OPHTHALMIC PHARMACEUTICAL COMPOSITIONS CONTAINING DORZOLAMIDE, METHODS FOR THEIR PREPARATION, AND FOR THEIR USE.

TECHNICAL FIELD.

[0001] The present application is related to the field of ophthalmic compositions, more specifically to novel pharmaceutical compositions with enhanced performance and stability, containing Dorzolamide or pharmaceutically acceptable salts thereof either as the sole active pharmaceutical ingredient (API), or in combination with one or more of other ophthalmic APIs or their pharmaceutically acceptable salts. Also, the present application refers to novel methods to prepare such compositions, and those methods or pharmaceutical uses of the compositions intended to decrease intraocular pressure in patients suffering glaucoma, ocular hypertension or any other ocular disease that increases the normal levels of intraocular pressure.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient

BCD: hydroxypropyl beta-cyclodextrin

BLD: below the limit of detection

Dorzolamide: any form of Dorzolamide

DZL: neutral solid phase of Dorzolamide; or Dorzolamide base

DZL-HCl: hydrochloride salt of Dorzolamide; or Dorzolamide hydrochloride

D1: formulation D1-B88A

D2; formulation D2-B84A

D3: formulation D3-B81E1

D4: formulation D4-B84B

D5: formulation D5-B81E2

IOP: intraocular pressure

FDA: Food and Drug Administration

HPLC: high performance liquid chromatographic

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

NaOH: sodium hydroxide

NOM: Official Mexican standards

q.s.: amount which is enough

RH: Relative humidity

T1: formulation T1-B88B

T2: formulation T2-B82K1

T3: formulation T3-B82K2

T4: formulation T4-B81K3

T5: formulation T5-B84G

T6: formulation T6-B84E

T7: formulation T7-B82K4

T8: formulation T8-B84F

T9: formulation T9-B81K5

UV: Ultraviolet

VAS: Visual analog scale

BACKGROUND.

[0002] The eye contains internally two types of fluids: within the posterior chamber there is a jelly-like substance called vitreous humor, whereas a more-watery liquid called aqueous humor fills the anterior chamber, which is delimited by the cornea and the lens. In the healthy eye, a small amount of aqueous humor is produced constantly whereas a similar amount drains out mainly through the iridocorneal angle. These mechanisms maintain a constant volume of aqueous humor throughout the day. This production-drainage equilibrium is determinant to maintain a stable intraocular pressure (IOP) (*American Association of Ophthalmology*). The imbalance of this equilibrium leads to increased IOP, also known as ocular hypertension, which is the most important risk factor for glaucoma. According to recent data from the Bright Focus Foundation "there are 80 million people worldwide with glaucoma, and this number is expected to increase to over 111 million by 2040".

[0003] Carbonic anhydrase inhibitors have been recognized as one of the therapeutic strategies used to regulate IOP as well as the blockage of beta-adrenergic receptors. However, the regulating mechanisms of IOP are complex and some of their key factors are still under exploration. For instance, recent studies have found potential additional regulation mechanisms for IOP control such as the 2,3,7,8-tetrachlorodibenzo-p-dioxin inducible by poly-adenosine diphosphate ribose polymerase (*Zhang, Commun. Biol 5, 1386 (2022*)).

[0004] Therefore, combining different APIs that target different routes to control, decrease or prevent the elevation of the IOP are a common therapy nowadays.

[0005] (4S,6S)-4-(ethylamino)-6-methyl-7,7-dioxo-5,6-dihydro-4H-thieno-[2,3-β]-thiopyran-2-sulfonamide, whether in its base form or any of its salts (thereafter referred as Dorzolamide), is known in the art for being a carbonic anhydrase inhibitor, which is an enzyme involved in the production of aqueous humor. Thus, by inhibiting the activity of this enzyme, Dorzolamide is able to decrease IOP (*Martens-Lobenhoffer J., Clin Pharmacokinet. 2002*).

[0006] Dorzolamide-containing ophthalmic compositions as well as other APIs to control IOP have some recognized side effects such as, among others, cytotoxicity, decreased bioavailability, decreased stability, burning, stinging, itching, redness, swelling, other signs of irritation on the eye or eyelid, or discomfort when medicine is applied. All these side effects have a final impact of non-compliance of the prescribed treatments for glaucoma or ocular hypertension.

[0007] Prior art technologies have tried to solve the aforementioned disadvantages by different approaches that aim to generate compositions for the control of IOP. For instance, the document JP2015120035A teaches pharmaceutical compositions formulated with fluorosilicone acrylate, sodium carboxymethylcellulose, hydroxypropyl methylcellulose, tetrahydrozoline hydrochloride, sodium carboxymethylcellulose, propylene glycol, zinc sulfate, Dorzolamide hydrochloride, Timolol maleate, azithromycin, Brimonidine tartrate and combinations thereof. This document also teaches that toxicity during administration can be reduced if the solubility-enhancing substance is easily degraded in the cellular environment.

[0008] Document WO 2018/226942 teaches ophthalmological compositions for the treatment of glaucoma comprising Brimonidine at an affixed concentration from about 0.01% to about 0.050% w/v which is combined with a second glaucoma drug that, in some embodiments is Dorzolamide. The document also focuses in achieving formulations that solve the existing technical problems by achieving low-Brimonidine concentration formulations that are still therapeutically effective.

[0009] The document "*Novel Drug Delivery Systems Fighting Glaucoma: Formulation Obstacles and Solutions*", published by Rahic, et al. (Pharmaceutics, 2021), describes a thorough and updated review of strategies used to solve technical problems of drugs intended to control IOP. The document describes solutions to reduce the frequency of APIs administration, including *in situ* gel systems, nanosystems, ocular inserts, contact lenses, collagen corneal shields, ocular implants, microneedles, and iontophoretic devices, among others.

[0010] Document WO 2009/125246 describes an ophthalmic composition for the treatment of glaucoma or ocular hypertension. The formulation contains a cyclodextrin and a therapeutically effective amount of a therapeutic component, wherein the composition has a pH between 5.8 and 6.5, and wherein the therapeutic components comprise Dorzolamide and latanoprost or a pharmaceutically acceptable salt thereof.

[0011] Also, document MX 295 966 describes a pharmaceutical composition comprising a triple combination of Dorzolamide with two other APIs. The compositions contain a very specific mixture of excipients that were able to solve partially some of the technical problems described above. The compositions described in MX 295 966 were constructed with an affixed base of excipients, namely polyoxyl 40 stearate, sodium borate, sodium chloride and benzalkonium chloride, resulting this particular combination in some stability of the compositions, but the main problem for patient compliance (burning sensations) remains unresolved.

[0012] Different approaches have been tested in the art to try to lower the side effects that accompany the use of the different molecules that are known to be useful in the control of IOP. However, there is still a wide need for technologies that solve the stability of formulations containing Dorzolamide, alone or in combination with other APIs, while at the same time lower or even eliminate the side effects associated to the use of those molecules without affecting their recognized therapeutic performances.

[0013] **OBJECT OF THE INVENTION.**

[0014] The applicants for the present invention have found a completely different approach to those known in the art to solve some of the most important side effects that are related to the use of APIs to control IOP, which would help patients to adhere and comply with their IOP treatments. The technical solution proposed by the present application is based on a special treatment of the known APIs for the control of IOP and their complexation afterwards in specially designed solubilizing carrier matrixes.

SUMMARY.

[0015] The present application provides ophthalmic pharmaceutical compositions for the control of IOP, which are formulated through a specially designed method of preparation that allow the known APIs and their salts to be treated in such a way that a sufficient stable base form is obtained. Once in the base form, APIs are introduced into different carrier matrixes producing a complex that is physically and chemically stable at critical pH values, and allows the use of APIs at concentrations never explored before under such conditions. Additionally, this technology allows an adequate bioavailability and avoids the side effects collectively known as "*burning sensation*".

[0016] Due to the broad design of the technology object of the present application, one of its major advantages is that it is applicable and effective in all the aspects described above for almost every single small molecule known or to be known and/or salts of such molecules; either if the therapeutic molecule is utilized alone in a single-API product or in a product that incorporates a combination of more than one API for the control of IOP.

[0017] During the development of the present application, the aforementioned technical advantages, *i.e.,* stability, bioavailability and avoidance or decrement of "*burning sensation*" were demonstrated even for complex formulations comprising a double combination of APIs: Dorzolamide/Timolol or salts thereof; and even in a triple combination of APIs for a formulation comprising: Dorzolamide/Timolol/Brimonidine or salts thereof.

[0018] Therefore, there is sufficient evidence to support that the scope of the technology developed for the formulations of the present invention applies to ophthalmic products designed for the control of IOP that incorporate from 1 to *n* APIs in its design.

[0019] The formulations of the present application optimally work at a pH value interval ranging from 5 to 8; but further pH intervals are possible following the same basic technical principles of the technology. This is relevant because prior to the development of the technology object of the present application, solubility of Dorzolamide and other of the most common drugs utilized for the control of IOP was only possible at low pH values.

[0020] The pharmaceutical compositions of the present invention utilize different types and different mixtures of pharmaceutically acceptable carriers and polymers to be constructed, together with the APIs and/or their pharmaceutically acceptable salts that were treated previously to their introduction to such

mixtures of carriers and polymers. In some embodiments, the treatment can be done after the APIs or their pharmaceutically acceptable salts are introduced to the mixtures of carriers and polymers.

[0021] Osmotic agents, solubilizing agents, buffer systems and water are also included in the compositions of the present application.

