International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
December - January, 20116, Vol. 5, No.1, pp 1955-1962
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

IN VITRO CORNEAL PERMEATION OF CELECOXIB FROM OIL DROPS

Ajit Kumar Acharya[1]*, Gitanjali Mishra[2] and Dipak Kanti Majumdar[3]

1. Royal College of Pharmacy and Health Sciences, Berhampur-76002 (Ganjam), Odisha, India
2. P.G. Department of Zoology, Berhampur University, Berhampur- 760007 (Ganjam), Odisha, India
3. Delhi Institute of Pharmaceutical Sciences and Research, Formerly College of Pharmacy, (University of Delhi), Pushp Vihar, Sector-III, New Delhi-10017, India

***Corresponding author's Email: ajit47@gmail.com**

(Received: October 18, 2015; **Accepted:** November 21, 2015**)**

ABSTRACT

The objective of present investigation was to study the in vitro permeation characteristics of celecoxib from oil drops through freshly excised goat corneas. Celecoxib ophthalmic solutions (0.3 to 1.0% w/v in arachis, castor and mustard oil or 0.3 to 0.5% w/v in olive, sesame and 0.3% w/v in sunflower oil) were prepared with or without benzyl alcohol (0.5% v/v). Permeation studies were conducted by placing 1 ml oil formulation on cornea (0.64 cm2) fixed between donor and receptor compartment of an all-glass modified Franz diffusion cell and the drug permeation in receptor (containing 10 ml bicarbonate ringer, pH 7.4 at 370C) was measured by spectrophotometer at 248 nm, after 120 minutes. 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. The maximum corneal permeation (0.099 mg) was obtained from 0.5% (w/v) celecoxib drops in sesame oil with benzyl alcohol, while minimum (0.016 mg) from 0.3% (w/v) formulation in castor oil without benzyl alcohol. Addition of benzyl alcohol, a preservative, to oil drops increased permeation of celecoxib from each oil drops. This could be due to increased partitioning of celecoxib in the aqueous phase in the presence of benzyl alcohol. Corneal hydration obtained with all the formulations was between 75 to 80% indicating no corneal damage. In conclusion, increasing celecoxib concentration increased the corneal permeation from oils but among all, celecoxib (0.5% w/v) in sesame oil containing benzyl alcohol showed the maximum permeation.

Keywords: Celecoxib, solubility, partition coefficient, corneal permeation, corneal hydration.

INTRODUCTION

Topical administration of rugs is the most preferred route for management of ocular inflammation as it provides higher ocular drug concentrations, avoiding the systemic side effects associated with the oral administration. Corticosteroids used to be the mainstay of topical therapy in the management of ocular inflammations [1]. Their use is associated with increase in intraocular pressure, cataract formation and risk of infections [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin [3], flurbiprofen [4], ketorolac [5] and diclofenac [6] have been found to be viable alternatives to corticosteroids in the management of ocular inflammation.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of COX enzymes and thereby the synthesis of all downstream PGs. Within the eye, PGs disrupt the blood–ocular barrier, increase vasodilation, and facilitate leukocyte migration. Consequently, topical formulations of NSAIDs have been shown in several well-designed clinical studies to reduce intraocular inflammation and macularedema after cataract and vitreoretinal surgery [7-9]. Celecoxib, chemically 4-[5-(4-methylphenyl)-3-(trifluoromethyl) 1H-pyrazol-1-yl] benzenesulphonamide, is a selective cyclooxygenase-2 (COX-2) enzymes inhibitor. It is used for the treatment of osteoarthritis, rheumatoid arthritis, and management of pain.

