ORIGINAL ARTICLE

Effect of Formulation Factors on Transcorneal Permeation of Acetazolamide from Aqueous Drops: *In vitro* and *In vivo* Study

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Abstract

Aim: The purpose of this research was to evaluate the effect the formulation factors on *in vitro* permeation of acetazolamide (ACZ) through freshly isolated goat corneas. **Materials and Methods:** Different formulation factors were studied. The concentration of drug was raised from 0.025% to 0.1% (w/v) and pH was also varied within the range of 6-8. Several viscosity modifiers and preservatives were also added to study their effect on permeation. Finally, the formulation with the optimized factors was used to study ocular hypotensive activity of the drug in rabbits. **Results and Discussion:** Raising concentration of the drops from 0.025% to 0.1% (w/v) significantly ($P < 0.05$), increased drug permeation, but decreased the percent permeation. An increase in the pH of the drops from 6.0 to 8.0 resulted in significant ($P < 0.05$) change in drug permeation. Eye drops containing benzalkonium chloride (BKC) showed significantly ($P < 0.05$) higher permeation as compared with control formulation. Compared with control formulation, ACZ 0.1% (w/v) drop containing viscosity modifier produced significant ($P < 0.05$) decrease in permeation. A formulation having BKC showed increased hypotensive activity as compared to the control formulation. **Conclusion:** The study reflects that the permeation of ACZ is greatly influenced by formulation factors and permeation was found to be concentration and pH dependent.

Key words: Acetazolamide, cornea, formulation, hypotensive, permeation

INTRODUCTION

Eye is one of the vital and complicated organs of human body. Being a complex organ, it might get affected by several disorders. Glaucoma is a disorder of eye and is characterized by increased intra ocular pressure (IOP) and optic nerve damage which may cause permanent blindness if left untreated. Increase in IOP >21 mmHg is threatening[1,2] and may be termed as ocular hypertension. Several categories of drug are being used for the treatment of ocular hypertension and ultimately Glaucoma, all alone or as an adjunct with a number of drugs. Carbonic anhydrase inhibitors (CAIs) are one such category. Among the available CAIs, acetazolamide (ACZ) is one of the most effective drugs for the treatment of glaucoma. To obtain the desired lowering in IOP, large oral doses of ACZ are used, and this usually leads to numerous systemic side effects, the most common of which are diuresis and metabolic acidosis. As a result, topical administration of ACZ is usually preferred over systemic administration.[3]

Designing an ophthalmic delivery system is one of the most challenging tasks for the researchers[4] as there are several barriers, e.g., low residence period, lachrymal drainage, etc., which pose different challenges. 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.

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

MATERIALS AND METHODS

Materials

ACZ was purchased from Sigma-Aldrich. Preservatives were received from Central Drug House (New Delhi, India). All other chemicals used were of analytical reagent grade. Fresh whole eyeballs of goat were obtained from butcher's shop (Ambedkar Nagar, New Delhi, India) within 1 h of slaughtering of the animal. The method of dissection of cornea and the apparatus used in permeation studies were the same as published elsewhere.[5] Animals for the ocular hypotensive activity were obtained from the DIPSAR animal house after approval from Institutional Animal Ethical Committee (IAEC).

Methods

ACZ ophthalmic solutions (pH 7.2) of increasing concentration

Required amount of ACZ was dissolved in sufficient distilled water; sodium chloride was added to make the final solution isotonic; and pH of the solution was adjusted to 7.2 using 0.1 N HCl or 0.1 N NaOH and final volume were made up to 100 ml with distilled water, to have solutions of 0.025, 0.05 and 0.1% (w/v) concentrations.

Preparation of test solutions ACZ ophthalmic solutions (0.1% w/v) of different pH

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

ACZ ophthalmic solutions (0.1% w/v, pH 7.2) containing preservatives

ACZ (0.1 g) was dissolved in sufficient distilled water; sodium chloride 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), phenylmercuric acetate (PMA, 0.002% w/v), phenylmercuric nitrate (0.002% w/v), and ethylene diamine tetra acetic acid disodium salt (0.01% w/v). The final volume of each solution was made up to 100 ml with distilled water.

