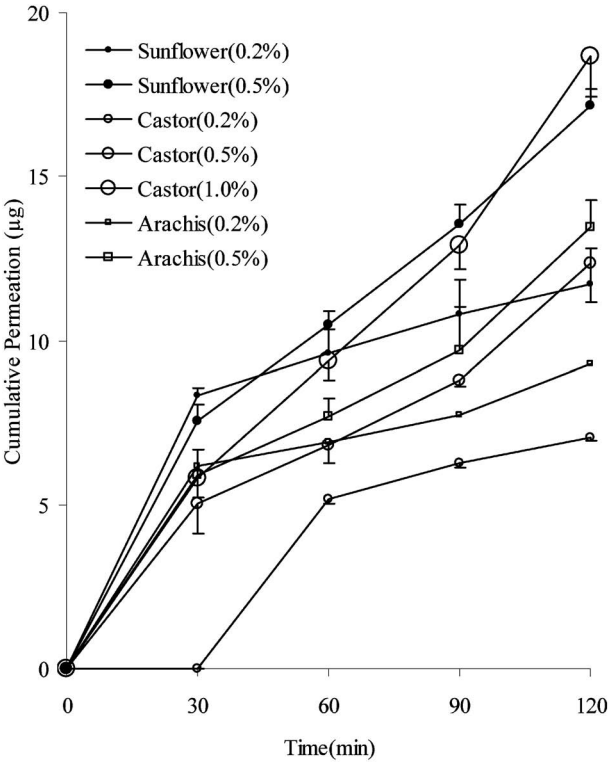

Fig. 4. Effects of Concentration on *in vitro* Transcorneal Permeation of Diclofenac from Oil Drops Containing Benzyl Alcohol

crease in drug concentration. Further, the use of higher drug concentrations was associated with higher corneal hydration levels, indicating corneal damage. The addition of benzyl alcohol to arachis and castor oil drops significantly ($p<0.05$) increased Papp compared with formulation without the preservative. The formulation containing ≥0.5% drug and benzyl alcohol increased corneal hydration to 83–84%. Among all the formulations, diclofenac 0.2% (w/v) drops in sesame oil containing 0.5% (v/v) benzyl alcohol showed maximum permeation. The formulation showed corneal hydration of 82%, indicating corneal damage. Since the hydration level is less than 83%, the damage appears to be reversible. The saturation solubility of diclofenac in sesame oil at 4℃ is 0.33% (w/v) (Table 1). Hence diclofenac 0.2% (w/v) solution in sesame oil, being below the saturation level, will not precipitate at 4℃ and the chances of crystallization of diclofenac from the solution due to climatic change leading to physical instability appear to be remote.

The most convenient way of delivering drug to the eye is topical application of an aqueous solution. Drug from the aqueous solution partitions through the corneal epithelium, stroma, and endothelium into the aqueous humor. One principle demerit of topically applied aqueous drug solution is the loss of drug due to drainage which results in lower ocular availability of drug and a therapeutic effect of shorter duration. One way of overcoming the problem is to apply the drug in the form of an oily solution. Vegetable oils like olive, castor, and sesame oil are used as vehicles for oil-based drops.[19] It has been reported that in healthy individuals pilocarpine dissolved in castor oil has a greater degree and duration of effect on the pupil than the same amount of drug given in aqueous solution. Statistically significant drug effects have been noted as long as 24 h after administration of oil-based drops.[20] Keeping this in view, oil-based drops of diclofenac were formulated in a number of vegetable oils. The concentration of diclofenac in the oil drops was decided depending on the solubility of drug in the respective oils. Permeation studies of oil drops with or without benzyl alcohol were conducted with paired corneas, *i.e.*, one cornea of an animal received formulation without benzyl alcohol while the contralateral cornea received formulation with benzyl alcohol, to avoid biological variation. The results suggest that the addition of benzyl alcohol to diclofenac oil drops increases the permeation of diclofenac from all drops. To ascertain the reason, partition characteristics of diclofenac between oil and aqueous phosphate buffer (pH 7.4) were evaluated. The results indicated lower partitioning of diclofenac in the oil phase in the presence of benzyl alcohol (Table 1) which means that there would be greater tendencies for the drug to enter the aqueous phase from oil drops containing benzyl alcohol compared with drops without the preservative. It would be appropriate to mention here that in oil solutions the release rate of a drug is determined by partitioning of the drug out of the oil in the surrounding aqueous medium.[21] The partitioning phenomenon is an equilibrium process described by the apparent oil/water partition coefficient ($K=C_0/C_W$, where C_0 is the concentration of drug in the organic phase in equilibrium and C_W is the concentration of the drug in the aqueous phase in equilibrium). Only the fraction of the total drug concentration which is present in aqueous phase, f, could be absorbed

$$f=1+\alpha/1+K\alpha \quad (2)$$

where K is the apparent oil/water partition coefficient and α is the ratio V_o/V_w, the volume of the oil phase to that of the aqueous phase. The equation indicates that the fraction of drug available for absorption is controlled by the partition coefficient and the ratio of the volumes of the two phases (α) and that it remains constant as long as α is constant. Since V_w is a physiologic parameter, it is usually constant and therefore the value of α is determined solely by the volume of the oil phase. The rate of drug absorption is described by Eq. 3

$$d(C)/dt=K_a.f.(Dt) \quad (3)$$

where (Dt) is the total drug concentration in both phases and K_a is the absorption rate constant. The above discussion suggests that the rate of absorption of drug from oil solution would depend on f, which in turn depends on the partition coefficient (K). The partition coefficients of diclofenac between the oils and aqueous phase (phosphate buffer, pH 7.4) were higher compared with the K values obtained with oil with benzyl alcohol/buffer. Eq. 2 indicates that the higher the values of the partition coefficient, the smaller the fraction of drug in the aqueous phase, f, and the slower the rate of absorption (from Eq. 3). Thus theoretically, corneal permeation of diclofenac from oil drops without benzyl alcohol should be less than drops containing the preservative. The results of our permeation studies confirm this, and permeation of the drug from oil drops without benzyl alcohol was less. Thus the results of the permeation experiments correlate well with the partition characteristics of diclofenac.

CONCLUSIONS

On the basis of the present study it can be concluded that diclofenac 0.2% (w/v) solution in sesame oil provides the maximum *in vitro* apparent corneal permeability coefficient (Papp) while the formulation in castor oil provides minimal Papp. The addition of benzyl alcohol to oil drops increases drug permeation due to increased partitioning of drug in the aqueous phase. The solubility of diclofenac is higher in castor, arachis, and sunflower oils. But drug permeation from 0.5–1% (w/v) diclofenac drops in castor oil or 0.5% drops in arachis/sunflower oil is less than that observed with 0.2% (w/v) sesame oil drops. Thus diclofenac 0.2% (w/v) drops in sesame oil containing 0.5% (v/v) benzyl alcohol appears suitable from the

permeation point of view. The formulation increased corneal hydration indicating corneal damage. Since the corneal hydration is less than 83% the damage appears to be reversible. The saturation solubility of diclofenac in sesame oil at 4°C is 0.33% (w/v). Hence diclofenac 0.2% (w/v) solution in sesame oil will not precipitate at 4°C and therefore chances of crystallization of diclofenac from the solution due to climatic change leading to physical instability appear to be remote.

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