Celecoxib has a pK of 11.1, and has low aqueous solubility (3-7 µg/ml) [10]. The therapeutic efficacy of a topical formulation depends on its composition and the physicochemical properties of the vehicle. Use of an appropriate vehicle is critical to increase the optimal efficacy of the pharmacologically active drugs[11]. Commonly, all the ophthalmic formulations have been administered to the eye as aqueous solutions. About 90% of the dose applied topically from such solutions is lost due to precorneal losses (nasolacrimal drainage) and tearing results in poor availability as contact time is less between drug and ocular tissue. Majority of active components are lipophilic in nature viz.-cyclosporine, ketorolac and diclofenac. Both problems can be overcome by selecting appropriate vehicle. 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. Several vegetable oils like olive, sunflower, castor and sesame oil have been used as vehicle for oil based drops to improve ocular drug delivery [12-13]. Earlier studies with pilocarpine [14], tetracycline [15], and ketorolac [16] revealed higher ocular availability of drugs from oily solutions. However, no such information is available on corneal permeation of celecoxib from oily solution. In the present study, the corneal permeation of celecoxib from oily solutions was investigated.

MATERIALS AND METHODS

Materials

Celecoxib (purity 99.6% w/w) was obtained as a gift sample from Mylan Laboritories Limited, India. Refined food grade vegetable oils used in the experiment were arachis (Adani Wilmar Limited, Ahmedabad, India), castor (Arora & Company, New Delhi, India), olive (Rajesh Chemicals Co., Mumbai, India), sunflower (Rajesh Chemicals Co. Mumbai, India), mustard (National Diary Development Board, Gujarat, India) and sesame oils (Tilsona, Recon oil Industries Pvt Limited, New Delhi, India). All other chemicals purchased were of analytical grade and were used as received. Fresh whole goat eye were obtained from a local butcher shop (Berhampur, Odisha, India).

Solubility of celecoxib in oils

An excess amount of celecoxib was added to oils to prepare a saturated solution at 50°C. The solution of celecoxib 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). The celecoxib oil solution (10 mL) was subjected to five successive extractions with 10 mL of 0.1N NaOH solution (pH 12.6). The aqueous phases were pooled, filtered, and volume was made up to 100 mL using 0.1N NaOH solution (pH 12.6). The extract was analyzed for celecoxib at 248 nm using UV-visible spectrophotometer (1800 Shimadzu, Kyoto, Japan).

Preparation of oily formulations

The concentration of celecoxib in test solutions was based on solubility of drug in different oils. Required amount of celecoxib was dissolved in oily vehicles to give celecoxib (0.3% w/v) solution in arachis, castor, mustard, olive, sesame and sunflower oil and celecoxib (0.5% w/v) oily solutions in arachis, castor, mustard, olive and sesame oils. Similarly celecoxib (1.0% w/v) oily solutions in arachis, castor and mustard oils were also prepared.

Celecoxib oily formulations containing preservative: Celecoxib oily formulations were prepared in the same concentrations as mentioned above in the different oils and benzyl alcohol (0.5% v/v) was added as preservative.

Partition behaviour study

Equal volumes of Celecoxib oil formulation without or with benzyl alcohol and phosphate buffer (pH 7.4) were shaken for 2 hour at 37°C in a mechanical shaker at 200 rpm (Remi industries Ltd, Mumbai, India). The concentration of drug in aqueous phase was analyzed and the partition coefficient was calculated. The partition coefficient represents the ratio of celecoxib distribution between oil and aqueous phase. The experiment was done in triplicate and results were expressed as mean ±SD.

Permeation study

Freshly excised cornea was fixed between clamped donor and receptor compartments of an all-glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.64 cm^2. The receptor compartment was filled

with 10 mL freshly prepared bicarbonate ringer solution (pH 7.2), and all air bubbles were expelled from the compartment. An aliquot (1 mL) of oil drop formulation was placed on the cornea and the opening of the donor cell was sealed with a glass cover slip; receptor fluid was kept at 37^{0}C with constant stirring using a Teflon-coated magnetic stir bead. Permeation study was continued for 120 minutes and samples were withdrawn from receptor and analyzed for celecoxib at 248 nm using UV-visible spectrophotometer (1800 Shimadzu, Kyoto, Japan). Results were expressed as amount permeated and percentage permeation or *in-vitro* ocular availability. The permeation (%) or *in-vitro* ocular availability was calculated as follows:

$$\text{Permeation} = \frac{\text{Amount of drug permeated in receptor}}{\text{Initial amount of drug in donor}} \times 100 \quad \ldots\ldots (1)$$

