Glaucoma, a disease of the eye, is the third-most prevalent cause of visual impairment and blindness in the United States, after cataracts and macular degeneration from aging. Glaucoma is a progressive disease that causes optic-nerve head damage. Open-angle glaucoma affects ~0.5% of the total US population but affects a much higher percentage (about 3%) in people aged 70 and older (1,2).

Glaucoma is characterized chiefly by an increase in intraocular pressure (IOP) that, if sufficiently high and persistent, can damage the optic disk at the juncture of the optic nerve and retina, which can lead to irreversible blindness (3). IOP is the only undisputed risk factor in glaucoma that can be identified and treated. The goal of the treatment is to lower IOP to a level at which damage of the optic nerve ceases to progress (4). Other risk factors of glaucoma are diabetes, cardiovascular diseases, and myopia (5).

Glaucoma can be classified according to the reason for poor aqueous outflow such as open-angle, closed-angle, and congenital glaucoma. Each of these categories is further subdivided as primary and secondary types. Primary open-angle glaucoma (POAG) is the most common type with ~90% of glaucoma patients suffering from it. POAG is insidious in onset and shows a slow, progressive pathology unlike closed-angle glaucoma in which the appearance of symptoms is rapid and dramatic.

Treatment
The treatment of glaucoma is generally focused on lowering IOP. Three methods of lowering IOP exist, namely medications (both topical and oral), laser therapy, and surgery (6). Both laser and surgical methods require expertise and also are very expensive; hence, they are last lines of treatment, which may be followed by medication. The first line of treatment is always medication, which may be administered topically or systemically. Commonly used medications and their possible side effects are listed in Table I (7–10).

Carbonic anhydrase inhibitors (CAIs), especially acetazolamide, are a potent class of ocular hypotensive agents because they are specific and reduce IOP by inhibiting the carbonic anhydrase (CA) enzyme. The inhibition of this enzyme leads to a direct decrease in aqueous humor production and therefore IOP. In spite of this, the use of CAIs is limited because they are administered orally, and because a large distribution of CA enzyme

Acetazolamide has been a highly effective drug for the treatment of glaucoma for many years. However, its use has been limited because of its low solubility and poor permeability characteristics that cause it to be unsuitable for topical administration. Its current use as an oral dosage form has been associated with a large number of side effects. Therefore, the present need is to develop a topical acetazolamide formulation that would cause a minimum number of side effects. This article reviews how the problems encountered in developing desirable topical acetazolamide formulations have been approached with the use of various techniques.

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Acetazolamide is a potent and reversible CAI, is effective in the production of the aqueous humor by the ciliary processes but is also involved in the transport of CO₂ from the tissues to the lung, in the excretion and reabsorption of electrolytes and H⁺ ions in the kidney, in the secretion of the H⁺ ions in the gastric mucosa, and in the maintenance of the major buffer system of the human body. The most common side effects are diuresis and metabolic as well as respiratory acidosis. Other side effects of acetazolamide include gastrointestinal upset, lassitude, paraesthesia, anorexia, weight loss, malaise, depression, altered taste, decreased libido, blood dyscrasias, urolithiasis, and rare complications such as renal stones and death.

Various studies confirmed the ability of topically applied acetazolamide to lessen the ocular hypertensive response observed in water loaded rabbits. A topical acetazolamide formulation possessing similar efficacy to the oral formulation would be a significant advance in the treatment of glaucoma. A topical formulation of acetazolamide, when compared with a systemic delivery, can offer the following advantages:

- reduction in dose
- faster onset of action
- marked decrease in side effects
- increase in patient compliance.