[0022] The potential mixture of any of the pharmaceutically acceptable agents referred above should be altogether understood and referred as "the carrier matrix".

[0023] Stability and bioavailability of the compositions was demonstrated using *in vitro* and *in vivo* models. Furthermore, improvement of the "*burning sensation*" was demonstrated on a panel of healthy volunteers who tested the novel compositions of the present invention. Upon ocular instillation, the volunteers compared and ranked the "*burning sensation*" perceived with the novel formulations in comparison with known compositions in the art.

[0024] Also, the present application describes a novel process for treating/conditioning the APIs and the solution media containing those APIs prior to their introduction into the carrier matrix.

[0025] All the possible embodiments within the scope of the present application can be combined with any type of pharmaceutical systems that allow their use and storage without any added preservative molecule(s).

BRIEF DESCRIPTION OF TABLES AND FIGURES.

[0026] The detailed description refers to the accompanying tables and figures. Entities and/or embodiments represented in the tables and figures may be indicative of one or more entities and/or embodiments and thus, reference may be made interchangeably to single or plural forms of the entities in the discussion. Tables, figures and examples included in the present application are illustrative but not limitative. Foregoing aspects and other characteristics of the descriptions are explained as follows:

[0027] Tables 1 – 5 illustrate examples of compositions containing Dorzolamide and one additional API (*e.g.,* Timolol) that can be formulated using the teachings of the present application.

[0028] Table 6 shows the physicochemical characteristics of Dorzolamide compositions containing an additional API (*e.g.,* Timolol).

[0029] Tables 7 – 11 show the stability results of the Dorzolamide compositions containing an additional API (*e.g.,* Timolol) over a period of 3 months.

[0030] Tables 12 – 20 illustrate examples of compositions containing Dorzolamide and two additional APIs (*e.g.,* Timolol/Brimonidine) that can be formulated using the teachings of the present application.

[0031] Table 21 shows the physicochemical characteristics of Dorzolamide compositions containing two additional APIs (*e.g.,* Timolol/Brimonidine).

[0032] Tables 22 – 30 show the stability results of Dorzolamide compositions containing two additional APIs (*e.g.,* Timolol/Brimonidine) over a period of 3 months.

[0033] Figures 1 and 2 show the enhanced solubility of Dorzolamide at neutral pH using cyclodextrins.

[0034] Figures 3 and 4 show the results from the bioavailability tests performed on the Dorzolamide compositions containing an additional API (*e.g.,* Timolol).

[0035] Figures 5 – 7 show the bioavailability of Dorzolamide compositions containing two additional APIs (*e.g.,* Timolol/Brimonidine).

[0036] Figures 8 and 9 show the level of "*burning sensation*" of healthy volunteers after the use of Dorzolamide composition containing one or two additional APIs.

DETAILED DESCRIPTION

[0037] Ophthalmic pharmaceutical compositions directed to the control of IOP containing a "high concentration" of Dorzolamide, such as those containing 2% or more, tend to precipitate at pH values just above 6. This is because of the pH-solubility relationship (i.e., solubility of Dorzolamide decreases at a pH above 6). Therefore, pH values in these types of Dorzolamide-containing ophthalmic compositions play a major role on their thermodynamic stability and their performance.

[0038] Dorzolamide molecule is a base compound according to the Brønsted–Lowry acid–base theory, this means it can accept a proton from another molecule. The pKa of its conjugate acid is approximately 6.4. Consequently, when the pH of an aqueous solution is equivalent to the pKa of Dorzolamide, the percentage of ionized and unionized Dorzolamide forms in the solution is 50 % each. However, the percentage of the conjugated acid will increase proportionally when the pH of the solution is lower than 6.4. On the other hand, the percentage of the non-ionized Dorzolamide will increase proportionally when the pH of the solution is higher than its pKa. Since ionized species are by far more hydrophilic than their unionized counterparts, solubility (defined as the highest concentration of a solute achievable in a defined solvent at a specific temperature) increases in accordance with solute ionization. Therefore, solubility of Dorzolamide is significantly higher at pH values below its pKa and drastically lower at pH values above its pKa.

[0039] The ionization percent in solution of an acid/base molecule of Dorzolamide can be modified either i) by tailoring the solution chemistry or ii) by changing the ionic state of the solute. Both strategies were tested in the present application, as follows:

[0040] For the case of the first strategy, applicants utilized the hydrochloride salt of Dorzolamide (described also in this document as DZL-HCl). Dissolution of DZL-HCl in water acidifies spontaneously the solution in accordance with concentration, wherein a 2.22 % DZL-HCl can be readily dissolved but yields solutions with pH values lower than 6. To modify the solution chemistry, a basic agent such as sodium hydroxide (NaOH) must be added to neutralize the thereof acidic solution, in such amount that depends on the equivalents or molar number of Dorzolamide in solution and the desired pH value of the final solution. Addition of the base molecule utilized to neutralize the acidic solution that results from dissolving DZL-HCl can be added prior,

during or after the addition of the salt of Dorzolamide. However, increasing the pH of a 2.22% DZL-HCl solution to values near 7 (neutral) induces a supersaturate state wherein Dorzolamide will spontaneously precipitate from the solution due to thermodynamic constrains, yielding a Dorzolamide solution of a concentration of about 0.3 %.

[0041] For the second strategy, the neutral solid phase of Dorzolamide was utilized (described interchangeable in this document as Dorzolamide base form or DZL). Dissolution of DZL in water yields spontaneously a solution pH in the range of 7 – 8. However, the intrinsic solubility of DZL in water is ≈3 mg/mL (0.3 %); then, the addition of DZL above that concentration results in spontaneous precipitation of DZL in excess.

[0042] Applicants realize that due to the low solubility of Dorzolamide at neutral pH, a 2% drug product of Dorzolamide at pH values above 6 can be achieved in the form of an ophthalmic suspension (solid particles of Dorzolamide dispersed in an external phase, e.g., aqueous vehicle). However, formulation of suspensions has some disadvantages due to the nature of this pharmaceutical form. Suspensions have a natural tendency to flocculate or aggregate depending on the attractive and repulsive forces within the system, in other words, these systems are thermodynamically unstable. This is a major concern that hurdle different stages such as drug development, manufacturing, quality control, bioavailability, and patient compliance. For instance, developing suitable suspension formulations depends on the interrelated properties of the solid (e.g., crystalline form, solution-mediated phase transformation, solubility, polymorphism, crystal habit, particles size, particle distribution and wettability), and the external phase (e.g., pH, excipients, water content and osmolarity). On the other hand, at a manufacturing level, sedimentation and particle size of suspensions can hurdle reproducibility and uniformity among batches. Additionally, a big challenge for suspensions is to stablish a sterilization process for the terminal drug product, which is mandatory for ophthalmic medicaments. Generally, the sterilization of ophthalmic medicaments is preferentially achieved by filtration. This process is suitable for ophthalmic solutions (e.g., Trusopt®, Cosopt®), however, filtration of suspensions can be challenging due to the presence of dispersed particles; then, the formulation of ophthalmic solutions is advantageous over suspensions.

[0043] Nevertheless, it is a technical challenge to construct thermodynamically stable pharmaceutical solutions for the treatment of IOP that can support those high concentrations of Dorzolamide (between 0.3% and 2.5%) at neutral pH values (emphasis added), and even more when they are combined with additional APIs; without compromising their physical and chemical stability, their ocular bioavailability, and without showing the “burning sensation”.

[0044] To solve these technical problems, the applicants of the present application specially designed a solubilizing system in the form of an inclusion complex host – guest, wherein the host is a carrier matrix. The guest can be any known API for the control of IOP. A method for the preparation of such system, was also designed to keep the APIs dissolved without the risk of precipitating during storage (thermodynamically stable systems).

[0045] As mentioned above, Dorzolamide has a secondary amine with a pKa of approximately 6.4. Consequently, the solubility of Dorzolamide decreases drastically at pH values above 6. To solve this issue, applicants created an additional chemical equilibrium in solution to increase the apparent solubility of Dorzolamide by using the aforementioned complexation system, yielding a thermodynamically stable system.

[0046] Applicants then selected a strategy considering the structural and electronic properties of Dorzolamide molecule. Furthermore, applicants not only considered to increase Dorzolamide solubility at neutral pH, but also the ability to release the API to maintain equal or better bioavailability than the current marketed products, this is to ensure a similar therapeutic effect of the APIs.

[0047] Carrier herein used to solubilize DZL were cyclic oligosaccharides containing linked D-glucopyranose units (cyclodextrins). It was found by the inventors of the present application that these compounds contain a hydrophobic cavity wherein hydrophobic structural moieties of molecules can fit depending on their stoichiometric ratio, size, chemical structure, and physicochemical properties.

[0048] These APIs-cyclodextrin complex allow, for the first time, the generation of novel thermodynamically stable solutions, in conditions not viable before this invention (emphasis added).

[0049] Applicants interestingly found that among cyclodextrins, beta-cyclodextrin best improves the solubility of Dorzolamide (See Experiment 1). However, this invention does not exclude the use of other cyclodextrins to achieve thermodynamically stable formulations of Dorzolamide at neutral pH.

[0050] The carrier matrix used to formulate the pharmaceutical compositions of the present application and host the APIs includes pharmaceutically acceptable water, at least one pharmaceutically acceptable solubilizing agent, at least one pharmaceutically acceptable viscosity-modifying agent, at least one pharmaceutically acceptable pH regulating agent or pH regulating system of agents, which are used altogether, solely, or in any mixture of at least one of such agents.