Corneal hydration (%)

At the end of the experiment, each cornea (freed from adhering sclera) was weighed, soaked in 1mL methanol, dried overnight at 90°C, and reweighed. The percentage corneal hydration level (%) was calculated by the formula,

$$\text{Corneal hydration} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

The study was planned with paired corneas to avoid biological variations i.e. one cornea of an animal received formulation without benzyl alcohol while the contra lateral cornea received formulation with benzyl alcohol.

Statistical methods

Statistical analysis was done by one-way ANOVA followed by Dunnett's test using GraphPad Prism 5 software (GraphPad Software Inc., San Diego CA). Paired t-test was used for studies with paired cornea. A p value < 0.05 was considered significant.

RESULTS

The solubility of celecoxib in different vegetable oils was found to be between 0.39 to 1.173% w/v. Table 1 shows the solubility of celecoxib in different oils and its partition characteristics. Celecoxib was found to have maximum solubility (%w/v) in arachis oil (1.173) followed by castor (1.154) and mustard oil (1.46). In the rest of the oils like olive, sesame and sunflower oil, the solubility was between 0.39 to 0.98%. The partition coefficient of celecoxib 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 sesame oil. Addition of benzyl alcohol significantly ($p<0.05$) reduced the partition coefficient value of celecoxib from all the oils.

Table 2 present the permeation characteristics of celecoxib from 0.3% (w/v) oil solutions with or without benzyl alcohol through excised goat corneas (paired). Amount of celecoxib permeated or percentage permeation was found to be maximum with sesame oil drop (0.054 mg and 1.8%) followed by sunflower oil drop and minimum with castor oil (0.016 mg and 0.53%). The corneal hydration level is between 76 to 79%. Benzyl alcohol addition increased celecoxib permeation from all the formulations as compared to formulations without benzyl alcohol.

Table 3 shows the corneal permeation of celecoxib oil drops (0.5% w/v) with and without benzyl alcohol through excised goat cornea (paired). As compared to castor oil; mustard, olive and sesame showed significant ($p < 0.05$) higher amount of drug permeated. Maximum celecoxib permeated or percentage permeation was observed with sesame oil drop (0.082 mg and 1.64%), while minimum with castor oil (0.024 mg and 0.48%). Benzyl alcohol addiction increased celecoxib permeation from all the formulations as compared to formulations without benzyl alcohol. Corneal hydration was found to be in acceptable range with all the oil drops.

Table 4 shows the effects of celecoxib concentration on the corneal permeation. Increasing celecoxib concentration in arachis, castor, and mustard oils from 0.3% to 1.0% (w/v) resulted in a significant ($p<0.05$) increase in drug permeation. Further, the use of higher drug concentrations was associated with higher corneal hydration. The addition of benzyl alcohol significantly ($p<0.05$) increased drug permeation compared with formulation without the preservative. The formulations containing 1.0 % (w/v) drug and benzyl alcohol increased

Table 1: Solubility of celecoxib in different oils and it's partition characteristics from oil drops (0.3% w/v) with and without benzyl alcohol (0.5% v/v)

Oils	Solubility (% w/v)	Partition coefficient	
		Without benzyl alcohol	With benzyl alcohol
Arachis	1.173± 0.11	1.402 ± 0.07 †	1.104 ± 0.02* ‡
Castor	1.154±0.073	1.676 ± 0.016 †	1.451 ± 0.027* ‡
Mustard	1.146±0.054	0.895 ± 0.021 †	0.781 ± 0.012* ‡
Olive	0.988±0.09	0.920 ± 0.19 †	0.904 ± 0.13*
Sesame	0.531±0.047	0.687 ± 0.024	0.403 ± 0.043 ‡
Sunflower	0.39±0.038	0.866 ± 0.047†	0.79± 0.023* ‡

Values are mean ±SD (n=3)

†Statistically significant ($p<0.05$) compared with sesame oil (0.3%w/v) without benzyl alcohol, as determined by 1-way ANOVA followed by Dunnett's test.