Despite these advantages, some practical problems associated with topical formulations of acetazolamide do exist and are discussed in the following paragraphs.
**Insolubility in water and aqueous fluids.** Acetazolamide is slightly soluble in water at room temperature and sparingly soluble in almost-boiling water. The solubility of the drug in water is \(\sim 0.72 \text{ mg/mL}\). The pH-solubility profile indicates that the solubility is higher on the basic side because of sodium salt formation (solubility ranges from 0.8 to 2.8 mg/mL between pH values of 1.7 and 8.2) (38). However, degradation increases by many folds on the basic side. Hence, the solubility of acetazolamide cannot be increased by increasing the pH of the solution because the pH of tear fluid is \(\sim 7.4\) (13).

**Limited ocular penetration.** The penetrability coefficient of acetazolamide is very low (4.1 \(\times\) 10\(^{-6}\) cm/s). Therefore, limited ocular penetration of the drug limits its ocular bioavailability through a topical route (39). The topical formulation is highly favorable for ophthalmic preparation, but it has not been possible to successfully formulate a topically effective preparation of acetazolamide because of the previously stated reasons. To develop an ideal topical ophthalmic delivery system of acetazolamide, these problems must be overcome.

**Approaches to developing a desirable topical formulation**

Topically effective CAIs offer a potential treatment for glaucoma without the undesirable side effects associated with systemic drug administration. In earlier studies, topical administration of acetazolamide was ineffective in lowering IOP in animals used for experimentation. Other commercially available CAIs such as methazolamide and ethoxazolamide were equally ineffective when administered topically; therefore, no further research was conducted in this area for more than two decades (40–42). In the 1980s, however, interest was renewed, and it was concluded that the previous failure to develop a topical formulation might have been a result of poor penetration of the drug moiety into the cornea (39). Hence, during the past few years, the approach has been directed toward improving both penetration and aqueous solubility of the drug to improve its therapeutic effectiveness by means of the topical route. Several attempts have been made to formulate topically active acetazolamide to minimize its systemic side effects. These include the use of a higher concentration of the drug, multiple dosing, a salt or modified form of the drug, or various drug delivery systems such as impregnated contact lenses, gels, cycloexitrimis, liposomes, and increased viscosity of the vehicle.

**Higher concentration of the drug.** Corneal penetration enhancement can be achieved by increasing the concentration of a drug in a solution, thereby resulting in an improved therapy. In a study conducted by Flach et al., topical application of acetazolamide (10% w/v solution, pH 9.5) led to a decrease in IOP (1–3 mm Hg) in water loaded pigmented rabbits and correlation with low plasma acetazolamide concentration (from 0 to 0.7 µg/mL) (43). In another study they observed a significant reduction in IOP (6–8 mm Hg) after a topical application of the solution (10% w/v, pH 9.15) and intravenous administration of 25 mg/mL of the drug. They have also shown that pigmented rabbits may be more suitable for testing sympathomimetic and sympatholytic drugs for antiglaucomatous activity (44). Although the solubility of acetazolamide is higher at basic pH because of sodium salt formation, leading to small scale in IOP, its degradation also increases many fold. Therefore, increase in pH to increase drug solubility is not a solution. Also, an increase in concentration can result in a hypertonic solution that causes discomfort and may accelerate the drainage rate by increasing lacrimation and thereby reducing absorption. It was concluded that perhaps a low or intermittent oral dose of acetazolamide supplemented with a topical application may be more efficacious.

**Modified form of the drug.** Thieno-(2,3-B)-thiopyran-2-sulfonamide derivative MK-927 has shown prolonged reduction of IOP as a monotherapy or as an add-on therapy to timolol in patients with glaucoma or ocular hypertension. No clinically significant systemic–ocular side effects were encountered. Sezolamide, which is an S-enantiomer of racemic mixture MK-927 and coded as MK-417, was developed later (45–47). Both MK-927 and MK-417 were assessed as safe and effectively lowered IOP in humans (48). Furthermore, structure activity relationship (SAR) studies resulted in the development of MK-507 (dorzolamide) with improved spectrum of activity and increased water solubility. Subsequently, another drug molecule, brinzolamide, which is structurally similar to dorzolamide, was also developed (49). Dorzolamide (Trusopt, Merck & Co., Inc., Rahway, NJ) and brinzolamide (Azopt, Alcon Laboratories, Inc., Houston, Texas) are the first CAIs commercially available as topical formulations (50). Although the incidence of side effects was greatly reduced with these formulations, Maus et al. found that topical (2%) dorzolamide hydrochloride was not as effective as systematically administered acetazolamide in their clinical studies (51).

