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SUBSTANTIATION OF THE pH RANGE FOR STABILITY OF TIMOLOL MALEATE AND TAURINE IN THE AQUEOUS SOLUTION

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Key words: combined eye drops; timolol maleate; taurine; aqueous solution; molar fractions of ions; pH; stability

At the stage of pharmaceutical development of the combined eye drops based on timolol maleate and taurine the behaviour of drugs in the aqueous solution depending on their chemical nature and pH of the solution have been analyzed. Based on the molar fractions of ions calculated the optimal area of pH where drugs are present in the form of ions has been proven. The results of studying appearance of both the freshly prepared aqueous solution of timolol maleate in the concentration of 0.34% and the solution of taurine in the concentration of 4.0% have shown that the test solutions are transparent at the pH range from 3.5 to 8.5, which is acceptable to eye drops. It has allowed to substantiate the optimal pH range, at which the stability of drugs in the form of aqueous solutions is preserved. A preliminary assessment of compatibility of timolol maleate in the concentration of 0.34% and taurine in the concentration of 4.0% in their combined presence in the aqueous solution depending on pH of the medium by such parameters as transparence, colour, pH and specific conductivity has been carried out. In the pH range of 3.5 to 8.5 the aqueous solution of timolol maleate and taurine in their combined presence is transparent and colourless; it indicates the presence of both drugs in the form of water-soluble ions. The value of specific conductivity of the solution of timolol maleate and taurine in their combined presence, which is equal to the sum of the values of electric conductivity of individual solutions of each drug, indicates no interaction between the drugs studied. The research conducted has allowed to prove scientifically the pH range, which provides stability and comfort during application of combined eye drops with medicinal substances of different chemical nature.

Pharmaceutical development (PD) covers various stages of drug creation, among them one of the first is the study of physicochemical properties of a medicinal substance (MS) concerning prospective dosage form (DF) and its way of administration. In order to identify critical quality indexes of MS, which may have an impact on the quality of the finished product, at this stage it is necessary to determine the class of substances, which the MS belongs to, functional groups contained in its structure, to analyze possible destructive transformations of the MS and factors affecting these transformations. When creating combined drug formulations it is also necessary to determine compatibility of medicinal substances using similar approaches.

The key parameter of stability for ophthalmic medicines when performing the manufacturing operations cycle and storage of the finished DF in the primary packaging is the value of ionization constant of MS and pH of the medium, which are responsible for equilibrium concentrations of ions that are present in aqueous solutions of the MS. Therefore, to avoid possible destructive processes, it is necessary to determine dependence of solubility and chemical stability on the ionization constant of MS and pH of the medium. The aim of this paper is to analyze the possible behaviour of MSs of timolol maleate and taurine in aqueous solutions depending on their chemical nature and pH of the medium to study the stability of the combined eye drops at the PD stage.

Materials and Methods

The objects chosen for investigation are:

1. Timolol maleate [7] produced by "Centaur Chemicals Pvt. Ltd.", India.

2. Taurine [5] produced by the State Plant for Chemical Reagents STC of "Institute for Single Crystals", Ukraine.

To assess the quality of model mixtures the following methods were used: clarity and degree of opalescence of liquids (SPU, 2.2.1.), degree of coloration of liquids (SPU, 2.2.2, Method II), potentiometric determination of pH (SPU, 2.2.3.) with the help of Seven Easy pH pH-meter manufactured by "Mettler Toledo", China [3], conductivity (SPU, 2.2.38.) with the help of Seven Easy S30 conductometer manufactured by "Mettler Toledo", China [4].

Calculation of the molar fractions of ions in MS solutions at different values of pH was carried out using the following formulas according to [6]:

- for timolol-base, taurine in cationic and protonated forms by the sulfonate group

$$\alpha = 1 / (1+10^{pH - pKa})$$

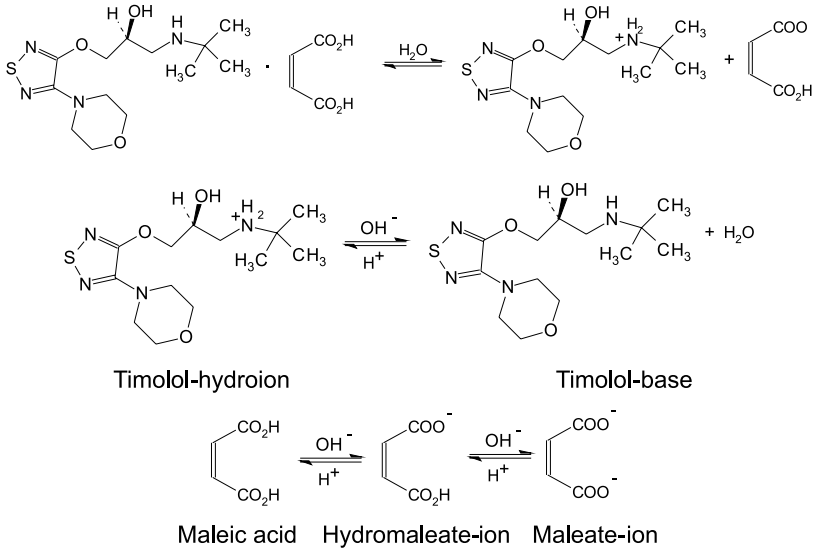

Fig. 1. Equilibrium processes in aqueous solutions of timolol maleate depending on pH of the medium.

