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In vitro permeation characteristics of moxifloxacin from oil drops through excised goat, sheep, buffalo and rabbit corneas

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The objective of present investigation was to study the *in vitro* permeation characteristics of moxifloxacin from oil drops through freshly excised goat, sheep, buffalo and rabbit corneas. Moxifloxacin, 0.043 to 0.048% (w/v) ophthalmic solutions with or without (0.5% v/v) benzyl alcohol were made in arachis, castor, cottonseed, olive, soybean, sunflower and sesame oils. Permeation studies were conducted by putting 1 ml oil formulation on cornea (0.50 cm^2) fixed between donor and receptor compartments of an all glass modified Franz diffusion cell and measuring the drug permeated in receptor (containing 10 ml bicarbonate ringer, pH 7.4 at 37 °C under stirring) by spectrophotometry at 291 nm, after 120 min. Post permeation corneal hydration was measured to assess corneal damage. 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. Moxifloxacin ophthalmic solution in castor oil showed maximum permeation with all the corneas. Addition of benzyl alcohol, a preservative, to oil drops reduced permeation of moxifloxacin from each oil drop, with corneas of all the species. Partition experiments with moxifloxacin oil drops and phosphate buffer (pH 7.4) indicated higher partitioning of drug in the oil phase, in presence of benzyl alcohol. Thus results of permeation are consistent with the partition characteristics of drug between oil and aqueous phase. Corneal hydration obtained with all the formulations was between 75 to 80% indicating no corneal damage.

1. Introduction

Fluoroquinolones elicit their bactericidal effect by inhibiting bacterial DNAgyrase and topoisomerase (Hardman and Limbird 2001). Moxifloxacin is a fourth generation fluoroquinolone with a methoxy group in C-8 position and a bulky C-7 side chain. This fourth-generation fluoroquinolone has *in vitro* activity similar to that of ciprofloxacin and ofloxacin against gram-negative bacteria but enhanced activity against gram-positive bacteria including *S. aureus* (Biedenbach and Jones 1996; Dalhoff et al. 1996; Davis and Bryson 1994). The fourth generation of fluoroquinolones, gatifloxacin and moxifloxacin have been reported to have increased susceptibility to *S. aureus* (isolated from clinical cases of keratitis) compared with older generation fluoroquinolones such as ciprofloxacin, levofloxacin or ofloxacin (Kowalski et al. 2003). Moxifloxacin has demonstrated greater effectiveness than ciprofloxacin or levofloxacin in experimental keratitis in rabbits (Dajcs et al. 2004). Experiments in rabbits suggest that surgical prophylaxis with topical moxifloxacin could be effective for prevention of bacterial endophthalmitis (Mather et al. 2004, Kowalski et al. 2004). Moxifloxacin penetration into inflamed ocular tissue of rabbit has been found to be better than ciprofloxacin, lomefloxacin, ofloxacin or levofloxacin (Yagci et al. 2006). In a recent study moxifloxacin penetration in aqueous humor of human subjects has been reported to be higher than that of gatifloxacin following topical dosing of drops (McCulley et al. 2006). This laboratory is developing ocular formulations of moxifloxacin and has earlier reported transcorneal permeation of the drug from aqueous drops (Pawar and Majumdar 2006).

The major hindrance to ocular bioavailability of topically applied ophthalmic drugs is incomplete absorption due to nasolacrimal drainage. One approach to overcome this problem has been through prolonging the ocular contact time of the medication. Increased contact time of the drug may be achieved by formulating the drug as oil solution. The oil drop formulation provides prolonged therapeutic action due to increased contact time and sustained release of medicament.

Thus, the purpose of this research was to formulate moxifloxacin in oily vehicles and evaluate the transcorneal permeation characteristics of drug from oil drops through freshly excised goat, sheep, buffalo, and rabbit corneas.

2. Investigations, results and discussion

The solubility of moxifloxacin (base) in different vegetable oils was found to be between 0.043 to 0.048%. Table 1 shows permeation characteristics of moxifloxacin from oil drops with or without benzyl alcohol through excised goat corneas (paired). Amount of moxifloxacin permeated from castor oil drop was found to be highest followed by soybean and cottonseed oil drops. The percentage permeation was however maximum with moxifloxacin drop in castor oil followed by soybean and cottonseed oils. The corneal hydration level of normal mammalian cornea is between 75 to 80% (Maurice and Riley 1970). Post permeation corneal hydration was found to be in the normal range with all the oil drops. Addition of benzyl alcohol, a commonly used preservative in oil formulation, significantly ($p < 0.05$) reduced the permeation of moxifloxacin from all oil drops compared with the formulation without benzyl alcohol. The amount of moxifloxacin permeated from castor oil drop containing benzyl alcohol was found to be maximum, followed by soybean oil drop. The percentage permeation of moxifloxacin from oil drops containing benzyl alcohol was also found to be higher with castor oil and soybean oil drops. Corneal hydration was in the normal range with all the formulations.