[0051] The water used for the formulations of the present application is selected from the group comprising purified water, water for injection, sterile water for injection, sterile water, bacteriostatic water, ultra-microfiltered and/or nano-filtered water, deuterated water, or any combination or mixture thereof.

[0052] Preferred pharmaceutically acceptable solubilizing agents are selected from the group comprising dextrans, dextrins, cyclic oligosaccharides including but not limited to cyclodextrins comprising alpha-cyclodextrin, beta-cyclodextrin, gamma-cyclodextrin, hydroxypropyl alpha-cyclodextrin, hydroxypropyl beta-cyclodextrin, hydroxypropyl gamma-cyclodextrin; more preferably hydroxypropyl beta-cyclodextrin; surfactants such as fatty alcohol esters, fatty acid esters, polyoxyethylene sorbitan fatty acid esters (e.g., polyoxyethylene (20) sorbitan monooleate or Tween 80, polyoxyethylene (20) sorbitan monostearate or Tween 60, polyoxyethylene (20) sorbitan monolaurate or Tween 20, and other Tweens), sorbitan esters, glycerol esters, polyethylene glycols, cetyl alcohol, calcium carboxymethylcellulose, sodium carboxymethylcellulose, polyoxyethylene (40) stearate, polyoxyethylene castor oil, derivatives thereof, and any combination thereof.

[0053] The concentration of at least one pharmaceutically acceptable solubilizing agent in the pharmaceutical compositions of the present application ranges from 0.5 to 60 % w/v.

[0054] Preferred osmotic agents used for the compositions of the present application are selected from the group comprising pharmaceutically accepted agents such as salts (e.g., sodium chloride); polyols such as glycerol, polyethylene glycol and propylene glycol; and carbohydrates (e.g., mannitol, trehalose).

[0055] The concentration of at least one pharmaceutically acceptable osmotic agent in the pharmaceutical compositions of the present application ranges from 0.1 to 10 % w/v.

[0056] Preferred viscosity-modifying agents utilized for the compositions of the present application are selected from the group comprising cellulose derivatives such as methylcellulose, microcrystalline cellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hypromellose or hydroxypropyl methylcellulose, natural gums such as acacia, guar gum, tragacanth, xanthan gum, alginates, carrageenan and locust bean gum; synthetic polymers such as carbomers, polyethylene oxide, propylene glycol alginate, povidone or polyvinyl pyrrolidone, polyvinyl alcohol and poloxamers.

[0057] The concentration of at least one viscosity-modifying agent in the pharmaceutical compositions of the present application ranges from 0.1 to 10 % (w/v).

[0058] Preferred pH-regulating agents or pH regulating systems utilized for the compositions of the present application are selected from the group comprising of hydroxide and acid forms of metallic and non-metallic elements such as sodium hydroxide, potassium hydroxide, borates, phosphates, acetic acids, sodium acetates, ammonium hydroxides, ammonium chlorides, citric acids, and sodium citrates either as individuals or as a system, carbonic acids with bicarbonate ions either as individuals or as a system, and potassium-based systems.

[0059] The concentration of at least one pH-regulating agent or pH regulating system in the pharmaceutical compositions of the present application ranges from 0.01 to 2 % (w/v).

[0060] Dorzolamide can be present alone or in combination with at least one other API for the treatment of IOP in the different embodiments of the present application; and it can be in its native form, or in the form of any of their pharmaceutical basic or acid salt forms.

[0061] The pharmaceutical system utilized herein is a solution-based platform suitable for sterilization by filtration with 0.2 µm pore size filters. Furthermore, this system is thermodynamically stable at different pH values, particularly in the range of 6.4 – 7.4 (emphasis added) wherein Dorzolamide displays low solubility.

[0062] When Dorzolamide is formulated with at least one other API for the treatment of IOP; such other API can be selected from the group comprising prostaglandin analogues and/or any salts or derivatives thereof, miotic molecules and/or any salts or derivatives thereof, beta blockers and/or any salts or derivatives thereof, alpha-adrenergic agonists and/or any salts or derivatives thereof, carbonic anhydrase inhibitors and/or any salts or derivatives thereof and Rho kinase inhibitors and/or any salts or derivatives.

[0063] The manufacturing process of the compositions described in the present application is performed by dissolving API(s) and excipients in sufficient aqueous volume to achieve the desired final concentration of components and solution pH. An example of one of the embodiments of the process is as follows:

[0064] A 1-liter solution comprising only DZL-HCl is prepared by dissolving 22.2 g of DZL-HCl in certain amount of water at room temperature, approximately 400 – 600 mL. Afterwards, 200 g of hydroxypropyl beta-cyclodextrin is added and dissolved in the foregoing solution mixture by stirring. The pH of solution is further adjusted preferentially to 6.8 – 7.2 through the addition of NaOH, added as an aqueous solution or as a solid.

Lastly, enough water is added to the solution mixture to complete 1 L. The resulting final product is a homogeneous solution suitable for terminal sterilization using 0.2 µm pore size filters. Formulation can be further packaged in suitable ophthalmic bottles, whether in single or multidose presentation.

[0065] In another embodiment of the process of the present application, when Dorzolamide base (DZL) is used, a 1-liter solution comprising DZL is prepared by dissolving 200 g of hydroxypropyl beta-cyclodextrin in about 600 mL of water. After dissolution, 20.0 g of DZL is added under stirring. Afterwards, enough water is added to the solution mixture to complete 1 L. The resulting final product is a homogeneous solution with a pH in the range of 6.8 – 7.2, suitable for terminal sterilization using 0.2 µm pore size filters. Formulation can be further packaged in suitable ophthalmic bottles, whether in single or multidose presentation.

[0066] A person skilled in the art will be able to identify that the aforementioned processes can be subtle of modifications such as changing the order of addition of API and excipients, the solid-state form of the API (e.g., molecular, salt, cocrystal, polymorphs, adducts), the mechanism of dissolution, the volume of water used to generate the first solution mixture, the neutralizing agent, the final pH, the sterilization mechanism, the temperature used for dissolving the solutes, and the batch size, among others.

[0067] The use of preservatives can also be avoided in some embodiments of the process by packaging the formulation in preservative-free containers. Additionally, the ophthalmic drug product can be tailored by adding other excipients such as solubilizers, pH-regulating systems, osmotic agents, viscosity-modifying agents, preservative, or other APIs; which can be added indistinctly since the final solution-based system is thermodynamically stable.

[0068] The technology described above is based on the treatment of APIs either in its native form or when they are already in the form of any acceptable salt.

[0069] pH values of the pharmaceutical compositions of the present application range in some of their embodiments from 5.0 to 8.0, more preferably from 5.4 to 7.8, even more preferably from 6.0 to 7.6, and ideally from 6.8 to 7.2.

[0070] Additional embodiments of the present application that also showed improved stability values in comparison with known formulations in the prior art are based on the use of a very specific mixture of excipients comprising hypromellose, mannitol, citric acid, sodium citrate and water, in combination with Dorzolamide either alone as the sole API or in double or triple combinations with other APIs such as prostaglandin analogues and/or any salts or derivatives thereof, miotic molecules and/or any salts or derivatives thereof, beta blockers and/or any salts or derivatives thereof, alpha-adrenergic agonists and/or any salts or derivatives thereof, carbonic anhydrase inhibitors and/or any salts or derivatives thereof, and Rho kinase inhibitors and/or any salts or derivates thereof. Even more preferably, Dorzolamide was combined with Timolol (double combinations) or with Timolol and Brimonidine (triple combinations).

[0071] Further embodiments that also showed improved stability values in comparison with known formulations in the prior art are based on the use of another very specific mixture of excipients comprising polyoxyl 40 stearate, sodium chloride, mannitol, sodium borate decahydrate, sodium hydroxide and water for injection, in combination with Dorzolamide either alone as the sole API or in double or triple combinations with other APIs such as prostaglandin analogues and/or any salts or derivatives thereof, miotic molecules and/or any salts or derivatives thereof, beta blockers and/or any salts or derivatives thereof, alpha-adrenergic

agonists and/or any salts or derivatives thereof, carbonic anhydrase inhibitors and/or any salts or derivatives thereof, and Rho kinase inhibitors and/or any salts or derivates thereof. Even more preferably, Dorzolamide was combined with Timolol (double combinations) or with Timolol and Brimonidine (triple combinations).

[0072] These additional embodiments of the present invention that showed fairly good stability values might utilize benzalkonium chloride as a preservative, but the use of such preservatives can also be avoided as in the embodiments described previously by packaging the formulation resulting from these particular embodiments in preservative-free containers.

[0073] Additionally, the ophthalmic drug product can be tailored by adding other excipients such as solubilizers, pH-regulating systems, osmotic agents, viscosity-modifying agents, preservatives, or other APIs, which can be added indistinctly since the final solution-based system is thermodynamically stable.

[0074] The scope of the aforementioned embodiments and methods of the present application will be better understood with the following non-limiting examples.

EXAMPLES

EXAMPLE 1. ENHANCED SOLUBILITY OF DORZOLAMIDE AT NEUTRAL PH USING CYCLODEXTRINS

Methods:

[0075] Applicants decided to test the hydroxypropyl derivative of cyclodextrins since these compounds share the geometrical and cyclic configuration of the parent linked D-glucopyranose units but they are functionalized with hydroxypropyl groups to enhance its own aqueous solubility.

