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(54) Title: OPHTHALMIC COMPOSITION

(57) Abstract: The present invention provides an ophthalmic composition comprising a hyperbranched polyester. The ophthalmic compositions may also comprise carbonic anhydrase inhibitors, wherein the hyperbranched polyester increases the aqueous solubility of the carbonic anhydrase inhibitor, and increases corneal permeation of the active agent. The ophthalmic compositions may also comprise non-ionic surfactants, such as PEG, Polysorbate, HPMC or HEC, and beta-blockers,such as Carteolol, Levobunolol, Betaxolol, Metipranolol, Timolol or Propranolol. The concentration of the hyperbranched polyester in the ophthalmic formulation should be less than or equal to 4% (w/v) in order to avoid any cytotoxic effects on human corneal cells and thus the eye irritation.

OPHTHALMIC COMPOSITION

FIELD OF THE INVENTION

The present invention relates to an ophthalmic composition comprising a hyperbranched polymer. The hyperbranched polymer of the present invention may be any hyperbranched polymer which is pharmaceutically acceptable, e.g., a hyperbranched polymer with a Polyethyleneimine, Polypropyleneimine or Polyester.

BACKGROUND OF THE INVENTION

COSOPT® and TRUSOPT® are commercially available topical ophthalmic solutions developed by Merck for treating an eye disease called glaucoma. In the case of TRUSOPT®, the active ingredient is Dorzolamide exclusively. In the case of COSOPT®, the active ingredients are Dorzolamide and Timolol (beta blocker). Dorzolamide is a carbonic anhydraze inhibitor with the aqueous solubility of 40 mg/mL at pH 4.0-5.5. It is a white to off-white, crystalline powder, which is soluble in water and slightly soluble in methanol and ethanol.

However, these formulations contain 2% (w/v) Dorzolamide, and are prepared at pH 5.65, due to the limited aqueous solubility of Dorzolamide at physiological pH. Consequently, the COSOPT® and TRUSOPT® formulations can lead to local irritation, due to the low pH. Dorzolamide has two pKa values of 6.35 and 8.5, which correspond to the protonated secondary amine group and the sulfonamide group, respectively. Dorzolamide is mainly in its hydrophilic cationic form at pH below 6.4, and in its hydrophilic anionic form above pH 8.5.

Thus, Dorzolamide has a relatively low aqueous solubility in solutions with pH between 6.4 and 8.5, mainly because of Dorzolamide's non-ionic behavior in that physiological pH

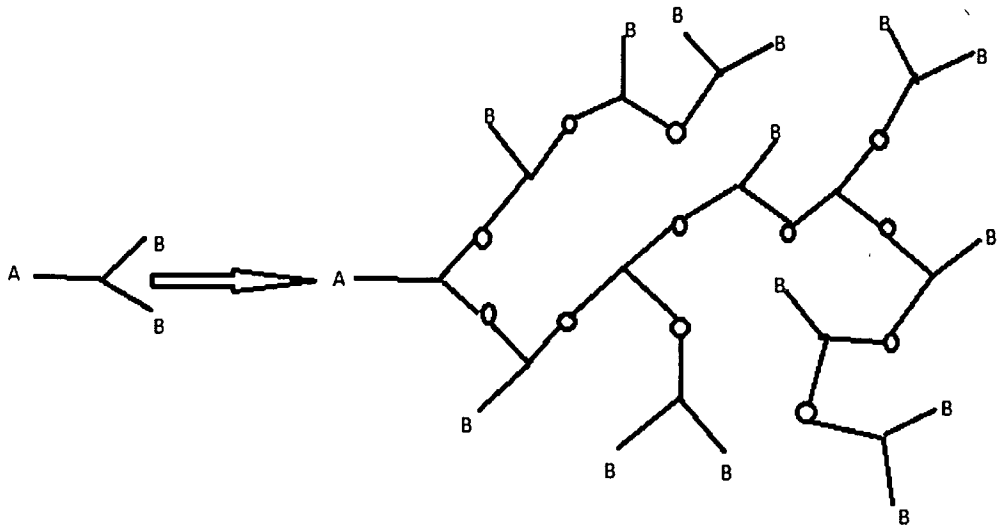
range.

AZOPT® (Brinzolamide ophthalmic suspension) 1% is a sterile, aqueous suspension of Brinzolamide, which has been formulated to be readily suspended and slow settling, following shaking. AZOPT® is developed by Alcon and contains Brinzolamide as active ingredient. The formulation has a pH of approximately 7.5 and an osmolality of 300 mOsm/kg. It is instilled for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Brinzolamide's pKa values are 5.9 (amine) and 8.4 (primary sulfonamide), allowing it to act as an acid or a base (ampholyte) depending upon the pH. It is mainly in its hydrophilic cationic form at pH below 5.9 and hydrophilic anionic form above pH 8.4. It is clear that Brinzolamide is significantly less protonated (<10%) at physiological pH. Thus, Brinzolamide has relatively low aqueous solubility in solutions with pH between 5.9 and 8.4, mainly because of Brinzolamide's nonionic (lipophilic) behavior in that pH range.

Dendritic polymers are tree-like polymers that can be classified into two main types based on their branching architecture as "perfectly branched" (dendrimers) and "imperfectly branched" (hyperbranched polymers or HP). Hyperbranched polymers are molecular constructions having a branched structure, generally around a core. Unlike dendrimers, the structure of hyperbranched polymers generally lacks symmetry, as the base units or monomers used to construct the hyperbranched polymer can be of diverse nature and their distribution is non-uniform. The branches of the polymer can be of different natures and lengths. The number of base units, or monomers, may be different depending on the different branching. While at the same time being asymmetrical, hyperbranched polymers can have: an extremely branched structure, around a core; successive generations or layers of branching; a layer of end chains. Hyperbranched polymers are generally derived from the polycondensation of one or more

monomers AB_x, A and B being reactive groups capable of reacting together, x being an integer greater than or equal to 2. However, other preparation processes are also possible. Hyperbranched polymers are characterized by their degree of polymerization DP=100-b, b being the percentage of non-terminal functionalities in B which have not reacted with a group A. Since the condensation is not systematic, the degree of polymerization is less than 100%. An end group T can be reacted with the hyperbranched polymer to obtain a particular functionality on the ends of chains.

Hyperbranched polymers are mainly identified by their core type and their terminal groups. Examples of a core type for a hyperbranched polymer are polyethylenimine, polypropylenimine, polyglycol, polyether, polyester, etc. A hyperbranched polymer with a polyester core may be referred to as a hyperbranched polyester. Examples of terminal or surface functional groups of hyperbranched polymers are amine, hydroxyl, carboxylic acid, a fatty acid, polyethylene glycol (PEG), polyester, etc. See U.S. Patent 6,432,423, U.S. Patent 7,097,856, and U.S. Patent Publication 2006/0204472, the contents of which are incorporated herein by reference.



Schematic showing the formation of a hyperbranched polymer from the polymerization of AB_2 monomers.

In contrast to the "structurally perfect" dendrimers prepared by multi-step synthesis, somewhat less perfect hyperbranched polymers can be synthesized in one-step reactions. Thus, unlike dendrimers, hyperbranched polymers are rapidly prepared with no purification steps needed for their preparation. Consequently, hyperbranched polymers are significantly less expensive than dendrimers. Thus it makes hyperbranched polymers amenable for large-scale *in vivo* trials and bringing highly branched polymers as candidates for drug delivery of even common drugs as ibuprofen (Kannan, R.M. et al., *Biomedical Applications of Nanotechnology,* 2007, John Wiley & Sons Inc., p. 105).

OBJECT OF THE INVENTION

An object of the invention is to provide an improved ophthalmic composition, with improved aqueous solubility and corneal permeation of the active agent.

SUMMARY OF THE INVENTION

The present inventors have studied ophthalmic compositions comprising hyperbranched polymers. The present inventors have discovered that hyperbranched polymers are muco-adhesive polymers with a high force of bioadhesion, which provide strong electrostatic interactions between the negatively charged cornea mucin membrane and the cationic hyperbranched polymers.

The present inventors have discovered that hyperbranched polymers increase the aqueous solubility of carbonic anhydrase inhibitors such as Dorzolamide or Brinzolamide for glaucoma therapy. Additionally, the present inventors have discovered that the aqueous solubility of Dorzolamide or Brinzolamide increases linearly with an increase in the concentration of the hyperbranched polymer. Furthermore, the present inventors have discovered that hyperbranched polymers, such as Bis-MPA hyperbranched polyester with hydroxyl functional groups (2^{nd} generation), can be safely employed up to 4% (w/v) with no cytotoxic or eye irritation, based on *in vitro* human corneal epithelial cell culture studies. Additionally, the present inventors have discovered that hyperbranched polymers increase the corneal permeation and partitioning of Dorzolamide and Timolol into intact cornea, and increase the partitioning of Dorzolamide and Timolol into the lipophilic cornea membrane.

Accordingly, the present invention provides:

(1) An ophthalmic composition comprising a hyperbranched polymer, wherein the hyperbranched polymer comprises a terminal functional group selected from the group consisting of an amine group, a hydroxyl group, a fatty acid group, and

Polyethylene Glycol (PEG).

(2) The ophthalmic composition according the above (1), further comprising a carbonic anhydrase inhibitor.

(3) The ophthalmic composition according to the above (1) or (2), further comprising a non-ionic surfactant.

(4) The ophthalmic composition according to the above (1), wherein the average molecular weight of the hyperbranched polymer is in the range from 1,000 to 750,000 Daltons (M_w).

(5) The ophthalmic composition according to the above (1) or (2), wherein the hyperbranched polymer comprises a core selected from the group consisting of Polyethylenimine, Polypropylenimine, and polyester.

(6) The ophthalmic composition according to the above (1), wherein the pH is in the range from 3.0 to 8.0.

(7) The ophthalmic composition according to the above (1), wherein the concentration of the hyperbranched polyester is in the range from 0.01% to 5% (w/v).

(8) The ophthalmic composition according to the above (2), further comprising a beta-blocker.

(9) The ophthalmic composition according to the above (2), wherein the carbonic anhydrase inhibitor is selected from the group consisting of Dorzolamide, Brinzolamide and Acetazolamide.

(10) The ophthalmic composition according to the above (3), wherein the non-ionic surfactant is selected from the group consisting of PEG, Polysorbate, Hydroxyl Propyl Methyl Cellulose (HPMC), and Hydroxy Ethyl Cellulose (HEC).

(11) The ophthalmic composition according to the above (8), wherein the beta-blocker is selected from the group consisting of Carteolol, Levobunolol, Betaxolol, Metipranolol, Timolol and Propranolol.

(12) The ophthalmic composition according to the above (5), wherein the hyperbranched polymer core is polyester, and wherein the hyperbranched polymer comprises a hydroxyl group, a fatty acid group, and PEG as terminal functional groups.

(13) The ophthalmic composition according to the above (12), wherein the average molecular weight of the hyperbranched polymer is in the range from 1,000 to 12,000 Daltons (M_w).

(14) The ophthalmic composition according to the above (12), wherein the concentration of the hyperbranched polymer is in the range from 0.001% to 4% (w/v).

(15) An ophthalmic composition comprising a hyperbranched polyester, Timolol, Dorzolamide, and Polysorbate 80, wherein the hyperbranched polyester comprises a terminal functional group selected from the group consisting of polyester hydroxyl group, a fatty acid group, and PEG.

(16) An ophthalmic composition comprising a hyperbranched polyester, Timolol, Brinzolamide, and Polysorbate 80, wherein the hyperbranched polyester comprises a terminal functional group selected from the group consisting of polyester hydroxyl group, a fatty acid group, and PEG.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the pH-solubility profile of Dorzolamide in 0.1% (w/v) phosphate buffer.

Figure 2 shows the dependence of hyperbranched polymer concentration on the aqueous solubility of Dorzolamide in 0.1% (w/v) phosphate buffer at pH 5.65.

Figure 3 shows the dependence of hyperbranched polymer concentration on the aqueous solubility of Dorzolamide in 0.1% (w/v) phosphate buffer at pH 7.

Figure 4 shows the effect of a combination of PEG 8000 and hyperbranched polymer (Lupasol® PS) with various concentrations on the aqueous solubility of Dorzolamide at pH 7.

Figure 5 shows the viscosity as a function of shear rate at 20 °C of different solutions in 0.1% (w/v) phosphate buffer.

Figure 6 shows the force of bioadhesion at pH 7 and shear rate of 80 s^{-1}.

Figure 7 shows the maximum aqueous solubility of Dorzolamide at pH 5.65 and pH 7 with addition of additives in the presence of 0.5% Timolol in the aqueous solution in all cases.

Figure 8 shows the maximum Dorzolamide solubility at pH 7 with different combinations of additives in the presence of 0.5% Timolol.

Figure 9 shows the schematic of a standard side by side diffusion cell.

Figure 10 shows the mean permeation profiles (n=2) of Dorzolamide through intact rabbit corneas for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 11 shows the mean permeation profiles (n=2) of Timolol through intact rabbit corneas for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 12 shows the mean percentage total corneal permeation of Dorzolamide and Timolol after 3 hours for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 13 shows the mean corneal permeability coefficients of Dorzolamide and Timolol

for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 14 shows the mean diffusion coefficients of Dorzolamide and Timolol for permeation through intact rabbit corneas for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 15 shows the mean partition coefficients of Dorzolamide and Timolol for permeation through intact rabbit corneas for a formulation containing Lupasol® PS hyperbranched polymer.

Figure 16 shows the aqueous solubility of Brinzolamide in 10 mM phosphate buffer at different pH values.

Figure 17 shows the maximum aqueous solubility of Brinzolamide at pH 7 with addition of additives in the absence and presence of 0.5% Timolol in the aqueous solution.

Figure 18 shows the mean permeation profiles of Dorzolamide (n=2) through rabbit corneas for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 19 shows the mean permeation profiles of Timolol (n=2) through rabbit corneas for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 20 shows the mean percentage total corneal permeation of Dorzolamide and Timolol after 2 hours for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 21 shows the mean corneal permeability coefficients of Dorzolamide and Timolol for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 22 shows the mean diffusion coefficients of Dorzolamide and Timolol for permeation through intact rabbit corneas for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 23 shows the mean partition coefficients of Dorzolamide and Timolol for permeation through intact rabbit corneas for a formulation containing Boltorn® H20 hyperbranched polymer.

Figure 24 shows the maximum aqueous solubility of Brinzolamide at pH 7.4 with addition of Boltorn® W3000 (amphiphilic HP) in the presence of 0.5% (w/v) Timolol in the emulsion solution.

Figure 25 shows the mean permeation profiles of Dorzolamide (n=2) through intact rabbit corneas for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 26 shows the mean permeation profiles (n=2) of Timolol through intact rabbit corneas for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 27 shows the mean percentage total corneal permeation of Dorzolamide and Timolol after 3 hours for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 28 shows the mean corneal permeability coefficients of Dorzolamide and Timolol for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 29 shows the mean diffusion coefficients of Dorzolamide and Timolol for permeation through intact rabbit cornea for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 30 shows the mean partition coefficients of Dorzolamide and Timolol for permeation through intact rabbit cornea for a formulation containing Boltorn® W3000 hyperbranched polymer.

Figure 31 shows the maximum aqueous solubility of Brinzolamide at pH 7.4 with addition of 2nd Bis-MPA hyperbranched polyester or 3rd Bis-MPA hyperbranched polyester in the presence of 0.5% (w/v) Timolol.

Figure 32 shows the *in vitro* human corneal epithelium cell viability of different concentrations of hyperbranched polyesters (hydroxyl groups generation 2 and 3), and AZOPT®.

Figure 33 shows the cytotoxicity of Bis-MPA hyperbranched polyester for different concentrations.

Figure 34 shows the solubility and stability of Dorzolamide at pH 7.4 with addition of Bis-MPA hyperbranched polyester and non-ionic surfactants in the presence of 0.5% (w/v) Timolol.

Figure 35 shows the maximum aqueous solubility of Dorzolamide at pH 7.4 with addition of different additives in the presence of 0.5% (w/v) Timolol.

Figure 36 shows intact cornea permeation profile of Dorzolamide for formulation containing Bis MPA hyperbranched polyester (2^{nd} generation).

Figure 37 shows intact cornea permeation profile of Timolol for formulation containing Bis MPA hyperbranched polyester (2^{nd} generation).

Figure 38 shows the mean percentage total corneal permeation of Dorzolamide and Timolol after 3 hours for formulation containing Bis MPA hyperbranched polyester (2^{nd} generation).

Figure 39 shows the permeability coefficients of Dorzolamide and Timolol for formulation containing Bis MPA hyperbranched polyester (2^{nd} generation).

Figure 40 shows the partition coefficients of Dorzolamide and Timolol for formulation containing Bis MPA hyperbranched polyester (2^{nd} generation).

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the present invention are topically administratable therapeutic

compositions for treatment of conditions of the eye. Such conditions of the eye include glaucoma, and ocular diseases such as cataract, conjunctivitis, infection, inflammation or retinopathy.

A detailed description of the invention is provided below.

The present invention includes an ophthalmic composition comprising a hyperbranched polymer.

The hyperbranched polymer according to the present invention may be any hyperbranched polymer which is pharmaceutically acceptable, e.g., a hyperbranched polymer with a Polyethyleneimine, Polypropylenimine or a polyester core. The molecular weight of the hyperbranched polymer in the ophthalmic compositions of the present invention is in the range of from 1,000 to 750,000 Daltons, preferably in the range of 1,000 to 12,000 Daltons. The molecular weight is weight average molecular weight measured by dynamic light scattering. The concentration of the hyperbranched polymer in the ophthalmic compositions of the present invention is in the range from 0.001% to 10% (w/v), preferably in the range from 0.001% to 5% (w/v), more preferably in the range from 0.001% to 4% (w/v), more preferably in the range from 0.01% to 4% (w/v), more preferably in the range of 0.01% to 3% (w/v).

The ophthalmic composition discussed above may also comprise a carbonic anhydrase inhibitor. Carbonic anhydrase inhibitors are a class of pharmaceuticals that suppress the activity of carbonic anhydrase, and are known to be useful as anti-glaucoma agents. Examples of carbonic anhydrase inhibitors which may be present in the ophthalmic compositions of the present invention are Dorzolamide, Brinzolamide or Acetazolamide.

The ophthalmic composition discussed above may also comprise a non-ionic surfactant. The non-ionic surfactant may be any non-ionic surfactant which is known as a pharmaceutically acceptable additive, for example, Polysorbate 80, PEG 8000, HPMC or HEC.

The ophthalmic compositions of the present invention are advantageously used after being adjusted to a pH range which is conventionally adopted for topical application to the eye, and is normally employed after being adjusted to a pH of 3 to 8, preferably a pH of 5 to 8. For the pH adjustment, hydrochloric acid, acetic acid, sodium hydroxide, etc. can be used.

The ophthalmic compositions of the present invention may also comprise a beta-blocker. Beta-blockers are known to reduce the pressure within the eye (the intraocular pressure), and thus, are used to lessen the risk of damage to the optic nerve and loss of vision in patients with glaucoma. The beta-blocker in the ophthalmic compositions of the present invention may be any beta-blocker which is known as acceptable in ophthalmic compositions, such as Carteolol, Levobunolol, Betaxolol, Metipranolol, Timolol and Propranolol.

A first specific embodiment of the present invention is an ophthalmic composition comprising a hyperbranched polymer, Timolol, Dorzolamide, PEG 8000 and Polysorbate 80.

Such compositions preferably comprise about 0.001% to 10% (w/v) of the hyperbranched polymer, most preferably about 1 to 5% (w/v), and 0.05 to 1% (w/v) of Timolol, most preferably about 0.5% (w/v), and about 0.05 to 5% (w/v) of Dorzolamide, most preferably about 0.5 to 2% (w/v), and about 0.05 to 5% (w/v) of PEG 8000, most preferably about 0.5 to 4% (w/v), and about 0.05 to 5% (w/v) of Polysorbate 80, most preferably about 0.5 to 4% (w/v), and are to be administered once or twice a day to each affected eye.

A second specific embodiment of the present invention is an ophthalmic composition comprising a hyperbranched polymer, Timolol, Brinzolamide, PEG 8000 and Polysorbate 80.

Such compositions preferably comprise about 0.001% to 10% (w/v) of the hyperbranched polymer, most preferably about 1 to 5% (w/v), and 0.05 to 1% (w/v) of Timolol, most preferably about 0.5% (w/v), and about 0.05 to 5% (w/v) of Brinzolamide, most preferably about 0.5 to 2% (w/v), and about 0.05 to 5% (w/v) of PEG 8000, most preferably about 0.5 to 4% (w/v), and about 0.05 to 5% (w/v) of Polysorbate 80, most preferably about 0.5 to 4% (w/v), and are to be administered once or twice a day to each affected eye.

A third specific embodiment of the present invention is an ophthalmic composition comprising a hyperbranched polyester, Timolol, Dorzolamide, PEG 8000 and Polysorbate 80.

Such compositions preferably comprise about 0.1% to 10% (w/v) of the hyperbranched polyester, most preferably about 1 to 5% (w/v), and 0.05 to 1% (w/v) of Timolol, most preferably about 0.5% (w/v), and about 0.05 to 5% (w/v) of Dorzolamide, most preferably about 0.5 to 2% (w/v), and about 0.05 to 5% (w/v) of PEG 8000, most preferably about 0.5 to 4% (w/v), and about 0.05 to 5% (w/v) of Polysorbate 80, most preferably about 0.5 to 4% (w/v), and are to be administered once or twice a day to each affected eye.

A fourth specific embodiment of the present invention is an ophthalmic composition comprising a hyperbranched polyester, Timolol, Brinzolamide, PEG 8000 and PEG 8000.

Such compositions preferably comprise about 0.1% to 10% (w/v) of the hyperbranched polyester, most preferably about 1 to 5% (w/v), and 0.05 to 1% (w/v) of Timolol, most preferably about 0.5% (w/v), and about 0.05 to 5% (w/v) of Brinzolamide, most preferably about 0.5 to 2% (w/v), and about 0.05 to 5% (w/v) of PEG 8000, most preferably about 0.5 to 4% (w/v), and about 0.05 to 5% (w/v) of Polysorbate 80, most preferably about 0.5 to 4% (w/v), and are to be administered once or twice a day to each affected eye.

The ophthalmic compositions according to the present invention may comprise a

pharmacologically acceptable carrier, excipient or diluent which is known *per se* and may be formulated by a method known *per se* for preparing ophthalmic compositions. The ophthalmic compositions of the present invention may be provided in any pharmaceutical dosage form that is conventionally used as an ophthalmic preparation, e.g., eye drops, emulsions, and eye ointments.

The eye drop formulation may, for example, be an aqueous formulation, such as ophthalmic solution which is clear solution, ophthalmic suspension, ophthalmic emulsion, as well as non-aqueous formulations, such as non-aqueous ophthalmic solution and non-aqueous ophthalmic suspension.

The ophthalmic solution formulation may contain various additives incorporated ordinarily, such as buffering agents (e.g., phosphate buffers, borate buffers, citrate buffers, tartarate buffers, acetate buffers, amino acids, Sodium acetate, Sodium citrate and the like), isotonicities (e.g., saccharides such as sorbitol, glucose and mannitol, polyhydric alcohols such as Glycerin, concentrated Glycerin, PEG and Propylene glycol, salts such as Sodium chloride), preservatives or antiseptics (e.g., Benzalkonium chloride, Benzethonium chloride, P-oxybenzoates such as Methyl p-oxybenzoate or Ethyl p-oxybenzoate, Benzyl alcohol, Phenethyl alcohol, Sorbic acid or its salt, Thimerosal, Chlorobutanol and the like), solubilizing aids or stabilizing agents (e.g., cyclodextrins and their derivative, water-soluble polymers such as polyvinyl pyrrolidone, surfactants such as tyloxapol, pH modifiers (e.g., Hydrochloric acid, Acetic acid, Phosphoric acid, Sodium hydroxide, Potassium hydroxide, Ammonium hydroxide and the like), thickening agents (e.g., HEC, Hydroxypropyl cellulose, Methyl cellulose, HPMC, Carboxymethyl cellulose and their salts), chelating agents (e.g., Sodium edetate, Sodium citrate, condensed Sodium phosphate) and the like.

The eye drop formulation in the form of an aqueous suspension may also contain suspending agents (e.g., Polyvinyl pyrrolidone, Glycerin monostearate) and dispersing agents (e.g., surfactants such as Tyloxapol, ionic polymers such as Sodium alginate) in addition to the additives listed above, whereby ensuring that the eye drop formulation is a further uniform microparticulate and satisfactorily dispersed aqueous suspension.

The eye drop formulation in the form of an aqueous suspension preferably contains Sodium citrate or Sodium acetate as a buffering agent, concentrated Glycerin and/or Propylene glycol as an isotonicity and Polyvinyl pyrrolidone as a suspending agent. A preferred dispersing agent is a surfactant and/or Sodium alginate. Such surfactant is preferably Tyloxapol.

The ophthalmic composition of the present invention may be administered to a mammal which is or may be suffering from an ophthalmic disease, such as glaucoma (e.g., a human, rabbit, dog, cat, cattle, horse, monkey).

While the administration route and the dose may vary depending on a symptom, age and body weight of a subject, the concentration of the active agent in the ophthalmic composition of the present invention is about 0.001 to 5 (w/v) %, preferably about 0.01 to 3 (w/v) % contained in an aqueous eye drop formulation when given to an adult, and is given preferably 1 to 8 times a day with a single dose being one to several drops.

Unless the intended purpose of use is affected adversely, the ophthalmic compositions of the present invention may contain or may be used together with other appropriate pharmacologically effective substances, for example, steroidal anti-inflammatory agents (Dexamethasone, Prednisolone, Loteprednolm Fluorometholone, Fluocinolone and the like), non-steroidal anti-inflammatory agents (Diclofenac sodium, Pranoprofen, Bromfenac, Ketorolac tromethamine, Napafenac, Flurbiprofen Sodium and the like), antiallergic agents (Tranilast,

Ketotifen fumarate, Olopatadine hydrochloride, Sodium Cromoglicate, Potassium Pemirolast, Sodium Nedocromil and the like), antihistamic agents (Epinastine hydrochloride, Azelastine hydrochloride, Azalastine hydrochrilidem, Diphenhydramine hydrochloride and the like), glaucoma-treating agents (Pilocarpine hydrochloride, Physostigmine salicylate, Timolol, Isopropylunoprostone, Latanoprost, Betaxolol hydrochloride, Apraclonidine, Brimonidine Tartrate, Carbacol, Dipivefrin, Bimatoprost, Travoprost, Brimonidine tartrate and the like), antibiotics (Azithromycin, Gentamycin sulfate, Fradiomycin sulfate, Tobramycin, Sulbenicillin, Cefmenoxime, Erythromycin, Colistin, Oxytetracycline, Polymyxin B, Chloramphenicol, Micronomicin, Dibekacin, Sisomicin and the like), antibacterial agents (Sulfamethizole, Sulfamethoxazole, Ofloxacin, Norfloxacin, Lomefloxacin hydrochloride, Moxifloxacin hydrochloride, Enoxacin, Ciprofloxacin hydrochloride, Cinoxacin, Sparfloxacin, Tosufloxacin tosylate, Nalidixic acid, Pipemidic acid Trihydrate, Pipemidic acid, Fleroxacin, Levofloxacin, Gatifloxacin and the like), and antiviral agents (Idoxuridine, Acyclovir and the like), and antimycotic agents (Pimaricin, Fluconazole, Miconazole, Amphotericin B, Flucytosine, Itraconazole and the like), anti VEGF antibody (Pegaptanib and the like).

The ophthalmic compositions of the present invention may be produced by dissolving or dispersing the active agent(s), hyperbranched polymer and optionally the non-ionic surfactant in a solution appropriately containing pharmaceutically acceptable additives, such as isotonicity agents, buffers, preservatives, suspending agents, thickeners, stabilizers, pH adjusting agents, and the like.

The present inventors hereby incorporate by reference prior filed U.S. Application No. 12/774,419, in its entirety. The present invention is further illustrated in detail by the following

Experimental Examples. These Experimental Examples are merely illustrative, and are not intended to limit the scope of the present invention.

EXPERIMENTAL EXAMPLE 1

pH-solubility profile of Dorzolamide in aqueous solution containing different concentrations of Hyperbranched Polymer (HP) (Lupasol® G20, Lupasol® G 35, Lupasol® PS) and PEG.

Methods

Suspensions of Dorzolamide hydrochloride in 0.1% (w/v) phosphate buffer solution at pH 5.5, pH 6, pH 6.5, pH 7, pH 7, pH 8 and pH 8.5 were prepared. Similar suspensions were also prepared in aqueous solution containing different concentrations of different HP and PEG with a molecular weight of 8000. A combination of Polysorbate 80 and PEG 8000 was also attempted. The pH was measured accurately with micro-pH electrode (Thermo Scientific). The desired pH was adjusted using either 1 M NaOH or 1 M HCl. The suspension solutions were first stirred for 10 min at room temperature (with heating up to 60 °C for 5 minutes). After allowing the suspensions to equilibrate at room temperature for an additional 30 minutes, the suspension solutions were then sonicated for 10 minutes and finally filtered through 0.45 μm syringe filters. The filtrates were analyzed for Dorzolamide concentration using UPLC. Dorzolamide detection was performed using: a gradient 1% (v/v) Triethylamine (TEA) in water:acetonitrile method, performed at room temperature, with the flow rate of 0.7 mL/min, at 254 nm wavelength and 10 μL injection volume, on BEH C18 1.7 μm, 2.1x 50 mm column. A calibration curve was prepared to find Dorzolamide concentration. The properties of polymers used are listed in Table 1.

Polymer name	Viscosity (cP)	Molecular weight	pKa	Solid content (% w/v)
Lupasol® G 20	200-500	1300	7-10	>98%
Lupasol® G 35	250-650	2000	7-10	48-52%
Lupasol® PS	1000-2500	750,000	7-10	33%

Table 1: Properties of HPs in EXPERIMENTAL EXAMPLE 1.

Results and Discussion

Figure 1 demonstrates that the aqueous solubility of Dorzolamide decreases as the pH increases from 5.65, and reaches a bottom at pH 7. Since COSOPT® is formulated at pH 5.65, Dorzolamide solubility in 0.1% (w/v) phosphate buffer was quantified in the presence of different HP of different concentrations at pH 5.65. The result is presented in **Figure 2**. The solubility of Dorzolamide increased at pH 5.65 with the increase in concentrations of HP from 0.1% to 1% (w/v). Similarly at pH 7, as shown in the bar graph of **Figure 3**, Dorzolamide solubility increased linearly with the increase in concentration of HP from 0.1% (w/v) to 4% (w/v).

As shown in **Figure 4**, combinations of various concentrations of PEG 8000 and 0.5% and 1% (w/v) of HP (Lupasol® PS) were applied at pH 7. It is clear from Figure 4 that 2% (w/v) solubility of Dorzolamide (similar to COSOPT®) in phosphate buffer at pH 7 can be achieved by using about 20% PEG 8000 and 0.5% of Lupasol® PS, or 17% of PEG 8000 and 1% Lupasol® PS.

Conclusion

The present inventors discovered that the aqueous solubility of Dorzolamide increased with an increase in the concentration of HP and PEG. In the case of PEG, the solubility also increased linearly with an increase in the molecular weight of the PEG. Further, the Polysorbate

80 assists in dispersing the Dorzolamide molecules and inhibits the precipitation in water in presence of PEG.

From these results, it is concluded that HP significantly enhances the solubility of Dorzolamide. Additionally, hydrophilic polyethylene glycol was discovered to be a Dorzolamide solubility enhancer. The results demonstrate the advantages of using hyperbranched polymers and PEG as Dorzolamide solubility enhancing additives at pH values closer to physiological pH.

EXPERIMENTAL EXAMPLE 2

A simple rheological method for the *in vitro* assessment of mucin-hyperbranched polymer bioadhesive bond strength.

A simple viscometric method was adopted to quantify the mucin-polymer bioadhesive bond strength. In order to determine the muco-adhesive properties of commercially available HP called Lupasol® PS, the force of bioadhesion was calculated for different concentrations of HP with porcine gastric mucin at pH 7 in comparison with the market product COSOPT®. Porcine gastric mucin was used as a model mucin. However, since all mucins appear to share general physical, structural, and rheological properties, it is believed that porcine gastric mucin is a satisfactory model for primary evaluation of bioadhesive materials.

Methods

Brookfield Rotational L VDVE viscometer was employed for all measurements. Spindle with code number 18 was used for all viscosity measurements. A factor of 1.32 was used to convert rpm to shear rate (s^{-1}) as per the manual. A solution of 15% (w/v) of gastric mucin was prepared in 0.1% (w/v) phosphate buffer at pH 7. The individual viscosities 0.5% (w/v) and 1%

(w/v) of Lupasol® PS in phosphate buffer solution were measured. The viscosities of 15% mucin in phosphate buffer were also measured. The viscosity was measured at 20 °C at different shear rates D from 2.6 to 80 s^{-1}. (Hassan, E. et al., *Pharm Res.* 5 (1990) 491) Five samples of 10 mL each were prepared with different concentrations of Lupasol® PS, PEG and with and without 15% (w/v) gastric mucin in 0.1% (w/v) phosphate buffer at pH 7.

Content (% w/v)	#1	#2	#3	#4	#5	#6
Lupasol® PS	-	0.5	0.5	1	1	1
Gastric mucin	15	-	15	-	15	15
PEG 8000	-	-	-	-	-	2
1M NaOH	Adjust pH to 7.0	Adjust pH to 7.0	Adjust pH to 7.0	Adjust pH to 7.0	Adjust pH to 7.0	Adjust pH to 7.0

Table 2: Contents (% w/v) of Test Samples.

Sample #7 is the original COSOPT® market product. The viscosity at 20 °C was measured at different shear rates. The force of bioadhesion was calculated using equations (1) and (2), discussed above.

The force of bioadhesion (F) was calculated as per the following equation (1):

$$F = \eta_b \sigma \qquad (1)$$

where σ is the rate of shear per second, and η_b is based on experimental measured values as per the following equation (2):

$$\eta_b = \eta_t - \eta_m - \eta_p \qquad (2)$$

where η_t is the viscosity coefficient of the system, and η_m and η_p are the individual viscosity coefficients of mucin and the bioadhesive polymer (e.g., HP and PEG 8000), respectively.