Table 2 shows the permeation characteristics of moxifloxacin from oil drops with or without benzyl alcohol through excised sheep corneas (paired). Permeation of moxifloxacin from castor oil drop was found to be highest followed by olive oil drop. The percentage permeation was however maximum with moxifloxacin drop in olive oil followed by castor oil. Post permeation corneal hydration was found to be in the normal range with all the oil drops. Addition of benzyl alcohol to oil drops significantly ($p < 0.05$) re-

Table 1: Permeation characteristics of moxifloxacin from oil drops (0.043 to 0.048% w/v) with or without benzyl alcohol (0.5% v/v) through excised goat cornea (paired)

Oils	Without benzyl alcohol				With benzyl alcohol			
	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
Cottonseed oil	0.471 ± 0.0093	0.024 ± 0.0012*	5.10	78.74 ± 0.074	0.478 ± 0.0076	0.014 ± 0.0005†	2.93	78.95 ± 0.220
Castor oil	0.484 ± 0.0038	0.027 ± 0.0003*	5.58	78.74 ± 0.326	0.473 ± 0.0055	0.018 ± 0.0005†**	3.80	79.02 ± 0.409
Olive oil	0.434 ± 0.0029	0.022 ± 0.0008	5.07	78.69 ± 0.230	0.449 ± 0.0063	0.013 ± 0.0011†	2.90	78.94 ± 0.086
Arachis oil	0.431 ± 0.0097	0.020 ± 0.0008	4.64	78.83 ± 0.334	0.440 ± 0.0079	0.014 ± 0.0006†	3.18	78.52 ± 0.290
Soybean oil	0.454 ±0.0109	0.024 ± 0.0013*	5.29	79.15 ± 0.037	0.447 ± 0.0011	0.016 ± 0.0003†**	3.58	78.81 ± 0.243
Sunflower oil	0.438 ± 0.0036	0.021 ± 0.0003	4.79	79.17 ± 0.192	0.441 ± 0.0014	0.014 ± 0.0005†	3.17	79.00 ± 0.298
Sesame oil	0.463 ± 0.0021	0.022 ± 0.0008	4.75	79.03 ± 0.208	0.458 ± 0.0046	0.012 ± 0.0012†	2.62	78.98 ± 0.185

Values are mean ± SE of 3 corneas in each group
† Statistically significant ($P < 0.05$) compared with oil drop without benzyl alcohol as determined by paired t test
* Statistically significant ($P < 0.05$) compared with arachis oil drop, as determined by one-way ANOVA followed by Dunnett's test
** Statistically significant ($P < 0.05$) compared with sesame oil drop, as determined by one-way ANOVA followed by Dunnett's test

Table 2: Permeation characteristics of moxifloxacin from oil drops (0.043 to 0.048% w/v) with or without benzyl alcohol (0.5% v/v) through excised sheep cornea (paired)

Oils	Without benzyl alcohol				With benzyl alcohol			
	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)	Drug content in oil drops (mg/ml)	Amount Permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
Cottonseed oil	0.471 ± 0.0093	0.018 ± 0.0003	3.82	79.17 ± 0.248	0.478 ± 0.0076	0.013 ± 0.0006†	2.72	78.71 ± 0.104
Castor oil	0.484 ± 0.0038	0.021 ± 0.0006*	4.34	79.20 ± 0.373	0.473 ± 0.0055	0.015 ± 0.0006†**	3.17	78.82 ± 0.087
Olive oil	0.434 ± 0.0029	0.020 ± 0.0011*	4.61	79.13 ± 0.267	0.449 ± 0.0063	0.013 ± 0.0012†	2.90	78.43 ± 0.215
Arachis oil	0.431 ± 0.0097	0.018 ± 0.0006	4.18	78.70 ± 0.349	0.440 ± 0.0079	0.012 ± 0.0009†	2.72	78.65 ± 0.214
Soybean oil	0.454 ± 0.0109	0.016 ± 0.0009	3.52	79.10 ± 0.103	0.447 ± 0.0011	0.012 ± 0.0007	2.68	78.95 ± 0.127
Sunflower oil	0.438 ± 0.0036	0.017 ± 0.0009	3.88	79.08 ± 0.116	0.441 ± 0.0014	0.011 ± 0.0007†	2.49	78.51 ± 0.280
Sesame oil	0.463 ± 0.0021	0.016 ± 0.0006	3.45	78.70 ± 0.269	0.458 ± 0.0046	0.012 ± 0.0008†	2.62	78.22 ± 0.176