[0076] Solubility experiments were performed on different concentrations of either hydroxypropyl beta-cyclodextrin or hydroxypropyl gamma-cyclodextrin wherein an excess amount of Dorzolamide base (DZL) was added into the dissolution media. Slurries were stirred at least 18 hours at 25 °C to assure equilibrium. Samples were filtered through 0.2 µm pore size filters and the concentration of Dorzolamide in the filtered solutions were assessed using a validated high performance liquid chromatographic (HPLC) method.

Results:

[0077] The pH of solutions using either hydroxypropyl beta-cyclodextrin or hydroxypropyl gamma-cyclodextrin was 7.0 ± 0.2.

[0078] As noted in Figure 1, the solubility of Dorzolamide increased accordingly with the concentration of hydroxypropyl beta-cyclodextrin found in solution. It is important to mention that the intrinsic solubility of Dorzolamide in water is approximately 3 mg/mL whereas a 20 % hydroxypropyl beta-cyclodextrin solution increased DZL solubility to 22 mg/mL.

[0079] On the other hand, a similar set of experiments were performed on solution containing 5, 10, 15 and 20 % of hydroxypropyl gamma-cyclodextrins. As noted in Figure 2, solubility of Dorzolamide was 4.5, 5.8, 7.3 and 8.6 mg/mL respectively, which demonstrates that hydroxypropyl gamma-cyclodextrin can also increase solubility of DZL in accordance with concentration.

[0080] It was concluded therefore, that complexation of Dorzolamide with cyclodextrins allows the formulation of thermodynamically stable Dorzolamide solutions at neutral pH values and at therapeutically relevant concentrations. A vis-à-vis comparison among cyclodextrins clearly show that the solubilizing ability of hydroxypropyl beta-cyclodextrin was significantly higher. This indicates that the complexation of Dorzolamide molecules is more favorable with beta-cyclodextrin than with gamma-cyclodextrin.

[0081] Additionally, further experiments demonstrated that the solubilizing effect of hydroxypropyl beta-cyclodextrin is conserved even though when a 2.2 % DZL-HCl solution is adjusted to neutral pH; and even in the presence of other excipients and APIs found in the formulation. This demonstrate that the interaction formed between the Dorzolamide and cyclodextrins is conserved despite other components added into the formulation.

EXAMPLE 2. EVALUATION OF ACCELERATED STABILITY OF FORMULATIONS COMBINING TWO APIs AT 40°C AND 25% OF RELATIVE HUMIDITY

Methods:

[0082] A series of pharmaceutical compositions according to the teachings of the present application were prepared with hydroxypropyl beta-cyclodextrins (BCD) and a double combination of APIs, *i.e.,* Dorzolamide/Timolol containing a concentration of Dorzolamide 2% and Timolol 0.5% (Tables 1 – 5). Formulations were named D2-B84A, D3-B81E1, D4-B84B and D5-B81E2; also described in this document as D2, D3, D4 and D5, respectively. All formulations were adjusted to a final pH of 7.0 ± 0.4 (Table 6). These formulations were compared with a solution containing also the same fixed combination of Dorzolamide and Timolol but formulated without BCD and showing pH values below 6.0 (D1-B88A or only D1; Table 1).

[0083] Formulations D2 and D3 contained DZL and were prepared according to the present application, whereas D1, D4 and D5 were formulated with DZL-HCl. All proposed formulations contained the maleate salt of Timolol.

[0084] 1 L of formulation D1 was prepared by dissolving 70 g of polyoxyl 40 stearate in approximately 900 mL (±5 %) of water at 80 – 90 °C. Afterwards, the solution was cooled down to 20 – 35 °C, followed by the addition and dissolution of 22.2 g of DZL-HCl. Upon dissolution, 6.8 g of Timolol maleate was added and dissolved, followed immediately by the sequential addition and dissolution of 5.0 g of mannitol, 2.0 g of sodium chloride and 3.2 g of sodium borate decahydrate. pH was further adjusted to 5.5 with sodium hydroxide, and enough water was added to reach 1 L. Lastly, the formulation was terminally sterilized by passing the solution through a 0.2 µm pore size membrane filter (Table 1).

Table 1

Formulation D1	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Polyoxyl 40 stearate	70.0
Sodium chloride	2.0
Mannitol	5.0
Sodium borate decahydrate	3.2
Sodium hydroxide	q.s. to pH 5.5
Water for injection	q.s. to 1 L

[0085] 1 L of formulation D2 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 20.0 g of DZL were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4 followed by the addition of enough water to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 2).

Table 2.

Formulation D2	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide Base	20.0
Timolol maleate	6.8
Hydroxypropyl beta-cyclodextrin	200.0
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0086] 1 L of formulation D3 of the present application was prepared by adding under mechanical stirring 200 g of hydroxypropyl beta-cyclodextrin in approximately 500 mL of room-temperature water. After dissolution, 20.0 g of DZL were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4, followed by the addition of 50.0 g of povidone K30. Enough water was further added to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 3).

Table 3.

Formulation D3	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide base	20.0
Timolol maleate	6.8
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Mannitol	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0087] 1 L of formulation D4 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4 followed by the addition of enough water to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 4).

Table 4.

Formulation D4	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Hydroxypropyl beta-cyclodextrin	200.0
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0088] 1 L of formulation D5 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4, followed by the addition of 50.0 g of povidone K30. Enough water was further added to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 5).

Table 5.

Formulation D5	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Mannitol	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0089] All sterile formulations were packaged in individual 5 mL multidose preservative-free containers. These were stored in chambers set at 40°C/25% relative humidity (RH) to determine whether formulations could withstand low relative humidity environments, in accordance to accelerated tests described in guidelines and regulations such as ICH, FDA and NOM. Analysis of APIs and impurities were performed at time 0, 1 month, 2 month, and 3 months by UV-HPLC.

Results:

[0090] Formulation D1 has physicochemical characteristics like the commercial double combination of Dorzolamide/Timolol (e.g., Cosopt) with a lightly acidic pH (Table 6). Both APIs remained stable through the time at the condition of 40°C and 25% RH. Known and unknown related substances of Dorzolamide and Timolol were no detected or remained below the limits declared by international guidelines. Additionally, no adducts of maleic acid were detected in this formulation (Table 7). D1 is a stable formulation due to the addition of excipients such as polyoxyl 40 stereate, and due to its pH below 6.0. It is commonly known that APIs as Dorzolamide and Timolol are stable at slightly acidic pH values.

[0091] BCD was the main excipient of the four proposed Dorzolamide/Timolol formulations (D2, D3, D4 and D5). It was demonstrated that BCD in combination with the two APIs could increase the solubility of the poorly soluble Dorzolamide, showing the ability of having a thermodynamically stable Dorzolamide-solution at pH values where the intrinsic solubility of Dorzolamide is very low, and even in the presence of a second API e.g., Timolol (Table 6).

[0092] Equivalent ophthalmic solutions containing similar fixed combination of Dorzolamide/Timolol have been reported in the prior art to be only achievable at pH values below 6.5.

Table 6.

Formulation	pH	Osmolality (±5%)
D1	5.5	311
D2	6.6	235
D3	7.0	415
D4	7.4	424
D5	7.0	599

[0093] Applicants of the present application found that the use of DZL or DZL-HCl inherently impacts the electrolyte content of the formulation, which determines the osmolality of the finished drug product. Osmolality of the DZL-HCl formulation is higher than that of DZL formulation, as can be observed in Table 6 (the reader can compare the differences between formulations D2 and D4) and table 21 (see for instance characteristics of formulations T2 and T6). However, osmolality of formulations using either DZL-HCl or DZL complies with USP <771> specification for ophthalmic formulations (171 – 1711 mOsm/kg).

[0094] The presence of other polymers such as polyvinylpyrrolidone or povidone synergically acted with BCD while at the same time slightly increased the viscosity of the solution, which could increase the residence time of the active ingredients on the ocular surface and therefore favor their bioavailability (as demonstrated in the rest of the examples).

[0095] These results also demonstrate that BCD alone is sufficient to endow a thermodynamically stable solution system regardless of pH. Thus, the choice of povidone as an additional excipient can be made as an optional choice for the search for a benefit in the ocular biodistribution of the active pharmaceutical ingredients.

[0096] The four BCD formulations tested (D2, D3, D4 and D5) achieved a pH within close range to 7 (Table 6). This stability study showed that pH remained constant even after the solution was exposed to the accelerated condition of 40°C and 25% of relative humidity for 3 months. Also, all formulations meet the specification for drug content, and known, unknown and total impurities of Dorzolamide and Timolol, in agreement with quality attributes included in national and international drug compendium such as, but not limited to, United States and Mexican pharmacopeia, among others (Tables 7 - 11). This indicates that the chemical stability of APIs is conserved through time within acceptable intervals, which is critical to stablish expiration dates in solution-based ophthalmic drug products.

[0097] Unknown impurities or maleic acid adducts were not identified in any of the formulations, which demonstrates that despite the high concentration of Dorzolamide utilized, the compositions object of the present application achieved for the first time ever, at neutral pH values, that neither Dorzolamide nor Timolol showed signs of degrading or precipitating from the solution.

Table 7.