For equations (1) and (2) to be valid, η_t, η_m and η_p should be measured at the same concentration, temperature, time, and rate of shear. The bioadhesive phenomenon plays a dominant role in the contact time of aqueous tear that substitute in the precorneal area.

Results & Discussion

As shown in **Figure 5**, the low concentrations of HP in phosphate buffer have relatively less viscosity compared to COSOPT® (Sample #7) and mucin (Sample #1). The viscosities of HP (0.5%, Sample #2 and 1 %, Sample #4) are relatively close to water at high shear rates. In addition, at high shear rates the difference between the viscosities of 0.5% (w/v) HP and 1% (w/v) are negligible. The result clearly suggests the advantage of using HP as an additive with rheological properties that may be very compatible for topical ophthalmic solutions since the addition of HP to a formulation may not change the rheological properties of final formulation.

The force of bioadhesion was quantified based on the data available from Figure 5 at shear rate of 80 s^{-1}. High shear rate was chosen since the polymers typically exhibit bioadhesive properties at high shear rates (close to 100 s^{-1}).

As shown in **Figure 6**, the bioadhesive bond strength of low concentrations (0.5% (w/v) and 1% (w/v)) of HP-mucin system is almost more than two times to that of COSOPT®-mucin system. The addition of 2% (w/v) PEG did not change the force of bioadhesion of 1% (w/v) HP-mucin system, suggesting that 2% PEG may not have influence on force of bioadhesion caused by the HP at pH 7. Overall, the results shown in Figure 6 indicate that the bioadhesive strengths of low concentrations of HPs are relatively significant compared to the polymers present in COSOPT® formulation. The bioadhesive phenomenon may be very conducive for increasing the ocular bioavailability of the drug.

Conclusion

In conclusion, data generated by the viscometric assessment method of bioadhesion suggests that the hyperbranched polymers are bio-adhesive additive materials that could strongly interact with ocular mucin. These bioadhesive forces between mucin and HP could eventually lead to enhancement of the ocular bioavailability of the drug.

EXPERIMENTAL EXAMPLE 3

Aqueous solubility of Dorzolamide in the presence of Timolol for a novel formulation containing HP (Lupasol® PS) and Polysorbate 80 or a combination of PEG and Polysorbate 80 at pH 5.65 and pH 7.

Methods

A suspension of Dorzolamide hydrochloride and 0.5% (w/v) Timolol in citrate buffer solution at pH 5.65 was prepared (Control sample). A similar suspension was also prepared in aqueous solution containing 2% (w/v) of HP in citrate buffer of pH 3. The final pH was adjusted to 5.65 with 1 M NaOH after addition of HP (sample 1). The combination of different molecular weight PEG and Polysorbate 80 at pH 5.65 as per Table 3 were also attempted. Table 3 shows all the different test samples suspensions to be prepared in 10 mM citrate buffer.

Content (%v/w)	Control Sample	S #1	S #2	S #3	S #4	S #5	S #6	S #7	S #8
Dorzolamide HCl	>2.22	>2.22	>2.22	>2.22	>2.22	>2.22	>2.22	>2.22	>2.22
Timolol Maleate	0.683	0.683	0.683	0.683	0.683	0.683	0.683	0.683	0.683
Lupasol® PS (MW=750k)	-	2	-	-	-	-	-	-	-
PEG 200	-	-	2	-	-	-	-	-	-
PEG 400	-	-	-	2	-	-	-	-	-
PEG 2000	-	-	-	-	2	-	-	-	-
PEG 3350	-	-	-	-	-	2	-	-	-
PEG 4000	-	-	-	-	-	-	2	-	-
PEG 8000	-	-	-	-	-	-	-	2	-
PEG 20000	-	-	-	-	-	-	-	-	2
Polysorbate 80	-	-	1	1	1	1	1	1	1
In 10 mM citrate or phosphate buffer	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7	Adjust pH to 5.65/7

Table 3: Different Test formulations prepared at pH 5.65 in citrate buffer, and at pH 7 in phosphate buffer.

Similarly, the formulations were again prepared in 10 mM phosphate buffer (Table 3) for the formulations to be tested for Dorzolamide solubility at pH 7 in phosphate buffer rather than citrate buffer. The suspension solutions were first stirred for 10 min at room temperature (with heating up to 60 °C for 5 minutes). After allowing the suspensions to equilibrate at room temperature for an additional 30 minutes, the suspension solutions were then sonicated for 10 minutes and finally filtered through 0.45 μm syringe filters. The filtrates were analyzed for Dorzolamide and Timolol concentration using UPLC with the same condition as EXPERIMENTAL EXAMPLE1.

Results and Discussion

In this experiment, the present inventors used HP, PEG, and Polysorbate 80 as solubility enhancer additives. Different combinations were attempted at pH 5.65 and pH 7. As shown in **Figure 7**, the solubility of Dorzolamide was shown to increase with the addition of additives, compared to the control sample without additives, at pH 5.65 and pH 7 in the presence of Timolol. At pH 5.65, in all cases the solubility of Dorzolamide was above 2%, and therefore the addition of HP or PEG and Polysorbate 80 combination increased solubility of Dorzolamide in the presence of Timolol.

While the market COSOPT® product has 2% (w/v) Dorzolamide at pH 5.65, the enhancement of solubility at pH 5.65 with more than 2% (w/v) Dorzolamide solubility by addition of HP or PEG will not have useful contribution to efficacy enhancement of drug by increasing the dosage. Thus, the solubility data at pH 7 is more vital, where Dorzolamide has poor solubility (less than 0.5% w/v solubility) in 10 mM phosphate buffer. It was also noted that the solubility of Timolol in the formulation samples (each containing exactly 0.5% w/v Timolol) did not change at pH 5.65 and pH 7 with the addition of additives. Since COSOPT® is formulated at pH 5.65, the Dorzolamide solubility in the presence of Timolol was quantified by the addition of different HP of different concentrations at pH 5.65 to the formulation sample. The result is presented in Figure 7. As shown in the bar graph, Dorzolamide solubility increases linearly with the increases in concentration of HP from 0.5% to 2% (w/v) at pH 5.65 and pH 7. However, the impact of HP to solubility enhancement of Dorzolamide is more pronounced at pH 5.65 than pH 7. As shown in Figure 8, the addition of Polysorbate 80 to HP increases Dorzolamide solubility.

The improvement in aqueous solubility of Dorzolamide in the presence of Timolol was significant with the additions of a HP or a combination of PEG and Polysorbate 80 at pH 5.65. In this case, the Polysorbate 80 helped in dispersing Dorzolamide molecules and inhibited the precipitation in water in the presence of PEG. A combination of HP and Polysorbate 80 was the best combination for enhancement of Dorzolamide solubility in presence of Timolol at pH 7. From the results, it can be concluded that HP and Polysorbate 80 significantly enhance the solubility of Dorzolamide in the presence of Timolol at pH 7. Hydrophilic PEG also turned out to be Dorzolamide solubility enhancer. Furthermore, a combination of low concentrations of Polysorbate 80 and PEG 8000 also proved to be a very useful additive for enhancement of solubility of Dorzolamide. Overall, a formulation at pH 7 with optimized concentration of HP and Polysorbate could be very useful for increasing the ocular bioavailability.

Conclusion

The results clearly indicate the advantages of using HP and Polysorbate 80 as Dorzolamide solubility enhancing additives at pH values closer to physiological pH that are more conducive for penetration of close to 1% (w/v) Dorzolamide through cornea membrane. Polysorbate 80 also proved to be an effective emulsifier, suppressing the precipitation of poorly soluble Dorzolamide at pH 7 in the presence of a HP.

EXPERIMENTAL EXAMPLE 4

In vitro corneal permeation study of Dorzolamide and Timolol for novel topical formulations containing HP (Lupasol® PS) and Polysorbate 80.

In vitro experiments on the corneal permeation of Dorzolamide and Timolol (active ingredients of COSOPT®) were carried out to investigate the effect of the addition of 0.5% (w/v) HP, or the addition of 0.5% (w/v) HP and 1% (w/v) Polysorbate 80, in comparison to the original market topical formulation (only active ingredients) at pH 5.65.

Materials and Methods

Experimental Method

1. Formulation Preparation: The following three solutions in 10 mM citrate buffer were formulated for examining the *in vitro* corneal permeation of Dorzolamide and Timolol, as well as determining the corneal hydrolysis effect.

Content	Composition (% w/v)		
	Test sample 1 (n=2)	*Test sample 2 (n=2)*	*Test sample 3 (n=2)*
Dorzolamide	2	2	2
Timolol	0.5	0.5	0.5
Lupasol® PS	-	0.5	0.5
Polysorbate 80	-	-	1
1M NaOH	Adjust pH to 5.65	Adjust pH to 5.65	Adjust pH to 5.65

The samples were filtered by 0.45 μm filter syringe. The initial concentration of both the samples was determined by UPLC analysis. From the experimental data, the following inferences were made:

a) HP exclusively (from Test 1 and Test 2 data comparison).

b) Polysorbate 80 (Test 2 & Test 3 comparison) significance on cornea permeation.

c) HP + Polysorbate 80 combination significance (from Test 1, Test 2, Test 3 data comparison).

2. In vitro rabbit corneal permeation experiment

Composition	Chemical Formula	[g/100mL]
Calcium chloride	$CaCl_2$	0.0132
Potassium chloride	KCl	0.04
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	0.02
Sodium dihydrogen phosphate dehydrate	$NaH_2PO_4 \cdot 2H_2O$	0.0187
Sodium chloride	NaCl	0.787
Glucose	Glucose	0.1
Sodium hydroxide	NaOH	q.s.
Water	Purified Water	q.s
pH	pH	7.2

Table 4: Composition of receptor solution for in vitro cornea permeation experiment.

Three male rabbits (New Zealand) weighing 3-4 pounds. The age of the rabbits was 11-12 weeks. Immediately after sacrifice by an overdose of carbon dioxide gas, the eyes were enucleated, saline washed, and the corneas were separated for the use in permeation experiments. Each cornea was rinsed with freshly prepared receptor solution (Table 4) to remove excess stain. The six intact and fresh corneas were fixed between clamped donor and receptor compartments of an all glass side-by-side diffusion cell in such a way that its epithelial surface faces the donor compartment. Figure 9 shows the schematic of a simple diffusion cell used in this experiment.

The corneal area available for permeation was 0.211 cm^2. The receptor compartment was filled with freshly prepared receptor solution at pH 7.2, as per the composition described in Table 4. An aliquot (5 mL) of sample #1 was placed on the two intact corneas, and the opening of the donor cells was sealed with a glass cover slip. After 10 minutes of applying sample #1, an aliquot (5 mL) of sample #2 was applied on the next two intact corneas. Again, after 10 minutes, sample #3 aliquot (5 mL) was applied on the remaining two intact corneas. The receptor fluid (5 mL in each receptor cell) was kept at constant temperature of 34 °C using constant stirring through water jacket in all the six cases. At predetermined time intervals of 10, 20, 40, 60, 80, 100, 120, 140, 160, and 180 minutes, 200 µL samples were withdrawn from the receptor

solution. Thereafter, the same amount of the phosphate buffer solution was added to the receptor cell. The drug concentrations were assayed by UPLC.

3. Analysis

The Dorzolamide and Timolol maleate detection conditions were a gradient 1% (v/v) Triethylamine (TEA) in water: acetonitrile method, performed at room temperature, with the flow rate of 0.7 mL/min, at 254 nm and 298 nm wavelength and 1 µL injection volume, on BEH C 18 1.7 µm, 2.1 x 50 mm column.

4. Corneal permeation parameters calculation

At the end of the experiment, each cornea (free from adhering sclera) was weighed after soaking in de-ionized water. The wet cornea was dried overnight in oven, and reweighed. From the difference of weights, corneal hydration was calculated. The final results of drug permeation were expressed as cumulative amount permeated (Q). The parameters that were calculated are as follows:

$$\text{Cumulative amount permeated (Q, ng/cm}^2\text{) (t}_i\text{)} = \frac{\text{Conc.(t}_i\text{)} \times \text{Cell volume (mL)} + Conc.(t_{i-1}) \times 0.2(samplingvolume(mL))}{Effective\ area(cm^2)}$$

i= sampling number (1-10), Conc (t_0) = 0

dQ/dt [ng/cm^2/min]	Slope of cumulative amount curve
t_d [min]	Intercept on the time axis
Diffusion coefficient (D) [cm^2/sec]	$\frac{h^2}{6 \times t_d \times 60}$
Partition Coefficient (K) [-]	$\frac{dQ}{dt} \times \frac{h}{D. \times C_d} \times \frac{1}{60}$
h [cm]	Thickness of cornea: 0.04 [cm]
C_d [ng/mL]	Initial drug concentration in donor solution

Results and Discussion

The initial concentrations of Dorzolamide and Timolol determined by UPLC are given in Table 5.

Samples	Dorzolamide(mg/mL)	Timolol (mg/mL)
Test 1	23.02	4.70
Test 2	21.48	4.51
Test 3	22.21	4.72

Table 5: Initial concentration of test formulations

The corneal hydration was measured based on the net wet weight and dry weight of cornea. Typically, the % (w/w) hydrations for cornea in normal mammalian are in the range of 75-80%. Overall, there was no significant change in the % hydrations for all the test samples, and they were within the desired range in all the cases. Thus, the HP or Polysorbate 80 did not have impact on corneal hydration.

Sample	Final net wet weight (g)	Final net dry weight (g)	% (w/w) corneal hydration
Test 1	0.0107	0.0017	84.11
Test 1	0.0112	0.0019	83.06
Test 2	0.0123	0.0023	80.16
Test 2	0.0133	0.0023	82.70
Test 3	0.0150	0.0024	84.00
Test 3	0.0053	0.0012	77.40

Table 6: Percentage corneal hydration calculation.

Figures 10 and **11** reveal the corneal permeation profiles of Dorzolamide and Timolol, respectively. The time dependent permeation of Dorzolamide and Timolol was carefully

examined across the isolated rabbit cornea at 34 °C. The Dorzolamide cumulative total amount permeated through the cornea, and the total amount permeated after 3 hours was relatively higher for the test formulation containing 0.5% (w/v) HP compared to the control sample with no additives. Furthermore, the addition of Polysorbate 80 along with HP enhanced the corneal permeation with more amount of Dorzolamide permeated than the formulation containing only HP. Overall, the addition of 0.5% (w/v) HP and 1% (w/v) Polysorbate 80 enhanced the corneal permeation rate of Dorzolamide and Timolol by about 25-30%. A similar trend was also observed for Timolol (Figure 11). Thus, the combination of HP and Polysorbate 80 improved the corneal penetration of active ingredients.

Figure 12 shows the percentage total permeation of Dorzolamide and Timolol. Clearly, the presence of HP and Polysorbate 80 increased the percentage of active ingredients (Dorzolamide and Timolol) permeated through the cornea. It should be noted that all test formulations had similar initial concentrations in case of Dorzolamide and Timolol (less than 10% change). Thus, it was easy to determine the influence of each additive under similar pH conditions. In comparing test 2 with test 1, the significance of using HP as an additive is clearly demonstrated.

Figure 13 shows the corneal permeability coefficients of Dorzolamide and Timolol. The permeability coefficient was inversely proportional to the initial concentration of the drug in the donor solution. In the case of Dorzolamide, the permeability coefficients for test 2 and test 3 were higher, suggesting that Dorzolamide in the presence of 0.5% HP has enhanced corneal permeability rate compared to pH 5.65 control formulation (Test 1) containing no HP. Test 3 had relatively higher corneal permeability than Test 2, thereby indicating the influence of Polysorbate 80. The Polysorbate 80 may possibly act as a viscosity enhancer, thereby increasing

the bioavailability of Dorzolamide and Timolol for corneal permeation. Overall, the data from Figure 13 clearly indicates that the permeability coefficients of Timolol and Dorzolamide were higher for formulation tests 2 and 3 containing HP, and HP & Polysorbate 80, respectively, in comparison to the control test 1 without HP at pH 5.65 (similar to COSOPT® active ingredient formulation).

The diffusion coefficient of Dorzolamide and Timolol, which is inversely proportional to the lag time, did not change significantly by the addition of HP and Polysorbate 80 (see **Figure 14**). Thus, HP and Polysorbate 80 do not have any impact on the corneal surface. If the diffusion coefficient would have increased or decreased significantly, it would indicate the change in corneal surface properties. Since the diffusion coefficient is the inherent property of drug compound, it should not change with the addition of additives.

HP promotes encapsulation of Timolol and Dorzolamide, and thus enhances the partitioning of Timolol into corneal epithelium. This theory is also supported by the data in **Figure 15**. The Timolol and Dorzolamide partition coefficient to the corneal surface for Test 3 is higher than Test 1, indicating the improvement in partitioning of Timolol and Dorzolamide into lipophilic corneal membrane in presence of 0.5% (w/v) highly functional HP. Thus, the improved permeation in the presence of HP is mainly because of improved portioning to the epithelium. The partitioning could be further enhanced by increasing the concentration of HP in the formulation solution. HP enhances corneal permeation mainly because a) molecular encapsulation within the branched structures of highly functional Polyethyleneimine, b) electrostatic interactions between the drug molecules and ionic functional amine groups of HP, and c) the muco-adhesive behavior of charged HP.

The addition of 0.5% (w/v) HP and 1% (w/v) Polysorbate 80 enhanced the corneal permeation rate of Dorzolamide and Timolol by about 25-30%. The presence of HP increased the partitioning of Dorzolamide and Timolol at pH 5.65 into the corneal membrane. There was insignificant change in the corneal diffusion rate and corneal hydration rate by the addition of HP and Polysorbate 80, suggesting that these additives did not have a harmful impact on the cornea surface. The corneal permeability coefficients of Dorzolamide and Timolol were relatively higher in the presence of HP, suggesting the significance of HP as an effective drug carrier additive. Thus, the present inventors discovered a novel formulation with enhanced corneal permeation compared to the current market product. The corneal permeation could be further enhanced by increasing the concentration of HP.

Conclusion

The cumulative amount permeated of Dorzolamide and Timolol at pH 5.65 in the presence of additives such as HP was relatively high, compared to the control formulation with no additives (COSOPT® active ingredients formulation). The 0.5% (w/v) HP and 1% (w/v) Polysorbate 80 addition to the formulation enhanced the corneal permeation rate of Dorzolamide and Timolol by about 25-30%. The partitioning of active ingredients into the corneal epithelium increases in presence of HP. Thus, the combination of HP and Polysorbate 80 could be very effective for increasing the ocular bioavailability of COSOPT® active ingredients.

EXPERIMENTAL EXAMPLE 5

Solubility enhancement of Brinzolamide in aqueous solution containing HP (Lupasol® PS) or a combination of HP and Polysorbate 80, or PEG and Polysorbate 80 combinations at pH 7 in phosphate buffer.

The aqueous solubility of Brinzolamide in the presence of Timolol at pH 7 in 10 mM phosphate buffer was studied.

Methods

Content (%v/w)	Control Sample	S #1	S #2	S #3	S #4	S #5	S #6	S #7	S #8
Brinzolamide	1	>1	>1	>1	>1	>1	>1	>1	>1
Lupasol® PS (MW=750k)	-	0.5	1	2	0.5	1	2	-	-
PEG 400	-	-	-	-	-	-	-	2	-
PEG 8000	-	-	-	-	-	-	-	-	2
Polysorbate 80	-	-	-	-	1	1	1	1	1
In 10 mM citrate or phosphate buffer (add 1M NaOH)	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7	Adjust pH to 7

Table 7: Different Test formulations prepared in phosphate buffer at pH 7.

A suspension of Brinzolamide in phosphate buffer containing 1% (w/v) was prepared for the control sample. Similar suspensions containing excess of Brinzolamide (> 1% (w/v)) were also prepared in aqueous solution (10 mM phosphate buffer) containing different combinations of HP, PEG and Polysorbate 80 as per Table 7 above. The final pH was adjusted to 7 with 1 M NaOH. The suspension solutions were first stirred for 10 min at room temperature (with heating up to 60 °C for 5 minutes). After allowing the suspensions to equilibrate at room temperature for additional 30 minutes, the suspension solutions were then sonicated for 10 min and finally

filtered through 0.45 μm syringe filters. The filtrates were analyzed for Brinzolamide concentration using UPLC with the same condition as EXPERIMENTAL EXAMPLE1.

Results and Discussion

Figure 16 shows the Brinzolamide solubility in 10 mM phosphate buffer at different pH values. It is clear that the aqueous solubility of Brinzolamide decreases as the pH increases from 4 towards 7. The solubility of Brinzolamide is least at pH 7, consistent with the complete non-ionic behavior at pH 7. As % ionization of Brinzolamide increases with the increase in pH from 8.4 towards 10, the solubility increases steeply consistent with the anionic nature of Brinzolamide in that pH range. The solubility properties are very similar to Dorzolamide. Therefore, it is important to develop a lipophilic Brinzolamide drug with enhanced solubility close to pH 7.4 (pH of tear fluid is 7.44) in order to enhance ocular bioavailability and to decrease eye irritation appearance of Brinzolamide.

In this study, the present inventors used HP, PEG, and Polysorbate 80 as solubility enhancer additives. Different combinations were attempted at pH 7. In **Figure 17**, the solubility of Brinzolamide is shown to increase with the addition of additives.

As shown in Figure 17, the solubility of Brinzolamide increases with the increase in the concentration of HP in both the cases (with and without Timolol). The solubility of Brinzolamide in absence of Timolol with 0.5% (w/v) HP and 1% (w/v) Polysorbate 80 is about 11 mg/mL. The addition of PEG 8000 over PEG 400 seems to enhance the solubility of Brinzolamide. However, the solubility for control solution as well as all the formulations with additives containing 0.5% (w/v) Timolol was relatively lower. Thus, Timolol, which is relatively more soluble in water than Brinzolamide at pH 7, makes an impact on aqueous

solubility of Brinzolamide by its presence in the topical formulation sample. These results are very similar to the results (EXPERIMENTAL EXAMPLE 1) regarding another carbonic anhydrase called Dorzolamide. The decrease in solubility by highly soluble ionic Timolol at pH 7 could be due to change in ionic strength of the solution by addition of Timolol or salting out effect. While the market AZARGA® product has Brinzolamide 10 mg/mL+Timolol 5 mg/mL ophthalmic suspension at pH 7.4, the enhancement of solubility at pH 7 by addition of HP or PEG will have useful contribution to efficacy enhancement of drug by increasing the dosage to greater than 1%.

The addition of Polysorbate 80 to HP increases the Brinzolamide solubility by preventing the precipitation. Polysorbate 80 may act as a surfactant thereby reducing the aggregation of Brinzolamide after phase separation in presence of HP. A combination of 0.5% (w/v) HP and 1% (w/v) Polysorbate could be very effective in the presence of 0.5% (w/v) Timolol formulation at pH 7.

The improvement in aqueous solubility of Brinzolamide in presence of Timolol was significant with the additions of HP or a combination of PEG and Polysorbate 80 at pH 7. The Polysorbate 80 helps in dispersing the Brinzolamide molecules and inhibits the precipitation in water in presence of PEG. A combination of HP and Polysorbate 80 could be good combination for enhancement of Brinzolamide solubility in presence of Timolol at pH 7. From the results, it can be concluded that HP and Polysorbate 80 significantly enhance the solubility of hydrophobic Brinzolamide in presence of Timolol at pH 7. Hydrophilic PEG also turned out to be a Brinzolamide solubility enhancer. Furthermore, a combination of low concentrations of Polysorbate 80 and PEG 8000 also proved to be a very useful additive for enhancement of solubility of hydrophobic Brinzolamide. Overall, a formulation at pH 7 with optimized

concentration of HP and Polysorbate 80 could be very useful for increasing the ocular bioavailability.

Conclusion

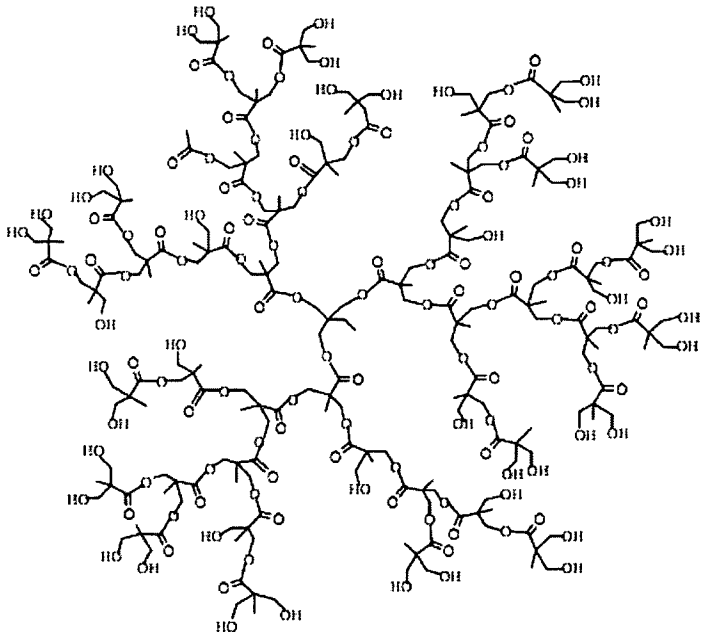
The results clearly indicate the advantages of using HP and Polysorbate 80 as hydrophobic Brinzolamide solubility enhancing additives at pH values closer to physiological pH. Polysorbate 80 also proved to be an effective emulsifier suppressing the precipitation of poorly soluble Brinzolamide at pH 7 in presence of HP. Timolol may have an effect on the solubility of Brinzolamide by changing the ionic strength of the solution.

EXPERIMENTAL EXAMPLE 6

In vitro corneal permeation study of Dorzolamide and Timolol containing a HP with terminal hydroxyl groups.

In vitro experiments on corneal permeation of Dorzolamide and Timolol (active ingredients of COSOPT®) were carried out to investigate the effect of the addition of a hyperbranched polyester with hydroxyl functional groups in comparison to the original market topical formulation (only active ingredients).

A novel formulation containing the commercially available HP called Boltorn® H20. The generic definition of Boltorn® H20 is a HP with polyester core and 16 terminal hydroxyl functional groups. It enhances the solubility of non-ionic (lipophilic) Dorzolamide that is formulated at pH 7 or pH 7.4.



Generic structure of hyperbranched polyester with hydroxyl terminal groups.

The properties of HP used in this example are listed in Table 8. It has 16 primary hydroxyl groups per molecule. The solid content is 100% (w/v).

Polymer name	Viscosity (cP)	Molecular weight (Daltons)	Polydispersity	pH	Partition Coefficient
Boltorn® H20	7	2100	1.3	2.5-4	-0.2 log POW

Table 8 : Properties of HP used in this example.

The *in vitro* transcorneal permeation of Dorzolamide and Timolol was determined from a novel formulation containing up to 2% (w/v) HP. The effect of the concentration of HP on the active ingredients was also determined. A standard solution containing COSOPT® active ingredients at pH 7.4 was used as a control sample.

Materials and Methods

Formulation Preparation

The following three solutions in 0.1% (w/v) phosphate buffer (Table 9) were formulated for examining the *in vitro* corneal permeation of Dorzolamide and Timolol, as well as for determining the corneal hydrolysis effect.

Content (% w/v)	Control Sample #1	Test Sample #2	Test Sample #3
Dorzolamide HCl	1	1	1
Timolol Maleate	0.683	0.683	0.683
Boltorn® H20	---	0.5	2
1M NaOH/ 1M HCl	Adjust pH to 7.4	Adjust pH to 7.4	Adjust pH to 7.4
Appearance	Suspension	Suspension	Suspension

Table 9: Composition of Test Formulations.

First, the 10 mM phosphate buffer was added to the appropriately weighed mass of solid active ingredients and stirred thoroughly for 15 minutes. Secondly, the effective volume of 5% (w/v) HP suspension solution was added to Test Sample 2 and Test Sample 3 to make up the exact concentrations described in Table 9.

The three test solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 5 minutes. Apart from the control solution (Control Sample #1), the solutions with HP were white slurry suspensions before adjusting the pH. After allowing the complete dilution of all the active and non-active ingredients, the pH was adjusted to 7.4 by using 1 M NaOH or 1 M HCl, and additional buffer was added to make up the exact composition as in Table 8. With the adjustment of pH, suspension solutions were formed in all cases, which were equilibrated by

stirring for an additional 15 hours or more at room temperature. The pH of all the sample solutions was measured again to confirm the final desired pH.

These suspension solutions were used directly as sample donor solutions for the cornea permeation study. In order to determine the solubility, the suspensions were filtered through 0.45 μm syringe filters. The filtrates were analyzed for Dorzolamide and Timolol concentrations using UPLC, after diluting each sample with ultrapure water (dilution factor = 1000). The *in vitro* cornea permeation profile results were also compared to the data obtained at pH 5.65 for the control sample containing active ingredients from EXPERIMENTAL EXAMPLE 4.

Three male rabbits (New Zealand) weighing 2 to 3 kg. Immediately after sacrifice by an overdose of inhaler isoflurane, the eyes were enucleated, and the corneas were separated for use in the permeation experiments. The details of the experimental procedure are described in previous EXPERIMENTAL EXAMPLE 4.

The calculated parameters that were calculated are as described in EXPERIMENTAL EXAMPLE 4, with C_d [ng/mL] being the initial drug concentration of active pharmaceutical ingredient in donor solution (Table 10).

Results and Discussion

The initial concentrations of Dorzolamide and Timolol determined by UPLC are given in Table 10. The percentage corneal hydration calculations are given in Table 10.

Samples	Dorzolamide (mg/mL)	Timolol (mg/mL)
Test 1	4.69	4.7
Test 2	6.6	4.5
Test 3	8.9	4.7

Table 10: Initial concentration of test formulations.

Sample	Final net wet weight (g)	Final net dry weight (g)	% (w/w) Corneal hydration
Test 1	0.0104	0.0020	80.7
Test 1	0.0124	0.0022	82.2
Test 2	0.0117	0.0025	78.6
Test 2	0.0126	0.0024	80.9
Test 3	0.0146	0.0029	80.1
Test 3	0.0113	0.0026	76.9

Table 11: Percentage corneal hydration calculation.

The corneal hydration was measured based on the net wet weight and dry weight of the cornea. Typically, the % hydrations for a cornea in a normal mammal are in the range of 75-80%. Overall, there was no significant change in the % hydrations for all the test samples, and they were within the desired range in all the cases. Thus, the HP did not have impact on corneal hydration.

Figures 18 and **19** reveal the corneal permeation profiles of Dorzolamide and Timolol, respectively. The control sample permeation profile at pH 5.65 from EXPERIMENTAL EXAMPLE 4 was also plotted along with the permeation profiles obtained for Test sample 1, 2 and 3. The time dependent permeation of Dorzolamide and Timolol was carefully examined across the isolated rabbit cornea at 34 °C. The Dorzolamide cumulative total amount permeated through the cornea, and the total amount permeated after 2 hours was relatively higher for the test formulation containing 0.5% (w/v) HP (Test 2) and 2% (w/v) HP (Test 3) compared to the control sample with no additives. Furthermore, the increased concentration from 0.5% HP to 2% HP showed an increase in the corneal permeation of both active ingredients. In the formulation containing 2% (w/v) HP (Test 3), the corneal permeation of active ingredients is higher than the market product COSOPT® (only active ingredients in the formulation) at pH 5.65 (see Figure 19

and Figure 20). In addition, the formulation containing 2% (w/v) HP provided significant enhancement in corneal permeation after 2 hours with a higher permeation rate (change in the slope).

Overall, the addition of a HP with hydroxyl functional groups enhances the corneal permeation rate of Dorzolamide and Timolol significantly, with an increase in the concentration of HP. Thus, HP improved the corneal penetration of active ingredients, when compared to the market products known as COSOPT® or TRUSOPT® or AZOPT®, which are used for glaucoma treatment.

Figure 20 shows the percentage total permeation of Dorzolamide and Timolol after 2 hours. Clearly, the presence of HP increased the percentage of active ingredients (Dorzolamide and Timolol) permeated through the cornea. It should be noted that all test formulations had different initial concentrations in the case of Dorzolamide, and similar concentrations of Timolol (less than 10% change). Different initial solubility of Dorzolamide is mainly because of the increased solubility by HP. In Test 2 and Test 3 in comparison with Test 1, the significance of using HP as an additive it is clear from Figure 20. The slopes from Figure 19 and 20 up to 2 hours were used in order to determine the corneal permeability, partition coefficient and diffusion coefficient.

Figure 21 shows the corneal permeability coefficients of Dorzolamide and Timolol. The permeability coefficient was inversely proportional to the initial concentration of the drug in the donor solution. In the case of both active ingredients, the permeability coefficients for Test 2 and Test 3 are higher, suggesting that dorozamide in the presence of HP has an enhanced corneal permeability rate compared to the control formulation (Test 1) containing no HP. Test 3 had a relatively higher corneal permeability than Test 2, thereby indicating the influence of increasing

the concentration of HP. Overall, the data from Figure 21 clearly indicates that the permeability coefficients of Timolol and Dorzolamide were higher for formulation Tests 2 and 3 containing HP, in comparison to the Control Test 1 without HP at pH 7.4, and the COSOPT® control formulation at pH 5.65 (similar to COSOPT® active ingredient formulation).

The diffusion coefficient of Dorzolamide and Timolol, which is inversely proportional to the lag time, did not change significantly by the addition of HP (see **Figure 22**). Thus, HP does not have any impact on the corneal surface. If the diffusion coefficient would have increased or decreased significantly, it would indicate the change in corneal surface properties. Since the diffusion coefficient is an inherent property of the drug compound, it should not change with the addition of additives.