*Statistically significant ($p<0.05$) compared with sesame oil (0.3%w/v) with benzyl alcohol, as determined by 1-way ANOVA followed by Dunnett's test.

‡ Statistically significant ($p<0.05$) compared without BA, as determined by 1-way ANOVA followed by Dunnett's test.

Table 2: Permeation characteristics of celecoxib from oil drops (0.3% w/v) with and without benzyl alcohol (0.5% v/v) through excised goat cornea. (Paired)

Oils	Without benzyl alcohol			With benzyl alcohol		
	Amount permeated (mg)	Permeation (%)	Corneal hydration (%)	Amount permeated (mg)	Permeation (%)	Corneal hydration (%)
Arachis	0.025 ± 0.0100	0.83±0.34	75.72±0.31	0.036±0.00040*‡	1.22±0.13	76.28±0.30
Castor	0.016±0.0032	0.53±0.10	78.49±0.24	0.031±0.0017‡	1.03±0.58	79.15±0.51
Mustard	0.032±0.0034†	1.06±0.11	77.17±0.68	0.046±0.0012*‡	1.53±0.41	78.61±0.26
Olive	0.026±0.0017	0.86±0.59	76.63±0.43	0.041±0.00073*‡	1.36±0.024	77.67±0.73
Sesame	0.054 ±0.0110†	1.8±0.39	75.96±0.09	0.078±0.0012*‡	2.6±0.42	77.39±0.38
Sunflower	0.036±0.0100†	1.2±0.33	75.72±0.19	0.048±0.00096* ‡	1.6±0.56	77.52±0.82

Values are mean ±SD (n=3)

† Statistically significant ($p<0.05$) compared with castor oil (0.3%w/v) without BA, as determined by 1-way ANOVA followed by Dunnett's test.

* Statistically significant ($p<0.05$) compared with castor oil (0.3%w/v) with BA, as determined by 1-way ANOVA followed by Dunnett's test.

‡ Statistically significant ($p<0.05$) compared without BA, as determined by 1-way ANOVA followed by Dunnett's test.

Table 3: Permeation characteristics of celecoxib from oil drops (0.5% w/v) with and without benzyl alcohol (0.5% v/v) through excised goat cornea. (Paired)

Oils	Without benzyl alcohol			With benzyl alcohol		
	Amount permeated (mg)	Permeation (%)	Corneal hydration (%)	Amount permeated (mg)	Permeation (%)	Corneal hydration (%)
Arachis	0.036±0.0072	0.72±0.43	77.96±0.62	0.054±0.0137 ‡	1.08±0.03	78.63±0.07
Castor	0.024±0.0010	0.48±0.29	79.69±0.16	0.044±0.0125 ‡	0.88±0.53	80.84±0.28
Mustard	0.048±0.0054 †	0.96±0.01	78.74±0.008	0.066±0.0032 * ‡	1.32±0.24	79.36±0.11
Olive	0.04±0.0091†	0.81±0.99	77.93±0.36	0.061±0.0800 * ‡	1.22±0.46	79.06±0.47
Sesame	0.082±0.0116 †	1.64±0.37	77.68±0.29	0.099±0.0093 * ‡	1.98±0.19	78.50±0.34

Values are mean ±SD (n=3)

†Statistically significant ($p<0.05$) compared with castor oil (0.5%w/v) without BA, as determined by 1-way ANOVA followed by Dunnett's test.

*Statistically significant ($p<0.05$) compared with castor oil (0.5%w/v) with BA, as determined by 1-way ANOVA followed by Dunnett's test.

‡ Statistically significant ($p<0.05$) compared without BA, as determined by 1-way ANOVA followed by Dunnett's test.