Ophthalmic solutions (0.1% w/v, pH 7.2) containing viscosity modifier

Required amount of ACZ was dissolved in sufficient distilled water; sodium chloride (0.9 g/100 ml) was added to make the final solution isotonic; and pH of the solution was adjusted to 7.2 using 0.1 N HCl and 0.1N NaOH. To this solution methylcellulose (MC) (0.25%, w/v), hydroxy propyl methyl cellulose or HPMC (0.25%, w/v), polyvinyl alcohol (1.4%, w/v), or polyvinylpyrrolidone (1%, w/v) were added and the final volume of each solution was made up to 100 ml with distilled water.

Measurement of surface tension and viscosity

The surface tension of each ophthalmic solution (0.1% w/v) containing preservatives was measured using a stalagmometer and the viscosity of each ophthalmic solution (0.1% w/v) containing viscosity modifiers 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 receptor compartment was filled with 10 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 bead. Permeation study was continued for 120 min. The sample was withdrawn from the receptor compartment and analyzed for ACZ content by in house developed and validated HPLC method at 265 nm. For the analysis of ACZ by HPLC, C8H column (250 mm × 4.6 mm) was used as stationary phase and potassium dihydrogen phosphate buffer (pH 3), Acetonitrile and water in a ratio 30:20:50 as mobile phase with a flow rate of 0.8 ml/min, injection volume of 20 µL, run time of 10 min and optimized real-time of 6.8 [Figure 1]. The developed method was having limit of detection of 37.60 ng/ml and limit of quantitation of 0.11396 µg/ml.[6]

Results were expressed as an amount permeated and percentage permeation. The permeation (%) or *in vitro* ocular availability was calculated as follows:

$$\% \text{ Corneal permeation} = \frac{\begin{matrix}\text{Amount of drug}\\ \text{permeated in}\\ \text{receptor chamber}\end{matrix}}{\begin{matrix}\text{Initial amount of}\\ \text{drug in donor}\\ \text{compartment}\end{matrix}} \times 100 \quad (1)$$

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.

$$\% \text{ Corneal hydration} = \frac{\text{Weight of moist cornea} - \text{weight of dried cornea}}{\text{Weight of moist cornea}} \times 100 \quad (2)$$

Ocular hypotensive efficacy

The normotensive rabbits were used to compare the ocular hypotensive activity of ACZ-BKC solution with the aqueous solution containing no preservative. The experimental protocol was designed and approval of IAEC was obtained. A group of three animals weighing 2-2.5 kg was used for each study. Animals were housed in Institutional Animal House under standard conditions with free access to food and water. Each rabbit of Group I served as the control and received 50 µL of the normal saline (0.9% w/v) vehicle while the Group II and Group III received 50 µL of ACZ (0.1%, w/v) ophthalmic solution in normal saline or 50 µL of ACZ-BKC solution, respectively. Schiotz tonometer was used to measure the IOP in conscious rabbits. A volume of 50 µL of a solution of lignocaine HCl (2%) was used as a local anesthetic. The resting IOP level was measured in all the animals before drug administration. The dose of ACZ administered was 1 mg in the both solutions. A single 50 µL drop was instilled into the experimental eye at time 0, and the IOP was measured at 0.5, 1, 2, 3, 4 and 5 h after drug administration. The change in IOP (ΔIPO) was determined by the following equation:[7,8]

$$\Delta IPO = IOP_{\text{Dosed eye}} - IOP_{\text{Control eye}} \quad (3)$$

Statistical analysis

The statistical calculations were done by One way analysis of Variance (ANOVA) followed by Dunnett's test or Student's *t*-test using GraphPad Prism 5 software (Graph Pad Software Inc., San Diego, CA, USA). A $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effect of concentration

The results of permeation studies through goat cornea showed that an increase in drug concentration in the drops resulted in an increase in amount permeated after 120 minutes, but the percent permeation and *in vitro* ocular availability decreased [Figure 2]. The cornea has 3 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. Thus, 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,