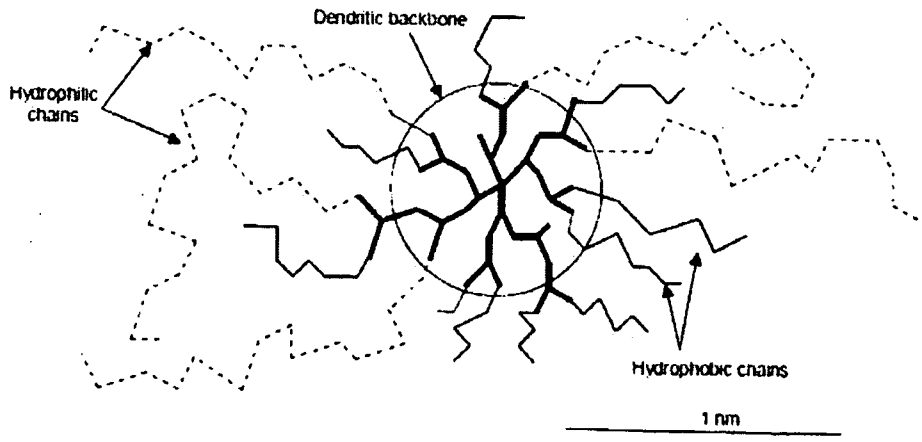
HP promotes the encapsulation of Timolol and Dorzolamide, and thus enhances the partitioning of Timolol and Dorzolamide into the corneal epithelium. This theory is also supported by the data suggested in **Figure 23**. The Timolol and Dorzolamide partition coefficient to the corneal surface for Tests 2 and 3 were higher than Test 1, indicating the improvement in partitioning of Timolol and Dorzolamide into lipophilic corneal membrane in the presence of highly functional (hydroxyl group) HP. Thus, the improved permeation in the presence of HP is mainly because of improved partitioning to the epithelium. The permeation was further enhanced by increasing the concentration of HP in the formulation solution from 0.5 % to 2% (w/v). However, the partition coefficient and permeability coefficient did not change significantly by increasing the concentration of HP, since these parameters will not be a function of the concentration of the material.

The cumulative amount permeated of Dorzolamide and Timolol at pH 7.4 in the presence of HP additives, such as commercially available Boltorn® H20 with hydroxyl functional group,

was relatively high, compared to the control formulation with no additives. The increase in concentration of such HP in the formulation enhanced the corneal permeation rate of Dorzolamide and Timolol significantly. The corneal permeability coefficients of Dorzolamide and Timolol were relatively higher in the presence of HP. The partitioning of active ingredients into the corneal epithelium increased in the presence of HP. Thus, an HP with hydroxyl functional groups could be very effective for increasing the ocular bioavailability of COSOPT® active ingredients.

EXPERIMENTAL EXAMPLE 7

The aqueous solubility of carbonic anhydrase inhibitors, such as Dorzolamide and Brinzolamide, in the presence of HP and Timolol at pH 7.4 in 10 mM phosphate buffer was studied. A HP called Boltorn® W3000 was used. The terminal functional groups of this HP are PEG (hydrophilic) and unsaturated long chain fatty acids. The model of the hyperbranched polyester used in this example is shown below.



Hyperbranched polyester with amphiphilic terminal groups.

The properties of HP used in this experiment are described in Table 12. The HP has 50 primary hydroxyl groups per molecule, and the solid content is 55% (w/w).

Polymer name	Viscosity (mPa-s)	Molecular weight (Daltons)	Polydispersity	pH	Acid number (mg KOH/g)
Boltorn® W3000	125	9000	1.3	3-5	10 (max)

Table 12: Properties of HP used in this study.

Table 13 shows the different test samples formulations which were prepared in 10 mM phosphate buffer at pH 7.4.

Content (%w/v)	Control Sample	S#1	S#2	S#3	S#4	S#5	S#6
CAI	>1	>1	>1	>1	>1	>1	>1
Boltorn® W 3000	-	0.1	0.5	1	2	5	2
Timolol maleate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC	-	-	-	-	-	-	0.5
In 10 mM phosphate buffer (add 1M NaOH)	Adjust pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4

Table 13: Different Test formulations prepared in phosphate buffer at pH7.

The emulsion was prepared by slowing dispersing water to the weighed amount of waxed Boltorn® W3000 to make 5% (w/v) emulsion with continuous stirring and heating at 60-70 °C for at least 30 minutes, followed by continuous vigorous stirring for an additional 15 hours, to obtain a homogeneous emulsion mixture in a flask.

10 mM phosphate buffer was added to the appropriately weighed mass of solid active ingredients and stirred thoroughly for 15 minutes. Secondly, the effective volume of 5% (w/v) HP emulsion solution was diluted appropriately to make up the exact concentrations described in Table 13. The sample test emulsion solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the emulsion solution was sonicated for 5 minutes. After allowing the complete emulsion of all the active and non-active ingredients, the pH was adjusted to 7.4 by using 1 M NaOH, and additional buffer was added to make up the exact compositions in Table 13. With the adjustment of the pH, the emulsion solutions were further equilibrated by stirring for an additional 15 hours or more at room temperature. The pH of all the sample emulsion solutions was measured again to confirm the final desired pH of 7.4. The filtrates were analyzed for CAI concentration using UPLC with the same condition as EXPERIMENTAL EXAMPLE1.

Results & Discussion

In this experiment, an amphiphilic self emulsifying HP was used as a solubility enhancer additive. Different concentrations of the HP were attempted at pH 7.4.

In **Figure 24**, the solubility of Brinzolamide and Dorzolamide is shown to increase with the increase in the concentration of HP. The solubility of Dorzolamide and Brinzolamide in the presence of 0.5% (w/v) Timolol with 5% (w/v) HP is about 2% (w/v) of CAI. The addition of HPMC to 2% (w/v) HP did not enhance the solubility. While the market AZARGA® suspension product has 1% (w/v) Brinzolamide at pH 7.4, and COSOPT® has 2% (w/v) Dorzolamide at pH 5.65, the enhancement of solubility at pH 7.4 by the addition of HP will have a useful contribution to the efficacy enhancement of the drug by increasing the dosage to greater than 1%

(w/v). Therefore, it is important to develop a Brinzolamide or Dorzolamide with enhanced solubility close to pH 7.4 (pH of tear fluid is 7.4) in order to enhance ocular bioavailability and to decrease eye irritation appearance of CAIs.

EXPERIMENTAL EXAMPLE 8

In vitro corneal permeation study of Dorzolamide and Timolol containing HP with amphiphilic functional groups (Boltorn® W3000).

In vitro experiments on corneal permeation of Dorzolamide and Timolol (active ingredients of COSOPT®) were carried out to investigate the effect of the addition of a HP with amphiphilic functional groups in comparison to the original market topical formulation (only active ingredients).

A new topical formulation containing Boltorn® W3000 (hyperbranched polyester) with non-ionic PEG as hydrophilic functional groups and unsaturated fatty acid as hydrophobic functional groups (commercially available), thus making it amphiphilic. The solubility of Dorzolamide was increased from 4.3 to 15 mg/mL by adding 2% (w/v) of this HP at pH 7.41. In this experiment, the *in vitro* transcorneal permeation of Dorzolamide and Timolol was determined from a novel formulation containing up to 2% (w/v) HP that was comparable to COSOPT®. The effect of concentration of HP on the active ingredients was also determined. A standard solution containing COSOPT® active ingredients at pH 7.4 was used as a control sample.

Materials and Methods

1. Formulation Preparation

The following three solutions in 0.1% (w/v) phosphate buffer (Table 14) were formulated for examining the *in vitro* corneal permeation of Dorzolamide and Timolol, as well as determining the corneal hydrolysis effect.

Content (% w/v)	Test 1	Test 2	Test 3
Dorzolamide HCl	1.5	1.5	2.22
Timolol Maleate	0.638	0.683	0.683
Boltorn® W3000	-	2	-
1 M NaOH/ 1 M HCl	Adjust pH to 7.4	Adjust pH to 7.4	Adjust pH to 5.65
Appearance	Suspension	Emulsion	Clear Solution

Table 14: Composition of test formulations

10 mM phosphate buffer was added to the appropriately weighed mass of the solid active ingredients, and stirred thoroughly for 15 minutes. Secondly, the effective volume of 5% (w/v) HP suspension solution was added to Test 2 to make up the exact concentrations as in Table 14. The three test solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 5 minutes. After allowing the complete dilution of all the active and non-active ingredients, the pH was adjusted to either 7.4 or 5.65 by using 1 M NaOH or 1 M HCl, and additional buffer was added to make up the exact compositions in the Table 15. With the adjustment of pH, the appearance was noted as per Table 14, and the formulations were further equilibrated by stirring for an additional 15 hours or more at room temperature. The pH of the all sample solutions was measured again to confirm the final desired pH. These formulations were used directly as sample donor solutions for the cornea permeation study. In order to determine the solubility, the suspension/emulsions were filtered through 0.45 μm syringe filters. The filtrates were analyzed for Dorzolamide and Timolol

concentration using UPLC after diluting each sample with ultrapure water (dilution factor = 1000).

2. In vitro rabbit corneal permeation experiment

The experimental procedure and analysis to be performed are described in detail in previous EXPERIMENTAL EXAMPLE 4. The parameters that were calculated are those described in EXPERIMENTAL EXAMPLE 4, where C_d [ng/mL] is the initial drug concentration of active pharmaceutical ingredient in donor solution (from Table 15).

Results and Discussion

The initial concentrations of Dorzolamide and Timolol determined by UPLC are given in Table 15. The percentage corneal hydration calculations are given in Table 16.

Samples	Dorzolamide (mg/mL)	Timolol (mg/mL)
Test 1	4.6	4.7
Test 2	15	4.7
Test 3	20	4.7

Table 15: Solubility of active pharmaceutical ingredient in test formulations.

Sample	Final net wet weight (g)	Final net dry weight (g)	% (w/w) corneal hydration
Test 1	0.0143	0.0028	80.41
Test 1	0.0281	0.0044	84.34
Test 2	0.0201	0.0035	82.59
Test 2	0.151	0.0028	81.46
Test 3	0.0265	0.0042	84.15
Test 3	0.0257	0.0043	83.27

Table 16: Percentage corneal hydration calculation.

The corneal hydration was measured based on the net wet weight and dry weight of the cornea. Typically, the % (w/w) hydrations for a cornea in a normal mammal are in the range of 75-80%. Tests 1 and 3 are both above 80%. However, there is no difference in the calculated values of partition and permeability coefficient, suggesting that there could not be any corneal damage due to higher hydration %. Overall, there was no significant change in the % hydrations for all the test samples, which were within the desired range in all the cases. Thus, the HP did not appear to have an impact on corneal hydration.

Figures 25 and **26** reveal the corneal permeation profiles of Dorzolamide and Timolol, respectively. The time dependent permeation of Dorzolamide and Timolol was examined carefully across the isolated rabbit cornea at 34 °C. The Dorzolamide cumulative total amount permeated through the cornea, and the total amount permeated after 2 hours was relatively higher for the test formulation containing 2% (w/v) HP (Test 2) compared to the control sample with no additive at pH 7.4 (Test 1). The total permeation of Dorzolamide of Test 2 (novel formulation) was comparable to the Test 3 permeation profile for Dorzolamide. However, it should be noted that Test 1 could be more comfortable for the patient since it is prepared at pH 7.4, compared to the market product which is prepared at pH 5.65. The permeation could be further increased by increasing the concentration of the HP.

Furthermore, the Timolol permeation significantly increased in the presence of HP for Test 2 compared to Tests 1 and 3 having similar aqueous solubility, unlike Dorzolamide (see Figure 26). In the formulation containing 2% (w/v) HP (Test 2), the corneal permeation of Timolol is almost two times higher than the market product COSOPT® (only active ingredients in the formulation) at pH 5.65 (see **Figure 27).** This result clearly demonstrates the importance of using HP as a drug carrier for a topical formulation, for both Dorzolamide and Timolol.

Overall, the addition of HP with amphiphilic functional group enhances the corneal permeation rate of Dorzolamide and Timolol significantly, with an increase in the concentration of HP. Thus, a dendritic polyester HP with amphiphilic functional groups improves the corneal penetration of active ingredients compared to the market products, known as COSOPT® or TRUSOPT® or AZOPT®, which are used for glaucoma treatment.

Figure 27 shows the percentage total permeation of Dorzolamide and Timolol after 3 hours. Clearly, the presence of HP increases the percentage of the active ingredients (Dorzolamide and Timolol) permeated through the cornea (see Test 2). Test 2, prepared at pH 7.4, had a Dorzolamide total % permeation which was slightly greater than Test 3, which is prepared at pH 5.65. It should be noted that all test formulations have different initial concentrations in case of Dorzolamide, and similar concentrations of Timolol. Different initial solubility of Dorzolamide is mainly because of the increased solubility by the HP. In Test 2, in comparison with Test 3 (pH 5.65) and Test 1 (pH 7.4), Figure 27 demonstrates the significance of using HP as an additive. The slopes from Figure 26 and 27 were used in order to determine the corneal permeability, partition coefficient and diffusion coefficient.

Figure 28 shows the corneal permeability coefficients of Dorzolamide and Timolol. The permeability coefficient is inversely proportional to the initial concentration of the drug in the donor solution. In the case of both active ingredients, the permeability coefficients for Test 2 are higher compared to the control samples at pH 7.4 and pH 5.65, thus suggesting that active pharmaceutical ingredient in presence of HP has enhanced corneal permeability rate compared to the control formulations (Tests 1 and 3) containing no HP. Test 3 had relatively higher corneal permeability than Test 2, thereby indicating the influence of pH. The active pharmaceutical ingredient at physiological pH is more conducive for permeation for similar solubilities. Overall,

the data from Figure 28 clearly indicates that the permeability coefficients of Timolol and Dorzolamide were higher for the formulation of Test 2 containing HP, in comparison to Test 1 without HP at pH 7.4, and COSOPT® control formulation at pH 5.65 (similar to COSOPT® active ingredient formulation).

The diffusion coefficient of Dorzolamide and Timolol, which is inversely proportional to the lag time did not change significantly by the addition of HP (see **Figure 29**). Thus, HP does not have any impact on the corneal surface. If the diffusion coefficient would have increased or decreased significantly, it would indicate the change in corneal surface properties. Since the diffusion coefficient is the inherent property of drug compound, it should not change with the addition of additives.

The Timolol and Dorzolamide partition coefficients to the corneal surface for Test 2 were higher than Tests 1 and 3, thus indicating the improvement in partitioning of Timolol and Dorzolamide into lipophilic corneal membrane in the presence of a highly functional (amphiphilic) HP. Thus, the improved permeation in the presence of HP is mainly because of improved partitioning to the epithelium. The permeation can be further enhanced by increasing the concentration of HP in the formulation solution from 2% to 5% (w/v).

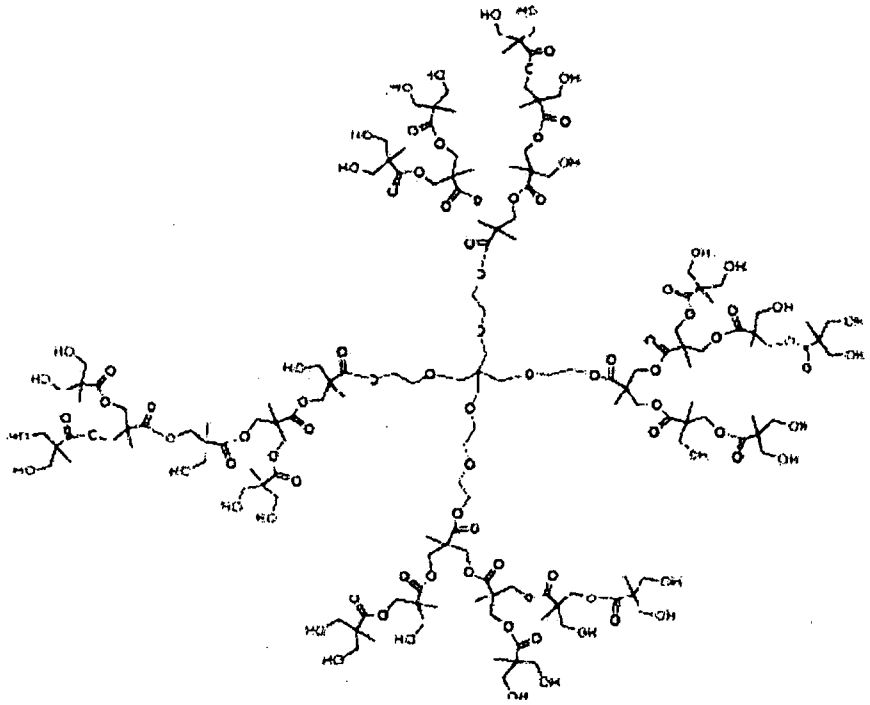
The cumulative amount permeated of Dorzolamide and Timolol at pH 7.4, in the presence of HP additives, such as commercially available Boltorn® W3000 with amphiphilic functional group (2% w/v), was almost 2 times higher compared to the control formulation at similar pH values, with no additives. The increase in concentration greater than 2% (w/v) of such HP in the formulation could further enhance the corneal permeation rate of Dorzolamide and Timolol significantly compared to the market product at pH 5.65. The novel topical formulation is prepared at pH 7.4, thus making it more conducive and comfortable for the

patients. The partitioning of active ingredients into the corneal epithelium increased in presence of HP. Thus, HP with amphiphilic functional groups could be very effective for increasing the ocular bioavailability of COSOPT® active ingredients.

EXPERIMENTAL EXAMPLE 9

Solubility enhancement of carbonic anhydrase inhibitor (CAI) by Bis-MPA polyester hyperbranched polymer (BMPA-HP) or a combination of PEG and BMPA-HP.

The influence of functionalized hyperbranched polymers on the aqueous solubility of a CAI, such as Dorzolamide and Brinzolamide, in the presence of Timolol at pH 7.4 in 10 mM phosphate buffer was studied. The generic definition of BMPA-HP is a hyperbranched polymer with dimethylolpropionic acid (Bis-MPA) polyester core and terminal hydroxyl (OH) functional groups. The number of terminal hydroxyl functional groups depends on the generation of the hyperbranched polyester. The generation is defined by the number of branching layers or the extent of branching from the core to the terminal functional groups. For example, the 2nd generation BMPA hyperbranched polyester contains 16 hydroxyl groups while the 3rd generation contains 32 hydroxyl groups. The structure of BMPA-HP is shown below.



Hyperbranched bis-MPA polyester generation 3. (3rd BMPA-HP)

The properties of the polyester HPs of different generations used in this research study are listed in Table 17.

Polymer name	Viscosity (Pa-s)	Molecular weight (Daltons)	Number of OH groups per monomer unit	pH
2nd BMPA-HP	0.007-0.25	1750	16	2.5-4
3rd BMPA-HP	0.007-0.25	3600	32	2.5-4

Table 17: Properties of HP used in this experiment

Materials and Methods

Table 18 shows the different test sample emulsions, except the control solution, to be prepared in 10 mM phosphate buffer at pH 7.4.

Content (%w/v)	Control Sample	S#1	S#2	S#3	S#4	S#5	S#6	S#7
CAI	>1	>1	>1	>1	>1	>1	>1	>1
2nd BMPA-HP	-	0.1	0.5	1	2	5	2	2
Timolol maleate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PEG 8000	-	-	-	-	-	-	2	4
In 10 mM phosphate buffer (add 1M NaOH)	Adjust pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4

Content (%w/v)	S#8	S#9	S#10	S#11	S#12	S#13	S#14
CAI	>1	>1	>1	>1	>1	>1	>1
3rd BMPA-HP	0.1	0.5	1	2	5	2	2
Timolol maleate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PEG 8000	-	-	-	-	-	2	4
In 10 mM phosphate buffer (add 1M NaOH)	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4

Table 18: Different test formulations prepared in phosphate buffer at pH 7.4

10 mM phosphate buffer was added to the appropriately weighed mass of solid active ingredients and stirred thoroughly for 15 minutes. Secondly, the effective volume of 5% (w/v) BMPA-HP solution was diluted appropriately to make up the exact concentrations described in Table 18. The sample test solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 5 minutes. After allowing the complete suspension of all the active and non-active ingredients, the pH was adjusted to 7.4 using 1 M NaOH, and additional buffer was added to make up the exact compositions described in Table 18. With the adjustment of pH, suspension solutions were further equilibrated by stirring for an additional 15 hours or more at room temperature. The pH of the all sample solutions was measured again to confirm the final desired pH of 7.4. The

filtrates were analyzed for CAI using UPLC with the same condition as EXPERIMENTAL EXAMPLE 1.

Results & Discussion

In this experiment, 2nd BMPA-HP or 3rd BMPA-HP were employed as solubility enhancer additives, and different concentrations of each were tested at pH 7.4. It is important to develop a lipophilic Brinzolamide or Dorzolamide drug with enhanced solubility close to pH 7.4 (pH of tear fluid is 7.4) in order to enhance ocular bioavailability and to decrease eye irritation appearance of CAIs. The solubility of Dorzolamide and Brinzolamide in the presence of 0.5% Timolol with 5% 3rd BMPA-HP is slightly about 1% (w/v) of CAI. With lower generation 2nd BMPA-HP, the solubility of CAI decreased by less than 10% in comparison to 3rd BMPA-HP for similar concentration of 2nd BMPA-HP used as an additive for all the samples.

When the combination of 2% (w/v) PEG 8000 and HP is used, the solubility of CAI increases dramatically (4 times more than the control). With the addition of 2% (w/v) PEG 8000 to 2% (w/v) BMPA-HP, the solubility of CAI almost doubled (see **Figure 31).**

While the market Azarga® suspension product has 1% Brinzolamide at pH 7.4 and COSOPT® has 2% (w/v) Dorzolamide at pH 5.65, the enhancement of solubility at pH 7.4 by the addition of BMPA-HP will have useful contribution to efficacy enhancement of the drug, by increasing the dosage to greater than 1% (w/v).

The results clearly indicate the advantages of using BMPA-HP as a hydrophobic CAI solubility enhancer at pH values closer to physiological pH. The addition of PEG to the solution containing BMPA-HP further improved the solubility of CAI.

EXPERIMENTAL EXAMPLE 10

Human Corneal Epithelium (HCE) tissue culture study of determining the eye irritancy of Bis-MPA hyperbranched polyester and the optimized sample application time for ophthalmic study based on the cytotoxicity of the cells.

Method and Materials

The samples were prepared in accordance with Table 19 below, in saline phosphate buffer (with the exception of AZOPT®) and sterilized using a 0.2 μm sterile syringe filter. 0.02% BAK was used as a positive control. The reconstructed human corneal epithelium was purchased from Skin Ethics laboratory (France).

Content (%w/v)	Blank Sample	+ve Control	-ve Control	S#1	S#2	S#3	S#4	S#5
Phosphate saline (pH 7.4)	Triplicates	-	-	-	-	-	-	-
0.02% Benzalkonium Chloride[i]	-	Triplicates	-	-	-	-	-	-
3% Bis MPA generation 3	-	-	Triplicates	-	-	-	-	-
4% Bis MPA generation 3	-	-	-	Triplicates	-	-	-	-
2% Bis MPA generation 2	-	-	-	-	Triplicates	-	-	-
3% Bis MPA generation 2	-	-	-	-	-	Triplicates	-	-
5% Bis MPA generation 2	-	-	-	-	-	-	Triplicates	-
Azopt®	-	-	-	-	-	-		Triplicates

Table 19: Sample set.

The Cell Culture Method is described below:

Transfer epithelium from agarose to maintenance medium 6-well plate
↓
Tissue conditioning: at least 2 hours at 37 °C, 5% CO_2, ≥ 95% humidity
↓
Transfer tissues to fresh maintenance medium in 24-well plates
↓

3 tissues each with 30 µL / 30 mg test substances
↓
Incubate at room temperature for 20 min or 40 min
↓
Rinse with PBS
↓
Transfer tissues to fresh maintenance medium
↓
Incubate at 37 °C, 5% CO_2, ≥ 95% humidity for 16 ± 1 hr
↓
Transfer tissues into MTT solution in culture medium (MTT incubation)
↓
Incubate tissues for 3 hours (37 °C, 5 % CO_2, ≥ 95%)
↓
Immerse the inserts in isopropanol (Formazan extraction)
↓
Extract formazan minimum 2 hours (at room temperature)
↓
Perforate the insert and homogenize
↓
Read OD in a plate spectrophotometer at 570 nm

The percentage viability of each of the treated cultures was calculated from the percentage MTT conversion in the test chemical treated cultures relative to the corresponding negative controls (100% viable).

The following equation was used:

Percentage viability = [individual $OD_{chemical}$/mean $OD_{negative\ control}$]x100.

HCE viability classification prediction model: NI (viability ≥ 60%), I (viability < 60%), i.e., the product is classified as an irritant (according to *in vivo* classifications) if the percentage of viability compared to the negative control obtained for the test product is < 60%.

Results and Discussion

As shown in **Figure 32**, the percentage viability of different samples (according to Table 19) was calculated using the equation for percentage viability described above, in the experimental section. Samples with viability of less than 60% were considered irritants. The standard deviation of the cell viability based on triplicates was less than 7% for all the samples. The results from the cytotoxicity study reveal that ophthalmic samples with up to 4% (w/v) of 3rd BMPA-HP will be a non-eye irritants, with greater than 60% cell viability. (See Figure 32.) The results from Figure 32 also reveal that AZOPT® could be cytotoxic against corneal epithelim cell with less than 50% cell viability for an application time of 1 hour. The sample containing 5% (w/v) Bis-MPA generation 2 also caused eye irritation with less than 60% cell viability.

Combining the results from previous studies, the cytotoxicity of Bis-MPA commercial hyperbranched polyester for different concentrations is revealed in **Figure 33**. It is evident from the Figure that the epithelial cell damage, and thus the eye irritation, caused by the 2nd BMPA-HP is less than the eye irritation caused by the 3rd BMPA-HP, for the same concentration in the ophthalmic solutions. It is known that the extent or length of branching and thus the molecular weight and number of terminal functional groups decrease with the decrease in the number of HP generations. The result from Figure 33 indicates that the decrease in cytotoxicity with the decrease in the number of generation could be mainly due to decrease in the prolonged interaction of the terminal functional groups with the epithelial cells.

Conclusions

The rate of epithelial cell death increased with the increase in the concentration dose and the generation (molar mass and extent of branching). In the case of the AZOPT® market product,

it causes eye irritation with less than 50% cell viability, possibly due to 0.01% (w/v) of BAK with exposure time of one hour.

EXPERIMENTAL EXAMPLE 11

Solubility enhancement of CAI containing BMPA-HP or a combination of non-ionic surfactants and BMPA-HP.

The aqueous solubility and stability of Dorzolamide was studied in the presence of Timolol at pH 7.4 in 10 mM phosphate buffer.

Method and Materials

First, the 10 mM phosphate buffer was added to the appropriate weighed mass of solid active ingredients and stirred thoroughly for 15 minutes. After complete dissolution of active pharmaceutical ingredient, hyperbranched 2nd BMPA-HP was added to the solution. After HP was dissolved, PEG 8000 was added as per the formulation concentration needed. The sample test solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 20 minutes. After allowing the complete dissolution of all the active and non active ingredients, the pH was adjusted to 7.4 by using 1 M NaOH and additional buffer was added to make up the exact compositions as per Tables 20 and 21. With the adjustment of pH, the formulations were further equilibrated by stirring for additional 15 hours or more at room temperature. The samples were filtered through 0.45 um syringe filter. Polysorbate 80 was added to the final formulation. In case of sample number 3, the formulation is equilibrated at 60 °C (24 hours) after pH adjustment, then Polysorbate 80 was added. The pH of all the sample solutions was measured again to confirm the final desired pH of 7.4. All samples were stored for 14 days at 25 and 60 °C, and the filtrates

were analyzed for Dorzolamide and Timolol using UPLC after diluting each sample with ultrapure water (dilution factor = 1000).

Results & Discussion

		Control Sample	S#1	S#2	S #3	S #4
Dorzolamide (mg/mL)	Initial	4.3	9.6	9.9	9.9	9.5
	1 W	4.3	8.9	9.5	9.9	8.2
	2 W	4.3	8.4	9.3	9.8	6.2
Timolol (mg/mL)	Initial	4.7	5.3	4.9	5.2	4.8
	1 W	4.6	5.0	4.7	5.1	4.8
	2 W	4.3	4.9	4.7	5.1	4.8
Appearance	Initial	Clear	Clear	Clear	Clear	Clear
	1 W	Clear	Suspension	Suspension	Clear	Suspension
	2 W	Clear	Suspension	Suspension	Clear	Suspension
pH	Initial	7.4	7.4	7.39	7.4	7.4
	1 W	7.4	7.4	7.38	7.4	7.39
	2 W	7.37	7.38	7.38	7.38	7.36

Table 20: Contents of Dorzolamide and Timolol, Appearance and pH of Samples at room temperature.

		Control Sample	S#1	S#2	S #3	S #4
Dorzolamide (mg/mL)	Initial	4.3	9.6	9.9	9.9	9.5
	1 W	4.3	8.9	9.5	9.9	8.2
	2 W	4.2	9.6	9.8	9.8	9.3
Timolol (mg/mL)	Initial	4.7	5.3	4.9	5.2	4.8
	1W	4.8	5.1	4.8	5.3	4.8
	2 W	4.8	5.4	4.8	5.6	4.6
Appearance	Initial	Clear	Clear	Clear	Clear	Clear
	1 W	Clear	Clear	Clear	Clear	Clear
	2 W	Clear	Clear	Clear	Clear	Clear
pH	Initial	7.40	7.40	7.39	7.40	7.40
	2 W	7.43	7.42	7.37	7.43	7.36

Table 21: Contents of Dorzolamide and Timolol, Appearance and pH of Samples at 60 °C.

In this study, 2nd BMPA-HP was studied as a solubility enhancer additive. Different combinations with non-ionic surfactants, such as PEG and Polysorbate 80, were attempted at pH 7.4. All the formulations were clear solutions at room temperature after sample preparation. In Figure 34, the solubility of Dorzolamide is shown to increase with the additions of 4% (w/v) HP and 2% (w/v) PEG. As shown in **Figure 34**, the concentration of Dorzolamide decreases steadily for the formulation containing 4% (w/v) HP and 2% (w/v) PEG from the 1st day up to 2 weeks. The formulation containing 4% (w/v) HP and 2% (w/v) PEG became a suspension after 2 weeks. The present inventors discovered that the rate of decrease of Dorzolamide concentration decreases with the addition of 1% (w/v) Polysorbate 80 (sample S#1). The concentration of Dorzolamide decreased from 9.6 mg/mL to 8.4 mg/mL over a time period of two weeks.

Thus, higher concentration of Polysorbate 80 prevents the precipitation and helps stabilize the new formulation. Negligible change was observed in the pH of all the formulations over a period of 2 weeks (See Table 20 and Table 21). The change in Timolol concentration was insignificant over a period of two weeks. While the market AZARGA® suspension product has 1% (w/v) Brinzolamide at pH 7.4, and COSOPT® has 2% Dorzolamide at pH 5.65, the enhancement of solubility at pH 7.4 by addition of HP and non-ionic surfactants will have useful contribution to efficacy enhancement of drug by increasing the dosage to greater than equal to 1% (w/v).

Conclusion

The results clearly demonstrate the advantages of using 2nd BMPA-HP in combination with PEG 8000 and Polysorbate 80 as CAI solubility enhancer at pH values closer to physiological pH.

EXPERIMENTAL EXAMPLE 12

A study to determine the topical formulation at pH 7.4, based on solubility and stability of carbonic anhydrase inhibitor (Dorzolamide and Brinzolamide) in the presence of Timolol in an aqueous solution containing different combinations of Hyperbranched bis-MPA polyester-16-hydroxyl, generation 2 (2nd BMPA-HP), PEG 8000 and Polysorbate 80 in phosphate buffer was performed .

Methods and Materials

Table 22 shows all the different test samples to be prepared in 10 mM phosphate buffer at pH 7.4.

Content (%w/v)	Control Sample	S#1	S#2	S #3	S #4	S #5	S#6
CAI	1	1	1	1	1	1	1
Timolol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2nd BMPA-HP	-	4	4	4	-	-	-
PEG 8000	-	2	-	2	2	2	-
Polysorbate 80	-	4	4	-	4	-	4
1M NaOH / 1 M HCl	Adjust pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4	pH to 7.4

Table 22: Different Test formulations prepared in phosphate buffer at pH 7.4.

First, the 10 mM phosphate buffer was added to the appropriately weighed mass of solid active ingredients and stirred thoroughly until the active pharmaceutical agent was dissolved. Secondly, solid BMPA-HP powder was added to the sample formulations 1, 2 and 3. After dissolution of the HP, the appearance was clear solution. The appropriate mass of non-ionic surfactants, such as PEG 8000, was added to the formulations to make up the exact concentrations as per the formulation content described in the above Table 22. All test solutions were then stirred for 10 minutes at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 5 minutes. After allowing the complete dissolution of all the active and non active ingredients, the pH was adjusted to 7.4 or 5.65 by using 1 M NaOH or 1 M HCl, and additional buffer was added to make up the exact compositions in accordance with the Table 22 sample compositions. With the adjustment of pH, test solutions were formed, which were equilibrated by stirring for an additional 24 hours or more at room temperature. For the samples that contain Polysorbate 80 and hyperbranched polyester (Table 2), Polysorbate 80 was only added after 24 hours of equilibration at 60 °C of the final formulation. The pH of the all the sample solutions was adjusted again to confirm the final desired pH.

The filtrates were analyzed for carbonic anhydrase inhibitors, Timolol and HP concentration using UPLC after diluting each sample with ultrapure water (dilution factor = 1000). The optimal conditions obtained from the EXPERIMENTAL EXAMPLE 1 for CAI and Timolol detection were used. The appearance and pH of each formulation is recorded over a period of 1 month at room temperature and 60 °C.

Results and Discussion

This study used a HP with hydroxyl terminal groups, PEG, and Polysorbate 80 as solubility enhancer additives. Different combinations were attempted at pH 7.4 in order to determine the best formulation based on the previous results. **Figure 35** demonstrates that the solubility of Dorzolamide increased significantly in the presence of 4% (w/v) HP and 2% (w/v) PEG or 4% (w/v) Polysorbate 80 (Sample 1, 2 and 3). However, the Dorzolamide solubility did not increase significantly in the presence of PEG 8000 (sample 5) or Polysorbate 80 (sample 6) exclusively, or their combination (sample 4). Thus, hyperbranched polyester addition to the formulation clearly indicates its advantage as a solubility enhancer. However, the formulation containing HP with hydroxyl group in combination with Polysorbate 80 (sample 2) has similar solubility in comparison to the formulation containing HP, Polysorbate 80 and PEG 8000 (sample 1). Thus, the addition of PEG 8000 could be avoided. Overall, it was discovered that sample 2 is the best formulation, based on the Dorzolamide and Timolol solubility data. The addition of surfactants such as Polysorbate 80 to HP also increases the Dorzolamide solubility by preventing the precipitation of Dorzolamide encapsulated within HP.