**Viscosity-imparting agents.** In general, increased vehicle viscosity in an ophthalmic preparation should correspond to the slower elimination from precorneal area and lead to greater transcorneal penetration of the drug into the anterior chamber. Moreover, an increased viscosity also should increase ocular bioavailability of the drug because of prolonged contact time of the drug instilled in the conjunctiva. Recently, Kaur et al. reported a topical suspension formulation of acetazolamide by using high-viscosity, water-soluble polymers such as polyvinyl alcohol (PVA) and hydroxypropyl methcellulose (HPMC) and by incorporating acetazolamide into an in situ–forming ophthalmic drug delivery system (52). Moreover, ethylenediaminetetraacetic acid was also used to increase the extent of absorption of the drug. A high concentration of the drug (10%) was used to overcome the problems of low water solubility and low partition coefficient of the drug. The formulation was therapeutically effective with a peak effect at 2 h. A decrease in IOP of as much as 46.4% was observed. Kaur et al. concluded that for a topical formulation of acetazolamide to be successful, it should contain a suitable viscosizer to increase the corneal residence, a penetration enhancer to help in the transport across the cornea, and finally the pH of the formulation should be maintained on the acidic side (pH <5) to prevent degradation of acetazolamide. This study was confined to rabbits, and Kaur et al. expect better results with viscous vehicles in the human eye.

Chrai and Robinson reported that the rate of solution drainage decreased with increasing viscosity but that the bioavailability was not proportional to contact time (53). It has also been reported that the optimal viscosity for use is in the range...
of 12–15 cP (54), beyond which the gain in ocular absorption would be minimal and the risk of inaccuracy of instillation and blurring of vision would increase (55). The most commonly used viscolizing agents are water-soluble polymers such as PVA, polyvinylpyrrolidone (PVP), and cellulosic polymers such as methylcellulose and HPMC. Studies of HPMC showed it to be superior to PVA and PVP because it does not blur vision.

Contact lenses. Friedman et al. observed that the use of high water contact lenses soaked in acetazolamide (5%) significantly decreased IOP (6.3 ± 0.4 mm Hg) in the treated eye of normotensive albino rabbits (56). The duration of the effect was as long as 7.5 h. They also inferred that the drug was effective only at a concentration of 2.5% but was ineffective at a concentration of 1%. However, a concentration 5% caused marked irritation, presumably because of the alkaline pH required for the dissolution of the drug. Both serum and aqueous humor analysis of pH, carbon dioxide pressure, bicarbonate, and base excess indicate that acetazolamide delivered by soft contact lenses can penetrate the cornea in a sufficient concentration to lower IOP by a local mechanism in rabbits without significant systemic absorption.

Gels. To prolong the contact time between the drug and the ocular surface and to enhance the ocular bioavailability of the drug, gels that contain soluble mucoadhesive polyanionic polymers (hyaluronic acid, carboxymethyl chitin, and polyacrylic acids) or gelling agents (PVA, HPMC, and carboxymethylcellulose) have been studied. Because the gel would remain dispersed over the entire eye, less localized toxicity would exist around the lower eyelid where a liquid suspension could accumulate.

According to one study, the gel formation did not significantly lower IOP during any time interval ≤12 h compared with results from the use of a placebo gel (57). However, another group of researchers reported that the use of a gel formulation greatly enhanced the corneal penetration and the resulting ocular hypotensive effect when a newly synthesized CAI, 6-hydroxyethoxzolamide (an ethoxzolamide analogue), was tested in normotensive albino rabbit eyes (58).