Formulation D1					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (Dorzolamide)	90 – 110%	102.5 ± 0.4	96.6 ±1.4	100.8 ± 0.1	100.9 ± 0.8
Drug content (Timolol)	90 – 110%	102.4 ± 0.5	98.9 ± 0.6	100.8 ± 0.5	100.2 ± 1.1
Dorzolamide related compound B	< 2.0 %	BLD	0.2 ± 0.0	0.3 ± 0.0	0.46 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	BLD	<0.1	<0.1
Any individual unspecified impurity of Dorzolamide	< 0.5%	BLD	BLD	BLD	BLD
Total impurities of Dorzolamide	< 3.0%	BLD	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
Timolol related compound B	< 1.0%	BLD	BLD	0.04 ± 0.0	0.04 ± 0.0
Timolol related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound G	< 0.5%	<0.1	<0.1	<0.1	<0.1
Any individual unspecified impurity of Timolol	< 0.6%	BLD	BLD	BLD	BLD
Total impurities of Timolol	< 2.0%	<0.1	<0.1	<0.1	<0.1
Dorzolamide maleic acid adducts	< 0.5%	BLD	BLD	BLD	BLD

Table 8.

Formulation D2					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	103.7 ± 3.5	99.8 ± 0.2	108.9 ± 0.5	109.3 ± 1.4
Drug content (Timolol)	90 – 110%	105.8 ± 0.8	102.1 ± 0.6	107.5 ± 0.3	108.5 ± 0.3
Dorzolamide related compound B	< 2.0 %	BLD	0.2 ± 0.0	0.51 ± 0.0	0.75 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	BLD	BLD	<0.1
Any individual unspecified impurity of Dorzolamide	< 0.5%	BLD	BLD	BLD	BLD
Total impurities of Dorzolamide	< 3.0%	BLD	0.2 ± 0.0	0.5 ± 0.0	0.8 ± 0.0
Timolol related compound B	< 1.0%	BLD	BLD	<0.1	BLD
Timolol related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound G	< 0.5%	BLD	<0.1	<0.1	<0.1
Any individual unspecified impurity of Timolol	< 0.6%	BLD	BLD	BLD	BLD
Total impurities of Timolol	< 2.0%	BLD	<0.1	<0.1	<0.1
Dorzolamide maleic acid adducts	< 0.5%	BLD	BLD	BLD	BLD

Table 9.

Formulation D3					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	108.2 ± 1.3	95.6 ± 3.0	108.6 ± 0.2	109.6 ± 3.1
Drug content (Timolol)	90 – 110%	108.7 ± 0.5	100.5 ± 0.4	106.9 ± 1.7	106.8 ± 3.4
Dorzolamide related compound B	< 2.0 %	BLD	0.2 ± 0.0	0.51 ± 0.0	0.7 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	0.4 ± 0.0	<0.1	<0.1
Any individual unspecified impurity of Dorzolamide	< 0.5%	BLD	BLD	BLD	BLD
Total impurities of Dorzolamide	< 3.0%	BLD	0.3 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
Timolol related compound B	< 1.0%	BLD	BLD	<0.1	<0.1
Timolol related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound G	< 0.5%	<0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Any individual unspecified impurity of Timolol	< 0.6%	BLD	BLD	BLD	BLD
Total impurities of Timolol	< 2.0%	<0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Dorzolamide maleic acid adducts	< 0.5%	BLD	BLD	BLD	BLD

Table 10.

Formulation D4					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	106.8 ± 0.8	99.0 ± 0.9	108.1 ± 0.5	110 ± 0.6
Drug content (Timolol)	90 – 110%	107.8 ± 0.6	101.7 ± 0.2	106.6 ± 0.8	108.4 ± 0.7
Dorzolamide related compound B	< 2.0 %	BLD	0.3 ± 0.0	0.53 ± 0.0	0.81 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	<0.1	<0.1	<0.1
Any individual unspecified impurity of Dorzolamide	< 0.5%	BLD	BLD	BLD	BLD
Total impurities of Dorzolamide	< 3.0%	BLD	0.3 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
Timolol related compound B	< 1.0%	BLD	BLD	<0.1	<0.1
Timolol related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound G	< 0.5%	BLD	<0.1	0.1 ± 0.0	0.2 ± 0.0
Any individual unspecified impurity of Timolol	< 0.6%	BLD	BLD	BLD	BLD
Total impurities of Timolol	< 2.0%	BLD	<0.1	0.2 ± 0.0	0.2 ± 0.0
Dorzolamide maleic acid adducts	< 0.5%	BLD	BLD	BLD	BLD

Table 11.

Formulation D5					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	106.9 ± 0.5	93.6 ± 2.9	108.2 ± 0.3	110 ± 0.2
Drug content (Timolol)	90 – 110%	110.8 ± 2.2	100.5 ± 1.1	106.2 ± 0.1	108.4 ± 1.4
Dorzolamide related compound B	< 2.0 %	BLD	0.2 ± 0.0	0.49 ± 0.0	0.75 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	<0.1	<0.1	0.1 ± 0.0
Any individual unspecified impurity of Dorzolamide	< 0.5%	BLD	BLD	BLD	BLD
Total impurities of Dorzolamide	< 3.0%	BLD	0.3 ± 0.0	0.6 ± 0.0	0.9 ± 0.0
Timolol related compound B	< 1.0%	BLD	BLD	<0.1	<0.1
Timolol related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound G	< 0.5%	<0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Any individual unspecified impurity of Timolol	< 0.6%	BLD	BLD	BLD	BLD
Total impurities of Timolol	< 2.0%	<0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Dorzolamide maleic acid adducts	< 0.5%	BLD	BLD	BLD	BLD

EXAMPLE 3. EVALUATION OF ACCELERATED STABILITY OF FORMULATIONS COMBINING THREE APIs AT 40°C AND 25% OF RELATIVE HUMIDITY.

Methods:

[0098] A series of pharmaceutical compositions according to the teachings of the present application were prepared with a triple combination of APIs, *i.e.,* Dorzolamide/Timolol/Brimonidine in its preferred salts forms and then compared with a solution containing the same fixed combination of Dorzolamide, Timolol and Brimonidine in its preferred salt forms, but formulated without BCD and at pH below 6 (formulation T1-B88B or T1; Table 12).

[0099] The studied formulations contained Dorzolamide, Timolol, and Brimonidine tartrate at concentrations of 2%, 0.5% and 0.2%, respectively. Formulations T2-B82K1, T3-B82K2, T4-B81K3, and T5-B84G (thereafter mentioned as T2, T3, T4 and T5) were formulated with DZL, whereas formulations T1-B88B, T6-B84E, T7-B82K4, T8-B84F, and T9-B81K5 (thereafter mentioned as T1, T6, T7, T8 and T9) were prepared with DZL-HCl. All proposed formulations contain the salts Timolol maleate and Brimonidine tartrate (Tables –12 - 20).

[0100] 1 L of formulation T1 of the present application was prepared by dissolving 6 g of hypromellose in approximately 900 mL (±5 %) of water at 80 – 90 °C. After 20 min, the solution was cooled down to 20 – 35 °C, followed by the addition and dissolution of 22.2 g of DZL-HCl. Upon dissolution, 6.8 g of Timolol maleate was added and dissolved, followed immediately by the sequential addition and dissolution of 10.0 g of mannitol, 0.2 g of citric acid and 10.0 g of sodium citrate dihydrate. Afterwards, 2.0 g of Brimonidine tartrate was added. Upon dissolution, pH was adjusted to 5.5-6.5 with 1 M sodium hydroxide, and enough water was added to reach 1 L. Lastly, the formulation was terminally sterilized using 0.2 µm pore size membrane filter (Table 12).

Table 12.

Formulation T1	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hypromellose	6.0
Mannitol	10.0
Citric acid monohydrate	0.2
Sodium citrate dihydrate	10.0
Water for injection	q.s. to 1 L

[0101] 1 L of formulation T2 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 20.0 g of DZL were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 2.0 g of Brimonidine tartrate was added and dissolved, followed by the addition of enough water to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 13).

Table 13.

Formulation T2	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide base	20.0
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0102] 1 L of formulation T3 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 20.0 g of DZL were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved, followed by the addition of 2.5 g mannitol. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 50.0 g of povidone K30 was dissolved, followed by the addition and dissolution of 2.0 g of Brimonidine tartrate. Lastly, enough water was added to reach 1 L. The formulation was terminally sterilized by passing through a 0.2 µm membrane filter (Table 14).

Table 14.

Formulation T3	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide base	20.0
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Mannitol	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0103] 1 L of formulation T4 of the present application was prepared by dissolving 2.5 g of Hypromellose under mechanical stirring in approximately 500 mL of water at 50 °C (± 5 °C). Afterwards, the solution was cooled down to room temperature, and 200 g of BCD was added and dissolved. Next, 20.0 g of DZL were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved, followed by the addition of 2.5 g mannitol. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 50.0 g of povidone K30 and 2.0 g of Brimonidine tartrate were added and dissolved. Lastly, enough water was added to reach 1 L. The formulation was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 15).

Table 15.

Formulation T4	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide base	20.0
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Mannitol	2.5
Hypromellose	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0104] 1 L of formulation T5 of the present application was prepared by dissolving 2.5 g of Hypromellose under mechanical stirring in approximately 500 mL of water at 50 °C (± 5 °C). Afterwards, the solution was cooled down to room temperature, and 200 g of BCD was added and dissolved. Next, 20.0 g of DZL and 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 50.0 g of povidone K30 and 2.0 g of Brimonidine tartrate were dissolved. Lastly, enough water was added to reach 1 L. The solution-based formulation was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 16).

Table 16.