		Control	S#1	S #2	S#3	S#4	S#5	S#6
Dorzolamide (mg/mL)	Initial	4.6	10.4	10.1	9.1	5.4	5.2	5.0
	1 W	4.6	10.1	10.0	8.2	5.2	4.9	4.9
	2 W	4.6	10.1	10.1	6.2	5.1	4.8	4.8
	4W	4.5	10.1	10.1	5.9	5.0	4.8	4.6
Timolol (mg/mL)	Initial	5.2	5.1	5.1	4.9	5.1	5.2	5.0
	1 W	5.1	5.0	5.1	4.9	5.1	5.1	5.0
	2 W	5.0	4.9	5.1	4.9	5.1	5.1	5.0
	4 W	5	4.9	5.1	4.8	5.1	5.0	4.9
Appearance	Initial	suspension	clear	clear	clear	suspension	suspension	suspension
	1 W	suspension	clear	clear	suspension	suspension	suspension	suspension
	2 W	suspension	clear	clear	suspension	suspension	suspension	suspension
	4 W	suspension	clear	clear	suspension	suspension	suspension	suspension
pH	Initial	7.40	7.40	7.40	7.39	7.40	7.40	7.39
	1 W	7.40	7.40	7.40	7.39	7.40	7.80	7.39
	2 W	7.37	7.38	7.38	7.37	7.38	7.37	7.39
	4 W	7.35	7.36	7.37	7.37	7.37	7.36	7.38

Table 23: Contents of Dorzolamide and Timolol, appearance and pH of samples at room temperature.

		Control Sample	S#1	S #2	S#3	S#4	S#5	S#6
Dorzolamide (mg/mL)	Initial	4.6	10.4	10.1	9.1	5.4	5.2	5.0
	1 W	4.6	10.3	10.2	9.0	5.3	5.1	4.9
	2 W	4.5	10.3	10.2	8.9	5.3	4.9	4.8
	4 W	4.5	10.1	10.2	8.7	5.3	4.9	4.8
Timolol (mg/mL)	Initial	5.2	5.1	5.0	4.9	5.1	5.2	5.0
	1W	5.1	5.1	5.1	4.9	5.1	5.1	5.0
	2 W	4.9	4.9	5.0	4.9	5.1	5.1	5.0
	4 W	4.9	5.0	5.0	4.8	5.1	5.0	4.9
Appearance	Initial	suspension	clear	clear	clear	suspension	suspension	suspension
	1 W	suspension	clear	clear	clear	suspension	suspension	suspension
	2 W	suspension	clear	clear	clear	suspension	suspension	suspension
	4 W	clear	clear	clear	clear	suspension	suspension	suspension
pH	Initial	7.40	7.39	7.40	7.39	7.40	7.40	7.39
	2 W	7.43	7.37	7.43	7.41	7.38	7.38	7.38
	4 W	7.40	7.35	7.38	7.40	7.38	7.38	7.38

Table 24: Contents of Dorzolamide and Timolol, appearance and pH of samples at 60 °C.

Tables 23 and 24 demonstrate the stability test results of Dorzolamide and Timolol over a period of 4 weeks for all the formulation samples. It is evident from the Table that sample #1 and sample #2 are relatively stable and clear solutions after 1 month. The presence of PEG in sample #1 could be avoided since sample #2 without PEG gives similar results.

Conclusion

The results clearly indicate the advantages of using HP with hydroxyl terminal functional groups in combination with surfactants such as Polysorbate 80 and PEG 8000. The surfactant behavior of Polysorbate 80 is very helpful for increasing the solubility of CAI by preventing the precipitation. The good formulation based on the solubility and 1 month stability results is a formulation containing active ingredients with a combination of 4% (w/v) 2nd BMPA-HP and 4% Polysorbate 80 only in phosphate buffer at pH 7.4.

EXPERIMENTAL EXAMPLE 13

In vitro corneal permeation study of Dorzolamide and Timolol for novel topical formulation containing Bis MPA hyperbranched polyester and Polysorbate 80.

Methods and Materials

The following three solutions in 0.1% (w/v) phosphate buffer (Table 27) were formulated for examining the *in vitro* corneal permeation of Dorzolamide and Timolol, as well as for determining the corneal hydrolysis effect.

Content (% w/v)	Test 1	Test 2	Test 3
Dorzolamide	2	1	1
Timolol	0.5	0.5	0.5
2nd BMPA-HP	-	4	4
Polysorbate 80	-	4	4
1M NaOH/ 1M HCl	Adjust pH to 5.65	Adjust pH to 5.65	Adjust pH to 7.4
Appearance	clear	clear	clear

Table 25: Composition of test formulations.

First, the 10 mM phosphate buffer was added to the appropriately weighed mass of solid active ingredients and stirred thoroughly for 15 minutes. In case of sample numbers 2 and 3, 2nd BMPA-HP powder was added to the solution as per the composition in Table 25. The three test solutions were then stirred for 10 min at room temperature (with heating up to 60 °C for 5 minutes). After stirring, the solution was sonicated for 5 minutes. After allowing the complete dissolution of all the active and non active ingredients, the pH was adjusted to 7.4 or 5.65 by using 1 M NaOH or 1 M HCl and additional buffer was added to make up the exact composition as per the Table sample compositions. With the adjustment of pH, test solutions were formed which were equilibrated by stirring for additional 24 hours or more at room temperature for samples 1 and 2. In sample numbers 2 and 3, the equilibration was conducted for 24 hours at 60 °C. Polysorbate 80 (4% (w/v)) was then added followed by another pH adjustment. The pH of the all the sample solutions was measured again to confirm the final desired pH.

These clear solutions were used directly as sample donor solutions for the cornea permeation study. In order to determine the solubility, the test solutions were filtered through 0.45 μm syringe filters. The filtrates were then analyzed for Dorzolamide and Timolol concentration using UPLC after diluting each sample with ultrapure water (dilution factor =

1000). The details of the materials and equipment used, as well as the rabbit cornea study procedure are given in EXPERIMENTAL EXAMPLE 4.

Results and Discussion

Samples	Dorzolamide (mg/mL)	Timolol (mg/mL)
Test 1	20.2	5.2
Test 2	10.2	5.1
Test 3	10.0	5.0

Table 26: Initial concentration of active ingredients in test samples.

The initial concentrations of Dorzolamide and Timolol determined by UPLC are given in Table 26.

Sample	Final net wet weight (g)	Final net dry weight (g)	% (w/v) corneal hydration
Test 1	0.0534	0.0534	80.42
Test 2	0.0471	0.0471	82.59
Test 3	0.0627	0.0550	84.15
Test 1	0.0684	0.0420	84.34
Test 2	0.0459	0.0365	81.46
Test 3	0.0421	0.0370	83.27

Table 27: Percentage corneal hydration calculation.

The corneal hydration was measured based on the net wet weight and dry weight of the cornea. Typically, the % (w/w) hydrations for cornea in normal mammalian are in the range of 75-85%. Overall, there was no significant change in the % hydrations for all the test samples,

and all were within the desired range, as shown in Table 27. Thus, the HP and Polysorbate 80 did not have impact on corneal hydration.

Figure 36 and **Figure 37** reveal the corneal permeation profiles of Dorzolamide and Timolol, respectively. The time dependent permeation of Dorzolamide and Timolol was examined carefully across the isolated rabbit cornea at 34 °C. From Figure 36, the Dorzolamide cumulative total amount permeated through the cornea and the total amount permeated after 3 hours was relatively higher for the test formulation containing 4% (w/v) HP and Polysorbate 80 at pH 7.4 (Test 3) compared to the same formulation at pH 5.65 (Test 2). Clearly, Dorzolamide penetration is enhanced at pH 7.4 because of its non-ionic behavior which is very conducive for cornea epithelial membrane that is lipophilic. Dorzolamide cornea permeation for Test 3 is comparable to the control solution with no additives at pH 5.65 (Test 1). Notice that Test 3 contains 1% (w/v) Dorzolamide while Test 1 contains 2% (w/v) Dorzolamide. While Test 3 contains half the Dorzolamide concentration of Test 1, the cornea permeation profiles are similar. Test 3 could be more comfortable for the patient since it is prepared at pH 7.4 compared to the market product at pH 5.65 that can cause eye irritation. Timolol permeation profiles from Figure 37 suggest that Test 3 has significantly higher cornea permeation compared to Test 1 (control solution). Clearly, Timolol permeation is enhanced by the presence of hyperbranched polyester and Polysorbate 80 in Test 3 at pH 7.4 compared to Test 1 having similar aqueous solubility. Overall, enhanced Timolol permeation and comparable Dorzolamide permeation to the market product are key advantages of new formulation containing HP and Polysorbate 80. From **Figure 38**, the total percentage permeation of Dorzolamide and Timolol after 3 hours in case of Test 3 (novel formulation) was at least 2 times higher than Test 1 (market product active

ingredients). Clearly, the presence of HP and Polysorbate 80 increase the percentage of active ingredients (Dorzolamide and Timolol) permeated through the cornea.

The slopes from Figure 36 and 37 were used in order to determine the corneal permeability coefficient and partition coefficient. The permeability coefficient is inversely proportional to the initial concentration of the drug in the donor solution. The permeability coefficients (see **Figure 39**) and partition coefficients (see **Figure 40**) of Test 2 are relatively higher than Test 1. Notice that both Test 1 and Test 2 solutions are at pH 5.65. The result suggests the influence of HP and Polysorbate 80 to enhance the partitioning and permeations of active ingredients. The permeability coefficient and partition coefficient of Test 3 is higher than Test 2 due to the pH effect. Test 3 formulation is prepared at pH 7.4 which is more conducive for permeation of non-ionic Dorzolamide and Timolol. Overall, the results clearly demonstrate the importance of using HP and Polysorbate 80 as drug carrier for a topical formulation for both Dorzolamide and Timolol.

Overall, the data from Figure 39 clearly indicates that the permeability coefficients of Timolol and Dorzolamide were higher for formulation Test 2 containing HP and Polysorbate 80, in comparison to the Test 1 without HP at pH 7.4 and COSOPT® control formulation at pH 5.65 (similar to COSOPT® active ingredient formulation).

HP and Polysorbate 80 promote encapsulation of Timolol and Dorzolamide, and thus enhance the partitioning of Timolol into corneal epithelium. This theory is also supported by the data in Figure 40. The Timolol and Dorzolamide partition coefficient to the corneal surface for Test 3 at pH 7.4 was the highest than Test 1 and Test 2 indicating the improvement in partitioning of Timolol and Dorzolamide into lipophilic corneal membrane in presence of highly

functional HP at pH 7.4 rather than pH 5.65. Thus, the improved permeation in presence of HP is mainly because of improved partitioning to the epithelium.

Conclusion

The cumulative amount permeated of active pharmaceutical agent at pH 7.4 in the presence of HP additives such as commercially available dendritic Bis-MPA HP and Polysorbate 80 was almost comparable to the market product in case of Dorzolamide and more than 2 times higher for Timolol permeation. This novel topical formulation is prepared at pH 7.4, thus making it more conducive for lipophilic epithelial cornea penetration and comfortable for the patients. Thus, HP with hydroxyl terminal groups and Polysorbate 80 could be very effective for increasing the ocular bioavailability of COSOPT® active ingredients.

INDUSTRIAL APPLICABILITY

According to the present invention, an ophthalmic composition comprising a HP, which shows increased aqueous solubility of carbonic anhydrase inhibitors, such as Dorzolamide or Brinzolamide, can be provided. The ophthalmic composition may also comprise a non-ionic surfactant and/or a beta-blocker. The ophthalmic compositions of the present invention result in increased permeation of the active agent into the cornea. Therefore, the overall ocular bioavailability and hence the therapeutic activity of the topical ophthalmic solution containing a carbonic anhydrase inhibitor and beta blocker (active ingredients) can be increased compared to current relevant ophthalmic market products available. The topical ophthalmic compositions presented in this invention provide more potent anti-glaucoma compositions that may increase patient compliance by increasing ocular bioavailability.

While some of the embodiments of the present invention have been described in detail in the above, those of ordinary skill in the art can enter various modifications and changes to the particular embodiments shown without substantially departing from the novel teaching and advantages of the present invention. Such modifications and changes are encompassed in the spirit and scope of the present invention as set forth in the appended claims.

The invention claimed is:

(1) An ophthalmic composition comprising a hyperbranched polymer, wherein the hyperbranched polymer comprises a terminal functional group selected from the group consisting of an amine group, a hydroxyl group, a fatty acid group, and PEG.

(2) The ophthalmic composition according to claim 1, further comprising a carbonic anhydrase inhibitor.

(3) The ophthalmic composition according to claim 1, further comprising a non-ionic surfactant.

(4) The ophthalmic composition according to claim 2, further comprising a non-ionic surfactant.

(5) The ophthalmic composition according to claim 1, wherein the average molecular weight of the hyperbranched polymer is in the range from 1,000 to 750,000 Daltons (M_w).

(6) The ophthalmic composition according to claim 1 or 2, wherein the hyperbranched polymer comprises a core selected from the group consisting of Polyethylenimine, Polypropylenimine, and polyester.

(7) The ophthalmic composition according to claim 1, wherein the pH is in the range from 3.0 to 8.0.

(8) The ophthalmic composition according to claim 1, wherein the concentration of the hyperbranched polymer is in the range from 0.01% to 5% (w/v).

(9) The ophthalmic composition according to claim 2, further comprising a beta-blocker.

(10) The ophthalmic composition according to claim 2, wherein the carbonic anhydrase inhibitor is selected from the group consisting of Dorzolamide, Brinzolamide and

Acetazolamide.

(11) The ophthalmic composition according to claim 3, wherein the non-ionic surfactant is selected from the group consisting of PEG, polysorbate, Hydroxyl Propyl Methyl Cellulose, and Hydroxy Ethyl Cellulose.

(12) The ophthalmic composition according to claim 4, wherein the non-ionic surfactant is selected from the group consisting of PEG, Polysorbate, Hydroxyl Propyl Methyl Cellulose, and Hydroxy Ethyl Cellulose.

(13) The ophthalmic composition according to claim 9, wherein the beta-blocker is selected from the group consisting of Carteolol, Levobunolol, Betaxolol, Metipranolol, Timolol and Propranolol.

(14) The ophthalmic composition according to claim 6, wherein the hyperbranched polymer core is polyester, and wherein the hyperbranched polymer comprises a hydroxyl group, a fatty acid group, and PEG as terminal functional groups.

(15) The ophthalmic composition according to the claim 14, wherein the average molecular weight of the hyperbranched polymer is in the range from 1,000 to 12,000 Daltons (M_w).

(16) The ophthalmic composition according to claim 14, wherein the concentration of the hyperbranched polymer is in the range from 0.001 to 4% (w/v).

(17) An ophthalmic composition comprising a hyperbranched polyester, Timolol, Dorzolamide, and Polysorbate 80, wherein the hyperbranched polyester comprises a terminal functional group selected from the group consisting of a polyester hydroxyl group, a fatty acid group, and PEG.

(18) An ophthalmic composition comprising a hyperbranched polyester, Timolol, Brinzolamide, and Polysorbate 80, wherein the hyperbranched polyester comprises a terminal functional group selected from the group consisting of a polyester hydroxyl group, a fatty acid group, and PEG.

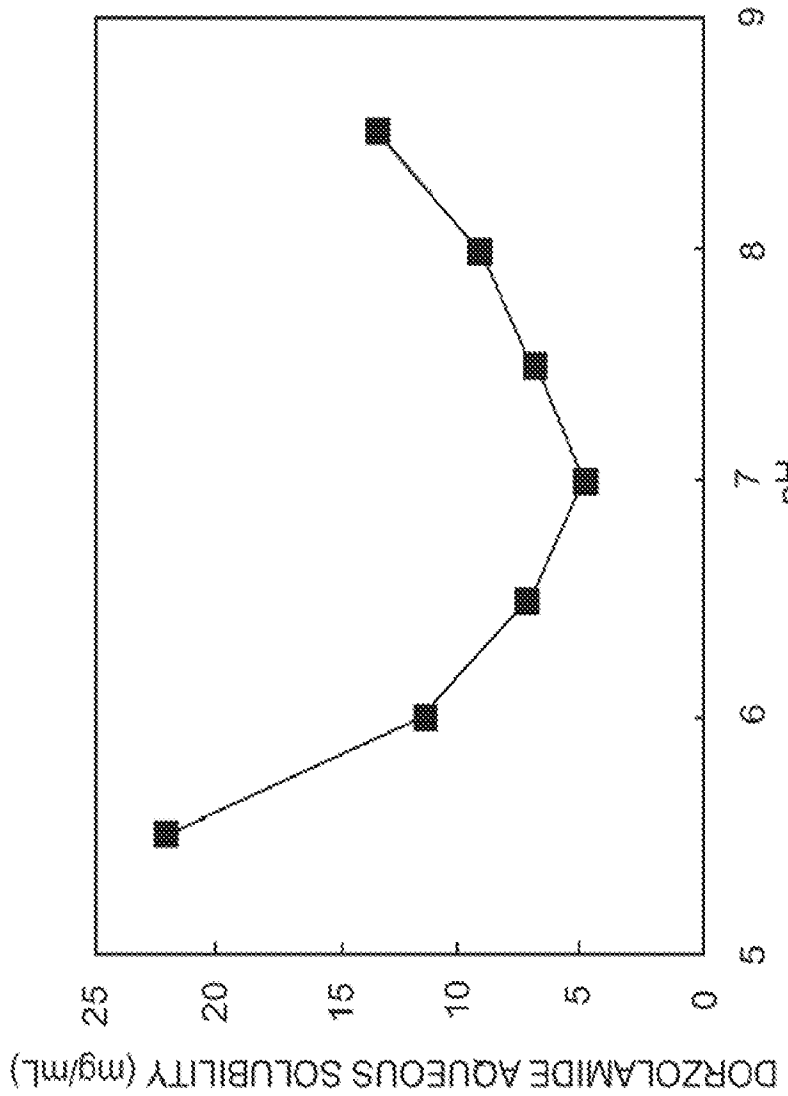


FIG. 1

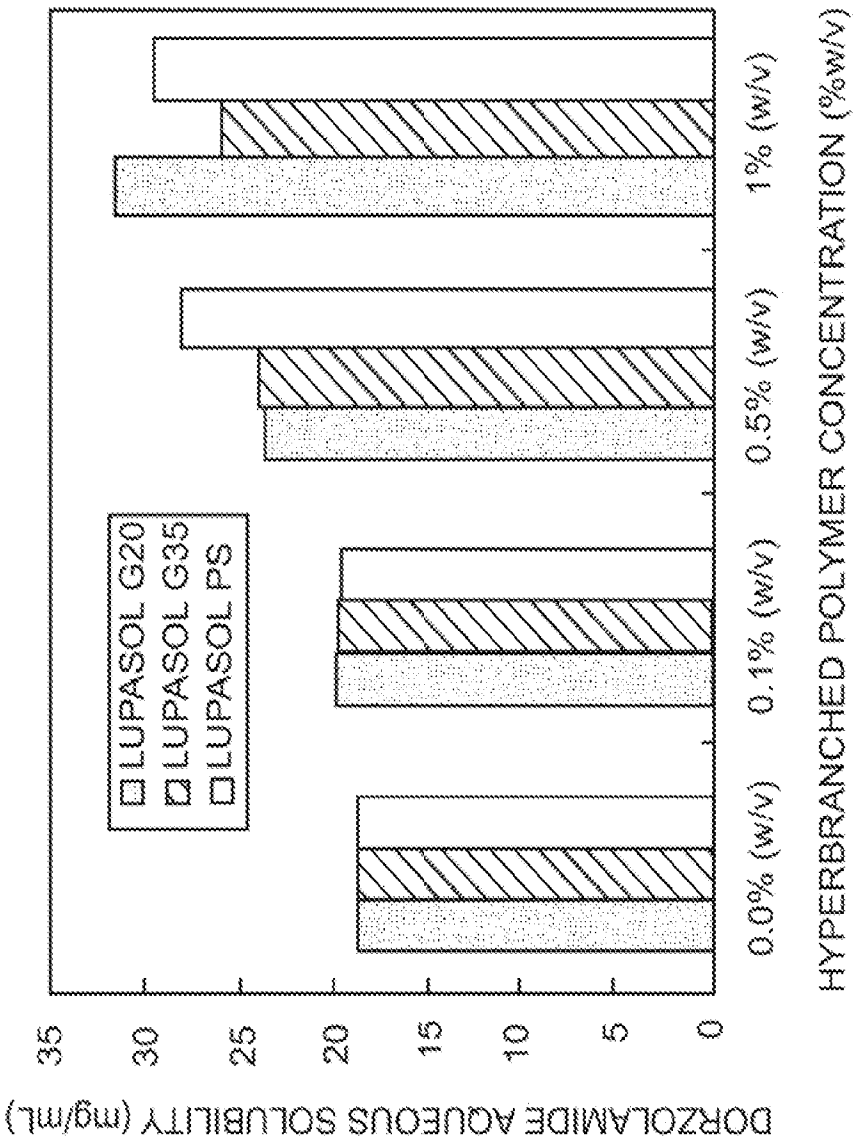

DORZOLAMIDE AQUEOUS SOLUBILITY (mg/mL)
0
5
10
15
20
25
30
35
LUPASOL G20
LUPASOL G35
LUPASOL PS
0.0% (w/v)
0.1% (w/v)
0.5% (w/v)
1% (w/v)
HYPERBRANCHED POLYMER CONCENTRATION (%w/v)

FIG. 2

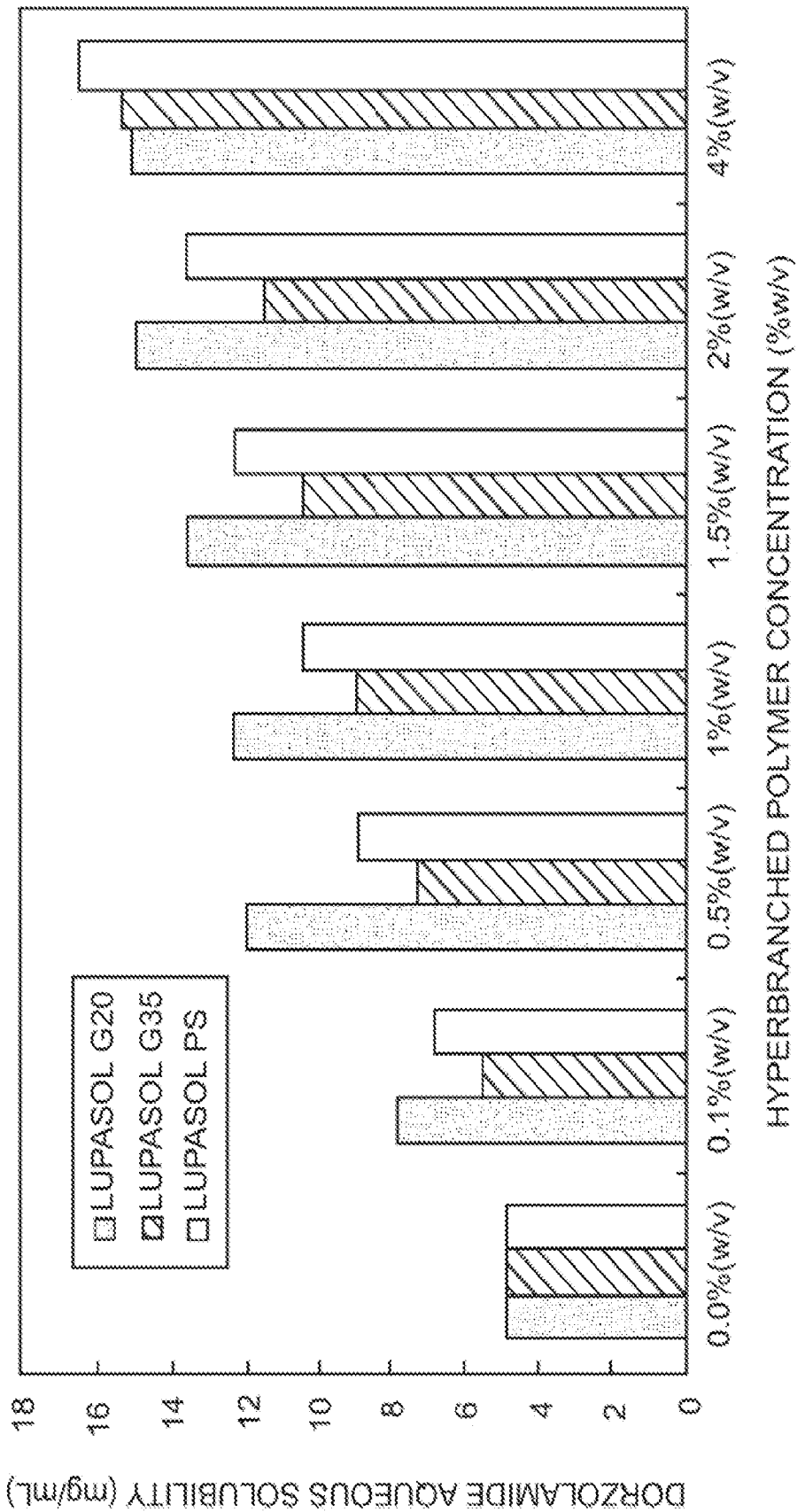

DORZOLAMIDE AQUEOUS SOLUBILITY (mg/mL)
0
2
4
6
8
10
12
14
16
18
HYPERBRANCHED POLYMER CONCENTRATION (%w/v)
0.0%(w/v)
0.1%(w/v)
0.5%(w/v)
1%(w/v)
1.5%(w/v)
2%(w/v)
4%(w/v)
LUPASOL G20
LUPASOL G35
LUPASOL PS

FIG. 3

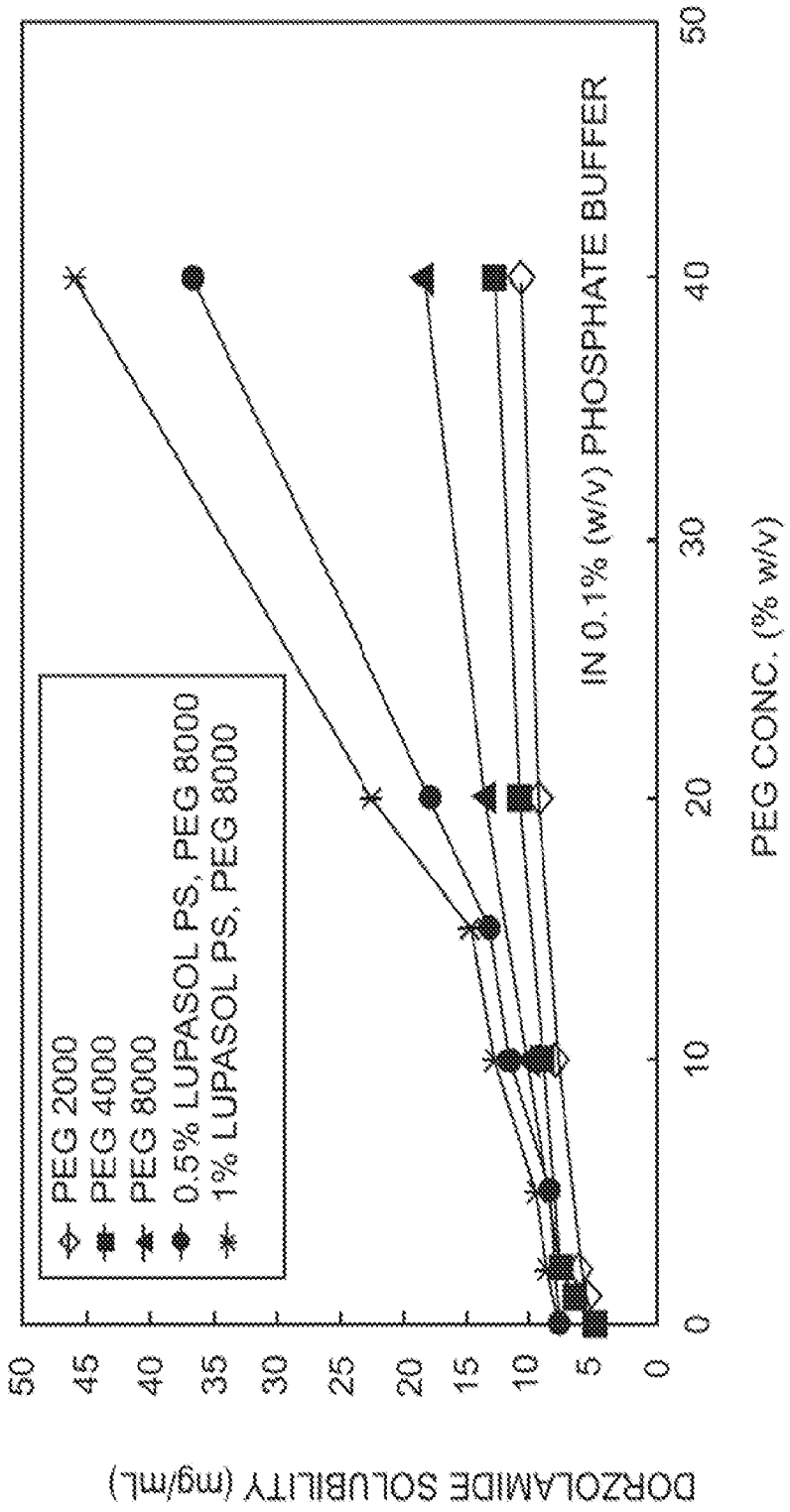

DORZOLAMIDE SOLUBILITY (mg/mL)
0
5
10
15
20
25
30
35
40
45
50
PEG CONC. (% w/v)
0
10
20
30
40
50
IN 0.1% (w/v) PHOSPHATE BUFFER
PEG 2000
PEG 4000
PEG 8000
0.5% LUPASOL PS, PEG 8000
1% LUPASOL PS, PEG 8000

FIG. 4

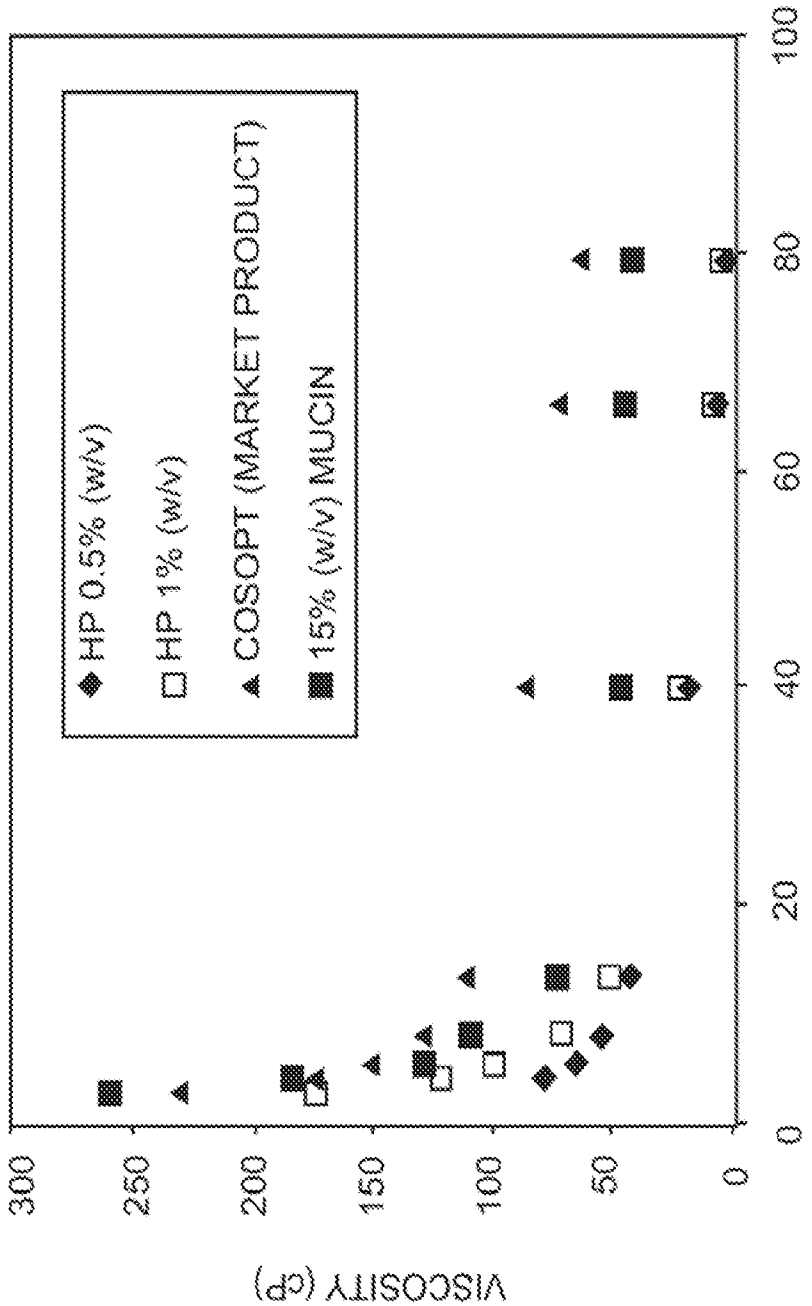

HP 0.5% (w/v)
HP 1% (w/v)
COSOPT (MARKET PRODUCT)
15% (w/v) MUCIN
VISCOSITY (cP)
SHEAR RATE (s^{-1})
0
50
100
150
200
250
300
0
20
40
60
80
100