Liposomes. Compared with other drug carrier systems, liposomes represent a mature, versatile technology with a considerable potential for encapsulation of both lipophilic and hydrophilic drugs and are in clinical trials for the treatment of a number of infectious and neoplastic diseases. Encapsulation in liposomes often creates distinct changes in the pharmacokinetic and pharmacodynamic properties of the drugs, in some cases causing a marked decrease in toxicity or increase in potency. Liposomes consist of one or more aqueous compartments contained within a lipid-membrane bilayer. Because liposomes contain a hydrophilic domain, a hydrophobic domain, and an interfacial region, they may accommodate therapeutic agents with diverse physical characteristics (59). Liposomes have gained considerable attention for ocular drug delivery. They are primarily used to enhance the corneal drug absorption, which is achieved through intimate contact with the corneal and conjunctival surfaces, thereby increasing the probability of ocular drug absorption. El-Gazayerly et al. prepared liposomes of acetazolamide using a reverse-phase evaporation technique to compare negatively charged, neutral, and positively charged liposomes and found that entrapment efficiency was 29.27,

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Category</th>
<th>Route of Administration</th>
<th>Mechanism of Action</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholinergics: pilocarpine, demecarium, epinephrine</td>
<td>Topical</td>
<td>Lower IOP as a result of various action on aqueous humor outflow</td>
<td>Miosis, ciliary spasms, headache, blurred vision, local irritation</td>
</tr>
<tr>
<td>2</td>
<td>CAIs: acetazolamide, methoxazolamide, ethoxzolamide, dorzolamide</td>
<td>Oral and topical (dorzolamide)</td>
<td>Reduce aqueous humor production</td>
<td>Lethargy, decrease in appetite, kidney stones, skin reactions</td>
</tr>
<tr>
<td>3</td>
<td>Beta-adrenergic antagonists: propranolol, atenolol, betaxolol, timolol</td>
<td>Topical</td>
<td>Decrease aqueous humor production and facilitate increased outflow through the uveoscleral pathway</td>
<td>Ocular irritation, bronchospasms, increase in coupled Hartree–Fock, bradycardia</td>
</tr>
<tr>
<td>4</td>
<td>Alpha-2-agonists: clonidine, breimonidine, apraclonidine</td>
<td>Topical</td>
<td>Reduce aqueous humor production</td>
<td>Local allergy, not effective for 8- and 12-h therapy</td>
</tr>
<tr>
<td>5</td>
<td>Prostaglandins: latanoprost</td>
<td>Topical</td>
<td>Enhance the uveoscleral outflow of aqueous humor</td>
<td>Mild irritation, foreign body stimulation, discomfort, burning and stinging in the eyes, increase in eyelash growth, irreversible darkening of iris</td>
</tr>
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</table>
41.06, and 49.5%, respectively (60). This can be explained on the basis that acetazolamide is a weak acid, and an electrostatic attraction exists between anion and positively charged stearylamine. The proportion of drug released was 13.36, 33.8, and 26.7% for negatively charged, neutral, and positively charged liposomes, respectively. Neutral liposomes had the highest rate and extent of drug release, followed by positively charged liposomes and then negatively charged liposomes. The charged lipid tightened the molecular packaging of the vesicle bilayer thereby decreasing drug release from the charged liposomes. Furthermore, positively charged liposomes produced a strong and sustained reduction in IOP in rabbits probably because these liposomes have a higher binding affinity to the corneal surface than do the neutral or negatively charged liposomes, presumably as a result of association with the polyanionic corneal and conjunctival mucoglycoproteins.

