ORIGINAL ARTICLE

Effect of formulation factors on *in vitro* transcorneal permeation of voriconazole from aqueous drops

Biswaranjan Mohanty, Sagar Kumar Mishra[1], Dipak K. Majumdar[2]

Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, [1]Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, [2]Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research, Formerly College of Pharmacy, University of Delhi, New Delhi, India

J. Adv. Pharm. Technol. Res.

ABSTRACT

The purpose of this research was to evaluate the effect the formulation factors on *in vitro* permeation of voriconazole through freshly isolated goat and sheep corneas. An increase in the pH of the drops from 4.0 to 8.0 resulted in significant ($P < 0.05$) increase drug permeation. Raising concentration of the drops from 0.05% to 0.2% (w/v) significantly, ($P < 0.05$) increased drug permeation, but decreased the percent permeation. Corneal transport of voriconazole is both pH and concentration dependent. Eye drops containing disodium edetate (ethylenediaminetetraacetic acid) alone or combination with benzalkonium chloride showed significantly ($P < 0.05$) higher permeation as compared with control formulation. Addition of beta-cyclodextrin to the formulation enhanced corneal permeation of voriconazole. Compared with control formulation, voriconazole 0.2% (w/v) drop containing viscosity modifier produced significant ($P < 0.05$) decrease in permeation. Most of the formulations showed higher zone of inhibition against *Candida albicans*.

Key words: Partition coefficient, permeation, preservative, voriconazole

INTRODUCTION

Current approaches for the treatment of fungal corneal infections trust on topical administration of antifungal agents.[1-4] Voriconazole (triazole antifungal) is a second-generation synthetic derivative of fluconazole.[5] Voriconazole acts as an enzyme inhibitor, blocking the synthesis of ergosterol, a constituent of fungal membranes, and thus the growth of the microorganism. It has a broad spectrum of activity with lower minimum inhibitory concentrations (MIC), in addition to a high systemic intraocular penetration profile.[6,7] Voriconazole is effective against a wide spectrum of keratitis-causative fungi.[7-9] Voriconazole is commercially available in oral and intravenous forms.[10] Oral voriconazole is highly bioavailable (96%) and has many side-effects with significant drug interactions.[11] Treatment of fungal keratitis with systemic voriconazole is costly.[12] For this reason, an efficient and economical approach of voriconazole delivery in the treatment of fungal keratitis is very much desirable. Topical voriconazole was reported to penetrate well through the cornea and achieve noticeable levels in rabbits[13] and in horses.[14] Eye drops are the most cost-effective and competent system for delivering a medicament into the eye.[15] Topical delivery of drugs to the ocular tissues is affected by a complex interplay of biological, physiochemical and formulation factors. Designing an ophthalmic delivery system is one of the most challenging tasks for the researchers.[16] Formulators usually have to design a dosage form, which provides a balance between the corneal penetration, ocular irritation and formulation stability. Manipulation of formulation parameters to enhance the corneal penetration is one of the approaches of increasing ocular availability. [17, 18] Animal Ethics Committees are discouraging experiments with rabbit cornea. Therefore, it appears logical to search for alternate mammalian corneas, especially from those animals that are slaughtered every day for meat (e.g., goat and sheep). In addition, such a study would also help in the development of veterinary ophthalmic formulation of the drug for the cattle population in the

Address for correspondence:
Dr. Dipak K. Majumdar,
Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research, Formerly College of Pharmacy, University of Delhi, Pushp Vihar,
Sector III, New Delhi - 110 017, India.
E-mail: dkmajumdar@gmail.com, dkmajumdaar@yahoo.com

Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/2231-4040.121416

Indian subcontinent. The present investigation was aimed to develop voriconazole eye drops and to evaluate the effect of pH, concentration of drug, viscosity modifier, preservative and stabilizers on the corneal permeation of voriconazole through freshly isolated goat and sheep cornea.