FIG. 5

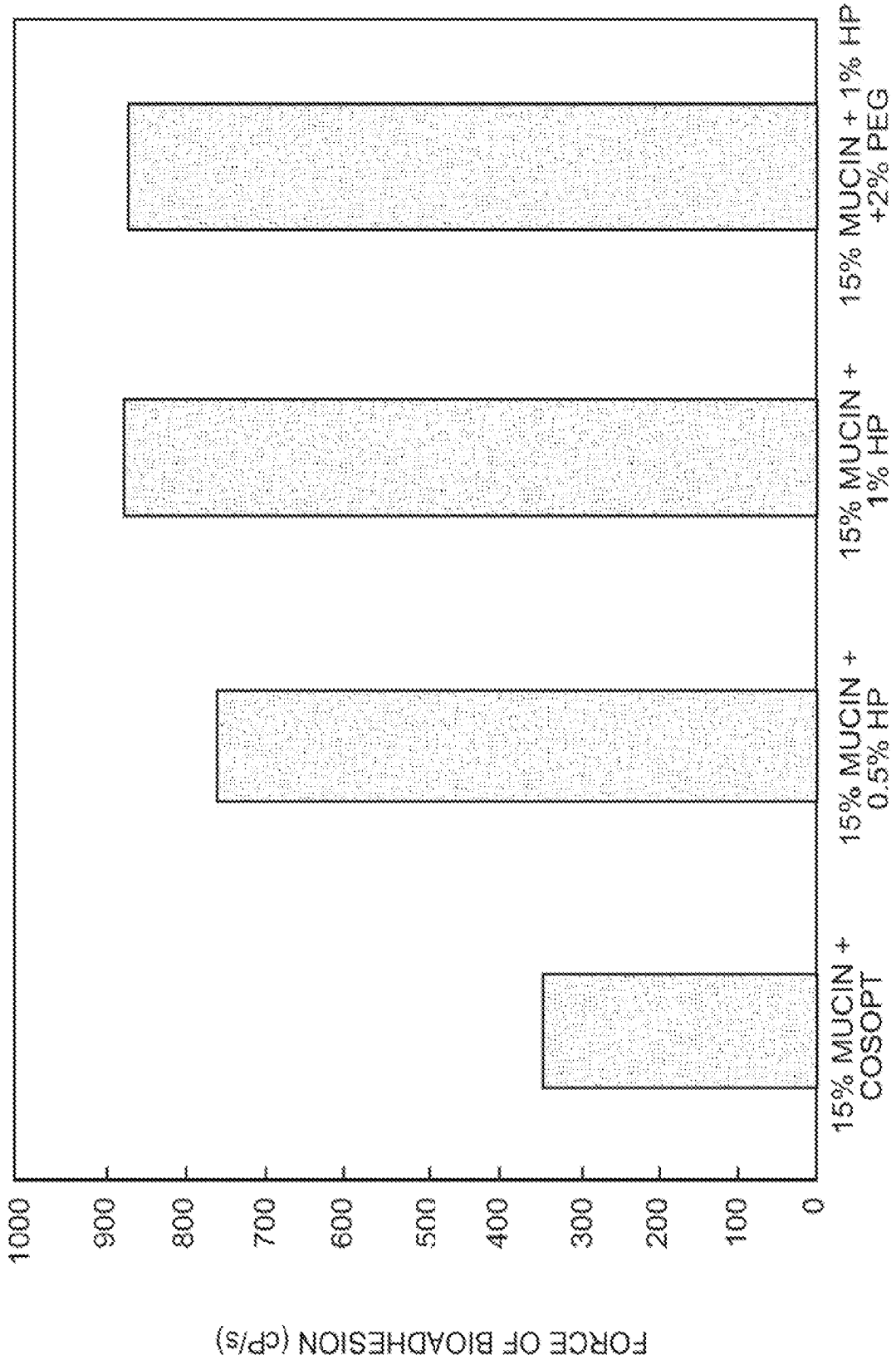

FORCE OF BIOADHESION (dP/s)
0
100
200
300
400
500
600
700
800
900
1000
15% MUCIN + COSOPT
15% MUCIN + 0.5% HP
15% MUCIN + 1% HP
15% MUCIN + 1% HP +2% PEG

FIG. 6

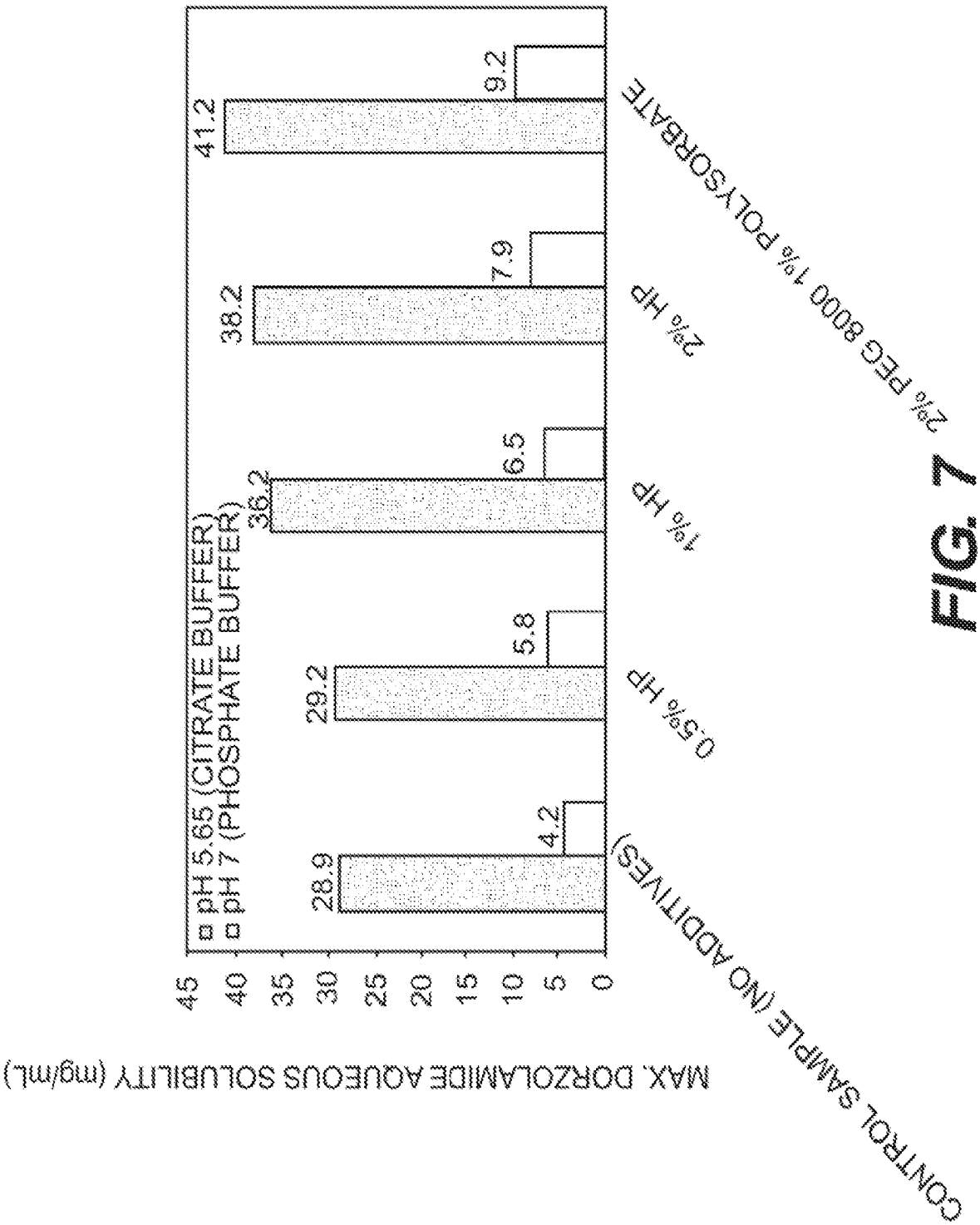

MAX. DORZOLAMIDE AQUEOUS SOLUBILITY (mg/mL)
0
5
10
15
20
25
30
35
40
45
pH 5.65 (CITRATE BUFFER)
pH 7 (PHOSPHATE BUFFER)
28.9
4.2
29.2
5.8
36.2
6.5
38.2
7.9
41.2
9.2
CONTROL SAMPLE (NO ADDITIVES)
0.5% HP
1% HP
2% HP
2% PEG 8000 1% POLYSORBATE

FIG. 7

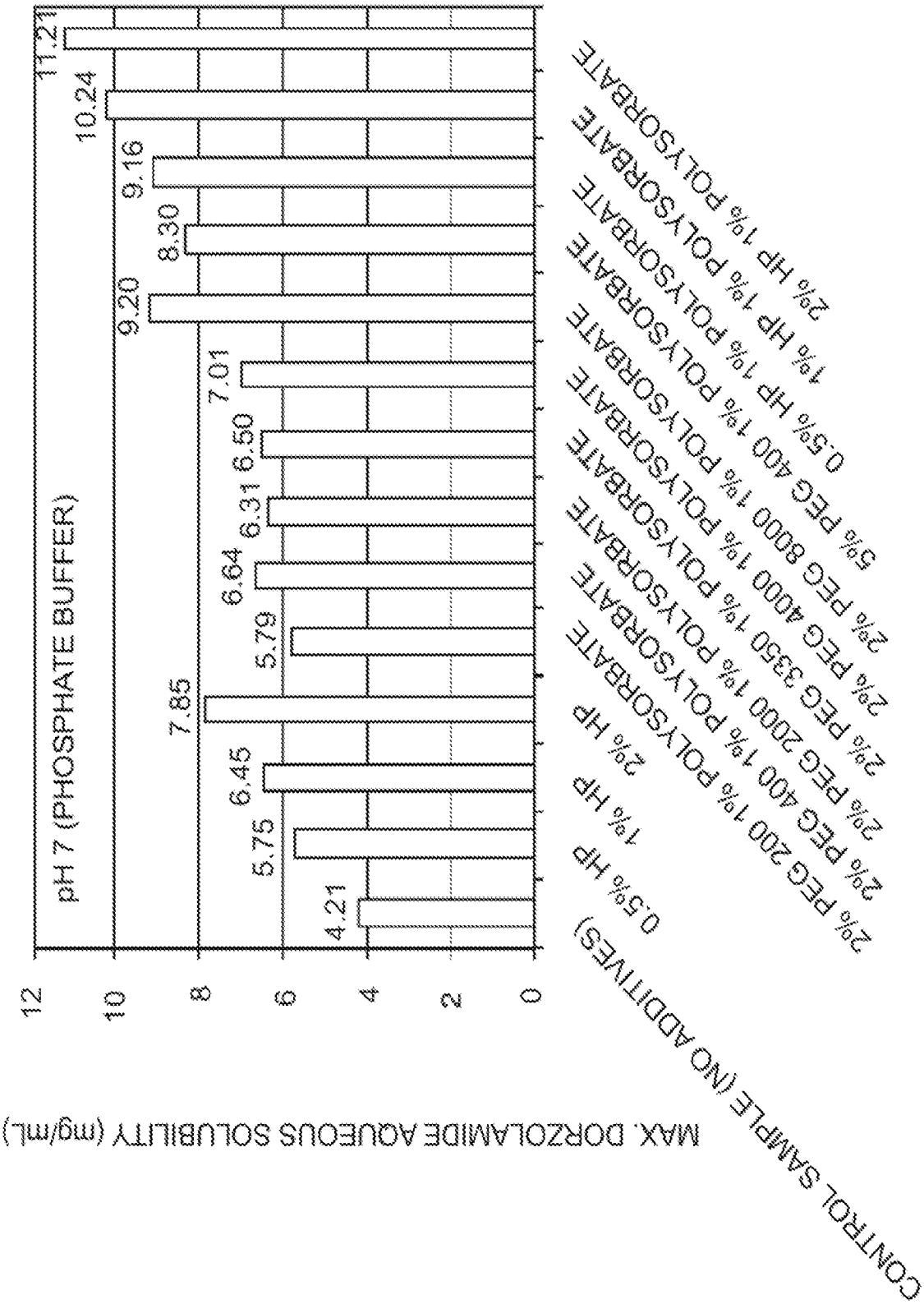

MAX. DORZOLAMIDE AQUEOUS SOLUBILITY (mg/mL)
12
10
8
6
4
2
0
pH 7 (PHOSPHATE BUFFER)
11.21
10.24
9.16
8.30
9.20
7.01
6.50
6.31
6.64
5.79
7.85
6.45
5.75
4.21
CONTROL SAMPLE (NO ADDITIVES)
0.5% HP
1% HP
2% HP
2% PEG 200 1% POLYSORBATE
2% PEG 400 1% POLYSORBATE
2% PEG 2000 1% POLYSORBATE
2% PEG 3350 1% POLYSORBATE
2% PEG 4000 1% POLYSORBATE
2% PEG 8000 1% POLYSORBATE
5% PEG 400 1% POLYSORBATE
0.5% HP 1% POLYSORBATE
1% HP 1% POLYSORBATE
2% HP 1% POLYSORBATE

FIG. 8

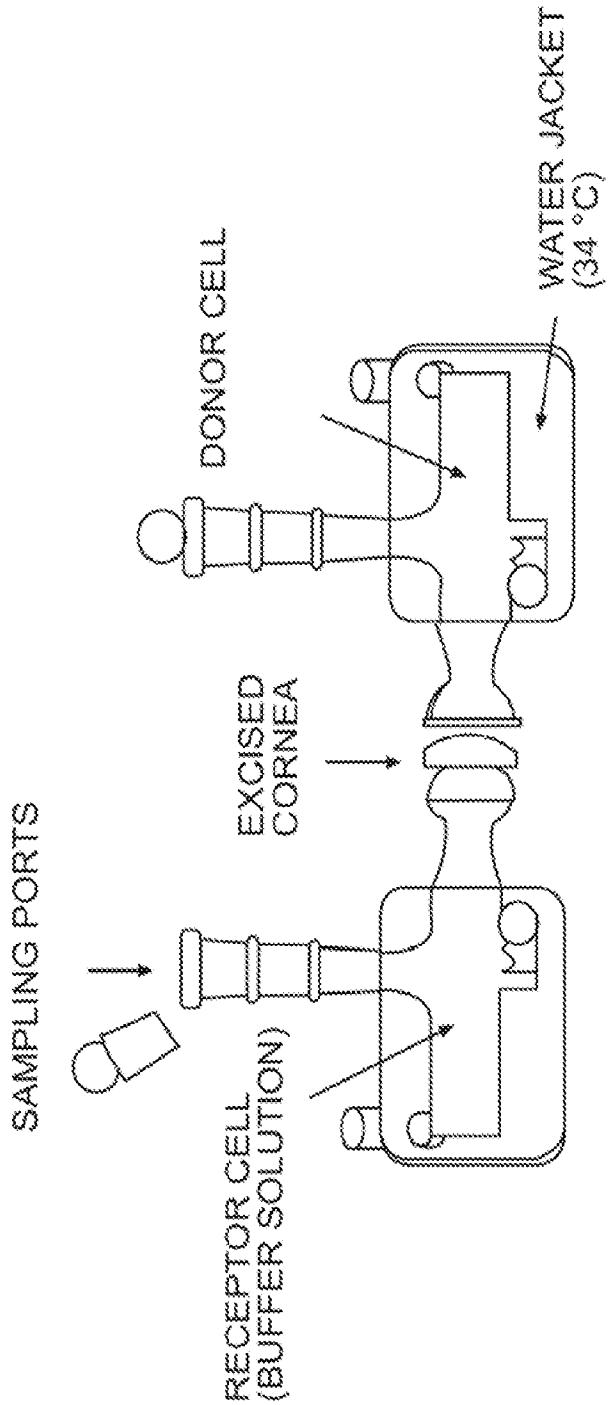

SAMPLING PORTS
RECEPTOR CELL
(BUFFER SOLUTION)
EXCISED
CORNEA
DONOR CELL
WATER JACKET
(34 °C)

FIG. 9

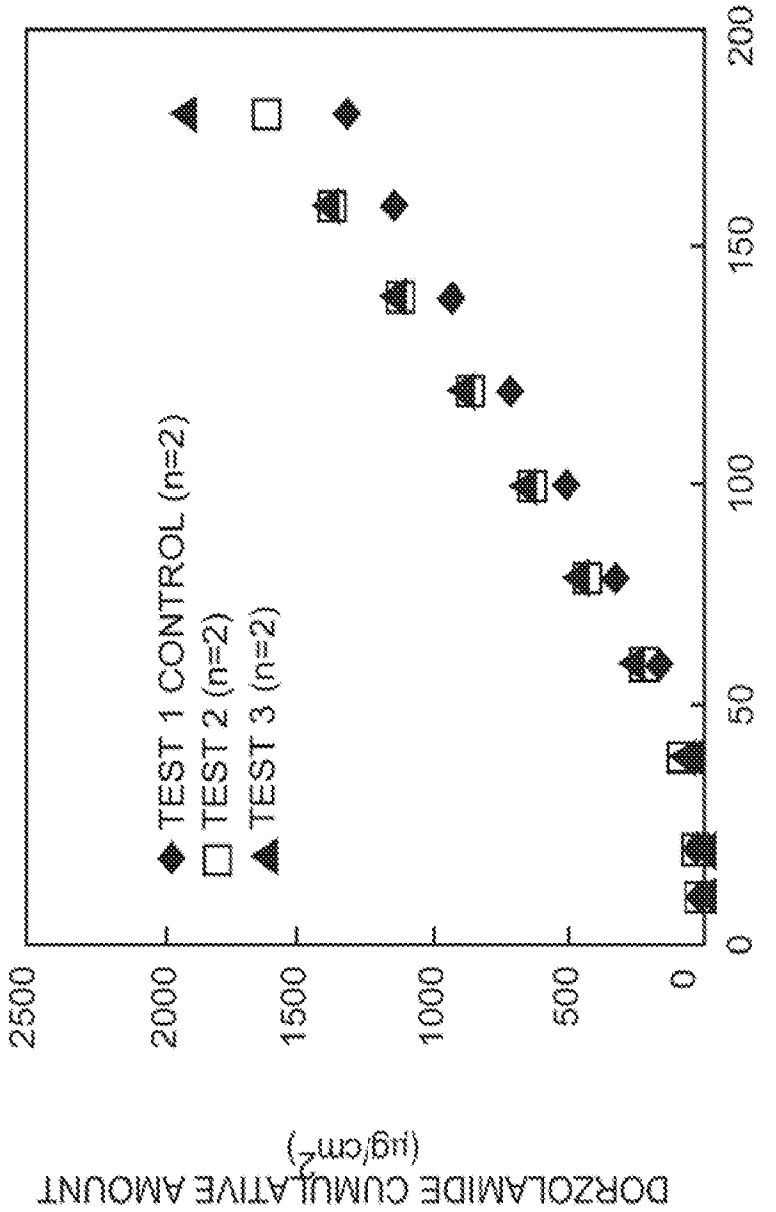

DORZOLAMIDE CUMULATIVE AMOUNT (μg/cm²)
2500
2000
1500
1000
500
0
0
50
100
150
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)

FIG. 10

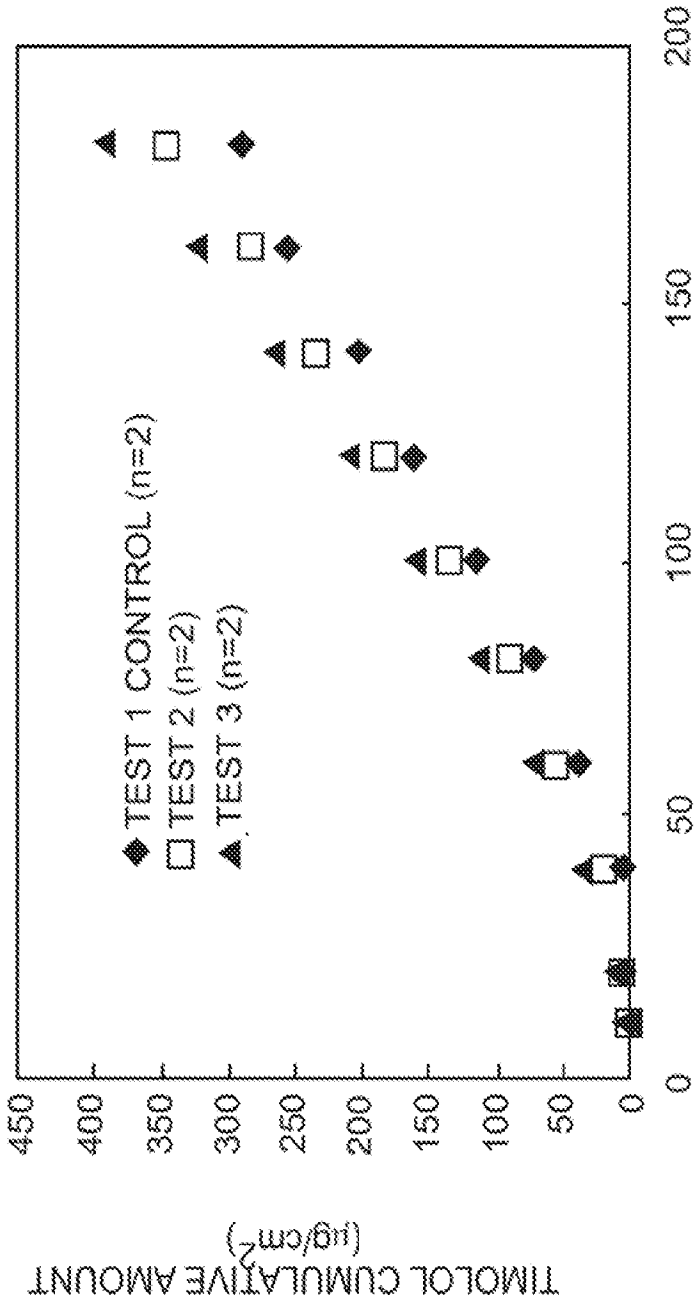

TIMOLOL CUMULATIVE AMOUNT (μg/cm²)
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)

FIG. 11

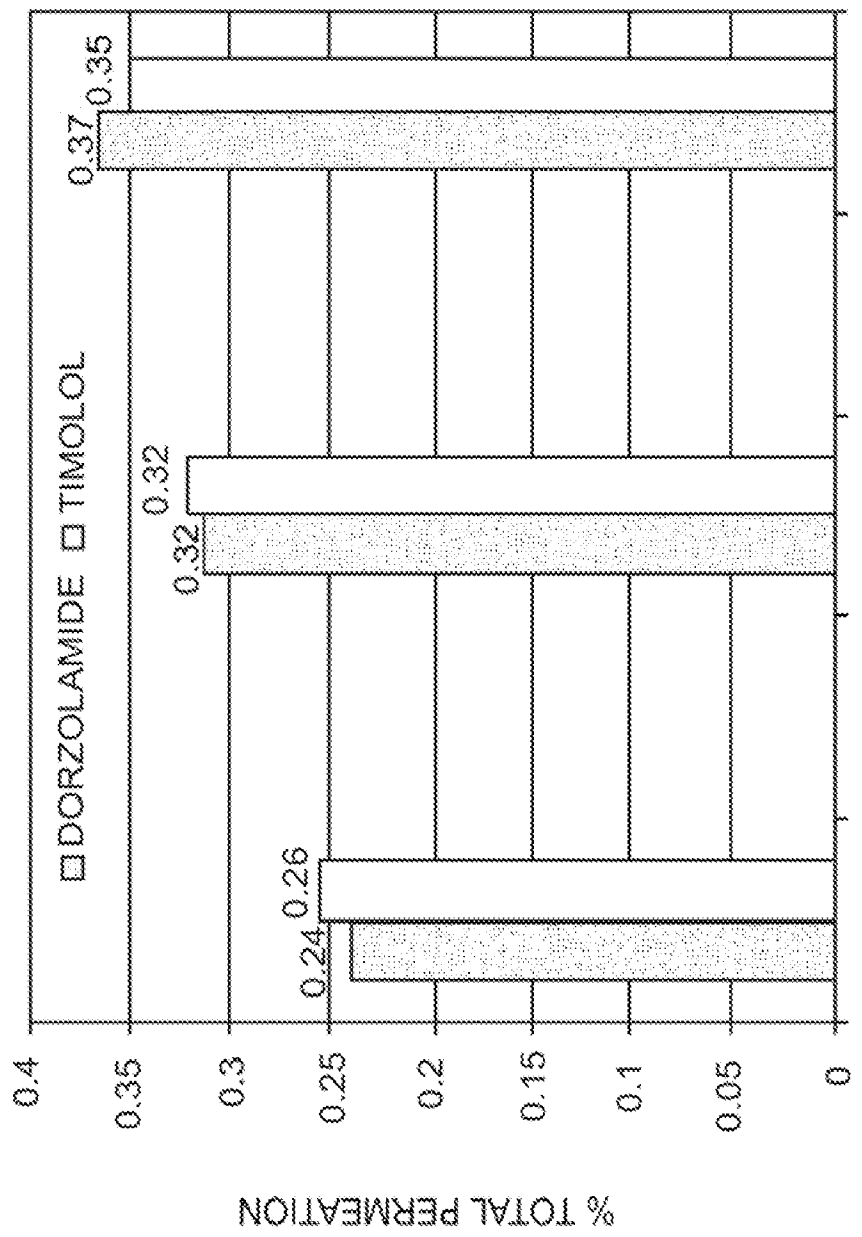

% TOTAL PERMEATION
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
DORZOLAMIDE
TIMOLOL
0.24
0.26
0.32
0.32
0.37
0.35
TEST 1
TEST 2
TEST 3

FIG. 12

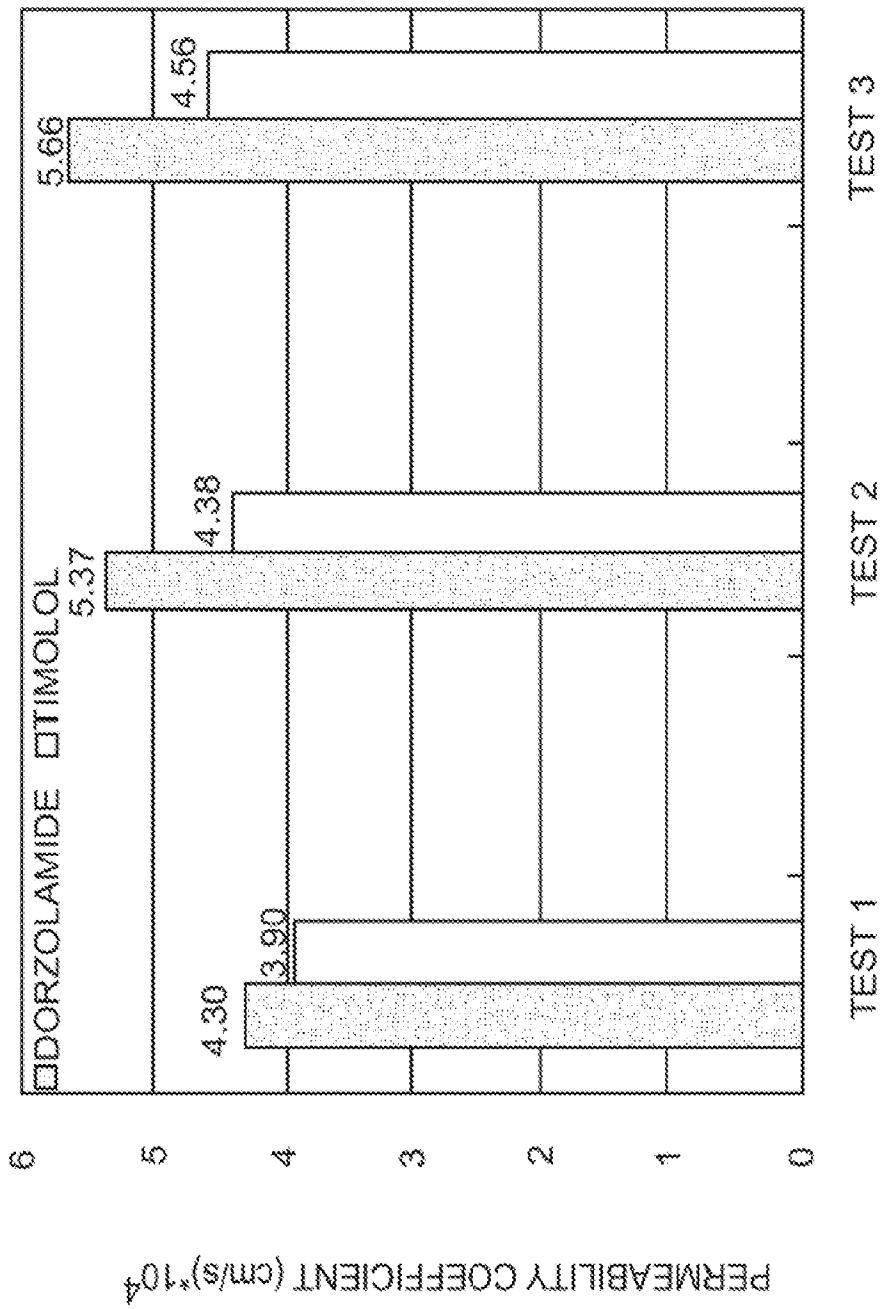

DORZOLAMIDE
TIMOLOL
4.30
3.90
5.37
4.38
5.66
4.56
TEST 1
TEST 2
TEST 3
0
1
2
3
4
5
6
PERMEABILITY COEFFICIENT (cm/s)*10⁴

FIG. 13

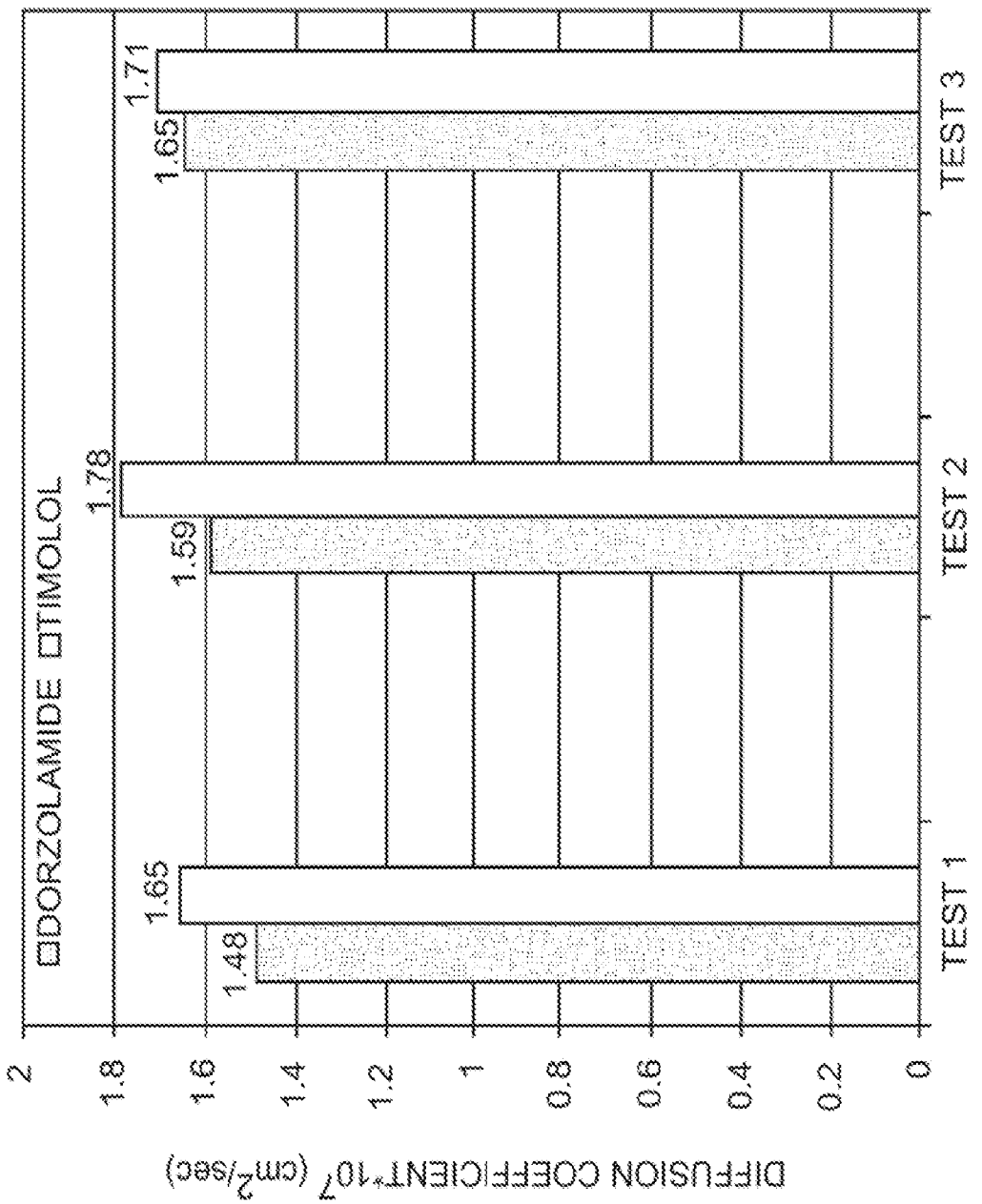

DORZOLAMIDE TIMOLOL
1.65
1.48
1.78
1.59
1.71
1.65
TEST 1
TEST 2
TEST 3
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DIFFUSION COEFFICIENT*10^7 (cm^2/sec)