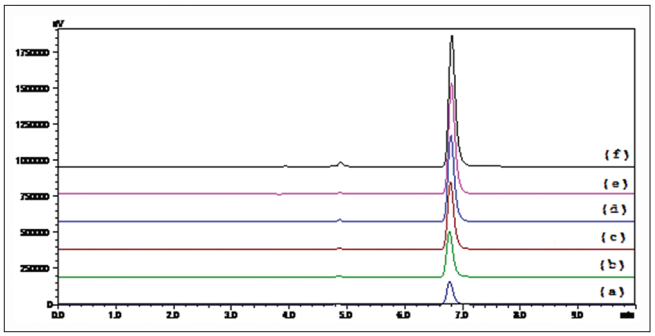

Figure 1: Overlay chromatogram of acetazolamide (a) 20 µg/ml (b) 40 µg/ml (c) 60 µg/ml (d) 80 µg/ml (e) 100 µg/ml (f) 120 µg/ml

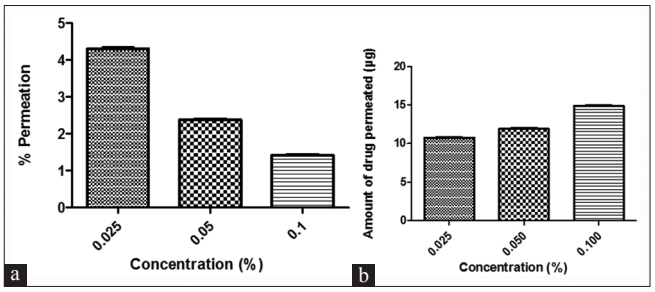

Figure 2: (a) Change in transcorneal permeation on changing drug concentration from 0.025 to 0.1%. (b) Amount of drug permeated on changing drug concentration from 0.025 to 0.1%

flurbiprofen[9,10] and moxifloxacin.[11] An increase in drug concentration did not affect the corneal hydration; however, it remained in the normal range of 75-80%. An increase in ACZ concentration from 0.025% to 0.1% (w/v) has been found to increase the amount permeated through excised goat cornea from 10.76 µg to 15 µg after 120 min, whereas % transcorneal permeation was decreased from 4.30% to 1.39% [Table 1].

Effect of pH

Fraction of ionized and unionized molecules affects the rate and extent of transcorneal transport, which in turn depends on the pKa of the drug and the pH of the formulation.[12] Table 2 shows the effect of pH of the formulation on permeation of ACZ through excised corneas. The increase in pH of the solution from 6 to 8 decreased drug permeation, indicating a pH-dependent transport of ACZ. ACZ, being weak acid, is having a pKa of 7.2[13] and when pH will be increased more fraction of the drug will get ionized, as per the Henderson – Hasselbach equation, resulting in decrease in transcorneal permeation [Table 2 and Figure 3].

Effect of preservatives

Eye drops are generally dispensed in multidose vials, each of which is intended to be used within a few days, once it

Table 1: Effect of concentration on transcorneal permeation

S.NO.	Concentration (%)	Amount of drug permeated (µg) ± SD *(n=3)*	% Permeation±SD *(n=3)*	% Corneal hydration±SD *(n=3)*
1	0.025	10.76±0.08	4.30±0.03	79.45±0.48
2	0.05	11.85±0.08*	2.38±0.03*	79.15±0.96
3	0.1	15.00±0.16*	1.39±0.01*	79.61±1.63

*Statistically significant *(p<0.05)* compared with 0.025% drug solution

Table 2: Effect of pH on transcorneal permeation

S.NO.	pH	Amount of drug permeated (µg) ± SD *(n=3)*	% Permeation±SD *(n=3)*	% Corneal Hydration±SD *(n=3)*
1	6	14.95±0.14	1.49±0.01	78.15±0.90
2	7.2	13.90±0.14	1.39±0.01*	79.61±1.63
3	8	11.52±0.19	1.15±0.01*	79.89±1.33