MATERIALS AND METHODS

Materials

Voriconazole (purity 99.9%) was obtained from Matrix laboratories, Hyderabad and beta-cyclodextrin (β-CD) was obtained from Dr.Reddy's Labs, Hyderabad as gift. Freeze-dried microbial cultures of *Candida albicans* (MTCC NO. 3017), *Candida glabrata* (MTCC NO. 3019), *Aspergillus flavus* (MTCC NO. 3306) and *Fusarium solani* (MTCC NO. 3763) were purchased from Institute of Microbial Technology, Chandigarh, India. All other chemicals were of analytical grade. Fresh eyeballs of goat and sheep were obtained from a local butchers shop (Baripada, Odisha, India) within 1 h of slaughtering of animals. The method of dissection of the cornea and the apparatus used in the permeation studies was the same as published elsewhere.[19]

Determination of Aqueous Solubility of Voriconazole

An excess amount of voriconazole was added to distilled water to prepare a saturated solution at room temperature and was shaken in a reciprocating shaker at 200 rpm (Satyam equipments, New Delhi) for 48 h at 25°C ± 0.5°C.[20] The solution was filtered, diluted and analyzed for voriconazole content by measuring absorbance in a spectrophotometer (ultraviolet-1700, Shimadzu) at 256 nm. The solubility was determined in triplicate.

Preparation of Test Solutions

Voriconazole ophthalmic solutions (0.05% w/v) of different pH

Voriconazole (0.05 g) was dissolved in sufficient distilled water; sodium chloride (0.895 g/100 ml) was added to make the final solution isotonic; the pH of the solution was adjusted to 4.0, 5.0, 6.0, 7.0 or 8.0 using 0.1N HCl or 0.1N NaOH and final volume were made up to 100 ml with distilled water to have solutions of different pH.

Voriconazole ophthalmic solutions (0.05% w/v, pH 7.2) containing preservatives

Voriconazole (0.05 g) was dissolved in sufficient distilled water; sodium chloride (0.895 g/100 ml) was added to make the final solution isotonic; and pH of the solution was adjusted to 7.2. To this solution benzalkonium chloride (BKC 0.01% w/v), thiomersal (THM, 0.005% w/v), phenyl mercuric acetate (PMA, 0.002% w/v), phenyl mercuric nitrate (PMN, 0.002% w/v), disodium edetate (ethylenediaminetetraacetic acid) (EDTA, 0.01% w/v), sodium metabisulfite (SMS, 0.1% w/v), combination of BKC (0.01% w/v) and EDTA (0.01% w/v) or combination of SMS (0.1% w/v) and EDTA (0.01% w/v) was added. The final volume of each solution was made up to 100 ml with distilled water.

Voriconazole ophthalmic solutions (pH 7.2) of increasing concentration

Required amount of physical mixture of voriconazole and β-CD in a ratio of 1:2 was dissolved in sufficient distilled water; sodium chloride (0.895 g/100 ml) was added to make the final solution isotonic; and pH of the solution was adjusted to 7.2 using 0.1N HCl or 0.1N NaOH and final volume were made up to 100 ml with distilled water, to have solutions of 0.05, 0.1, 0.15 and 0.2% (w/v) concentrations.

Ophthalmic solutions (0.2% w/v, pH 7.2) with β-CD containing viscosity modifier

Required amount of physical mixture of voriconazole and β-CD in a ratio of 1:2 was dissolved in sufficient distilled water; sodium chloride (0.895 g/100 ml) was added to make the final solution isotonic; and pH of the solution was adjusted to 7.2 using 0.1N HCl and 0.1N NaOH. To this solution methylcellulose (MC) (0.25%, w/v), hydroxyl propyl methylcellulose (HPMC) (0.25%, w/v), polyvinyl alcohol (PVA) (1.4%, w/v) or polyvinylpyrrolidone (PVP) (1%, w/v) was added and the final volume of each solution was made up to 100 ml with distilled water.

Determination of Partition Coefficient

Equal volumes of voriconazole ophthalmic solution (0.05% w/v, pH 7.2) with or without additive (control) and n-octanol were shaken at room temperature in a reciprocating shaker at 200 rpm (Satyam equipments, New Delhi) for 2 h.[21] Voriconazole content in the aqueous phase of each experiment was analyzed and partition coefficients were calculated. The experiments were done with triplicate sample of each formulation. The result was expressed as mean ± standard deviation.