FIG. 14

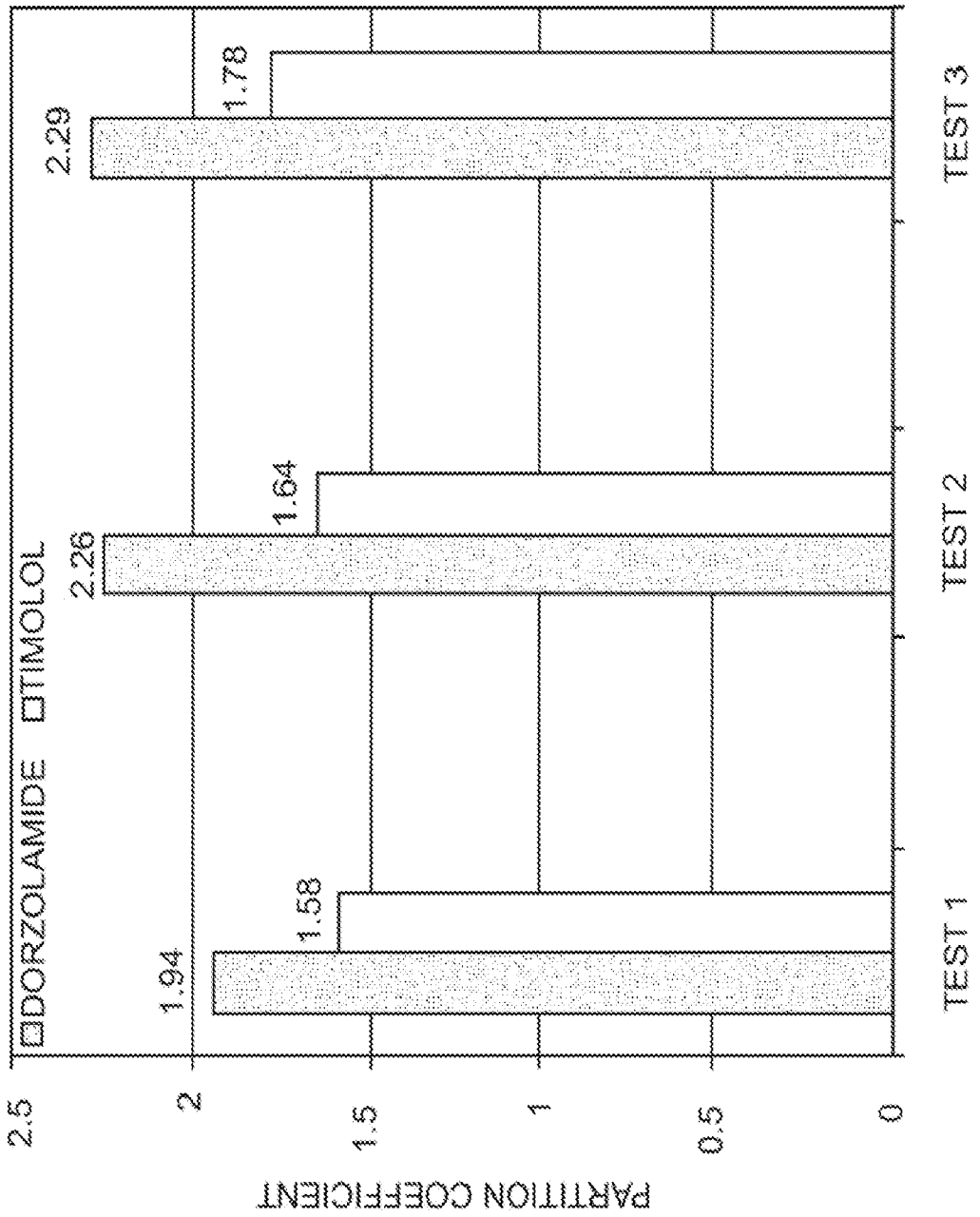

DORZOLAMIDE
TIMOLOL
PARTITION COEFFICIENT
2.5
2
1.5
1
0.5
0
TEST 1
TEST 2
TEST 3
1.94
1.58
2.26
1.64
2.29
1.78

FIG. 15

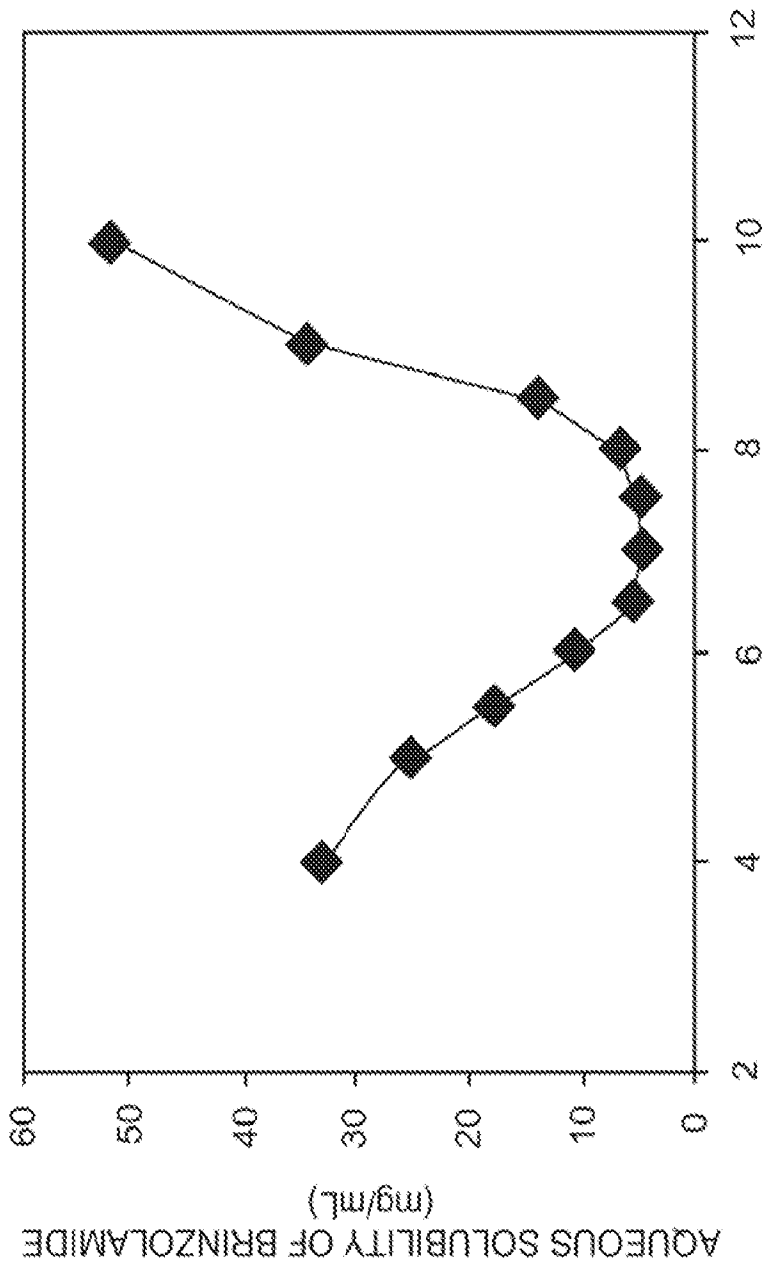

AQUEOUS SOLUBILITY OF BRINZOLAMIDE
(mg/mL)
0
10
20
30
40
50
60
2
4
6
8
10
12
pH

FIG. 16

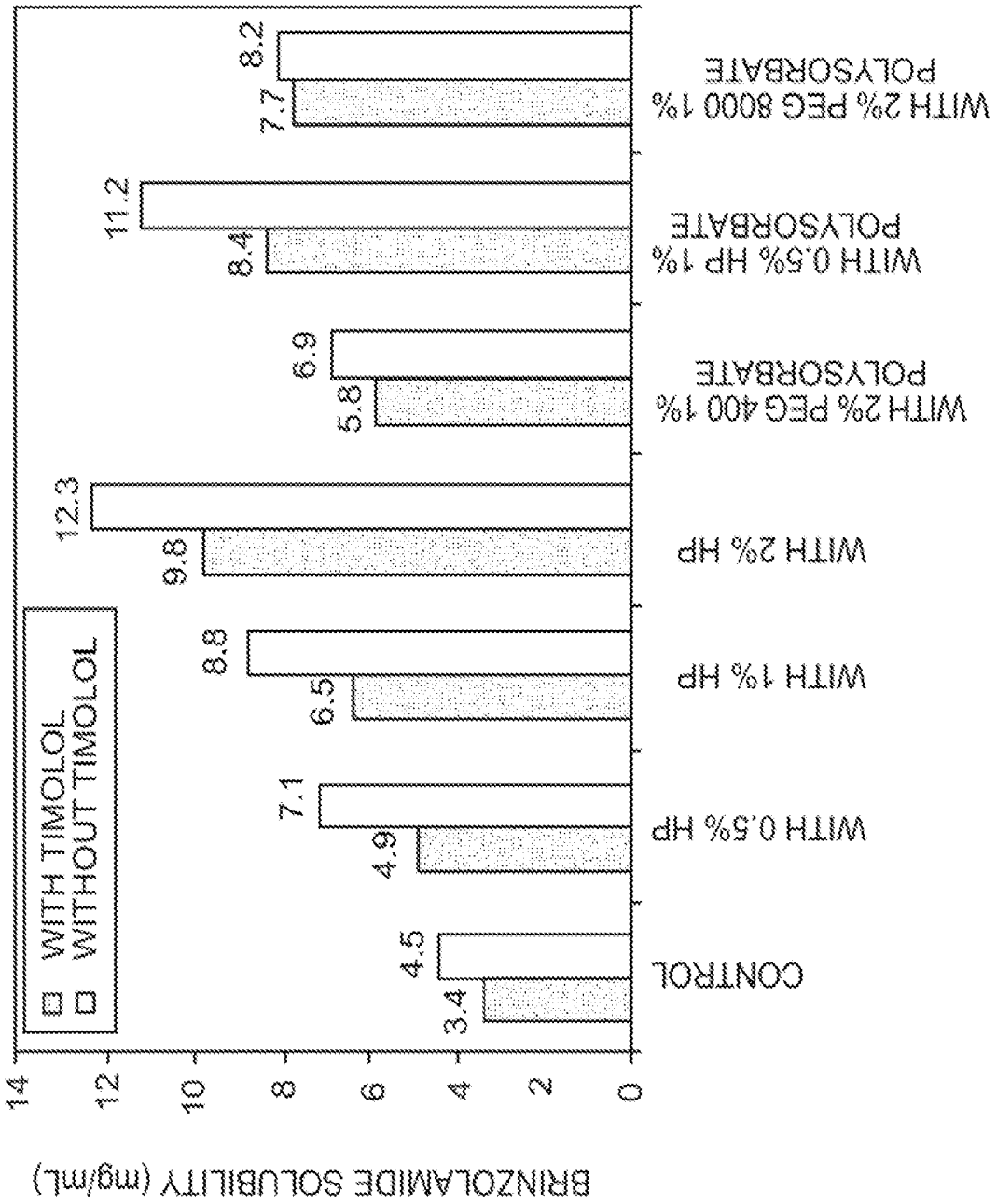

BRINZOLAMIDE SOLUBILITY (mg/mL)
0
2
4
6
8
10
12
14
WITH TIMOLOL
WITHOUT TIMOLOL
CONTROL
3.4
4.5
WITH 0.5% HP
4.9
7.1
WITH 1% HP
6.5
8.8
WITH 2% HP
9.8
12.3
WITH 2% PEG 400 1% POLYSORBATE
5.8
6.9
WITH 0.5% HP 1% POLYSORBATE
8.4
11.2
WITH 2% PEG 8000 1% POLYSORBATE
7.7
8.2

FIG. 17

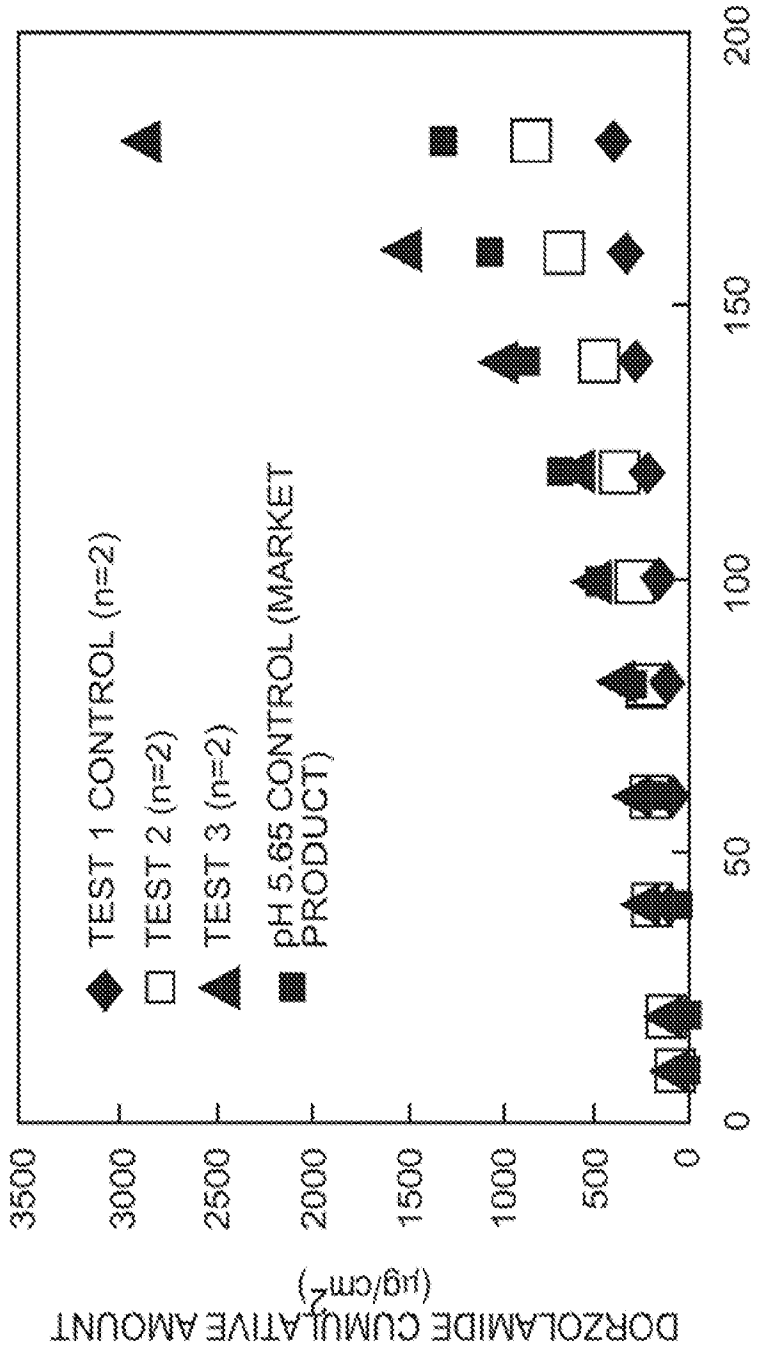

DORZOLAMIDE CUMULATIVE AMOUNT (μg/cm²)
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)
pH 5.65 CONTROL (MARKET PRODUCT)

FIG. 18

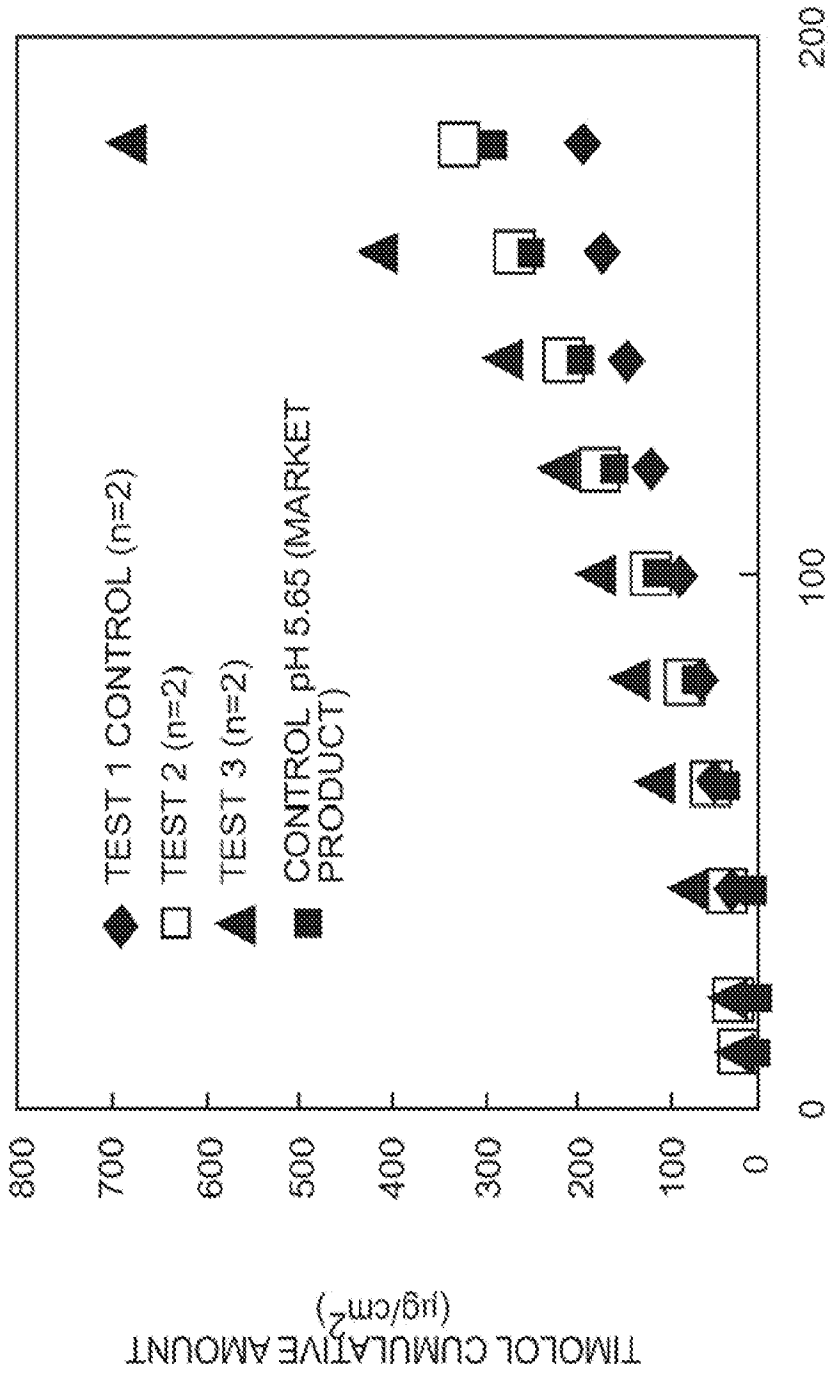

TIMOLOL CUMULATIVE AMOUNT (µg/cm²)
800
700
600
500
400
300
200
100
0
0
100
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)
CONTROL pH 5.65 (MARKET PRODUCT)

FIG. 19

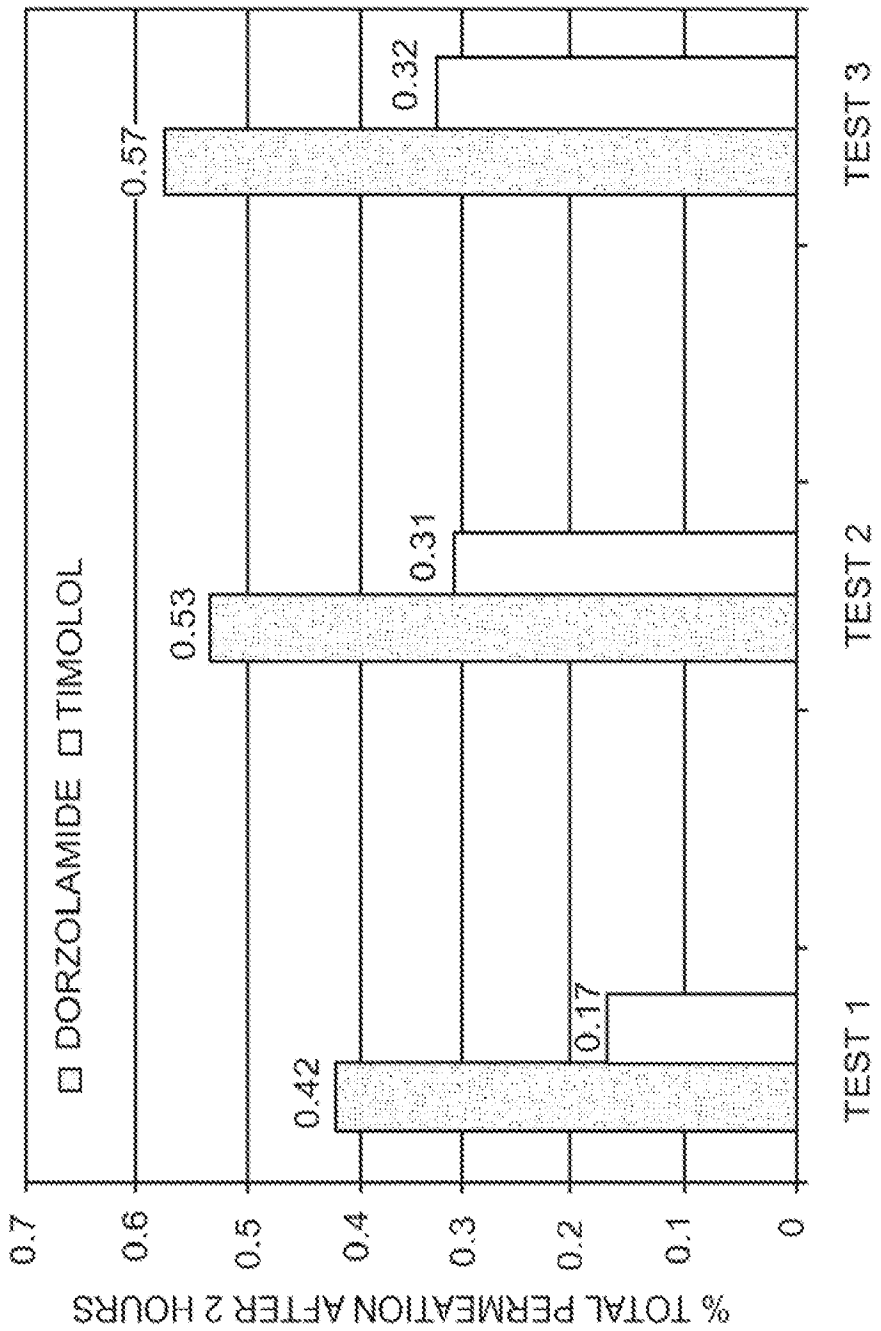

% TOTAL PERMEATION AFTER 2 HOURS
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
TEST 1
TEST 2
TEST 3
0.42
0.17
0.53
0.31
0.57
0.32
DORZOLAMIDE
TIMOLOL

FIG. 20

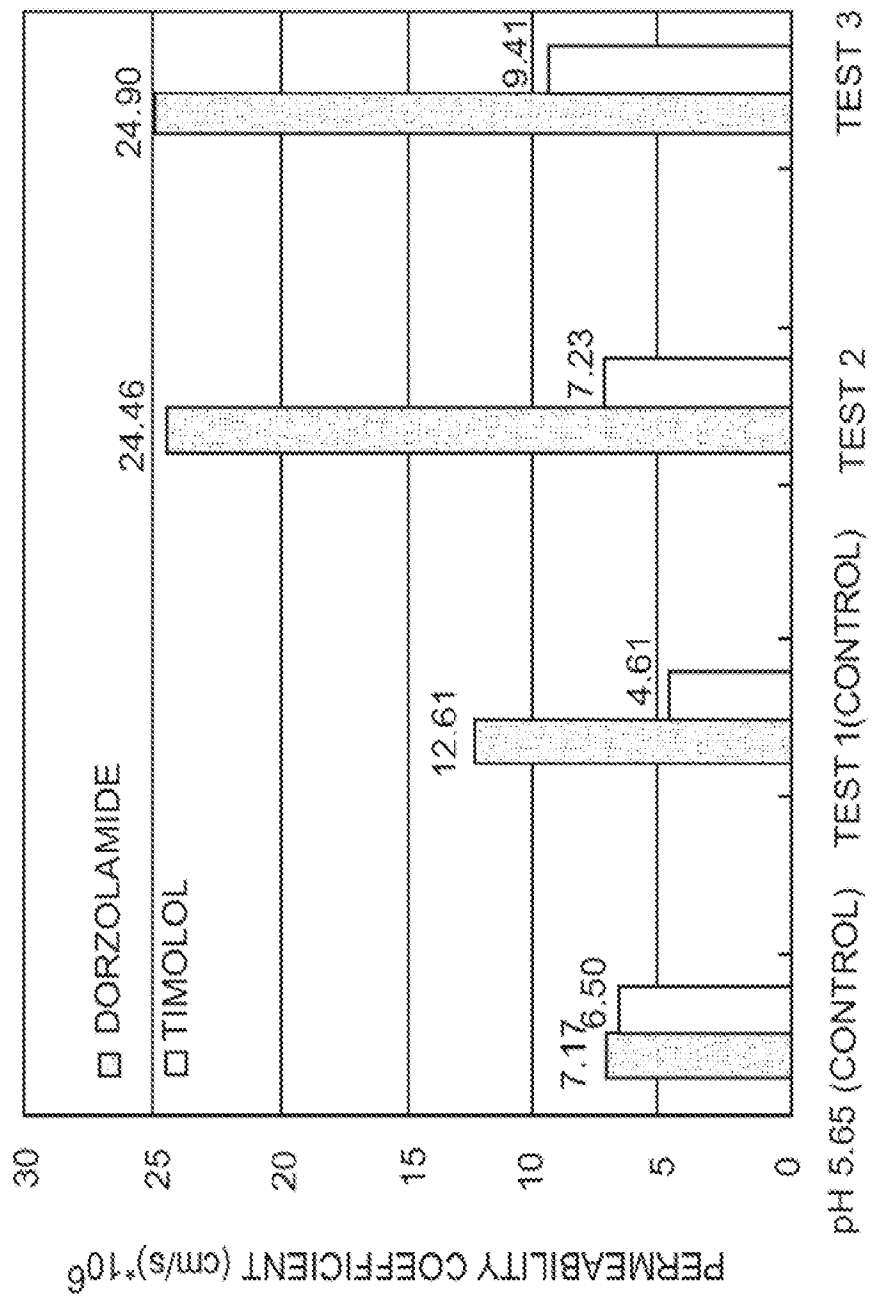

DORZOLAMIDE
TIMOLOL
PERMEABILITY COEFFICIENT (cm/s)*10^6
30
25
20
15
10
5
0
24.90
24.46
12.61
7.17
6.50
9.41
7.23
4.61
pH 5.65 (CONTROL)
TEST 1(CONTROL)
TEST 2
TEST 3

FIG. 21

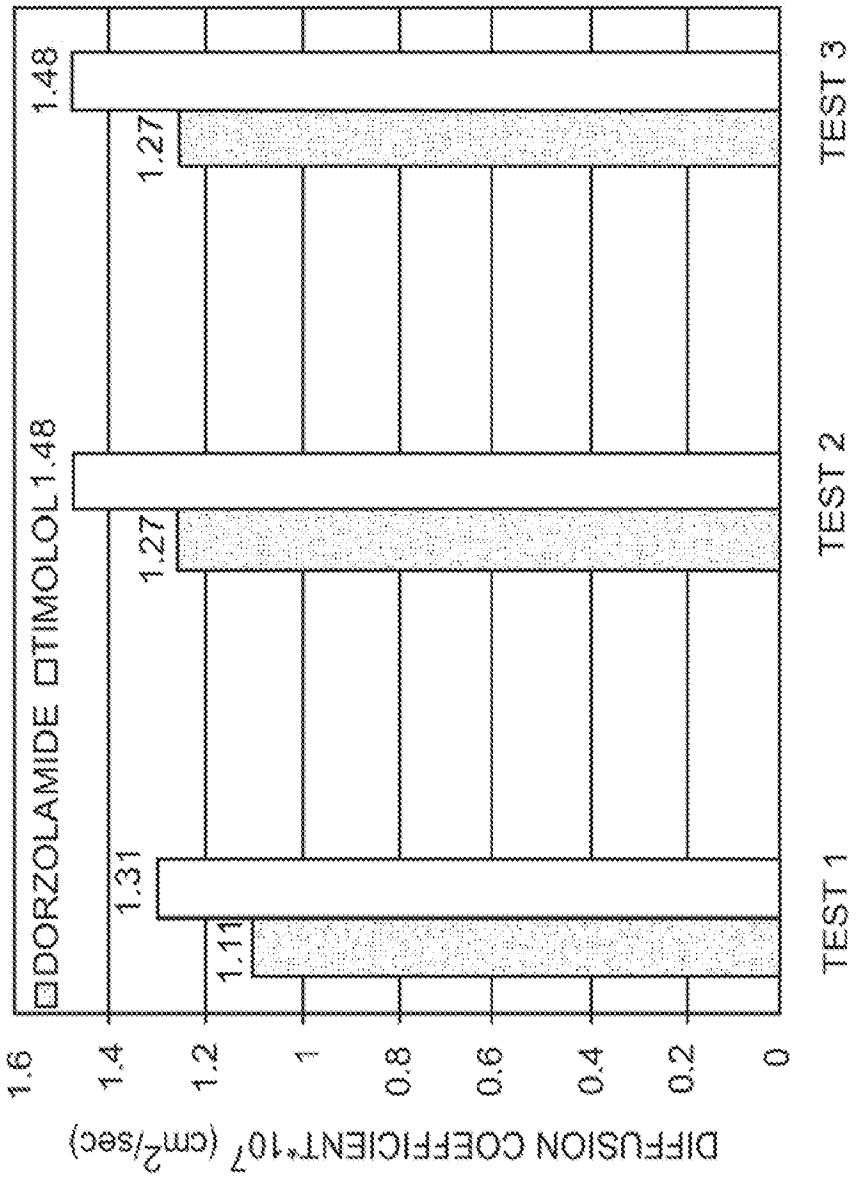

DORZOLAMIDE TIMOLOL 1.48
1.48
1.27
1.27
1.31
1.11
TEST 3
TEST 2
TEST 1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DIFFUSION COEFFICIENT*10^7 (cm^2/sec)

FIG. 22

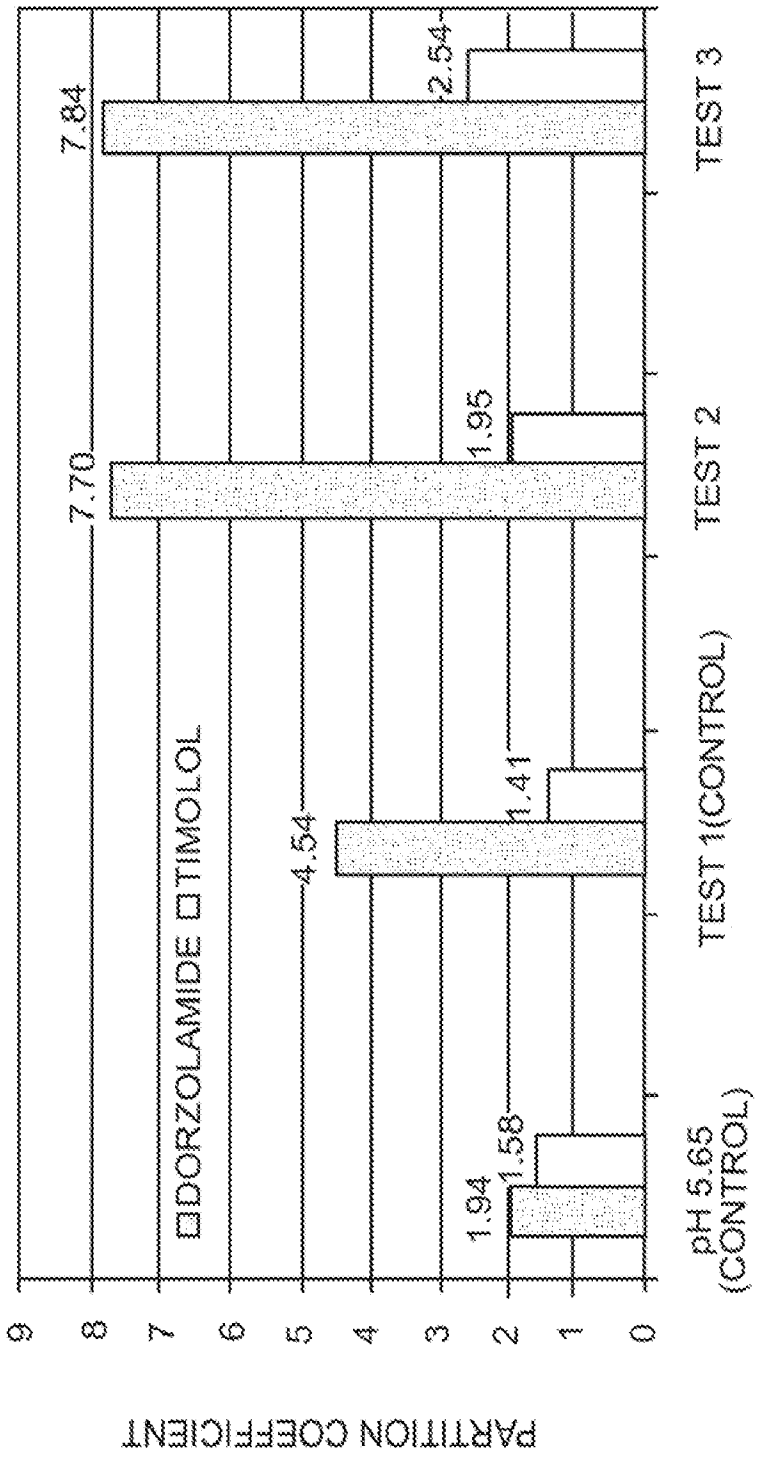

PARTITION COEFFICIENT
0
1
2
3
4
5
6
7
8
9
DORZOLAMIDE
TIMOLOL
pH 5.65 (CONTROL)
TEST 1(CONTROL)
TEST 2
TEST 3
1.94
1.58
4.54
1.41
7.70
1.95
7.84
2.54