Formulation T5	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide base	20.0
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Hypromellose	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0105] 1 L of formulation T6 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 2.0 g of Brimonidine tartrate was added and dissolved, followed by the addition of enough water to reach 1 L. Lastly, the solution was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 17).

Table 17.

Formulation T6	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0106] 1 L of formulation T7 of the present application was prepared by adding under mechanical stirring 200 g of BCD in approximately 500 mL of room-temperature water. After dissolution, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 50.0 g of povidone K30 and 2.0 g of Brimonidine tartrate were dissolved. Lastly, enough water was added to reach 1 L. The solution-based formulation was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 18).

Table 18.

Formulation T7	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0107] 1 L of formulation T8 of the present application was prepared by dissolving 2.5 g of hypromellose under mechanical stirring in approximately 500 mL of water at 50 °C (± 5 °C). Afterwards, the solution was cooled down to room temperature, and 200 g of BCD was added and dissolved. Next, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 2.0 g of Brimonidine tartrate were added and dissolved. Lastly, enough water was added to reach 1 L. The formulation was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 19).

Table 19.

Formulation T8	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Hypromellose	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0108] 1 L of formulation T9 of the present application was prepared by dissolving 2.5 g of hypromellose under mechanical stirring in approximately 500 mL of water at 50 °C (± 5 °C). Afterwards, the solution was cooled down to room temperature, and 200 g of BCD was added and dissolved. Next, 22.2 g of DZL-HCl were added under gentle stirring. Upon dissolution, 6.8 g of Timolol maleate were added and dissolved. Afterwards, a small volume of 1 M sodium hydroxide was added to fix pH to 7.0 ± 0.4. Subsequently, 50.0 g of povidone K30 and 2.0 g of Brimonidine tartrate were dissolved. Lastly, enough water was added to reach 1 L. The solution-based formulation was terminally sterilized by passing through a 0.2 µm pore size membrane filter (Table 20).

Table 20.

Formulation T9	
INGREDIENT	**CONCENTRATION (g/L)**
Dorzolamide hydrochloride	22.2
Timolol maleate	6.8
Brimonidine tartrate	2.0
Hydroxypropyl beta-cyclodextrin	200.0
Povidone-K30	50.0
Hypromellose	2.5
Sodium hydroxide	q.s. to pH 7.0
Water for injection	q.s. to 1 L

[0109] All sterile formulations were packaged in individual 5 mL multidose preservative-free containers. These were stored in chambers set at 40 °C/25% RH to determine that formulations herein can withstand low relative humidity environments, in accordance to accelerated tests described in international guidelines and regulations such as ICH, FDA and NOM. Analysis of APIs and impurities were performed at time 0, 1 month, 2 month, and 3 months by UV-HPLC.

Results:

[0110] Formulation T1 of the present application showed an adequate physical and chemical stability (tables 21, 22), and thus suitable to be commercialized. This formulation used a very specific mixture of excipients comprising hypromellose, mannitol, citric acid, sodium citrate and water of the second set of preferred embodiments of the present application.

[0111] On the other hand, BCD was the main excipient of eight of the proposed Dorzolamide/Timolol/Brimonidine formulations (T2, T3, T4, T5, T6, T7, T8, T9). It was demonstrated that BCD when acting as the host of the combination of APIs was capable to increase the solubility of Dorzolamide, endowing the ability of having a thermodynamically stable Dorzolamide-solution at pH values above its pKa, even in the presence of a second and even a third API (Table 21).

Table 21.

Formulation	pH	Osmolality (±5%)
T1	5.7	314
T2	7.0	270
T3	6.9	310
T4	6.9	449
T5	7.0	431
T6	7.0	428
T7	6.9	639
T8	6.9	413
T9	6.9	697

[0112] The presence of other polymers such as povidone synergically acted with BCD while at the same time povidone slightly increased the viscosity of the solution, which is believed would also increase the residence time of the active ingredients on the ocular surface and therefore favor their bioavailability (as demonstrated in the rest of the examples).

[0113] Furthermore, it was demonstrated that BCD alone is sufficient to ensure this stability independent of pH. Thus, the choice of povidone and HPMC as additional excipients can be made as an optional choice for the search for a benefit in bioavailability of the active pharmaceutical ingredients.

[0114] The eight BCD-based formulations (T2, T3, T4, T5, T6, T7, T8, T9) achieved a pH value near to neutrality, whereas BCD-free formulation T1 had a pH value of 5.7 (Table 21). This stability study showed that this pH value remains constant even after the solution has been exposed to the extreme testing conditions of 40°C and 25% of RH for 3 months. Also, all formulations meet the specification for drug content and known, unknown and total impurities (Tables 22 - 30). This indicates that the chemical stability of APIs is conserved through time within acceptable intervals, which is critical to stablish expiration dates in ophthalmic drug products.

[0115] Dorzolamide impurity II was identified in all formulations by day 28. This was the most abundant impurity found in the study; even so, the specification criterion is met for all samples (Tables 22 - 30).

[0116] Dorzolamide impurity III was identified only in four formulations at 28 days, these were: T3, T4, T7and T9. However, the results show that the concentration of this impurity is within the corresponding specification (Tables 22 - 30).

[0117] For Timolol, impurities I and II were below the quantification limit, and even when impurity VI was identified on day 28 of all the proposed formulations it was found in concentrations <0.1% and so it meets the corresponding specification. No unknown substances were found at 295 nm (Tables 22 - 30).

[0118] For Brimonidine, related substances III and IV were found to be below the quantification limit, while impurity V was found in all formulations at day 28 at concentrations that meet the corresponding specification. Additionally, in all the proposed formulations it was possible to identify two unknown substances at times 5.2 and 19.3 min. However, these concentrations were below the specification for unknown substances at 247 nm (Tables 22 - 30).

[0119] All formulations met the specification for total impurities at 247 nm and 295 nm (Tables 22 - 30). Therefore, the compositions object of the present application for the first time ever achieved, at neutral pH values, that any of the APIs degraded or precipitated from the solution.

Table 22.

Formulation T1					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	108.7 ± 6.1	105.2 ± 0.2	101.0 ± 0.6	99.2 ± 0.6
Drug content (Timolol)	90 – 110%	100.0 ± 4.9	90.0 ± 0.2	103.1 ± 0.5	101.6 ± 0.5
Drug content (Brimonidine)	90 – 110%	108.7 ± 6.1	105.2 ± 0.2	104.5 ± 0.5	103.3 ± 0.5
Dorzolamide related compound B	< 2.0%	BLD	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound I	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	BLD	BLD	BLD
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.3 ± 0.0	0.4 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	BLD	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	BLD	< 0.1	0.7	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	BLD	BLD	< 0.1

Table 23.

Formulation T2					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	100.0 ± 1.1	98.6 ± 0.6	100.6 ± 0.5	99.7 ± 0.5
Drug content (Timolol)	90 – 110%	100.0 ± 1.0	95.4 ± 0.6	101.4 ± 0.6	102.1 ± 0.6
Drug content (Brimonidine)	90 – 110%	100.0 ± 0.8	97.9 ± 0.2	104.8 ± 0.3	105.3 ± 0.3
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.5 ± 0.0	0.9 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	< 0.1	< 0.1
Brimonidine related compound III	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.3 ± 0.0	0.4 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.9	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	< 0.1	0.1

Table 24.

Formulation T3					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	100.0 ± 3.7	101.4 ± 0.1	103.5 ± 0.5	101.0 ± 0.5
Drug content (Timolol)	90 – 110%	100.0 ± 4.1	95.8 ± 0.1	101.1 ± 0.6	101.0 ± 0.6
Drug content (Brimonidine)	90 – 110%	100.0 ± 2.8	102.7 ± 0.0	103.5 ± 0.4	101.8 ± 0.4
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.5 ± 0.0	1.0 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	< 0.1	< 0.1	0.1 ± 0.0
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	0.2 ± 0.0	0.2 ± 0.0
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.3 ± 0.0	0.5 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.9	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	0.2	0.2

Table 25.

Formulation T4					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	107.1 ± 4.9	105.9 ± 0.9	101.6 ± 0.0	99.9 ± 0.0
Drug content (Timolol)	90 – 110%	107.8 ± 2.9	101.6 ± 0.7	99.7 ± 0.5	100.2 ± 0.5
Drug content (Brimonidine)	90 – 110%	107.1 ± 4.9	105.9 ± 0.9	102.4 ± 0.1	101.4 ± 0.1
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.5 ± 0.0	0.9 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	< 0.1	< 0.1	< 0.1
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	0.1 ± 0.0	0.2 ± 0.0
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.2 ± 0.0	0.5 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.8	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	0.2	0.2

Table 26.

Formulation T5					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	109.9 ± 0.5	107.6 ± 5.2	99.8 ± 1.5	100.3 ± 1.5
Drug content (Timolol)	90 – 110%	100.0 ± 0.6	95.4 ± 4.6	100.1 ± 0.5	99.8 ± 0.5
Drug content (Brimonidine)	90 – 110%	100.0 ± 0.5	100.3 ± 5.0	102.4 ± 0.3	99.2 ± 0.3
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.6 ± 0.0	0.9 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	< 0.1	< 0.1	< 0.1
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound VI	< 0.5%	< 0.1	< 0.1	0.2	0.2
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.3 ± 0.0	0.5 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	1.0	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	< 0.1	< 0.1	0.2	0.3

Table 27.