Table 4: Effects of Drug Concentration on Corneal Permeation of celecoxib from Oil Drops with and without benzyl alcohol (0.5% v/v) through excised goat cornea. (Paired)

Oils	Drug conc. (% w/v)	Without benzyl alcohol			With benzyl alcohol		
		Amount permeated (mg)	Permeation (%)	Corneal hydration (%)	Amount permeated (mg)	Permeation (%)	Corneal hydration (%)
Arachis	0.3	0.025 ± 0.0100	0.83±0.34	75.72±0.31	0.036±0.00040*	1.22±0.13	76.28±0.30
	0.5	0.036±0.0072†	0.72±0.43	77.96±0.62	0.054±0.0137 *	1.08±0.03	78.63±0.07
	1.0	0.059±0.0010 †	0.59±0.16	79.94±0.47	0.089±0.0183 *	0.89±0.03	80.82±0.48
Castor	0.3	0.016±0.0032	0.53±0.10	78.49±0.24	0.031±0.0017*	1.03±0.58	79.15±0.51
	0.5	0.024±0.0010†	0.48±0.29	79.69±0.16	0.044±0.0125 *	0.88±0.53	80.84±0.28
	1.0	0.038±0.0031†	0.38±0.31	80.98±0.42	0.069±0.0076 *	0.69±0.28	82.24±0.36
Mustard	0.3	0.032±0.0034	1.06±0.11	77.17±0.68	0.046±0.0012*	1.53±0.41	78.61±0.26
	0.5	0.048±0.0054†	0.96±0.01	78.74±0.008	0.066±0.0032 *	1.32±0.24	79.36±0.11
	1.0	0.069±0.0054 †	0.69±0.05	79.89±0.31	0.081±0.0079*	0.81±0.44	81.51±0.39

Values are mean ±SD (n=3)

† Statistically significant ($p<0.05$) compared with celecoxib 0.3% (w/v) drops, as determined by 1-way ANOVA followed by Dunnett's test.

* Statistically significant ($p<0.05$) compared with drops with out benzyl alcohol, as determined by 1-way ANOVA followed by Dunnett's test.