FIG. 23

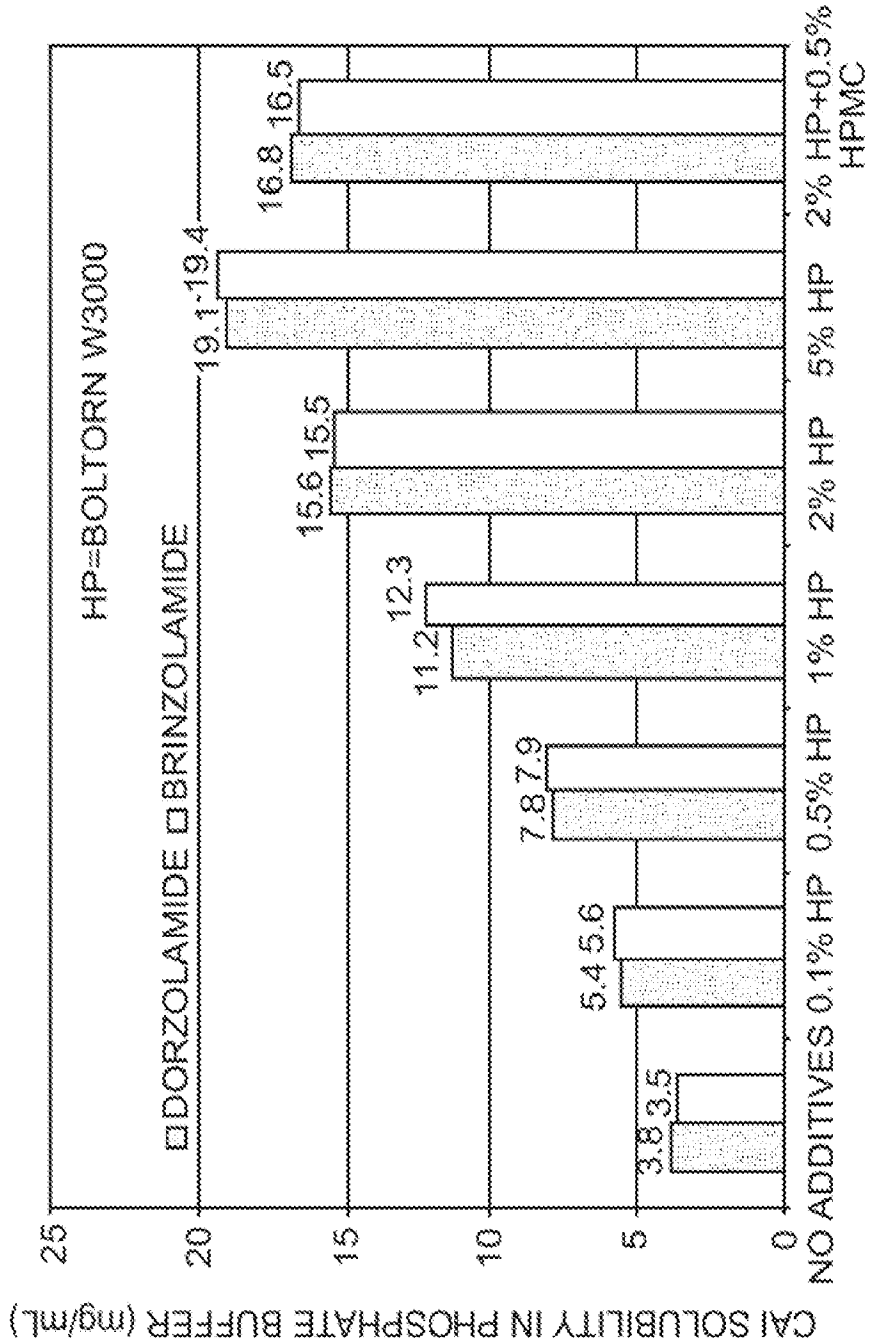

HP=BOLTORN W3000
DORZOLAMIDE BRINZOLAMIDE
CAI SOLUBILITY IN PHOSPHATE BUFFER (mg/mL)
25
20
15
10
5
0
NO ADDITIVES
0.1% HP
0.5% HP
1% HP
2% HP
5% HP
2% HP+0.5% HPMC
3.8
3.5
5.4
5.6
7.8
7.9
11.2
12.3
15.6
15.5
19.1
19.4
16.8
16.5

FIG. 24

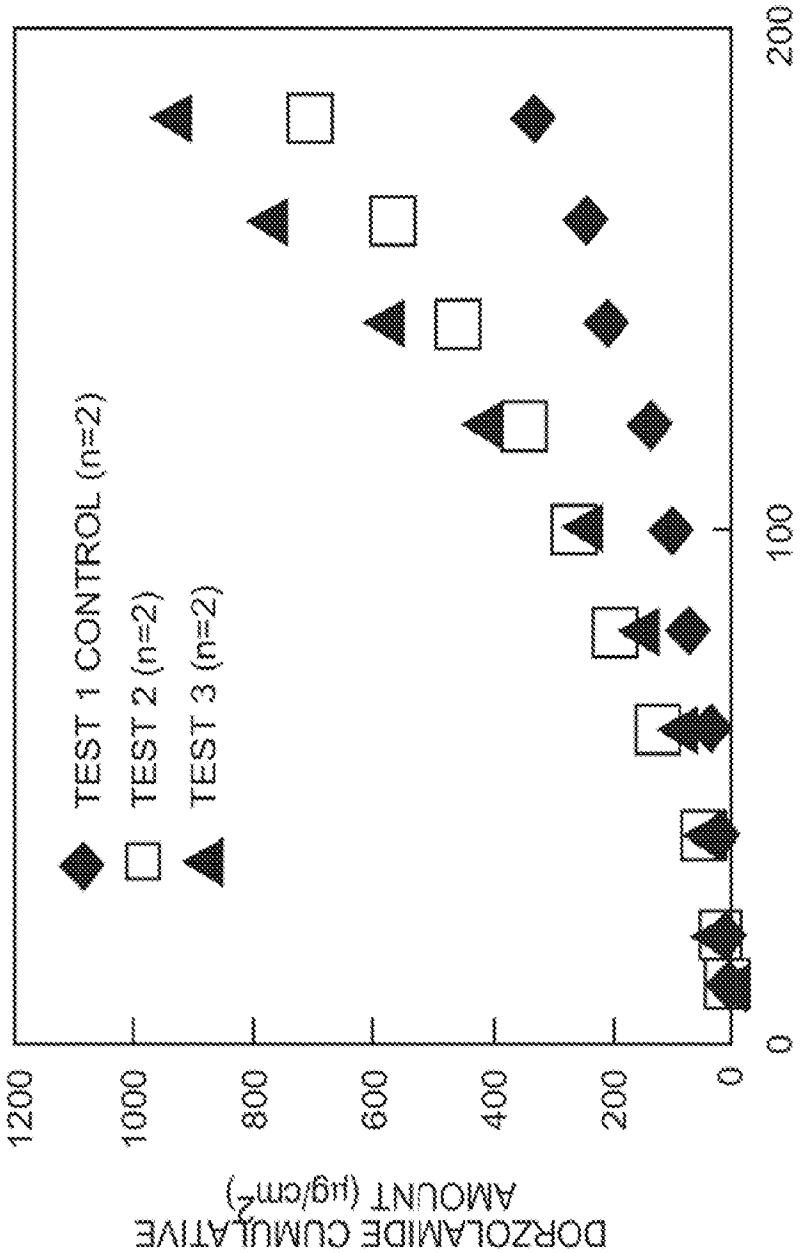

DORZOLAMIDE CUMULATIVE AMOUNT (μg/cm²)
1200
1000
800
600
400
200
0
0
100
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)

FIG. 25

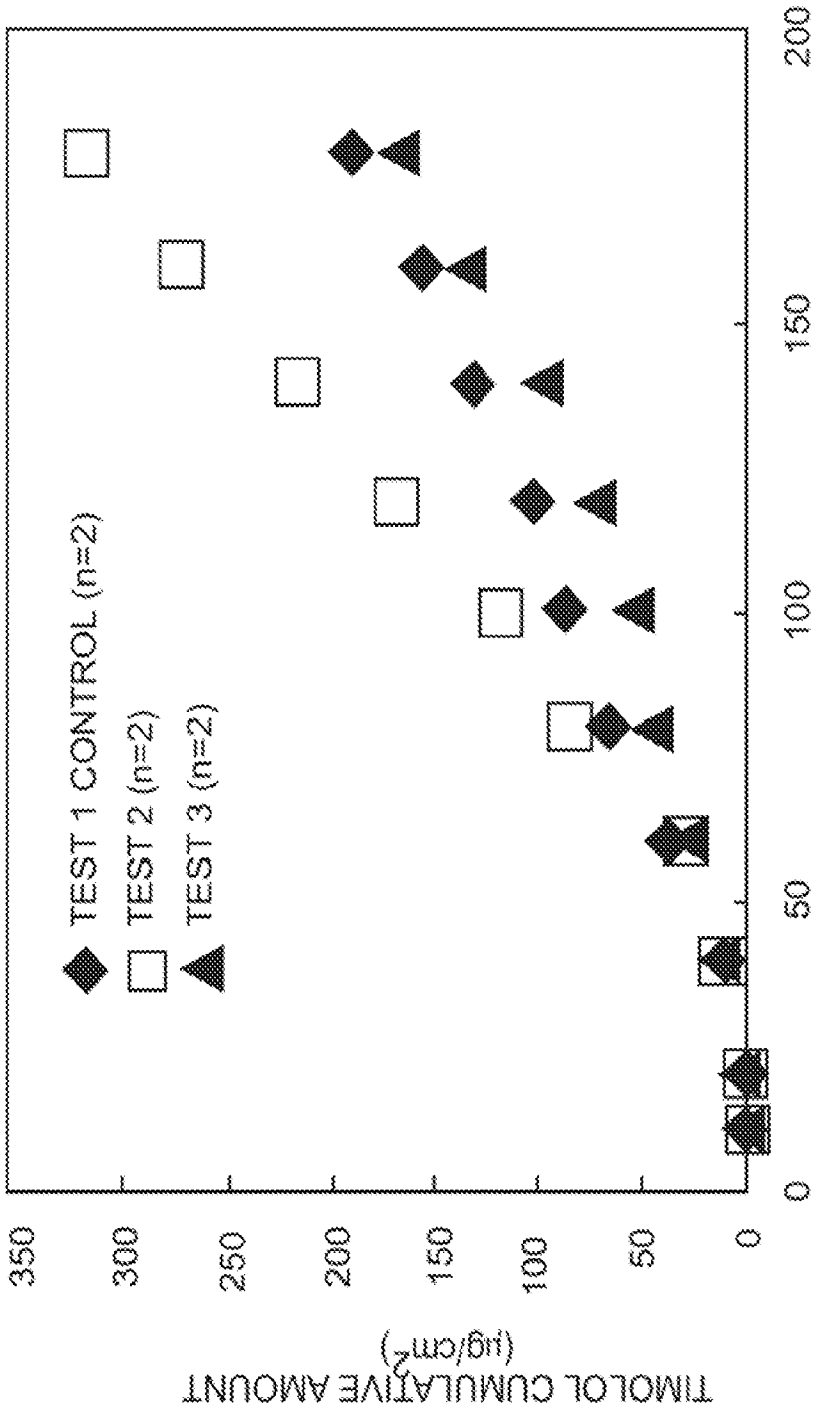

TIMOLOL CUMULATIVE AMOUNT (μg/cm²)
350
300
250
200
150
100
50
0
0
50
100
150
200
TIME (MIN)
TEST 1 CONTROL (n=2)
TEST 2 (n=2)
TEST 3 (n=2)

FIG. 26

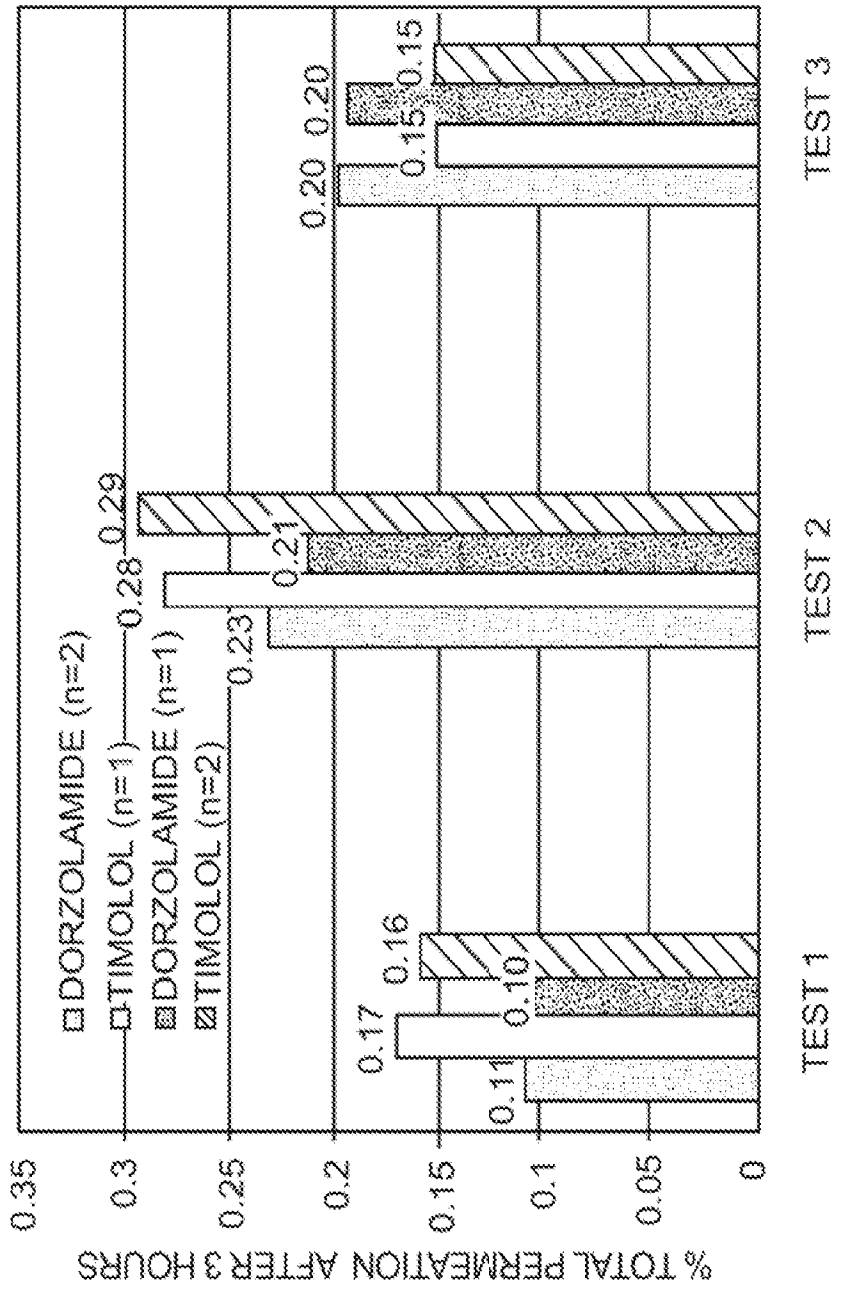

% TOTAL PERMEATION AFTER 3 HOURS
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
DORZOLAMIDE (n=2)
TIMOLOL (n=1)
DORZOLAMIDE (n=1)
TIMOLOL (n=2)
0.29
0.28
0.23
0.21
0.20
0.20
0.20
0.15
0.15
0.15
0.16
0.17
0.10
0.11
TEST 1
TEST 2
TEST 3

FIG. 27

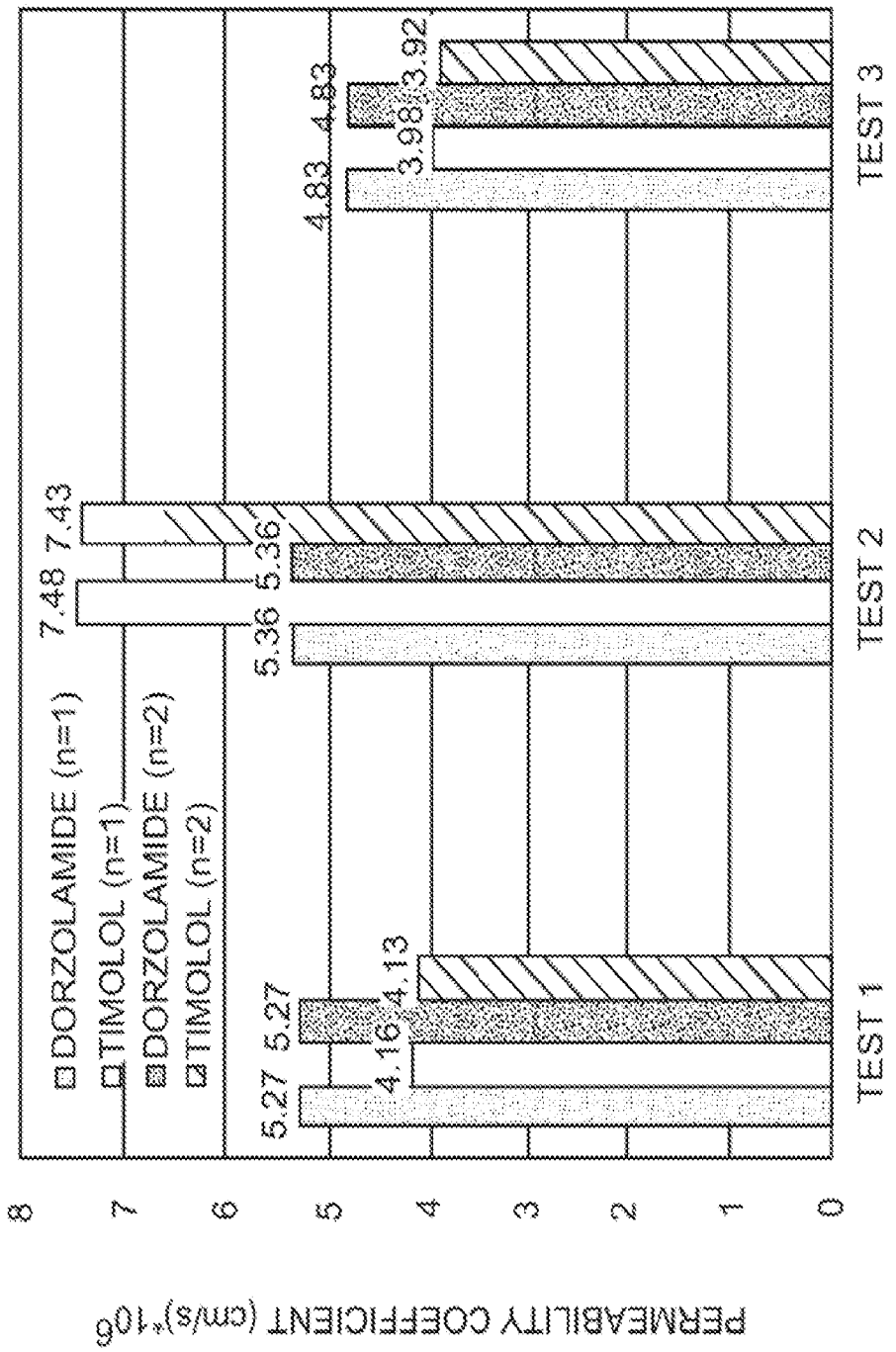

DORZOLAMIDE (n=1)
TIMOLOL (n=1)
DORZOLAMIDE (n=2)
TIMOLOL (n=2)
7.48 7.43
5.36 5.36
4.83 4.83
3.98 3.92
5.27 5.27
4.16 4.13
TEST 1
TEST 2
TEST 3
8
7
6
5
4
3
2
1
0
PERMEABILITY COEFFICIENT (cm/s)*10^6

FIG. 28

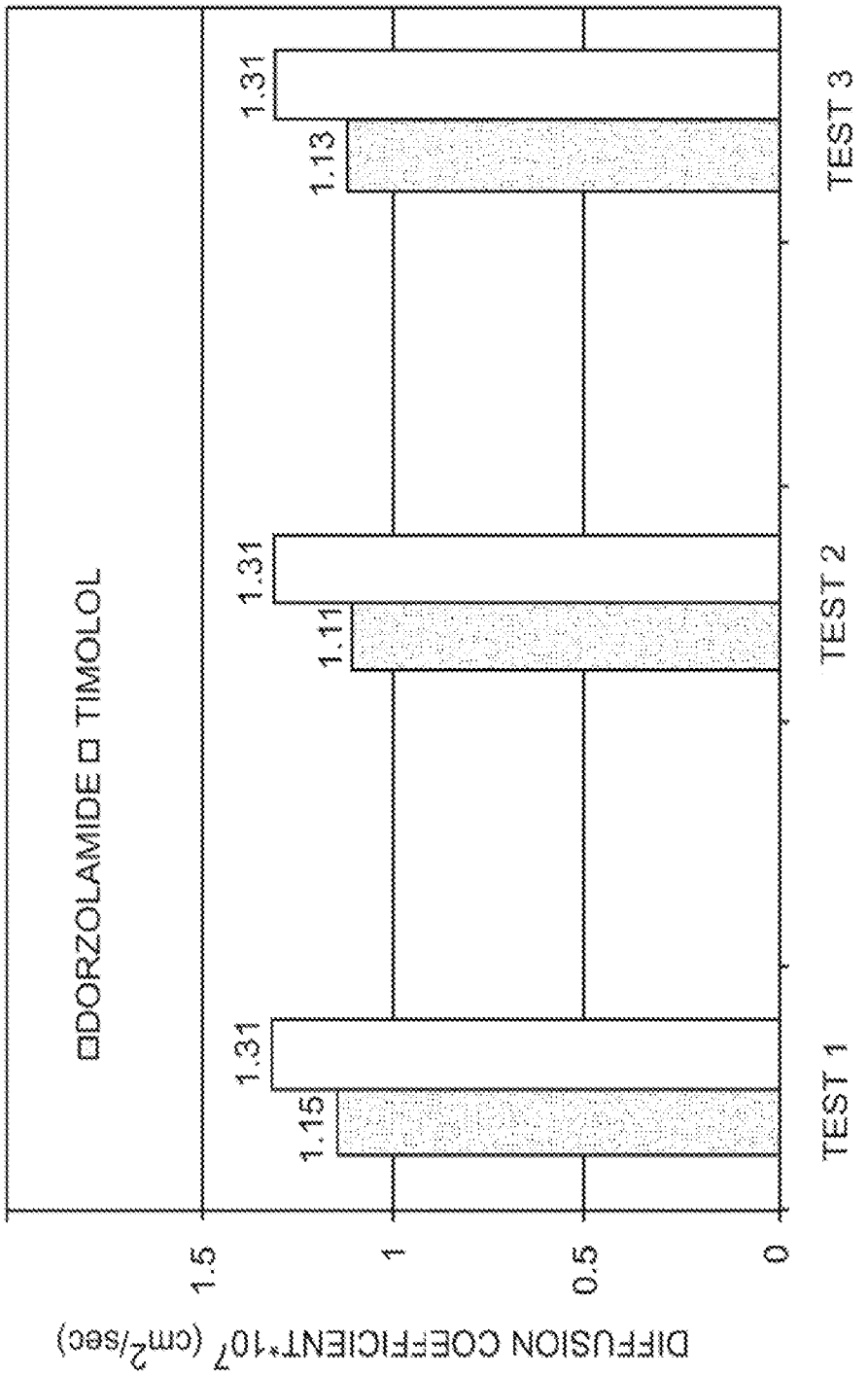

DORZOLAMIDE TIMOLOL
1.31
1.15
1.31
1.11
1.31
1.13
TEST 1
TEST 2
TEST 3
0
0.5
1
1.5
DIFFUSION COEFFICIENT*10^7 (cm^2/sec)

FIG. 29

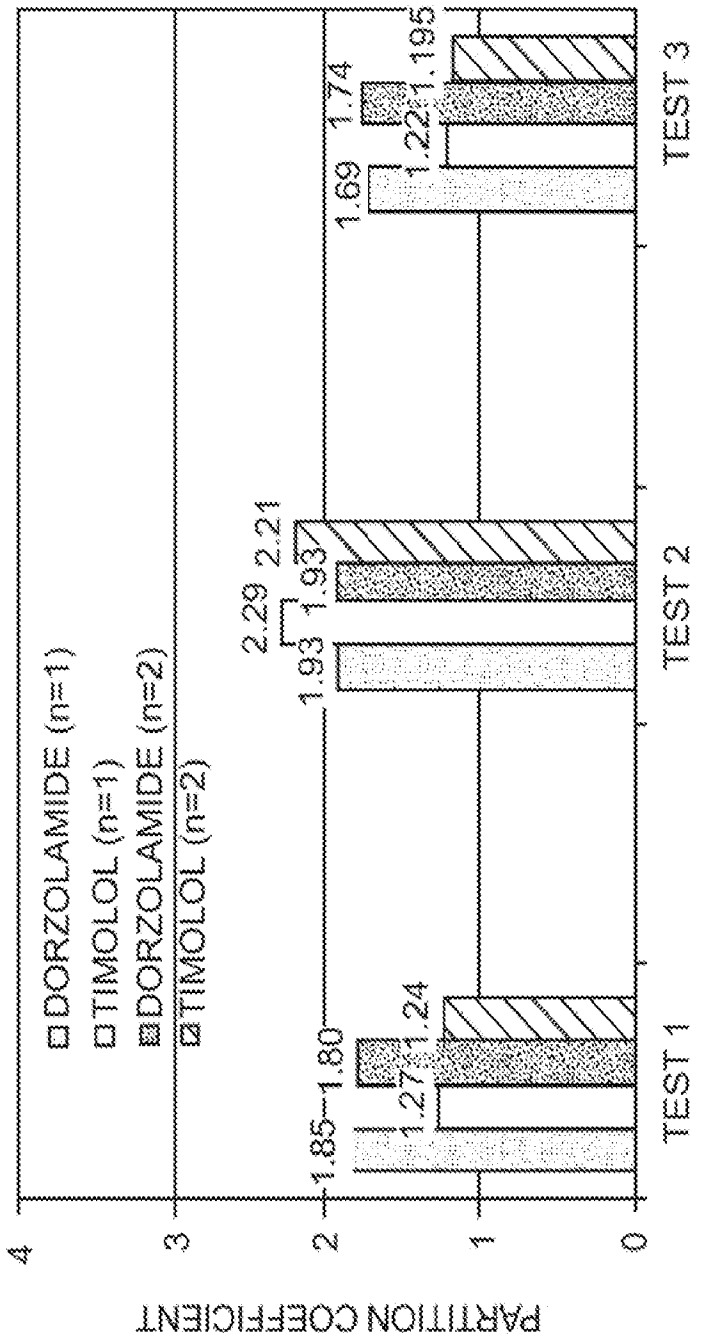

DORZOLAMIDE (n=1)
TIMOLOL (n=1)
DORZOLAMIDE (n=2)
TIMOLOL (n=2)
PARTITION COEFFICIENT
4
3
2
1
0
TEST 1
TEST 2
TEST 3
1.85
1.80
1.27
1.24
2.29
2.21
1.93
1.93
1.69
1.74
1.22
1.195

FIG. 30

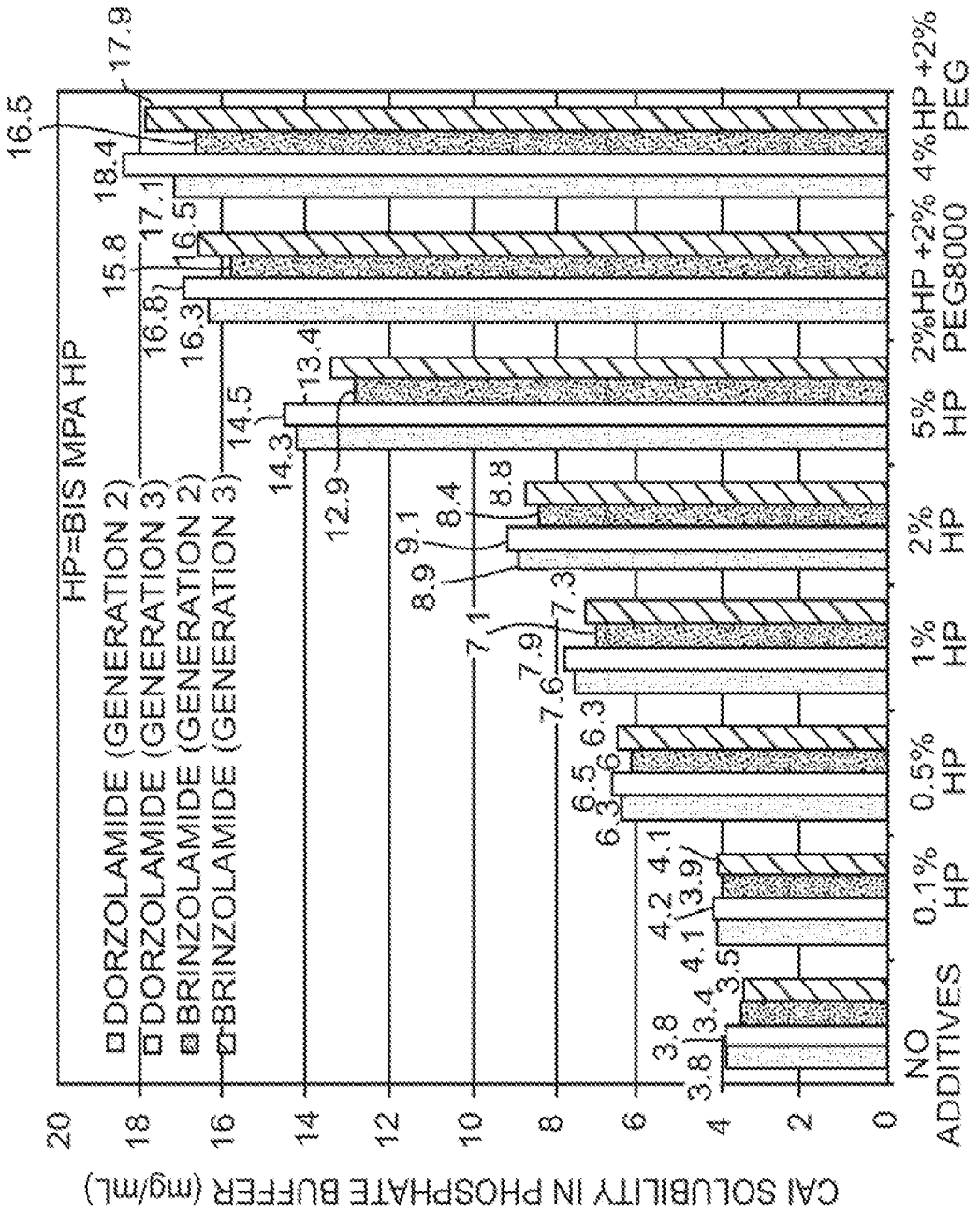

CAI SOLUBILITY IN PHOSPHATE BUFFER (mg/mL)
20
18
16
14
12
10
8
6
4
2
0
HP=BIS MPA HP
DORZOLAMIDE (GENERATION 2)
DORZOLAMIDE (GENERATION 3)
BRINZOLAMIDE (GENERATION 2)
BRINZOLAMIDE (GENERATION 3)
16.5
17.9
18.4
15.8
17.1
16.8
16.5
16.3
13.4
14.5
14.3
12.9
9.1
8.4
8.8
8.9
7.1
7.3
7.9
7.6
6.3
6.5
6
6.3
4.2
4.1
4.1
3.9
3.8
3.4
3.5
3.8
NO ADDITIVES
0.1% HP
0.5% HP
1% HP
2% HP
5% HP
2%HP +2% PEG8000
4%HP +2% PEG

FIG. 31

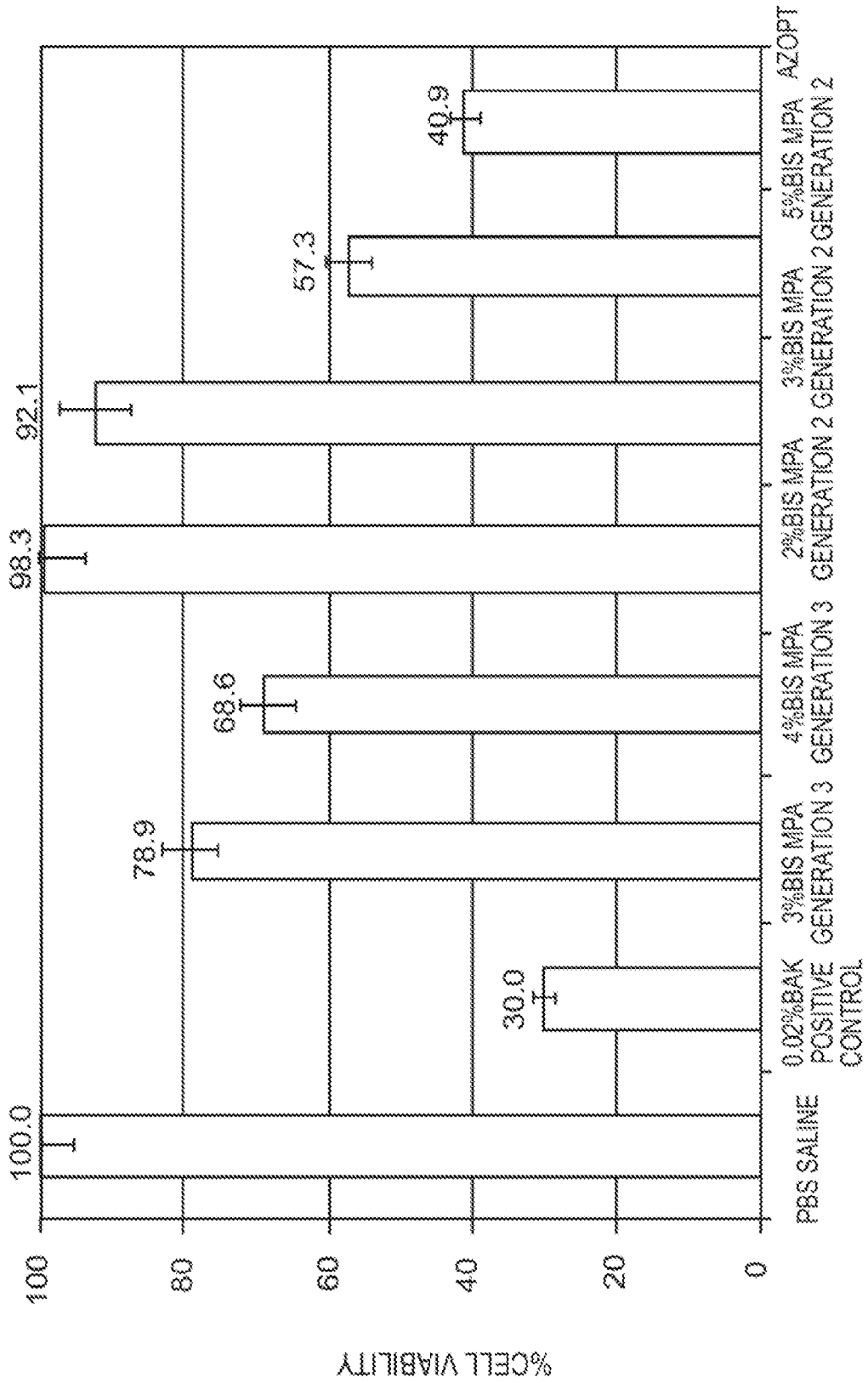

%CELL VIABILITY
100
80
60
40
20
0
100.0
98.3
92.1
57.3
40.9
78.9
68.6
30.0
PBS SALINE
0.02%BAK POSITIVE CONTROL
3%BIS MPA GENERATION 3
4%BIS MPA GENERATION 3
2%BIS MPA GENERATION 2
3%BIS MPA GENERATION 2
5%BIS MPA GENERATION 2
AZOPT