Measurement of Surface Tension and Viscosity

The surface tension of each ophthalmic solution (0.05% w/v) was measured by using a stalagmometer and the viscosity of each ophthalmic solution (0.05% and 0.2% w/v) was measured using an Ostwald viscometer.

Permeation Experiment

Freshly excised cornea was mounted 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 11.4 ml of freshly prepared bicarbonate ringer solution (pH 7.4). The donor sample (1 ml of drug solution) was placed on the cornea. The opening of the donor compartment was sealed with a cover slip and the receptor compartment was maintained at 37°C with constant stirring, using a Teflon-coated

magnetic stir bead. Permeation study was continued for 120 min. The sample was withdrawn from the receptor compartment and analyzed for voriconazole content using a spectrophotometer at 256 nm. Results were expressed as an amount permeated and percentage permeation. The permeation (%) or *in vitro* ocular availability was calculated as follows;

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

At the end of the experiment, the scleral tissue was removed from cornea; its epithelial surface was wiped with filter paper and weighed. The cornea was then soaked in 1 ml methanol, dried overnight at 90°C, and reweighed. From the difference in weight, corneal hydration (%) was calculated.

Antifungal Study

The antifungal activities of all formulations were evaluated against *C. albicans*, *C. glabrata*, *A. flavus* and *F. solani*. The fungal strains were maintained on agar slants (malt yeast agar media for *C. albicans* and *C. glabrata*, malt extract agar media for *A. flavus* and potato sucrose agar media for *F. solani).* Antifungal activity was evaluated by paper disc diffusion method (IP 1985). The medium used for the antifungal activities was same as the maintenance medium of respective fungi. The slant of the microorganism was washed with sterile saline and the cell suspension was further diluted with sterile saline. The cell suspension (0.1 ml) was used to inoculate 100 ml of molten media (sterile). This inoculated medium was poured in 20 ml quantities into 9 cm petridishes (borosil) and the medium was allowed to solidify. Sterile paper discs of 4 mm diameter (made from Whatman No.1 filter paper) were soaked in the eye drop formulation (sterile) and each disc in triplicate was placed in the inoculated media contained in the petridish. Each petridish was incubated at 25°C. As per the specification supplied by MTCC, Chandigarh, the incubation period was 2 days for *C. albicans* and *C. glabrata* and 5 days for *A. flavus* and *F. solani*. After the specified period of incubation, the clear zone of inhibition (ZOI) in each petridish was measured in cm.

RESULTS AND DISCUSSION

Table 1 shows the partition coefficient, viscosity and surface tension of voriconazole drops containing different preservative. Effect of pH on corneal permeation of voriconazole through excised goat corneas is shown in Table 2. Increase the pH of voriconazole formulation from pH 4.0 to 8.0 resulted in significant ($P < 0.05$) increase in permeation of voriconazole, indicating a pH-dependent transport of voriconazole. Corneal permeation depends mainly on the drug's molecular size,[22,23] on its oil/water partition coefficient[22,24-26] and its degree of ionization.[27,28] In addition, unionized form of ionizable acidic or basic compound penetrates corneal epithelium mainly due to its higher lipid solubility.[29,30] Fraction of ionized and un-ionized molecules affect the rate and extent of transcorneal transport, which in turn depends on the pKa of the drug and the pH of the formulation.[31] Voriconazole, being a basic drug, would be in unionized form as the pH of the formulation is shifted toward neutrality resulting in increased permeation. Increased permeation of voriconazole at physiological pH of tears (i.e., pH 7.2) might be because cornea contains both positively and negatively charged groups whose magnitude and polarity depend on the degree of protonation. The average pH of tears is 7.2 and eyes can tolerate pH of 6.5-8.0 without much discomfort.[17] The cornea carries a net negative charge at pH above the isoelectric point (pI = 3.2) and is selectively permeable to cations.[32] Permeation of moxifloxacin[21] across the excised goat, sheep, buffalo corneas and the permeation of levofloxacin across the excised rabbit cornea has also been reported to be pH-dependent.[33]

Table 3 shows the effect of concentration of voriconazole in ophthalmic solution on corneal permeability.