Values are mean ± SE of 3 corneas in each group
† Statistically significant ($P < 0.05$) compared with oil drop without benzyl alcohol as determined by paired t test
* Statistically significant ($P < 0.05$) compared with sesame/soybean oil drop, as determined by one-way ANOVA followed by Dunnett's test
** Statistically significant ($P < 0.05$) compared with sunflower oil drop, as determined by one-way ANOVA followed by Dunnett's test

Table 3: Permeation characteristics of moxifloxacin from oil drops (0.043 to 0.048% w/v) with or without benzyl alcohol (0.5% v/v) through excised buffalo cornea (paired)

Oils	Without benzyl alcohol				With benzyl alcohol			
	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
Cottonseed oil	0.471 ± 0.0093	0.015 ± 0.0003	3.18	78.68 ± 0.303	0.478 ± 0.0076	0.011 ± 0.0003†	2.30	79.40 ± 0.413
Castor oil	0.484 ± 0.0038	0.017 ± 0.0003*	3.51	78.12 ± 0.017	0.473 ± 0.0055	0.013 ± 0.0003†**	2.75	79.37 ± 0.279
Olive oil	0.434 ± 0.0029	0.016 ± 0.001*	3.69	78.13 ± 0.213	0.449 ± 0.0063	0.010 ± 0.0008†	2.23	78.68 ± 0.263
Arachis oil	0.431 ± 0.0097	0.015 ± 0.0008	3.48	78.45 ± 0.324	0.440 ± 0.0079	0.011 ± 0.0012†	2.50	79.20 ± 0.073
Soybean oil	0.454 ± 0.0109	0.015 ± 0.0005	3.30	78.02 ± 0.227	0.447 ± 0.0011	0.011 ± 0.0005†	2.46	78.89 ± 0.195
Sunflower oil	0.438 ± 0.0036	0.015 ± 0.0003	3.42	78.17 ± 0.262	0.441 ± 0.0014	0.010 ± 0.0003†	2.27	79.01 ± 0.118
Sesame oil	0.463 ± 0.0021	0.013 ± 0.0008	2.80	78.82 ± 0.143	0.458 ± 0.0046	0.009 ± 0.0006†	1.97	79.35 ± 0.131

Values are mean ± SE of 3 corneas in each group
† Statistically significant ($P < 0.05$) compared with oil drop without benzyl alcohol as determined by paired t test
* Statistically significant ($P < 0.05$) compared with sesame oil drop, as determined by one-way ANOVA followed by Dunnett's test
** Statistically significant ($P < 0.05$) compared with sesame oil drop, as determined by one-way ANOVA followed by Dunnett's test

Table 4: Permeation characteristics of moxifloxacin from oil drops (0.043 to 0.046% w/v) with or without benzyl alcohol (0.5% v/v) through excised rabbit cornea (paired)

Oils	Without Benzyl alcohol				With Benzyl alcohol			
	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)	Drug content in oil drops (mg/ml)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
Cottonseed oil	0.462 ± 0.0016	0.025 ± 0.0009	5.41	78.67 ± 0.289	0.457 ± 0.0082	0.016 ± 0.0006†**	3.50	78.77 ± 0.335
Castor oil	0.449 ± 0.0047	0.033 ± 0.0015*	7.35	78.62 ± 0.263	0.445 ± 0.0059	0.018 ± 0.0003†**	4.04	78.92 ± 0.404
Olive oil	0.435 ± 0.0053	0.023 ± 0.0003	5.29	79.28 ± 0.216	0.432 ± 0.0007	0.015 ± 0.0003†**	3.47	79.99 ± 0.329
Arachis oil	0.444 ± 0.0025	0.024 ± 0.0006	5.40	79.23 ± 0.247	0.432 ± 0.0073	0.013 ± 0.0007†	3.01	78.26 ± 0.132
Soybean oil	0.441 ± 0.0075	0.026 ± 0.0003*	5.89	79.39 ± 0.124	0.442 ± 0.0012	0.016 ± 0.0003†**	3.62	79.39 ± 0.124
Sunflower oil	0.437 ± 0.0042	0.024 ± 0.0006	5.49	79.32 ± 0.525	0.436 ± 0.0039	0.013 ± 0.0006†	2.98	78.54 ± 0.448
Sesame oil	0.433 ± 0.0042	0.023 ± 0.0007	5.31	79.03 ± 0.208	0.437 ± 0.0042	0.014 ± 0.0007†	3.20	79.48 ± 0.270