*Statistically significant *(p<0.05)* compared with pH 6 drug solution

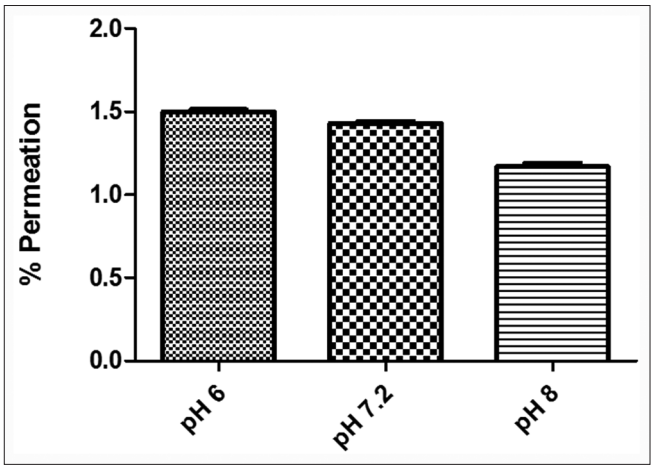

Figure 3: Effect change in pH (6-8) on transcorneal permeation

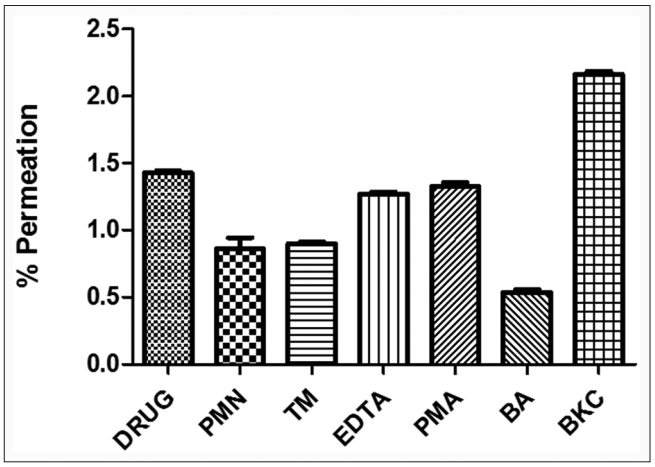

Figure 4: Effect of different preservatives on transcorneal permeation of acetazolamide

is opened. Thus, to maintain the sterility of the preparation during use, a preservative is generally added to the formulation. Some common ophthalmic preservatives in their conventional concentrations were used in making the ACZ 0.1% weight/volume or w/v formulation. The effect of preservatives on the transcorneal permeation of the drug was evaluated using excised goat cornea. The results are shown in Table 3. Formulation with BKC (0.01% w/v) showed significantly ($P < 0.05$) higher permeation than did the control formulation with no preservative [Figure 4]. Formulation with PMA (0.002% w/v), THM (0.005% w/v), and BA (0.5% v/v) produced lower permeation of drug than did the control formulation containing no preservative. The addition of BKC (a cationic surfactant) reduced the surface tension of the ACZ drop from 68.38 to 36.48 dynes/cm. BKC, a cationic surfactant has also been reported to increase the corneal permeation of drugs by emulsification and disruption of the corneal epithelium.[14]

Effect of viscosity modifiers

The effect of viscosity modifier on the transcorneal permeation of the drug evaluated using excised goat corneas are shown in Table 4 and Figure 5. Viscosity modifiers are used in eye drops to prolong the precorneal residence of drugs. The addition of different viscosity modifiers resulted in significant increase in the viscosity of the formulation and significant ($P < 0.05$) decrease in permeation of ACZ than control formulation containing no viscosity modifier.[15]

In vivo ocular hypotensive activity

In the case of plain ACZ solution the IOP was decreased for a period of 2 h and then started rising and same was observed in the case of BKC-Drug Solution but the intensity of decrease in IOP was more as compared to drug solution.

Table 3: Effect of preservatives on transcorneal permeation

S. No.	Preservative	Concentration of preservative (%)	Surface tension±SD *(n=3)*	Amount of drug permeated (µg) ± SD *(n=3)*	% permeation±SD *(n=3)*	% corneal hydration±SD *(n=3)*
1	None	--	68.38±2.47	13.90±0.14	1.39±0.01	79.61±1.63
2	PMN	0.002	51.72±3.28	9.57±0.82	0.95±0.08*	77.68±2.28
3	TM	0.005	62.31±2.00	9.14±0.14	0.91±0.01*	76.75±1.34
4	EDTA	0.01	63.36±2.08	12.57±0.14	1.25±0.01*	79.42±2.99
5	PMA	0.002	68.32±1.61	13.00±0.28	1.30±0.02*	75.72±3.42
6	BA	0.05	66.10±4.08	5.57±0.21	0.55±0.02*	77.49±0.47
7	BKC	0.002	36.48±1.38	21.85±0.21	2.18±0.02*	77.46±1.48