Table 1: Physicochemical properties of voriconazole aqueous drops

Formulations	Partition coefficient*	Viscosity (Cps)	Surface tension (dyne/cm)
Control	13.98±0.18	0.908	67.59
BKC	20.90±1.24	0.895	37.90
EDTA	16.38±0.25	0.916	68.22
PMA	12.82±0.16	0.888	61.47
PMN	12.42±0.15	0.937	72.04
SMS	9.26±0.09	0.942	69.14
THM	6.69±0.38	0.937	54.87
BKC+EDTA	18.13±0.17	0.915	36.61
SMS+EDTA	10.54±0.11	0.931	76.93

*values are mean±SD (n=3), BKC: Benzalkonium chloride, EDTA: Ethylenediaminetetraacetic acid, PMA: Phenyl mercuric acetate, PMN: Phenyl mercuric acetate, SMS: Sodium metabisulfite, THM: Thiomersal

Table 2: Effect of pH on permeation of voriconazole from 0.05% aqueous solution through excised goat cornea

pH	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
4.0	0.039±0.0015	7.44±0.297	83.16±0.224
5.0	0.050±0.0.002*	9.34±0.452	82.65±0.465
6.0	0.052±0.0021*	9.76±0.307	82.45±0.127
7.0	0.056±0.0013*	10.63±0.054	79.89±0.312
8.0	0.066±0.0039*	12.36±0.750	80.28±0.739

Values are mean±SD (n=3), *Statistically significant (P<0.05) compared with formulation (pH 4.0) as determined by one-way ANOVA followed by Dunnett's test

Voriconazole is a lipophilic compound with low aqueous solubility. The aqueous solubility of voriconazole could be enhanced by physical mixing of voriconazole and β-CD in a weight ratio of 1:1 and 1:2. Physical mixture of voriconazole and β-CD (1:2) was selected to prepare 0.2% (w/v) solution at pH 7.2 due to its higher solubility. Increase in drug concentration in the formulation resulted in significant ($P < 0.05$) increase in permeation of voriconazole after 120 min, but decreased the percentage permeation or *in vitro* ocular availability. The cornea has three layers: The epithelium, the stroma and the endothelium. Only the amount of drug needed to saturate the epithelium would be able to partition through the stroma and endothelium to the receptor. As a result, an increase in concentration would have a negative effect on the *in vitro* ocular availability of the drug. Similar findings of reduced *in vitro* ocular availability with an increase in drug concentration have been reported for ibuprofen, flurbiprofen[34] and moxifloxacin.[21]

Inclusion of β-CD enhanced the corneal permeation of voriconazole. CD is reported to enhance drug penetration into the eye by carrying the lipopilic drug molecules through the aqueous mucin layer and thus increasing drug availability at the lipophilic eye surface without disruption of ophthalmic barrier like BKC, a conventional penetration enhancer.[35] CDs have earlier been reported to enhance,[36] diminish[37] and have no effects[38] on the corneal transport of drugs. The result of corneal hydration was more than the normal range of 75% to 80% indicating slight damage to the corneas.[39] Since the corneal hydration is below 83%, the damage appears to be reversible.[40]

The results of corneal permeation of voriconazole from ophthalmic solution (0.05% w/v, pH 7.2) preserved with different preservatives are shown in Table 4. Formulation with EDTA (0.01% w/v) showed significantly ($P < 0.05$) higher permeation than did the control formulation containing no preservatives. EDTA has been reported to increase corneal absorption of various drugs through intact corneas.[32,41,42] EDTA, a known calcium-chelating agent, has been shown to act on cell junctions by interfering with calcium ions and altering intercellular integrity. EDTA also disrupts the plasma membrane and thereby increases intercellular permeability.[43] In addition, the anionic EDTA interacts with cationic voriconazole to form more lipid-soluble ion pair, which may increase the permeation of drug through the cornea. Formulation with BKC, the combination of BKC and EDTA, combination of SMS and EDTA resulted in significant ($P < 0.05$) increase in permeation of

Table 3: Effect of concentration on permeation of voriconazole from aqueous solution through excised goat cornea

Concentration (%w/v)	Amount permeated (mg) (120 min)	Permeation (%) (120 min)	Corneal hydration (%)
0.05	64.01±1.61	12.80±0.322	80.93±0.64
0.1	72.27±2.41*	7.23±0.241	81.69±0.64
0.15	79.66±1.58*	5.31±0.105	80.87±1.02
0.2	95.13±1.69*	4.76±0.084	82.14±1.94