Values are mean ± SE of 3 corneas in each group
† Statistically significant ($P < 0.05$) compared with oil drop without benzyl alcohol as determined by paired t test
* Statistically significant ($P < 0.05$) compared with sesame/olive oil drop, as determined by one-way ANOVA followed by Dunnett's test
** Statistically significant ($P < 0.05$) compared with sunflower/arachis oil drop, as determined by one-way ANOVA followed by Dunnett's test

duced permeation of drug from all oil drops, except the one made with soybean oil. Formulation with benzyl alcohol showed maximum permeation of drug from castor oil drop followed by olive and cottonseed oil drops while percentage permeation was highest with castor oil drop followed by olive, cottonseed and arachis oil drops. Corneal hydration was in the normal range with all the formulations.

Permeation studies with paired buffalo corneas showed maximum permeation of moxifloxacin with castor oil drop followed by olive oil drop while percentage permeation was highest with olive oil drop followed by the castor oil preparation. Addition of benzyl alcohol significantly ($p < 0.05$) decreased permeation of drug from all oil drops while formulation in castor oil showed maximum permeation. Corneal hydration remained in normal range with all the formulations (Table 3).

Similarly studies with paired rabbit corneas also showed maximum permeation of moxifloxacin with castor oil drop followed by soybean oil drop. Addition of benzyl alcohol to oil drops significantly ($p < 0.05$) reduced drug permeation but the trend remained the same i.e. castor oil drop showed highest permeation. Corneal hydration remained in normal range with all the formulations (Table 4).

Several vegetable oils like olive, castor and sesame oil are used as vehicle for oil based drops to improve dry delivery (Hecht et al. 1990). It has been reported that in healthy subjects 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 (Smith et al. 1978). Keeping this in view oil based drops of moxifloxacin was formulated in a number of vegetable oils. The

Table 5: Partition characteristics of moxifloxacin from oil drops (0.043 to 0.048% w/v) with or without benzyl alcohol (0.5% v/v) and viscosity of different oils

Oils	Partition coefficient*		Viscosity (cps)
	Without benzyl alcohol	With benzyl alcohol	
Cottonseed oil	1.61 ± 0.028	2.19 ± 0.134	43.3
Castor oil	1.35 ± 0.151	1.95 ± 0.071	620
Arachis oil	1.45 ± 0.087	2.03 ± 0.061	53.3
Olive oil	1.49 ± 0.185	2.05 ± 0.156	46.7
Soybean oil	1.37 ± 0.050	2.05 ± 0.255	33.3
Sunflower oil	1.43 ± 0.052	2.12 ± 0.057	40.0
Sesame oil	1.33 ± 0.059	1.99 ± 0.070	46.7

* Partition coefficient between oil and phosphate buffer (pH 7.4)
Values are mean ± SE of 3 observations in each group

concentration of moxifloxacin in the oil drops was decided depending on the solubility of drug in the respective oil. 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 contra lateral cornea received formulation with benzyl alcohol. This was done to reduce biological variation. The results suggest that addition of benzyl alcohol to moxifloxacin oil drops reduced permeation of moxifloxacin from all oil drops through all the four mammalian corneal species. In order to ascertain the reason, partition characteristics of moxifloxacin between oil and aqueous phosphate buffer (pH 7.4) were evaluated. The results indicated higher partitioning of moxifloxacin in oil phase in presence of benzyl alcohol (Table 5) which means that there would be fewer tendencies for the drug to come to aqueous phase from oil drops containing benzyl alcohol compared with drops without the preservative. It would be appropriate to mention here that, in case of oil solutions the release rate of a drug is determined by partitioning of the drug out of the oil in the surrounding aqueous medium (Longer and Robinson 1990). The partitioning phenomenon is an equilibrium process described by the apparent oil/water partition coefficient ($K = C_O/C_W$, where Co is the concentration of drug in organic phase in equilibrium and Cw is the concentration of the drug in aqueous phase in equilibrium). Only the fraction of total drug concentration which is present in aqueous phase, f, could be absorbed