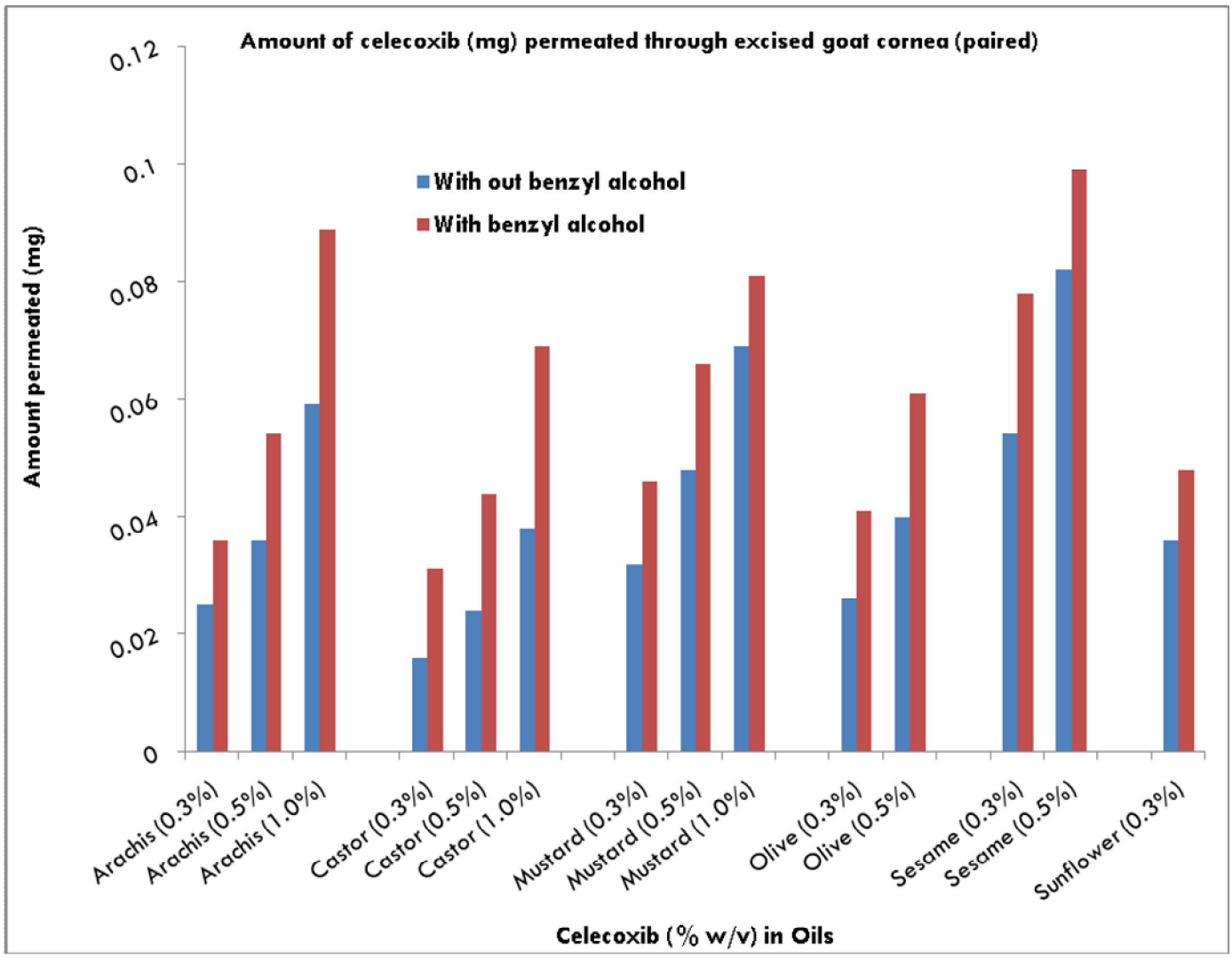

Figure 1: Effects of drug concentration on corneal permeation of celecoxib from Oil Drops with and without benzyl alcohol (0.5% v/v) through excised goat cornea. (Paired)

corneal hydration to 80-82%. Among all the formulations (Fig. 1), celecoxib 0.5% (w/v) drops in sesame oil containing 0.5% (v/v) benzyl alcohol showed maximum permeation with corneal hydration of 78.5%, which shows no corneal damage.

DISCUSSION

The saturation solubility of celecoxib was measured at 4°C. The lesser corneal permeability of celecoxib from castor oil, arachis oil and olive oil-based drops could be attributed to the higher partitioning of celecoxib in castor, arachis and olive oils. Similarly, higher permeability of the drug from sesame, sunflower and mustard oil drops could be due to the lower partition coefficient of drug between the oil and aqueous phase. Earlier studies with ketorolac [16] also reported less permeation of drug from castor oil-based Hence celecoxib 0.5% (w/v) solution in sesame oil, being below the saturation level, will not precipitate at 4°C and the chances of crystallization of celecoxib from the solution due to climatic change leading to physical instability appear to be remote. 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 celecoxib oil drops increases the permeation of celecoxib from all drops. To ascertain the reason, partition characteristics of celecoxib between oil and aqueous phosphate buffer (pH 7.4) were evaluated. The results indicated lower partitioning of

celecoxib 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 [18]. 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

$$f=1+a/1+Ka \quad \ldots\ldots\ldots\ldots \quad (3)$$

Where K is the apparent oil/water partition coefficient and a 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 (a) and that it remains constant as long as a is constant. Since V_W is a physiologic parameter, it is usually constant and therefore the value of a is determined solely by the volume of the oil phase. The rate of drug absorption is described by Eq. 4

$$d(C\ )/dt=K_a.f.(Dt) \quad \ldots\ldots\ldots \quad (4)$$

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 celecoxib 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.3 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.4). Thus theoretically, corneal permeation of celecoxib 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 celecoxib.