Values are mean±SD (n=3); *Statistically significant (P<0.05) compared with formulation (beta-cyclodextrin, 0.05% w/v) as determined by one-way ANOVA followed by Dunnett's test

Table 4: Effect of preservative on permeation of voriconazole from 0.05% (w/v) aqueous solution (pH 7.2) through excised goat and sheep cornea

Formulations	Amount permeated (mg) (120 min)		Permeation (%) (120 min)		Corneal hydration (%)	
	Goat	Sheep	Goat	Sheep	Goat	Sheep
Control	0.0584±0.0016	0.0536±0.0013	10.97±0.303	10.09±0.249	77.97±0.247	78.32±0.125
BKC	0.0633±0.0016*	0.0596±0.0005†	11.90±0.297	11.20±0.099	81.27±0.748	80.67±0.169
EDTA	0.0698±0.0013*	0.0661±0.0012†	13.12±0.250	12.43±0.229	82.34±0.622	79.46±0.225
PMA	0.0552±0.0016	0.0517±0.0019	10.38±0.303	9.72±0.358	80.51±0.773	79.36±0.412
PMN	0.0508±0.0040*	0.0499±0.0018	9.55±0.757	9.39±0.348	79.37±0.372	78.96±0.314
SMS	0.0564±0.0010	0.0520±0.0032	10.60±0.198	9.78±0.597	76.50±0.769	77.19±0.265
THM	0.0594±0.0008	0.0550±0.0017	11.17±0.151	10.34±0.318	81.09±0.312	80.36±0.133
BKC+EDTA	0.0675±0.0005*	0.0638±0.0013†	12.69±0.099	11.99±0.262	82.41±0.473	81.59±0.165
SMS+EDTA	0.0635±0.0022*	0.0531±0.0035†	11.93±0.413	9.98±0.660	81.22±0.476	79.87±0.691

Values are mean±SD (n=3); *Statistically significant (P<0.05) compared with control (goat cornea) as determined by one-way ANOVA followed by Dunnett's test. †Statistically significant (P<0.05) compared with control (sheep cornea) as determined by one-way ANOVA followed by Dunnett's test. BKC: Benzalkonium chloride, EDTA: Ethylenediaminetetraacetic acid, PMA: Phenyl mercuric acetate, PMN: Phenyl mercuric acetate, SMS: Sodium metabisulfite, THM: Thiomersal

Table 5: Effect of viscolizing agents on permeation of voriconazole from 0.2% (w/v) aqueous solution (pH 7.2) through excised goat cornea

Formulations (%)	Viscosity (Cps)	Amount permeated (mg) (120 min)*	Permeation (%) (120 min)*	Corneal hydration* (%)
Control	1.111	0.094±0.003	4.78±0.015	82.14±1.94
MC (0.5)	4.308	0.052±0.003†	2.63±0.108	80.41±1.08
HPMC (1)	2.406	0.085±0.002	4.38±0.108	80.61±0.47
PVP (1)	1.201	0.088±0.001	4.47±0.107	78.97±0.73
PVA (1.4)	3.327	0.062±0.002†	3.17±0.108	75.81±0.66

*Values are mean±SD (n=3), †Statistically significant (P<0.05) compared with control (beta-cyclodextrin formulation) as determined by one-way ANOVA followed by Dunnett's test. MC: Methylcellulose, HPMC: Hydroxyl propyl methylcellulose, PVP: Polyvinyl pyrrolidone, PVA: Polyvinyl alcohol

voriconazole than control formulation. Formulation with PMN produced significantly (P < 0.05) lower permeation of voriconazole than did the control formulation. BKC, a cationic surfactant has been reported to increase the corneal permeation of drugs by emulsification and disruption of the corneal epithelium.[44] Combination of BKC and EDTA has been observed to increase the corneal permeation of fluoroquinolones like moxifloxacin[21] and gatifloxacin.[45] The addition of BKC in the formulation reduced surface tension of voriconazole drop from 67.59 to 37.90 dynes/cm. Partitioning experiment indicated partition coefficient of voriconazole in n-octanol/voriconazole drop with EDTA or BKC was higher than the control formulation containing no preservative. Thus, EDTA or BKC increased the partitioning of voriconazole in the lipid phase. The result of corneal hydration showed higher than the normal range in the formulation with BKC (80.67%) and with the combination of BKC and EDTA (81.59%) indicating slight corneal damage.