$$f = \frac{1 + \alpha}{1 + k\alpha} \quad (1)$$

where K is the apparent oil/water partition coefficient and α is the ratio Vo/Vw, the volume of 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 while α is constant. Since Vw is a physiological parameter, it usually is constant and therefore the value of α is determined solely by the volume of oil phase. The rate of drug absorption is described by Eq. (2)

$$\frac{d(C)}{dt} = Ka \cdot f \cdot (Dt) \quad (2)$$

where (Dt) is the total drug concentration in both phases and Ka 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 partition coefficient (K). The partition coefficients of moxifloxacin between oils and aqueous phase (phosphate buffer, pH 7.4) were smaller compared to the K values obtained with oil with benzyl alcohol/buffer. Eq. (1) indicates that the smaller the value of partition coefficient the larger would be the fraction of drug in the aqueous phase, f and the faster would be the rate of absorption (from Eq. 2). Thus theoretically, corneal permeation of moxifloxacin from oil drops without benzyl alcohol should be higher than from drops containing the preservative. The results of our permeation studies confirm this, and permeation of drug from oil drops without benzyl alcohol was higher with all the four mammalian corneas. Thus the results of the permeation experiments correlate well with the partition characteristics of moxifloxacin.

The percentage permeation or *in vitro* ocular availability of moxifloxacin from castor oil drop decreased in the order of rabbit > goat > sheep > buffalo whereas the availability from drops containing benzyl alcohol could be ranked as rabbit = goat > sheep > buffalo (Fig.). The rabbit cornea being the thinnest is more permeable than a thicker buffalo cornea. Post permeation corneal hydration with oil drops, with or without benzyl alcohol, was in the normal range (i.e. 75–80%) with all the four mammalian corneas. Thus all the moxifloxacin oil drops appear to be eye friendly. Table 5 also shows the viscosity of different oily vehicles where castor oil possesses maximum viscosity which is 13 to 18 times higher than that of the other oils. As the viscosity of a liquid increases, its fluidity i.e. ability to flow, decreases; castor oil drop, being highly viscous, on instillation to the eye would provide longer residence time in conjunctival cul-de-sac compared with the other oil drops which could provide increased contact time and higher bioavailability. Addition of benzyl alcohol to oil drops would reduce the partitioning of the drug from the oil phase resulting in reduced permeation. However further studies, *in vivo*, are needed to comment more in this respect.

Thus it can be concluded from the present studies that moxifloxacin ophthalmic solution in castor oil provides maximum *in vitro* permeation through all the corneas. Ad-

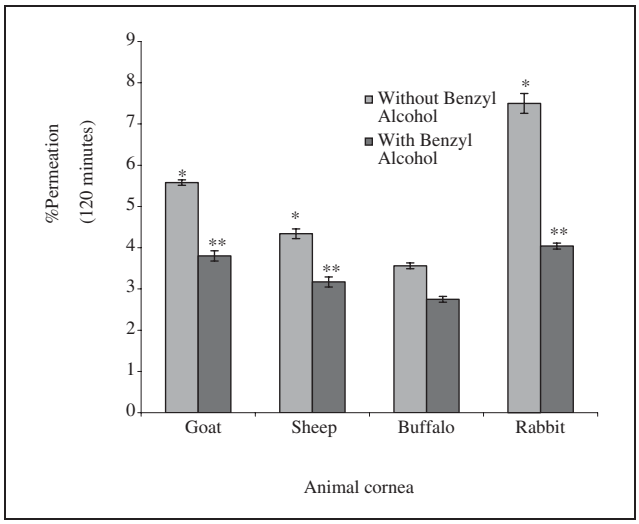

Fig.: Percentage permeation of moxifloxacin from castor oil drops (0.043 to 0.048% w/v) with or without benzyl alcohol (0.5% v/v) through excised goat, sheep, buffalo, and rabbit corneas. Values are mean ± SE of 3 corneas in each group.
* Statistically significant ($P < 0.05$) compared with buffalo cornea as determinded by oneway ANOVA followed by Dunnett's test.
** Statistically significant ($P < 0.05$) compared with buffalo cornea as determinded by oneway ANOVA followed by Dunnett's test

dition of benzyl alcohol, a preservative, to oil drop reduces drug permeation, due to reduced partitioning of the drug into the aqueous phase. The permeation or *in vitro* ocular availability of moxifloxacin from castor oil drops containing benzyl alcohol could be ranked as rabbit = goat > sheep > buffalo. All the moxifloxacin oil drops appear to be friendly to the different mammalian corneas.