*Statistically significant *(p<0.05)* compared with drug solution containing no preservative

Table 4: Effect of viscosity modifiers on transcorneal permeation

S.NO.	Viscosity enhancer	Concentration of viscosity enhancer (%)	Viscosity±SD *(n=3)*	Amount of drug permeated±SD *(n=3)*	% permeation±SD *(n=3)*	% corneal hydration±SD *(n=3)*
1	None	--	0.92±0.06	13.90±0.14	1.39±0.01	79.61±1.63
2	HPMC	0.25	1.04±0.00*	6.52±0.14	0.65±0.01*	76.99±2.05
3	PVP	1	1.08±0.01*	12.61±0.21	1.26±0.02*	78.12±1.55
4	PVA	1.4	3.57±0.04*	10.90±0.21	1.09±0.02*	77.56±0.97
5	MC	0.25	1.96±0.01*	7.76±0.42	0.77±0.04*	77.81±1.08

*Statistically significant *(p<0.05)* compared with drug solution containing no viscosity modifier

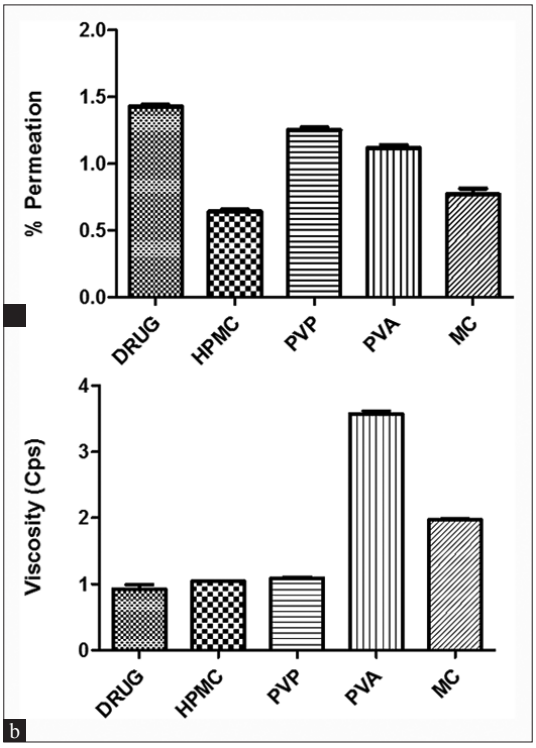

Figure 5: (a) Effect of different viscosity modifiers on transcorneal permeation of acetazolamide. (b) Amount of drug permeated on using different viscosity modifiers

The observation suggested that the hypotensive activity of BKC-drug solution was comparable to that of the plain drug solution, however, the decrease in IOP is not significant ($P > 0.05$) [Figure 6].

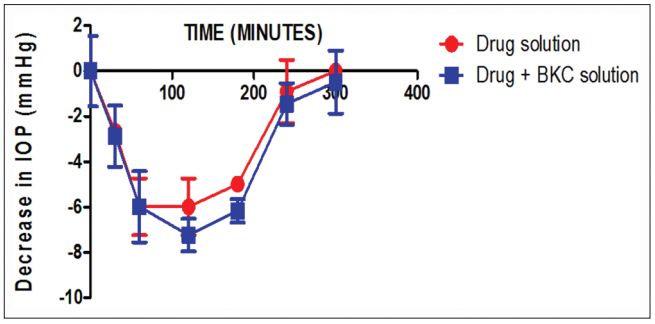

Figure 6: Ocular hypotensive activity of acetazolamide from plain drug solution and drug solution containing preservative

CONCLUSION

It can be concluded from the present studies that increase in the concentration of ACZ in aqueous drop causes a decrease in % permeation but at the same time increase the amount of drug permeated. On the other hand moving toward alkaline pH from pH of 6, % transcorneal permeation also got decreased. Corneal transport of ACZ is concentration and pH dependent. ACZ, 0.1% w/v aqueous drop containing BKC (0.01% w/v) provides maximum *in vitro* ocular availability and increased *in vivo* ocular hypotensive activity.

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