The effect of viscosity modifier on the transcorneal permeation of the drug evaluated using excised goat corneas are shown in Table 5. Viscosity modifiers are used in eye drops to prolong the precorneal residence of drugs. Formulation with MC and with PVA resulted in significant (P < 0.05) decrease in permeation of voriconazole than control formulation containing no viscosity modifier.

The antifungal study of the eye drops against *C. albicans*, *C. glabrata*, *A. flavus* and *F. solani* was evaluated. The diameters of clear ZOI are shown in Figures 1-3. Most of the formulations showed higher ZOI against *C. albicans*.

CONCLUSIONS

Based on the present study it can be concluded that voriconazole 0.05% (w/v) ophthalmic solution (pH 7.2) containing EDTA (0.01% w/v), BKC (0.01% w/v) and a combination of BKC and EDTA (each 0.01% w/v) provides significantly (P < 0.05) higher permeation of voriconazole than did the control formulation. Corneal transport of

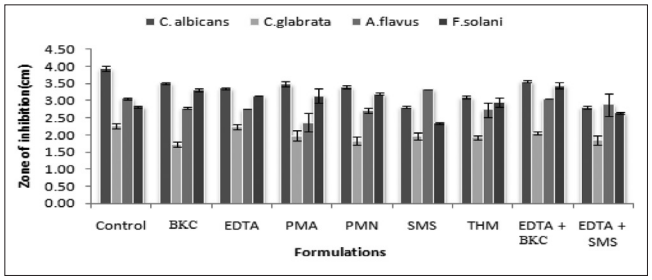

Figure 1: Comparison of the diameter of zone of inhibition (cm) of voriconazole drops (0.05% w/v) against *Candida albicans*, *Candida glabrata*, *Aspergillus flavus* and *Fusarium solani*

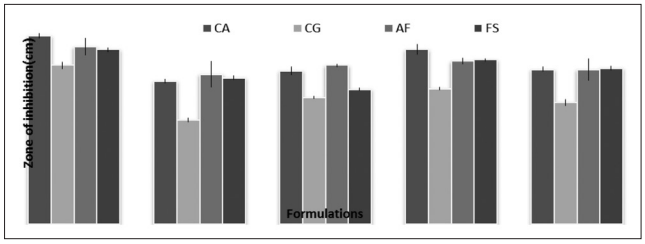

Figure 2: Comparison of the diameter of zone of inhibition (cm) of voriconazole drops (0.2% w/v) against *Candida albicans*, *Candida glabrata*, *Aspergillus flavus* and *Fusarium solani*

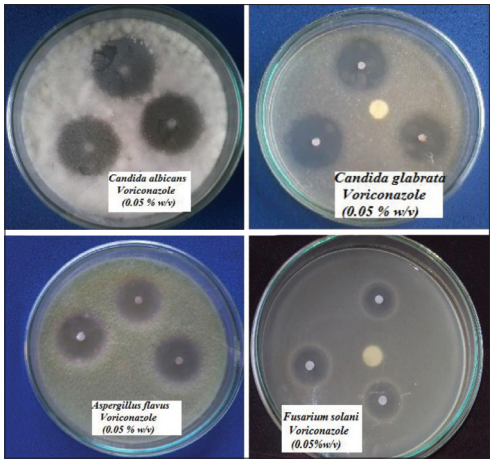

Figure 3: Photographs of zone of inhibition of voriconazole drops (0.05% w/v) against *Candida albicans*, *Candida glabrata*, *Aspergillus flavus* and *Fusarium solani*

voriconazole is both pH and concentration dependent. Most of the formulations showed higher ZOI against *C. albicans*.

ACKNOWLEDGMENT

Authors are thankful to Matrix laboratories (Hyderabad, India) for a gift sample of voriconazole.