3. Experimental

3.1. Materials

Moxifloxacin hydrochloride was obtained from Ranbaxy Laboratories Limited (Gurgaon, India) as a gift. Benzyl alcohol was received from Central Drug House (New Delhi, India). Refined food grade vegetable oils used in the experiment were arachis (Adani Wilmar Limited, Ahmedabad, India), castor (Arora & Company, New Delhi, India), cottonseed (Argo Tech Limited, Secunderabad, India), olive (Figaro), (S O S Cuetara S. A., Madrid, Spain), sunflower (Sundrop), (Agro Tech Foods Ltd, Secunderabad, India), soybean (Adani Wilmar Limited, Kutch, Gujrat, India) and sesame oils (Tilsona), (Recon oil Industries Pvt Limited, New Delhi, India). All other chemicals were of analytical grade. Fresh eyeballs of goat, sheep and buffalo were obtained from butchers shop (Ambedkar Nagar, New Delhi, India) within 1 hour after the animal's slaughtering. Albino rabbits (of either sex) weighing between 1.5 to 2.0 kg were received from Institute Animal House. Phenobarbital sodium injection (Samarth life Sciences Pvt.Ltd. Solan, Himachal Pradesh, India) was used.

3.2. Rabbit experiments

The protocol on the use of animals (Albino rabbit) was approved by the Institutional Ethics Committee. Rabbits were sacrificed with intraperitonial injection of a lethal dose of phenobarbital sodium. The eyeballs of rabbit were removed immediately. The method of dissection of cornea and the apparatus used in the permeation studies were described previously (Malhotra and Majumdar 1997).

3.3. Permeation experiment

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 0.50 cm^2. The receptor compartment was filled with 10 ml freshly prepared bicarbonate ringer solution (pH 7.4), 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 °C with constant stirring using a Teflon-coated magnetic stir bead. Permeation study was continued for 120 min, and samples were withdrawn from receptor and analyzed for moxifloxacin content by measuring absorbance at 291 nm in a spectrophotometer (1601 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:

$$\text{Permeation (\%)} = \frac{\text{Amount of drug permeated in receptor}}{\text{Initial amount of drug in donor}} \times 100 \qquad (3)$$

At the end of the experiment, each cornea (freed from adhering 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. Statistical calculations were done by one-way ANOVA followed by Dunnett's test. Paired t-test was used for studies with paired cornea. A P value less than 0.05 were considered significant.

3.4. Preparation of moxifloxacin (base)

Moxifloxacin hydrochloride was dissolved in distilled water. The pH of the solution was brought to 6.5 with 0.1 N NaOH to precipitate moxifloxacin free base. The precipitate of moxifloxacin base was collected by filtration and washed repeatedly with distilled water till it was completely free from chloride ions. The precipate was dried at 80 °C for 12 h. The product was characterized by spectroscopy and melting point.

3.5. Preparation of test solutions

The concentration of moxifloxacin in test solutions was determined by the solubility of drug in different oils. Moxifloxacin oily solutions (0.043–0.048% w/v) with or without benzyl alcohol (0.5% v/v) were formulated in arachis, cottonseed, castor, olive, soybean, sunflower and sesame oils. Each moxifloxacin oil formulation (10 ml) was subjected to five successive extractions with 10 ml of 0.1 N HCL. The aqueous phases were pooled, filtered, and volume was made up to 100 ml using 0.1 N HCL. The extract was analyzed for moxifloxacin content by measuring absorbance at 291 nm in a spectrophotometer (1601 Shimadzu, Kyoto, Japan) using 0.1 N HCl as blank.

3.6. Measurement of partition coefficient

Equal volumes of moxifloxacin oil formulation without benzyl alcohol or with benzyl alcohol and phosphate buffer (pH 7.4) were shaken for 2 h at room temperature in a mechanical shaker at 200 rpm (Adolf Kuhner, Basel, Switzerland). The experiment was done with triplicate samples of each formulation. The concentration of drug in aqueous phase was analyzed and the partition coefficient was calculated. The partition coefficient represents the ratio of moxifloxacin distribution between oil and aqueous phase. The result was expressed as mean ± SE.

3.7. Measurement of viscosity

Viscosity of oils was determined by Brookfield DV-1+ viscometer (Brookfield Engineering Laboratories, Mlddl Eobro, MA) at 25 °C using spindle 4 at 30 rpm.

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