**Cyclodextrins (CDs).** CDs are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity. They are generally used to increase the aqueous solubility of the drug by forming water-soluble drug cyclodextrin complexes (61). They act as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane. This approach has also been used in ocular drug delivery. The ocular availability of drugs in the aqueous cyclodextrin containing eye-drop solution depends on several factors such as the release of the drug from the cyclodextrin complex and the partitioning of the drug molecules into and through the cornea or the conjunctival epithelium. In general, it is believed that cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface, the drug molecules partition from the cyclodextrin cavity into the lipophilic barrier. Thus, the delivery from aqueous cyclodextrin solution is both diffusion and membrane controlled (62).

2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a nontoxic β-cyclodextrin derivative, which is capable of forming water-soluble drugs (63–65). Under normal conditions the large and very hydrophilic HP-β-CD molecules do not penetrate biological membranes but act as penetration enhancers by ensuring a high concentration of dissolved drug at the membrane surface (66,67). Therefore, HP-β-CD may improve ocular bioavailability of the drugs by keeping the water-insoluble drug molecules in solution and deliver them to the surface of the corneal barrier where the molecules partition into the eye.

Loftsson et al. showed that topically active formulations could be obtained by forming water-soluble inclusion complexes between HP-β-CD and CAIs such as acetazolamide and ethoxzolamide (68). Later, they improved the IOP-lowering effect of these inclusion complexes by formulating acetazolamide in a suspension or by combining it with timolol maleate and by combining HP-β-CD with polymers such as HPMC (69). In conscious normotensive English brown rabbits, both acetazolamide and ethoxzolamide had significant IOP-lowering effects. The maximum pressure reduction after instillation of one drop (50 μL) of 1% acetazolamide solution or one drop of 0.3% ethoxzolamide solution was 2.6 mm Hg. The maximum effect (I_{max}) was obtained at 1.25 h after the administration and the duration of the activity was ~4 h. The duration increased to ~8 h and the maximum decline in pressure (~3 mm Hg) was observed after one drop of suspension containing 2% acetazolamide (half the drug was in solution and the other half was in a solid form, resulting in sustained drug release) was administered. Timolol had a much longer IOP-lowering effect than the two CAIs. The maximum decline after administration of one drop of 0.5% timolol in the form of timolol maleate solution was ~5 mm Hg, but the duration of activity was as long as 4.5 h. However, in compound formulations, the IOP-lowering effects of the CAIs and of timolol appeared to be additive to some extent. Thus, the IOP decline after topical administration of one drop of an aqueous HP-β-CD formulation that contained both 1% acetazolamide and 0.5% timolol was significantly larger (4.70 mm Hg for 5 h) than the one obtained after a HP-β-CD formulation containing 1% acetazolamide only.

**Conclusion**

A new drug molecule requires several million dollars and several years of research to become commercially available. A more economical alternative to this process would be to modify existing drugs and their molecules.

The research described in this article suggests that systemic side effects could be dramatically reduced with a topical formulation compared with a systemic CAI therapy. If a topical acetazolamide formulation is developed, it could be used as a first-line treatment and an adjunctive therapy. It may be particularly helpful for patients who cannot tolerate cholinergic agents, which alter accommodation and pupil size, and for patients with asthma, low blood pressure, or cardiac disease who cannot tolerate beta-adrenergic blocking agents.

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**References**


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deliverables with storage location of the validation documents. In other words, the validation testing report is a place to document what happened and how it happened for each validation test. In addition, the report captures all deviations and out-of-specification results.

Procedures and policies manual. A computer system can have all the bells and whistles that will help it comply with applicable regulations. However, built-in functionality and validation documentation does not make a computer system compliant. Policies and procedures are an enforcement tool that companies use to protect their computer systems from fraudulent use. These written guidelines provide a framework for specifying how the system will be used and managed, how data will be recovered, and what disciplinary actions can be taken for violations. Procedures should be written and implemented and should include security and data integrity, system operation, and system maintenance.

Team development
The activities of team development are an integral part of any development activity — validated or nonvalidated. The planning process helps the validation team implement a validated system. Standard documents are required to prove that a system is validated, and policies and procedures are necessary to implement and maintain a computer system in a validated state.

References