REFERENCES

1. Johns KJ, O'Day DM. Pharmacologic management of keratomycoses. Surv Ophthalmol 1988; 33:178-88.
2. O'Day DM. Selection of appropriate antifungal therapy. Cornea 1987;6:238-45.
3. Jones DB. Decision-making in the management of microbial keratitis. Ophthalmology 1981;88:814-20.
4. Forster RK. Fungal diseases. In: Smolin G, Thoft RA, editors. The Cornea: Scientific Foundation and Clinical Practice. Boston: Little Brown & Co. 1987. p. 228-40.
5. Richardson K, Bell A, Dickinson R, Naryanaswami S, Ray S. UK-109, 496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: synthesis and SAR (abstract F69). Presented at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, September 17-20,1995.
6. Hariprasad SM, Mieler WF, Lin TK, Sponsel WE, Graybill JR. Voriconazole in the treatment of fungal eye infections: A review of current literature. Br J Ophthalmol 2008; 92:871-8.
7. Jurkunas UV, Langston DP, Colby K. Use of voriconazole in the treatment of fungal keratitis. Int Ophthalmol Clin 2007; 47:47-59.
8. Breit SM, Hariprasad SM, Mieler WF, Shah GK, Mills MD, Grand MG. Management of endogenous fungal endophthalmitis with voriconazole and caspofungin. Am J Ophthalmol 2005;139:135; 139:135-40.
9. Gao H, Pennesi M, Shah K, Qiao X, Hariprasad SM, Mieler WF, *et al*. Safety of intravitreal voriconazole: Electroretinographic and histopathologic studies. Trans Am Ophthalmol Soc 2003;101:183-9.
10. Bhartiya P, Daniell M, Constantinou M, Islam FM, Taylor HR. Fungal keratitis in Melbourne. Clin Experiment Ophthalmol 2007;35:124-30.
11. Vfend [Package insert]. New York, NY: Pfizer Inc.; March 2008.
12. Stewart A, Powles R, Hewetson M, Antrum J, Richardson C, Mehta J. Costs of antifungal prophylaxis after bone marrow transplantation. A model comparing oral fluconazole, liposomal amphotericin and oral polyenes as prophylaxis against oropharyngeal infections. Pharmacoeconomics 1995;8:350-61.
13. Sponsel W, Chen N, Dang D, Paris G, Graybill J, Najvar LK, *et al*. Topical voriconazole as a novel treatment for fungal keratitis. Antimicrob Agents Chemother 2006;50:262-8.
14. Clode AB, Davis JL, Salmon J, Michau TM, Gilger BC. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. Am J Vet Res 2006;67:296-301.
15. Davies NM. Biopharmaceutical considerations in topical ocular drug delivery. Clin Exp Pharmacol Physiol 2000;27:558-62.
16. Kumar D, Jain N, Gulati N, Nagaich U. Nanoparticles laden *in situ* gelling system for ocular drug targeting. J Adv Pharm Technol Res 2013;4:9-17.
17. Smolen VF, Bull L, editors. The control of drug bioavailability from ophthalmic dosage forms. In: Bioavailability Control by Drug Delivery System Design. Vol. 3. New York: John Wiley & Sons; 1985. p. 257-306.
18. Malhotra M, Majumdar DK. Permeation through cornea. Indian J Exp Biol 2001;39:11-24.
19. Malhotra M, Majumdar DK. *In vitro* transcorneal permeation of ketorolac tromethamine from buffered and unbuffered aqueous ocular drops. Indian J Exp Biol 1997;35:941-7.
20. Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instrum 1965;4:117-22.
21. Pawar PK, Majumdar DK. Effect of formulation factors on *in vitro* permeation of moxifloxacin from aqueous drops through excised goat, sheep, and buffalo corneas. AAPS Pharm Sci Tech 2006;7:E13.
22. Grass GM, Robinson JR. Mechanisms of corneal drug penetration. I: *In vivo* and *in vitro* kinetics. J Pharm Sci 1988;77:3-14.
23. Ahmed I, Gokhale RD, Shah MV, Patton TF. Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea. J Pharm Sci 1987;76:583-6.
24. Flynn GL, Yalkowsky SH, Roseman TJ. Mass transport phenomena and models: Theoretical concepts. J Pharm Sci 1974;63:479-510.
25. Grass GM, Robinson JR. Relationship of chemical structure to corneal penetration and influence of low-viscosity solution on ocular bioavailability. J Pharm Sci 1984;73:1021-7.
26. Yoshida F, Topliss JG. Unified model for the corneal permeability of related and diverse compounds with respect to their physicochemical properties. J Pharm Sci 1996;85:819-23.
27. Edward A, Prausnitz MR. Predicted permeability of the cornea to topical drugs. Pharm Res 2001;18:1497-508.
28. Huang HS, Schoenwald RD, Lach JL. Corneal penetration behavior of beta-blocking agents II: Assessment of barrier contributions. J Pharm Sci 1983;72:1272-9.
29. Benson H. Permeability of the cornea to topically applied drugs. Arch Ophthalmol 1974;91:313-27.
30. Mitra AK, Mikkelson TJ. Mechanism of transcorneal permeation of pilocarpine. J Pharm Sci 1988;77:771-5.
31. Goskonda VR, Khan MA, Hutak CM, Reddy IK. Permeability characteristics of novel mydriatic agents using an *in vitro* cell culture model that utilizes SIRC rabbit corneal cells. J Pharm Sci 1999;88:180-4.
32. Rojanasakul Y, Robinson JR. Transport mechanisms of the cornea: Characterization of barrier permselectivity. Int J Pharm 1989;55:237-46.
33. Kawazu K, Midori Y, Shiono H, Ota A. Characterization of the carrier-mediated transport of levofloxacin, a fluoroquinolone antimicrobial agent, in rabbit cornea. J Pharm Pharmacol 1999;51:797-801.
34. Gupta M, Majumdar DK. Effect of concentration, pH, and preservative on *in vitro* transcorneal permeation of ibuprofen and flurbiprofen from non-buffered aqueous drops. Indian J Exp Biol 1997;35:844-9.
35. Loftsson T, Masson M. Cyclodextrins in topical drug formulations: Theory and practice. Int J Pharm 2001;225:15-30.
36. Usayapant A, Karara AH, Narurkar MM. Effect of 2-hydroxypropyl-beta-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. Pharm Res 1991;8:1495-9.
37. Davies NM, Wavy G, Tucker IG. Evaluation of hydrocortisone/hydroxypropyl-β-cyclodextrin solution for ocular drug delivery. Int J Pharm 1997;56:201-9.
38. Ahuja M, Singh G, Majumdar DK. Effect of formulation parameters on corneal permeability of ofloxacin. Sci Pharm 2008;76:505-14.
39. Maurice DM, Riley MV. Ocular pharmacokinetics. In: Graymore CN, editor. Biochemistry of the Eye. London, UK: Academic Press; 1970. p. 1-103.
40. Schoenwald RD, Huang HS. Corneal penetration behavior of beta-blocking agents I: Physiochemical factors. J Pharm Sci 1983;72:1266-72.