FIG. 32

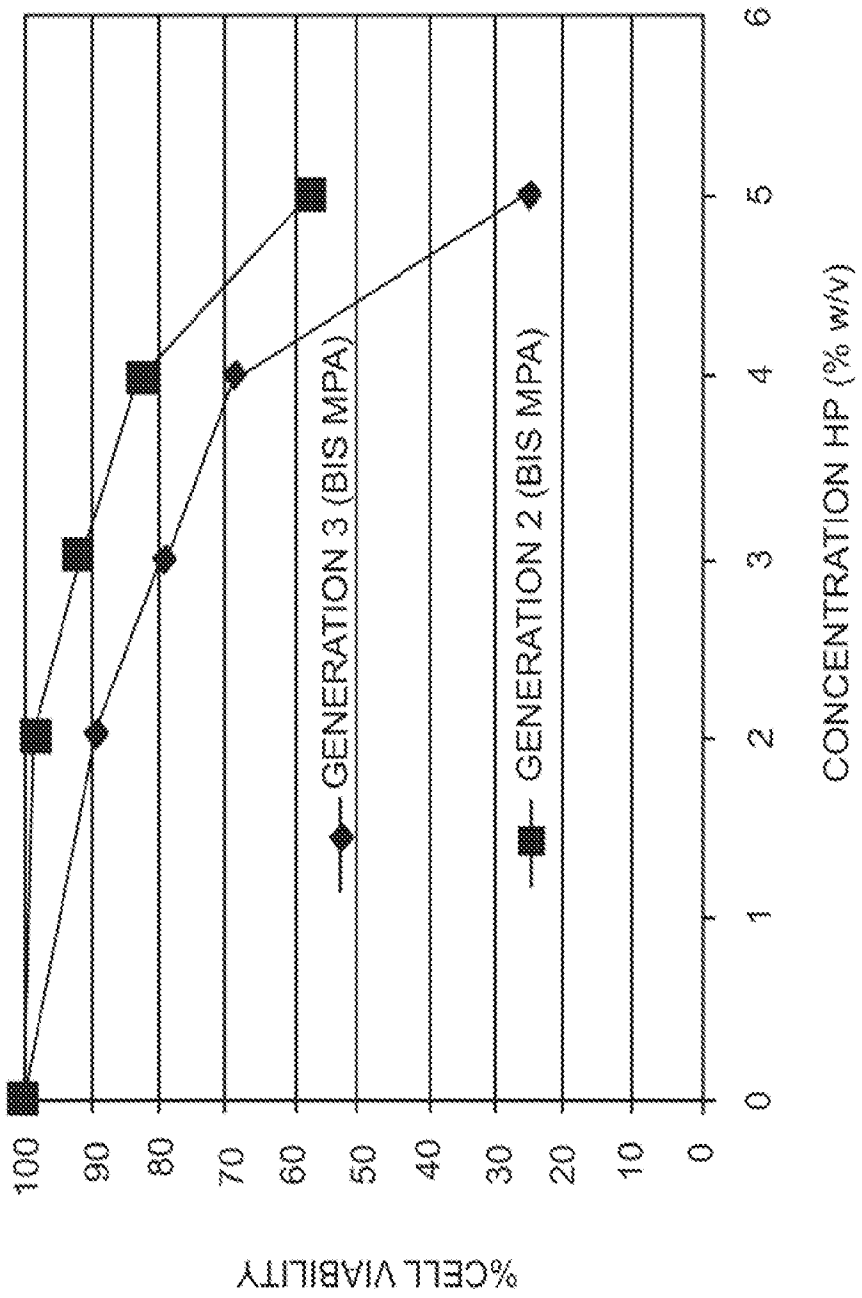

%CELL VIABILITY
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
CONCENTRATION HP (% w/v)
GENERATION 3 (BIS MPA)
GENERATION 2 (BIS MPA)

FIG. 33

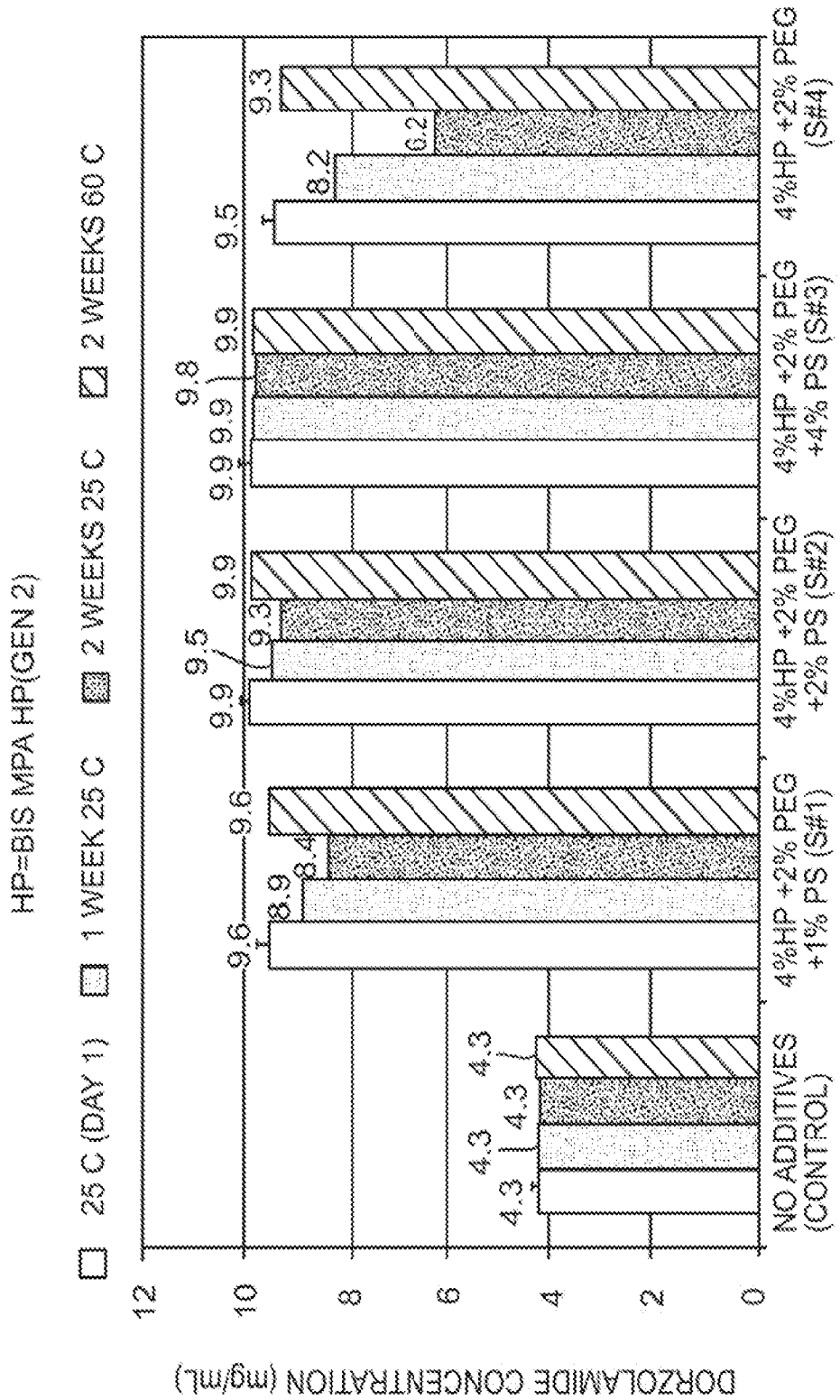

HP=BIS MPA HP(GEN 2)
25 C (DAY 1)
1 WEEK 25 C
2 WEEKS 25 C
2 WEEKS 60 C
DORZOLAMIDE CONCENTRATION (mg/mL)
12
10
8
6
4
2
0
NO ADDITIVES (CONTROL)
4%HP +2% PEG +1% PS (S#1)
4%HP +2% PEG +2% PS (S#2)
4%HP +2% PEG +4% PS (S#3)
4%HP +2% PEG (S#4)
4.3
4.3
4.3
4.3
9.6
9.6
8.9
8.4
9.9
9.5
9.3
9.9
9.8
9.9
9.9
9.5
8.2
6.2
9.3

FIG. 34

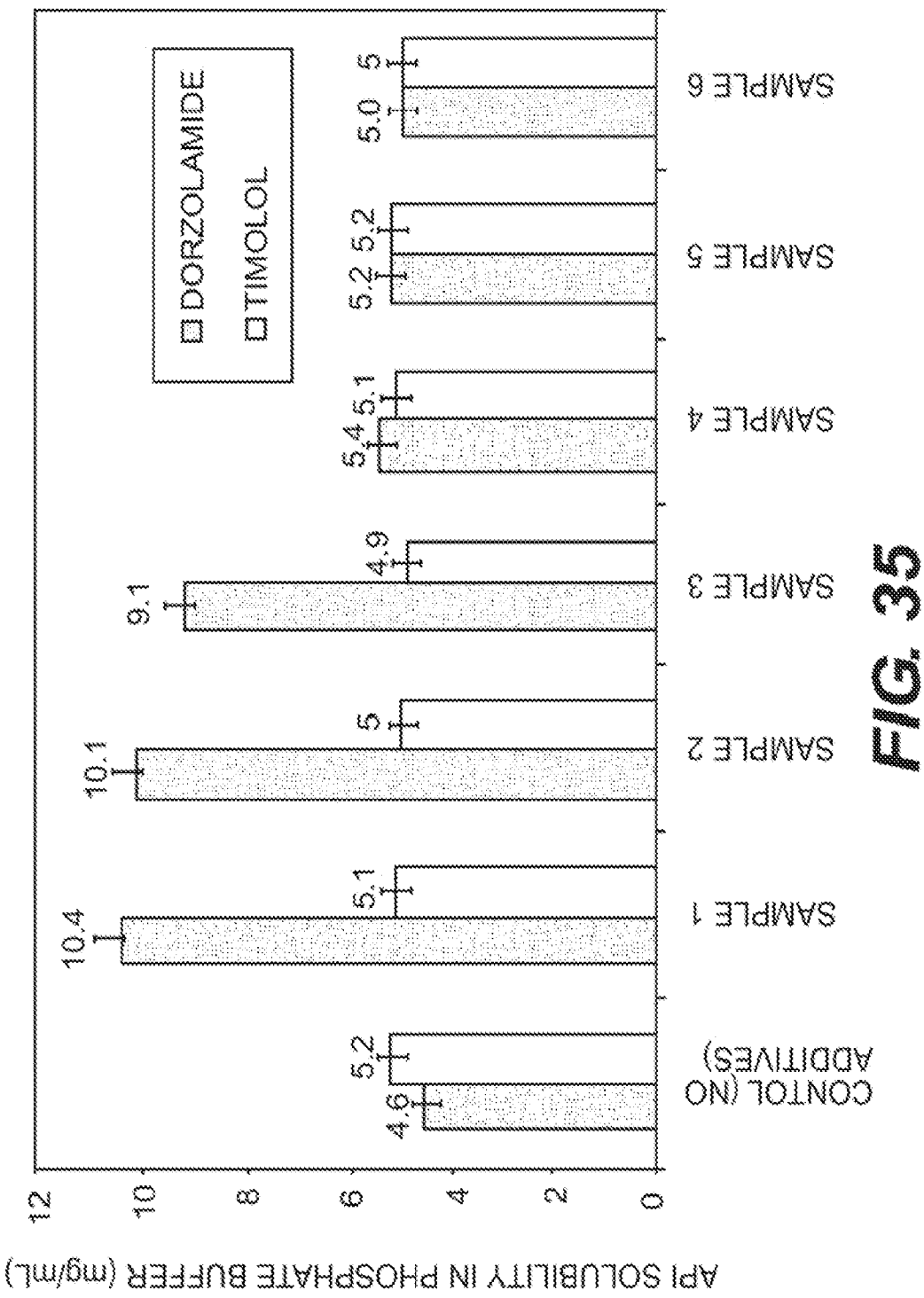

API SOLUBILITY IN PHOSPHATE BUFFER (mg/mL)
0
2
4
6
8
10
12
DORZOLAMIDE
TIMOLOL
CONTOL (NO ADDITIVES)
SAMPLE 1
SAMPLE 2
SAMPLE 3
SAMPLE 4
SAMPLE 5
SAMPLE 6
4.6
5.2
10.4
5.1
10.1
5
9.1
4.9
5.4
5.1
5.2
5.2
5.0
5

FIG. 35

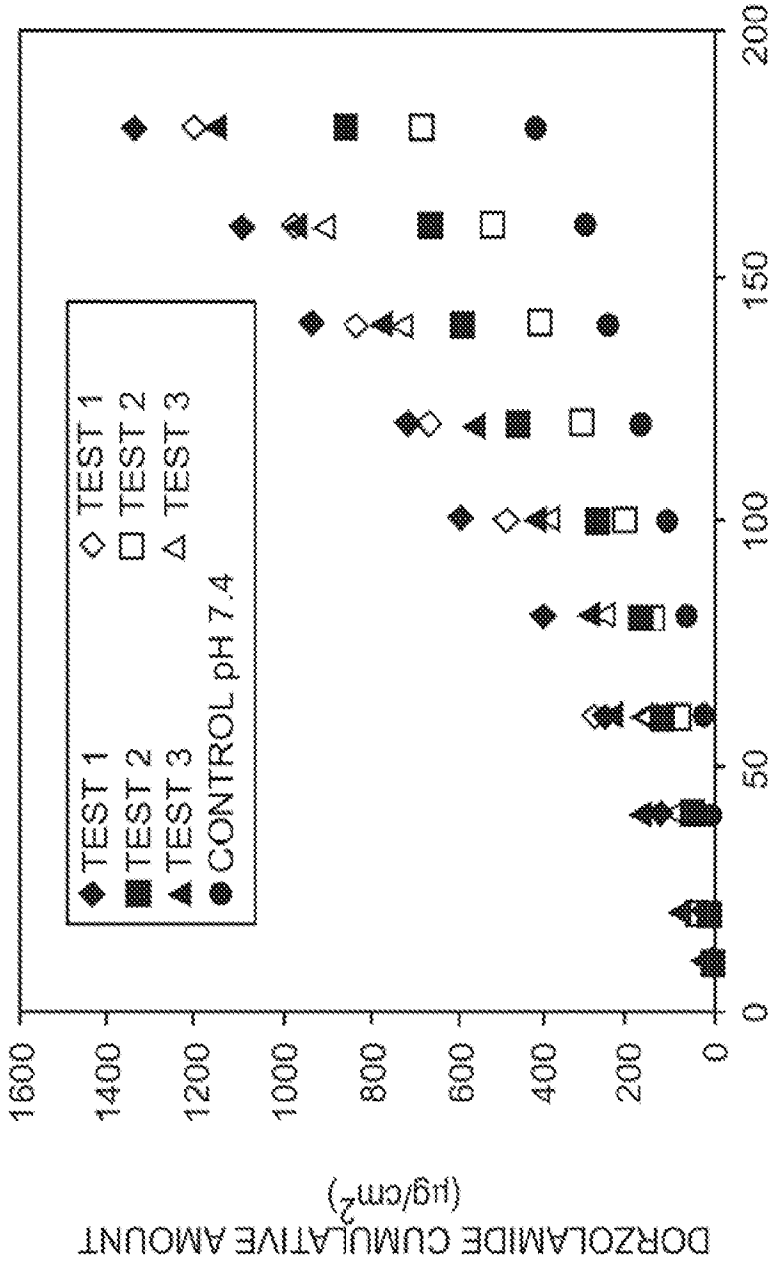

DORZOLAMIDE CUMULATIVE AMOUNT (µg/cm²)
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
200
TIME (MIN)
TEST 1
TEST 2
TEST 3
CONTROL pH 7.4
◇ TEST 1
□ TEST 2
△ TEST 3

FIG. 36

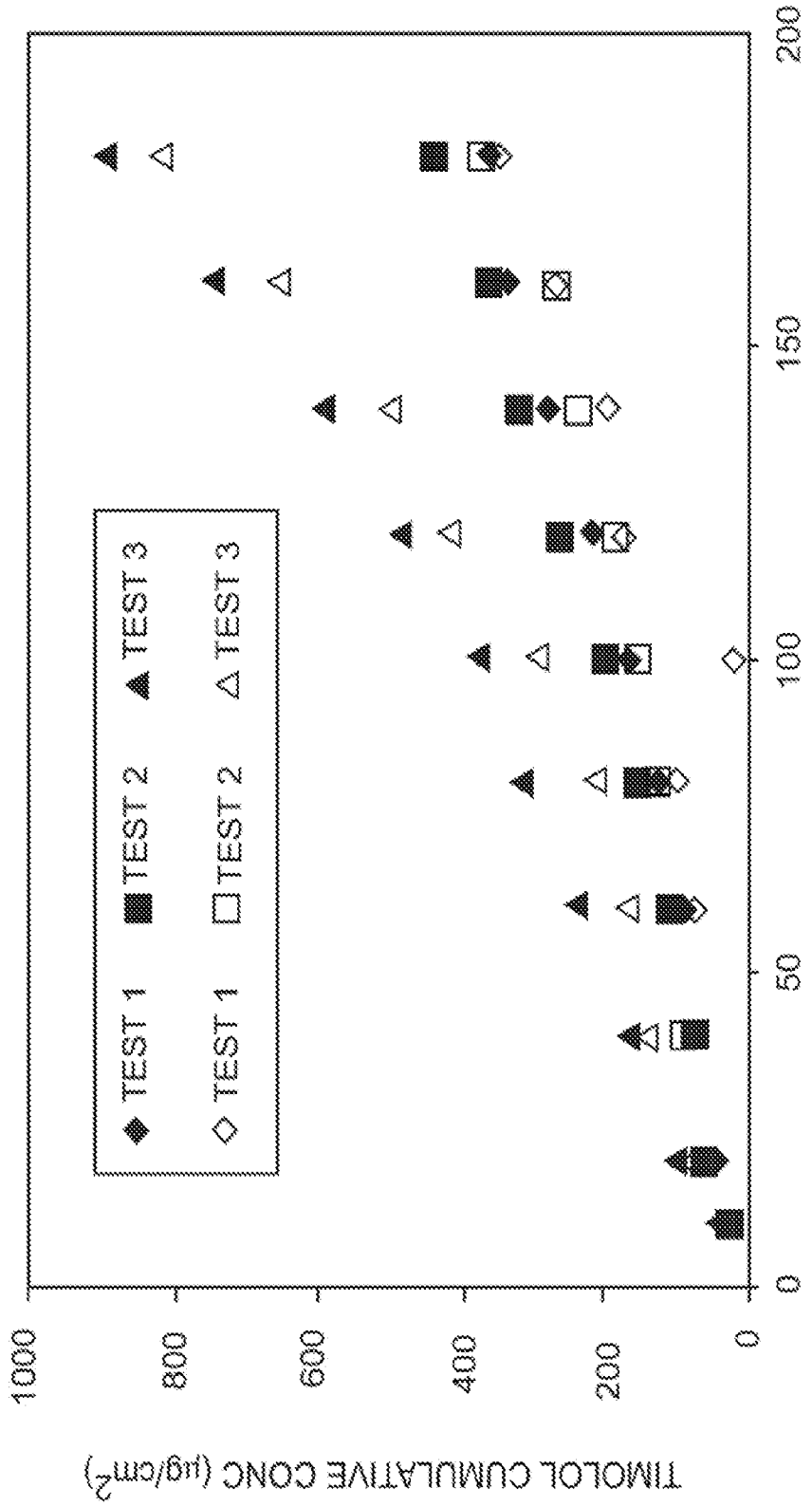

TIMOLOL CUMULATIVE CONC (μg/cm²)
1000
800
600
400
200
0
0
50
100
150
200
TIME (MIN)
TEST 1
TEST 2
TEST 3
TEST 1
TEST 2
TEST 3

FIG. 37

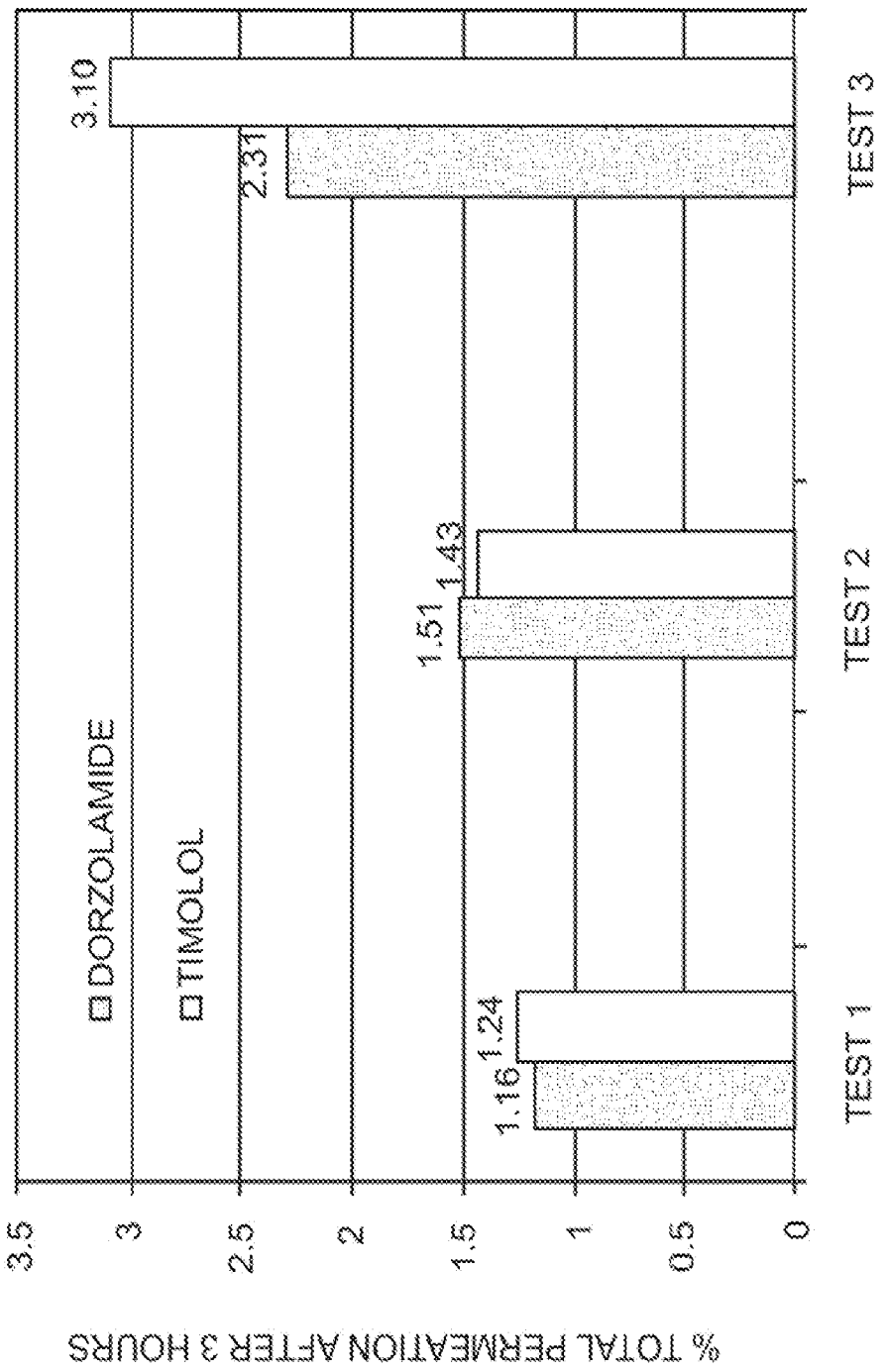

% TOTAL PERMEATION AFTER 3 HOURS
3.5
3
2.5
2
1.5
1
0.5
0
DORZOLAMIDE
TIMOLOL
3.10
2.31
1.51
1.43
1.16
1.24
TEST 3
TEST 2
TEST 1

FIG. 38

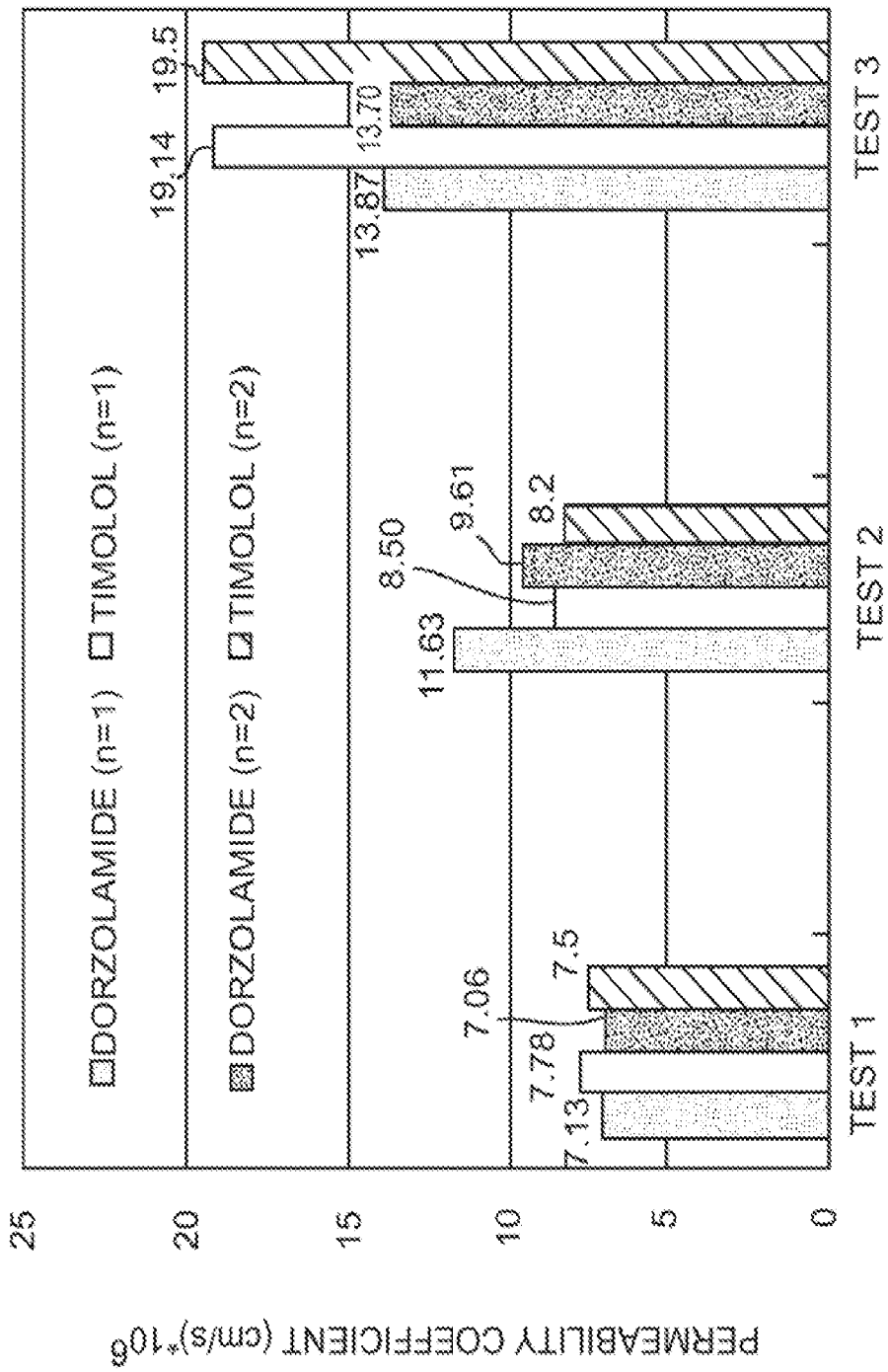

DORZOLAMIDE (n=1)
TIMOLOL (n=1)
DORZOLAMIDE (n=2)
TIMOLOL (n=2)
19.5
19.14
13.70
13.87
8.2
9.61
8.50
11.63
7.5
7.06
7.78
7.13
TEST 3
TEST 2
TEST 1
25
20
15
10
5
0
PERMEABILITY COEFFICIENT (cm/s)*10^6

FIG. 39

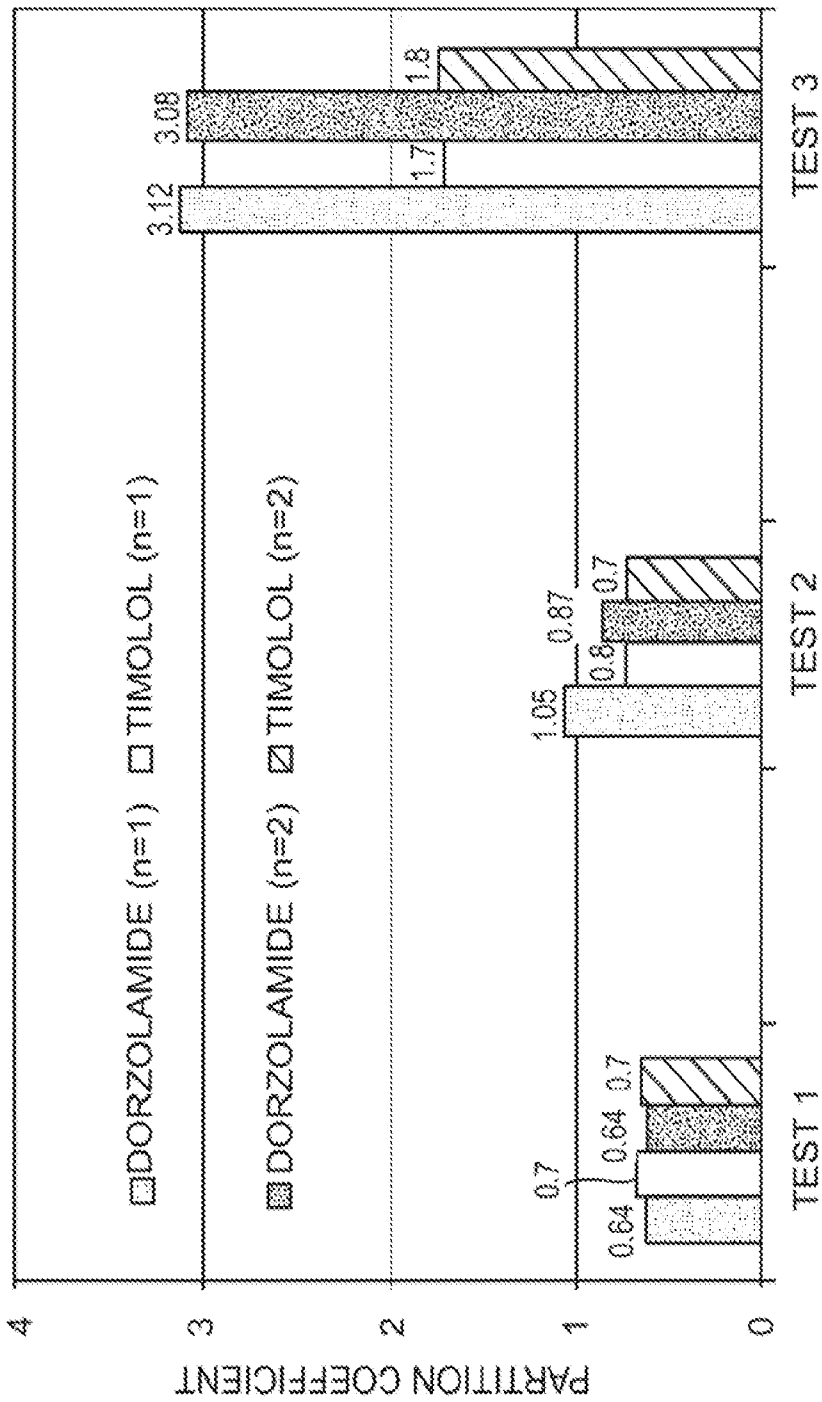

PARTITION COEFFICIENT
4
3
2
1
0
DORZOLAMIDE (n=1)
TIMOLOL (n=1)
DORZOLAMIDE (n=2)
TIMOLOL (n=2)
TEST 1
TEST 2
TEST 3
0.7
0.64
0.64
0.7
1.05
0.8
0.87
0.7
3.12
1.7
3.08
1.8

FIG. 40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/35147

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/74 (2011.01)
USPC - 424/78.08
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 424/78.08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 462, 482, 486, 497 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar
Search Terms Used: ophthalmic, hyperbranched polymer, highly branched polymer, dorzolamide, timolol, carbonic anhydrase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2008/0180803 A1 (Seybert et al.) 31 July 2008 (31.07.2008) para [0013], [0035]-[0036]	1, 6/(1), 14/(6/(1)) --- 2-5, 6/(2), 7-13, 14/(6/(2)), 15-16
Y	US 2007/0048337 A1 (Arthur) 01 March 2007 (01.03.2007) para [0010], [0052], [0074]-[0075], [0099], [0114], [0184]	2-4, 6/(2), 7-13, 14/(6/(2)), 15/(14/(6/(2))), 16-18
Y	US 2006/0204472 A1 (Paleos et al.) 14 September 2006 (14.09.2006) para [0008]-[0009], [0027], [0074]	5, 15, 17-18
Y	US 2010/0008993 A1 (Proksch et al.) 14 January 2010 (14.01.2010) para [0021]; Table 1	2, 4, 6/(2), 9-10, 12-13, 14/(6/(2)), 15/(14/(6/(2))), 16/(14/(6/(2))), 17-18

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 2011 (15.07.2011)	25 JUL 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)