Formulation T6					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	100.0 ± 1.7	99.6 ± 0.2	98.3 ± 0.4	99.5 ± 0.4
Drug content (Timolol)	90 – 110%	100.0 ± 2.0	94.3 ± 0.1	100.4 ± 0.7	99.0 ± 0.7
Drug content (Brimonidine)	90 – 110%	100.0 ± 1.7	99.7 ± 0.0	103.4 ± 0.9	101.6 ± 0.9
Dorzolamide related compound B	< 2.0%	BLD	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
Dorzolamide related compound D	< 0.5%	< 0.1	BLD	< 0.1	< 0.1
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	< 0.1	0.2 ± 0.0
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.2 ± 0.0	0.3 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.9	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	< 0.1	0.2

Table 28.

Formulation T7					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	100.0 ± 0.1	98.0 ± 1.0	102.6 ± 1.0	99.3 ± 1.0
Drug content (Timolol)	90 – 110%	100.0 ± 2.2	93.3 ± 0.7	100.9 ± 1.1	99.8 ± 1.1
Drug content (Brimonidine)	90 – 110%	100.0 ± 1.7	98.2 ± 0.9	103.4 ± 1.3	101.2 ± 1.3
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.5 ± 0.0	0.9 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	< 0.1	< 0.1	0.1 ± 0.0
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	0.1 ± 0.0	0.2 ± 0.0
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.1 ± 0.0	0.4 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.8	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	0.2	0.2

Table 29.

Formulation T8					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	108.1 ± 2.9	96.9 ± 1.0	98.6 ± 0.4	99.8 ± 0.4
Drug content (Timolol)	90 – 110%	105.4 ± 3.8	98.2 ± 1.1	100.8 ± 0.6	100.2 ± 0.6
Drug content (Brimonidine)	90 – 110%	108.1 ± 2.9	101.2 ± 1.2	104.3 ± 0.4	102.7 ± 0.4
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.6 ± 0.1	0.9 ± 0.1
Dorzolamide related compound D	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	< 0.1
Timolol related compound VI	< 0.5%	BLD	< 0.1	< 0.1	< 0.1
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.2 ± 0.1	0.4 ± 0.1
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	1.0	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	0.1	0.2

Table 30.

Formulation T9					
Parameter	Specification	Sampling time			
		0	1 month	2 months	3 months
Drug content (DZL)	90 – 110%	101.5 ± 5.5	104.2 ± 3.8	100.5 ± 2.8	97.6 ± 2.8
Drug content (Timolol)	90 – 110%	104.2 ± 3.2	101.4 ± 4.8	99.8 ± 2.9	97.4 ± 2.9
Drug content (Brimonidine)	90 – 110%	101.5 ± 5.5	104.2 ± 3.8	101.9 ± 3.0	99.0 ± 3.0
Dorzolamide related compound B	< 2.0%	BLD	0.5 ± 0.0	0.5 ± 0.0	0.9 ± 0.0
Dorzolamide related compound D	< 0.5%	BLD	< 0.1	< 0.1	0.1 ± 0.0
Timolol related compound I	< 0.5%	BLD	BLD	< 0.1	< 0.1
Timolol related compound II	< 0.5%	BLD	BLD	BLD	BLD
Timolol related compound VI	< 0.5%	BLD	< 0.1	0.2 ± 0.0	0.2 ± 0.0
Brimonidine related compound III	< 1.0%	BLD	BLD	BLD	BLD
Brimonidine related compound IV	< 1.0%	BLD	BLD	< 0.1	< 0.1
Brimonidine related compound V	< 1.7%	BLD	< 0.1	0.1 ± 0.0	0.4 ± 0.0
Any individual unspecified impurity at 247 nm	< 0.5%	< 0.1	< 0.1	< 0.1	< 0.1
Total impurities at 247 nm	< 5.0%	< 0.1	< 0.1	0.8	<1.5
Any individual unspecified impurity at 295 nm	< 0.6%	BLD	BLD	BLD	BLD
Total impurities at 295 nm	< 2.0%	BLD	< 0.1	0.2	0.2

EXAMPLE 4. BIOAVAILABILITY.

[0120] The technological platform, herein designed for ophthalmic solutions, showed the ability to enhance the solubility of Dorzolamide and endow suitable stability attributes. However, it is important to determine the impact on the bioavailability of APIs contained in the formulation. Particularly, considering that some studies have shown that cyclodextrins can limit or even reduce the absorption of APIs (*Westerberg, J Pharm Sci. 2004*); therefore, a preclinical study was performed to assess the ocular bioavailability of novel formulations herein described.

[0121] Bioavailability of formulations was evaluated in healthy white New Zealand rabbits of 2 – 3 months old. Double formulations D1, D2, D4, D5 and triple formulations T1, T2, T6 and T9 were tested. Each composition was evaluated in both eye from a total of four rabbits.

[0122] Study subjects were sacrificed with pentobarbital at 0.5, 1, 2 or 4 h after the instillation of one drop of the product on both rabbit's eyes. Then, aqueous humor was obtained from the anterior chamber of each eye using 1 mL syringes with 27G needles. Samples of aqueous humor were stored at -70°C until their use. Afterwards, APIs were quantified by HPLC-Mass/Mass.

[0123] Bioavailability of double and triple combinations were compared with a reference product that was described in MX 295966 and do not use BCD, neither the very specific mixture of excipients comprising hypromellose, mannitol, citric acid, sodium citrate and water of the second set of preferred embodiments of the present application; nor the other very specific mixture of excipients comprising polyoxyl 40 stearate, sodium chloride, mannitol, sodium borate decahydrate, sodium hydroxide and water for injection.

[0124] Bioavailability results for the series of formulations containing the double combination of APIs, *i.e.,* Dorzolamide and Timolol (D1, D2, D4 and D5) showed that the highest concentration of DZL was found at 1 h in most of the formulations tested. Moreover, the four compositions that were formulated according to the methodologies described in this document, presented a similar or higher concentration of Dorzolamide in aqueous humor in comparison to the reference product (Figure 3). On the other hand, Timolol presented its maximal concentration at 0.5 h in all the double formulations. The four compositions tested, D1, D2, D4 and D5 had a similar or higher concentration of Timolol in comparison to the reference product in all the times that were evaluated (Figure 4).

[0125] Bioavailability results for the series of formulations containing the triple combination of APIs, *i.e.,* Dorzolamide, Brimonidine and Timolol showed that the highest concentration of Dorzolamide was found at 1 h (Figure 5). The compositions T1, T2, T6 and T9, which were formulated according to the protocol described in this document, had similar or higher concentrations of Dorzolamide in comparison to the reference product at all the times tested (Figure 5). The highest concentrations of Timolol at 0.5 h. In addition, all samples had a similar or higher concentration of this API in comparison to the reference product at all the times tested (Figure 6). Finally, the highest concentrations of Brimonidine were found at times 0.5 h and 1 h. Additionally, all the compositions tested had a similar or higher concentration of Brimonidine at all times tested when compared to the reference product (Figure 7).

[0126] Results for both series of formulations (i.e., doubles and triple) demonstrate that the carrier matrix of the present application *i.e.,* BCD achieves all the benefits of stability without modifying bioavailability of the APIs. This benefit was observed for all the Dorzolamide formulations no matter the addition of one or two additional APIs (Timolol and Timolol/Brimonidine, respectively).

EXAMPLE 5. BURNING SENSATION IN VOLUNTEERS.

[0127] A single blind pilot study was carried out to compare the burning sensation among the compositions formulated based on the teachings of the present application. Visual analogue scale (VAS) from 0 to 10 was used to determine the grade of burning sensation felt by the volunteers with each of the compositions tested.

[0128] Formulation D1 and T1 described in the present application were used as reference to evaluate the burning sensation. D1 was compared with D2, D4 and D5, which contain BCD and the double combination of APIs Dorzolamide/Timolol. Formulation T1 was compared with formulation T2, T6 and T9, which contain BCD and the triple combination of APIs *i.e.,* Dorzolamide/Timolol/Brimonidine.

[0129] A first group of volunteers received selected test compositions of the present application all having a double combination of Dorzolamide and Timolol. Volunteers were instilled with formulation D1 one eye, whereas paired eye received a test formulation randomly selected among formulations D2, D4 and D5.

[0130] After D1 was applied to one of the eyes, volunteers rested their heads towards the back of their necks, and were further asked to rank the burning, itching, or any other uncomfortable sensation using the following scores of VAS: "**zero**" for the absence of burning sensations; "**1-3**" when a mild burning sensation was present, "**4-6**" when a moderate burning sensation was present; and "**7-10**" when a strong or severe burning sensation was present. Data was collected after 0, 15, 30 and 60 seconds following the drop application. Once the

volunteer referred that any sensation induced by D1 had ceased, one drop of D2, D4 or D5 was applied to the contralateral eye. Volunteers rested their heads towards the back of their necks, and were further asked to rank the burning, itching, or any other uncomfortable sensation using the following scores of VAS: "**zero**" for the absence of burning sensations; "**1-3**" when a mild burning sensation was present, "**4-6**" when a moderate burning sensation was present; and "**7-10**" when a strong or severe burning sensation was present. Data was collected after 0, 15, 30 and 60 seconds following the drop application.

[0131] Results show that formulation D1 produced a mild to moderate burning sensation while reactions to compositions D2, D4, and D5 were categorized as null or very low (Figure 8).

[0132] On the other hand, a second group of volunteers were administered with selected test compositions of the present application, all having a triple combination of Dorzolamide, Timolol and Brimonidine. All the volunteers had the formulation T1 applied in one eye, while the composition tested in the paired eye was randomly selected from the group of T2, T6 and T9.