41. Ashton P, Diepold R, Platzer A, Lee VH. The effect of chlorhexidine acetate on the corneal penetration of sorbitol from an arnolol formulation in the albino rabbit. J Ocul Pharmacol 1990;6:37-42.
42. Rojanasakul Y, Liaw J, Robinson JR. Mechanism of action of some penetration enhancers in the cornea: Laser scanning confocal microscopic and electrophysiology studies. Int J Pharm 1990;66:131-42.
43. Grass GM, Wood RW, Robinson JR. Effects of calcium chelating agents on corneal permeability. Invest Ophthalmol Vis Sci 1985;26:110-3.
44. Fu RC, Lidgate DM. *In vitro* rabbit corneal permeability study of ketorolac tromethamine, a non-steroidal anti-inflammatory agent. Drug Dev Ind Pharm 1986;12:2403-30.
45. Rathore MS, Majumdar DK. Effect of formulation factors on *in vitro* transcorneal permeation of gatifloxacin from aqueous drops. AAPS Pharm Sci Tech 2006;7:57.

How to cite this article: Mohanty B, Mishra SK, Majumdar DK. Effect of formulation factors on *in vitro* transcorneal permeation of voriconazole from aqueous drops. J Adv Pharm Technol Res 2013;4:210-6.

Source of Support: Nil, **Conflict of Interest:** Nil.