CONCLUSIONS

On the basis of the present study it can be concluded that celecoxib 0.5% (w/v) solution in sesame oil provides the maximum in-vitro permeation through goat cornea while the formulation in castor oil provides minimum permeation. The presence of benzyl alcohol to oil drops increases drug permeation due to increased partitioning of drug in the aqueous phase. The solubility of celecoxib was found to maximum (% w/v) in arachis oil (1.173) followed by castor (1.154) and mustard oil (1.46). In the rest of the oils like olive, sesame and sunflower oil, the solubility was between 0.39 to 0.98%. But drug permeation from 0.3-1.0 % (w/v) celecoxib drops in arachis, castor and mustard oil or 0.3-0.5% (w/v) drops in olive oil or 0.3%(w/v) sunflower/sesame oil is less than that observed with 0.5% (w/v) sesame oil drops. Among all the formulations, celecoxib 0.5% (w/v) drops in sesame oil containing 0.5% (v/v) benzyl alcohol showed maximum permeation (0.099 mg or 1.98%). The formulation showed corneal hydration of 78.5%, which is in the acceptable range. So there will not be any corneal damage. The saturation solubility of celecoxib in sesame oil at 4°C is 0.53% (w/v) (Table 1). Hence celecoxib 0.5%(w/v)solution in sesame oil, being below the saturation level, will not precipitate at 4°C and the chances of crystallization of celecoxib from the solution due to climatic change leading to physical instability appear to be remote.

Acknowledgements:

The authors are thankful to Mylan Laboratories Limited (India) for providing the gift sample of celecoxib. Authors are also thankful to Royal College of Pharmacy and Health Sciences, Berhampur, Odisha (India) for providing the necessary research facilities.

REFERENCES

1. Polansky J, Weinreb R. Pharmacology of the Eye. New York: Springer. 460–583: 1984.
2. Hersh PS, Rice BA, Baer JC, Wells PA, Lynch SE, Mcguigan LJB, Foster S. (1990) Arch. Ophthalmol.108:577-583.

3. Searle AE, Pearce JL, Shaw DE. (1990) Br. J. Ophthalmol. 74:19-21.
4. Copper LA, Bergamini MVW, Leopold IH. (1980) Arch. Ophthalmol. 98:1102-1105.
5. Solomen KD, Cheetham JK, Degryse R., Brint SF, Rosenthal A. (2001) Ophthalmology. 108:331-337.
6. Kraff MC, Saunders DR, Mcguigan L, Rannan MG. (1990) Arch. Ophthalmol. 108:380-383.
7. Kim SJ, Flach AJ, Jampol LM. (2010) Surv Ophthalmol. 55:108–133.
8. Reddy R, Kim SJ. (2011) Clin Ophthalmol. 5:751–758.
9. Kim SJ, Hubbard GB. (2008) Arch Ophthalmol. 126:1203–1208.
10. Paulson S, Vaughn M, Jessen S, Lawal Y, Gresk C, Yan B, Maziasz T, Cook C, Karim A. (2001) J. Pharmacol. Exp. Ther. 297:638–645.
11. Ozsoy Y, Gungor S, Cevher E. (2004) Farmaco. 59:563-566.
12. Wiederholt M, Kossendrup D, Schulz W, Hoffmann F. (1986) Invest Ophthalmol. Vis Sci. 27:519-524.
13. Banker GS, Rhodes CS: "Modern Pharmaceutics", Marcel Dekker, New York, Ed. 4th, 2007.
14. Tilmouth T, Briscoe J. (1984) Med. J. Australia. 140 (2):119.
15. Malhotra M, Majumdar DK. (1997) Indian J. Exp. Biol. 35: 1324-1330
16. Ahuja M, Dhake AS, Majumdar DK. (2006) Yakugaku Zasshi. 126(12):1369-1375.
17. Maurice DM, Riley MV. (1970) Biochemistry of the Eye, Academic Press, London, 6-16.
18. Longer MA, Robinson JR: "Remington's Pharmaceutical Sciences," Mack Publishing Company, Easton, PA, Ed. 19th, 1990.