[0133] After T1 was applied to one of the eyes, volunteers rested their heads towards the back of their necks, and were further asked to rank the burning, itching, or any other uncomfortable sensation using the following scores of VAS: "**zero**" for the absence of burning sensations; "**1-3**" when a mild burning sensation was present, "**4-6**" when a moderate burning sensation was present; and "**7-10**" when a strong or severe burning sensation was present. Data was collected after 0, 15, 30 and 60 seconds following the drop application.

[0134] Once the volunteer referred that any sensation induced by T1 had ceased, one drop of T2, T6 or T9 was applied to the contralateral eye. Volunteers rested their heads towards the back of their necks, and were further asked to rank the burning, itching, or any other uncomfortable sensation using the following scores of VAS: "**zero**" for the absence of burning sensations; "**1-3**" when a mild burning sensation was present, "**4-6**" when a moderate burning sensation was present; and "**7-10**" when a strong or severe burning sensation was present.

[0135] Results show that formulation T1 reaches a mild to moderate burning sensation in the times evaluated, while the triple compositions T2, T6 and T9 presented a reduced reaction that was categorized as null or mild in the different times (Figure 9).

-CLAIMS-

1. An ophthalmic pharmaceutical composition to control the intraocular pressure (IOP), comprising:

   i. Dorzolamide or a pharmaceutically acceptable salt, free form or basic form thereof,
   ii. a matrix comprising at least one ingredient selected from at least one pharmaceutically acceptable solubilizing agent; or at least one pharmaceutically acceptable viscosity-modifying agent; or at least one pharmaceutically acceptable osmotic agent;
   iii. at least one pharmaceutically acceptable pH-regulating agent or pH regulating system of agents, and
   iv. pharmaceutically acceptable water.

2. The ophthalmic pharmaceutical composition of claim 1, further comprising at least one additional active pharmaceutical ingredient (API) to control IOP or a pharmaceutically acceptable salt, free form, or basic form or any thereof combination.

3. The ophthalmic pharmaceutical composition of claim 1 or 2, wherein the pH of the composition ranges from 6.8 to 7.2.

4. The ophthalmic pharmaceutical composition of claim 1 or 2, wherein the concentration of Dorzolamide or a pharmaceutically acceptable salt, free form or basic form thereof is from 0.01% to 2.5%.

5. The ophthalmic pharmaceutical composition of claim 2, wherein the additional API is selected from the group comprising prostaglandin analogues and/or any salts or derivatives thereof, miotic molecules and/or any salts or derivatives thereof, beta blockers and/or any salts or derivatives thereof, alpha-adrenergic agonists and/or any salts or derivatives thereof, carbonic anhydrase inhibitors and/or any salts or derivatives thereof and Rho kinase inhibitors and/or any salts or derivatives.

6. The ophthalmic pharmaceutical composition of claim 5, wherein the additional API is Timolol, or a pharmaceutically acceptable salt, free form, or basic form or any thereof combination.

7. The ophthalmic pharmaceutical composition of claim 5, wherein the additional third API is Brimonidine, or a pharmaceutically acceptable salt, free form, or basic form or any thereof combination.

8. The ophthalmic pharmaceutical composition according to any previous claim, wherein the solubilizing agent is selected from the group comprising dextrans, dextrins, cyclic oligosaccharides including but not limited to cyclodextrins comprising hydroxypropyl alpha-cyclodextrin, hydroxypropyl gamma-cyclodextrin; and more preferably hydroxypropyl beta-cyclodextrin.

9. The ophthalmic pharmaceutical composition of claim 8, wherein the solubilizing agent is hydroxypropyl beta-cyclodextrin.

10. The ophthalmic pharmaceutical composition according to any previous claims, wherein the viscosity modifying agent is selected from the group comprising cellulose derivatives such as methylcellulose, microcrystalline cellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose or hypromellose, natural gums such as acacia, guar gum, tragacanth, xanthan gum, alginates, carrageenan and locust bean gum; synthetic polymers such as carbomers, polyethylene oxide, propylene glycol alginate, povidone o polyvinyl pyrrolidone, polyvinyl alcohol and poloxamers.

11. The ophthalmic pharmaceutical composition according to any previous claims, wherein the osmotic agent is selected from the group comprising pharmaceutically accepted agents such as salts (e.g., sodium chloride); polyols such as glycerol, polyethylene glycol and propylene glycol; and carbohydrates (e.g., mannitol, trehalose).

12. The ophthalmic pharmaceutical according to any previous claims, wherein the pH-regulating agent or pH regulating system are selected from the group comprising of hydroxide and acid forms of metallic and non-metallic elements such as sodium hydroxide, potassium hydroxide, borates, phosphates, acetic acids, sodium acetates, ammonium hydroxides, ammonium chlorides, citric acids, and sodium citrates either as individuals or as a system, carbonic acids with bicarbonate ions either as individuals or as a system, potassium-based systems.

13. The elaboration of an ophthalmic pharmaceutical product according to any previous claims, to prepare a medicament to control IOP.

14. A method to control IOP, comprising the administration an ophthalmic pharmaceutical composition, according to any previous claims, to a patient in need thereof.

15. An ophthalmic pharmaceutical composition according to claim 1 or 2, comprising Dorzolamide hydrochloride, Timolol maleate, hydroxypropyl beta-cyclodextrin, sodium hydroxide, and water for injection.

16. An ophthalmic pharmaceutical composition according to claim 1 or 2, comprising Dorzolamide hydrochloride, Timolol maleate, Brimonidine tartrate, Hydroxypropyl beta-cyclodextrin, sodium hydroxide and water for injection.

17. An ophthalmic pharmaceutical composition according to claim 1 or 2, comprising Dorzolamide hydrochloride; Timolol maleate; Polyoxyl 40 Stearate; sodium chloride; mannitol; sodium borate decahydrate; sodium hydroxide; and water for injection.

18. An ophthalmic pharmaceutical composition according to claim 1 or 2, comprising Dorzolamide hydrochloride; Timolol maleate; Brimonidine tartrate; hypromellose; mannitol; citric acid monohydrate/sodium citrate dihydrate; and water for injection.

19. A process for preparing an ophthalmic pharmaceutical composition, according to claims 1 or 2, comprising:

a) Adding, under mechanical stirring, at least one ingredient selected from at least one pharmaceutically acceptable solubilizing agent, at least one pharmaceutically acceptable viscosity-modifying agent; or at least one osmotic agent, or any combination thereof, to form a carrier matrix:
b) adding Dorzolamide or a pharmaceutically acceptable salt, free form or basic form thereof under gentle stirring;
c) optionally adding at least one second API useful to control IOP;
d) adding sodium hydroxide to fix pH to 7.0 ± 0.4,
e) optionally adding at least a third API useful for treating IOP;
f) adding water; and
g) mechanically sterilizing the thus obtained solution.

20. The process of claim 19, wherein the concentration of Dorzolamide or a pharmaceutically acceptable salt, free form, or basic form, of the obtained composition is from 0.01% to 2.5%.

21. The process of claim 19, wherein the pH of the obtained composition ranges from 6.8 to 7.2.

22. The process of claim 19, wherein the additional API is selected from the group comprising prostaglandin analogues and/or any salts or derivatives thereof, miotic molecules and/or any salts or derivatives thereof, beta blockers and/or any salts or derivatives thereof, alpha-adrenergic agonists and/or any salts or derivatives thereof, carbonic anhydrase inhibitors and/or any salts or derivatives thereof and Rho kinase inhibitors and/or any salts or derivatives.

23. The process of claim 19, wherein the second additional API is Timolol, or a pharmaceutically acceptable salt, free form or basic form thereof.

24. The process of claim 19, wherein the third additional API is Brimonidine, or a pharmaceutically acceptable salt, free form, or basic form thereof.

25. The process of claim 19, wherein the solubilizing agent is selected from the group comprising dextrans, dextrins, cyclic oligosaccharides including, but not limited to, cyclodextrins comprising hydroxypropyl alpha-cyclodextrin, hydroxypropyl gamma-cyclodextrin; and more preferably hydroxypropyl beta-cyclodextrin.

26. The process of claim 19, wherein the solubilizing agent is hydroxypropyl beta-cyclodextrin.

27. The process of claim 19, wherein the viscosity modifying agent is selected from the group comprising cellulose derivatives such as methylcellulose, microcrystalline cellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose or hypromellose, natural gums such as acacia, guar gum, tragacanth, xanthan gum, alginates, carrageenan and locust bean gum; synthetic polymers such as carbomers, polyethylene oxide, propylene glycol alginate, povidone or polyvinyl pyrrolidone, polyvinyl alcohol and poloxamers.

28. The process of claim 19, wherein the osmotic agent is selected from the group comprising pharmaceutically accepted agents such as salts (e.g., sodium chloride); polyols such as glycerol, polyethylenglycol and propilenglycol; and carbohydrates (e.g., mannitol, trehalose).

29. The process of claim 19, wherein the pH-regulating agent or pH regulating system are selected from the group comprising of hydroxide and acid forms of metallic and non-metallic elements such as sodium hydroxide, potassium hydroxide, borates, phosphates, acetic acids, sodium acetates, ammonium hydroxides, ammonium chlorides, citric acids, and sodium citrates either as individuals or as a system, carbonic acids with bicarbonate ions either as individuals or as a system, potassium-based systems.