- for timolol-hydroion, taurine in anionic and unprotonated forms by amino group

$$\alpha = 1 / (1+10^{pKa - pH})$$

- for maleic acid (H_2A), hydromaleate-ion (HA-) and maleate-ion (A^{2-})

$$\alpha (H_2A) = 1 / (1+10^{pH - pK_1}+10^{2\,pH - pK_1 - pK_2}),$$
$$\alpha (HA^-) = 10^{pH - pK_1}/(1+10^{pH - pK_1}+10^{2\,pH - pK_1 - pK_2}),$$
$$\alpha (A^{2-}) = 10^{2\,pH - pK_1 - pK_2}/(1+10^{pH - pK_1}+10^{2\,pH - pK_1 - pK_2})$$

Results and Discussion

The MS selected for development of eye drops are well-known, that is why their therapeutic concentrations that are used in ophthalmology are listed in the reference literature and are 0.34% or 0.68% for timolol maleate and 4% for taurine. According to the normative documents both MSs are "soluble in water" by the solubility scale, i.e. 10-30 ml of water is required for dissolving of 1 g of the substance; it corresponds to the concentration of 3-10% [7, 5]. Thus, the MS concentrations chosen are within the limits of their solubility in water and can be used to create eye drops.

Timolol maleate, (2S)-1-[(1,1-Dimethylethyl)amino]-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol (Z)-butenedioate refers to the group of salts formed by weak organic bases and weak organic acids: timolol-base and maleic acid by means of proton transfer from maleic acid to the amino group of the timolol-base.

Aqueous solutions of timolol maleate demonstrates weakly acidic properties (pH of 5% solution ranges from 3.5 to 4.5; 1-2% solution – from 3.8 to 4.3) [7, 11] and are stable to pH 12 [9]. The acidic medium promotes protonation of a weak base – timolol by the amino group to form more water-soluble form (pKa = 9.2 [11]), and maleic acid formation from maleate of maleic acid (pKa_1 = 1.92; pKa_2 = 6.22 [2]) that is easily soluble in water. In the alkaline medium a reverse process takes place leading to formation of timolol-base.

Equilibrium processes in aqueous solutions of timolol maleate depending on pH of the medium can be described by equations given in Fig. 1.

In our previous work [1] behaviour of timolol maleate in aqueous solutions was assessed at different pH values.

Calculation of molar fractions of ions conducted for this purpose has shown that at the pH range from 3.5 to 8.5, which is acceptable to eye drops [4], in the solution of timolol maleate there is timolol-hydroion and hydromaleate-ion and maleate-ion in different proportions depending on the medium of pH in the ionized form.

Appearance of freshly prepared aqueous solutions of timolol maleate has confirmed that at pH within the indicated above limits, the solutions are transparent. This is indicative of timolol-base and timolol-hydroion solubility in the pH range investigated. The pH value of timolol maleate solution in the concentration of 0.34% is 4.05, i.e. corresponds to the MS stability area; however, it is different from pH of the lacrimal fluid (pH = 7.4) [4]. To create the MS solution, which is comfortable for eyes by the pH value, it is necessary to add a buffer to it or substances for adjusting the pH value.

Taurine is aminoetan 2-sulfonic acid, a typical representative of amino sulfonic acids. By its physical properties taurine is a white crystalline powder, soluble in water, poorly soluble in most organic solvents. In its structure taurine has an acidic part in the form of the sulfonate group and an alkaline part in the form of the amino group. The presence in the chemical structure of this compound of groups having a dual nature characterizes it as a highly polar substance that is responsible for good water solubility and indicates that this MS is present in the solution in the form of zwitter ions depending on pH, and it determines its acid-base properties. The value of the ionization constant of taurine, which is responsible for the balance between the loss and addition of a proton, is pKa = 1.5 by the sulpho group, and pKa = 8.74 by the amino group [8]. Forms of taurine, which are present in the aqueous solution depending on pH, are shown schematically in Fig. 2.

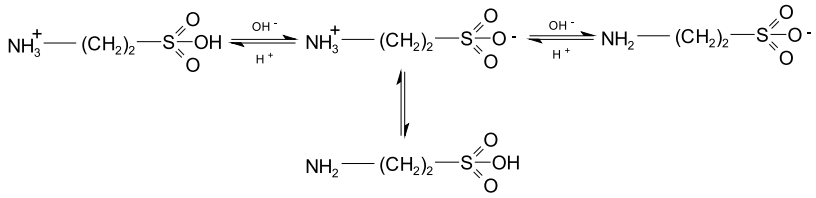

Fig. 2. Forms of taurine that are present in the solution depending on pH of the medium.

At pH = 5.12 that corresponds to the isoelectric point the MS solution contains electroneutral molecules (form II) and some identical number of anions and cations, which balance each other. From the isoelectric point toward the area of acidic pH values, the number of cations with the charge localized on the amino group nitrogen (form I) increases; in the alkaline area there is an increasing number of anions with the charge localized on the sulpho group (Form III). Changing pH values toward acidic or alkaline areas should not affect the solubility of taurine forms that are present in the solution since such substances as ethyl sulfuric acid and ethylamine, which can be regarded as integral parts of taurine, also have good solubility in water [2].

To confirm the aforementioned we have calculated molar fractions of ions in the solution of taurine at different pH values and studied the appearance of aqueous solutions of taurine in therapeutic concentrations at different pH values. The results are presented in Table and Fig. 3.

In Fig. 3 it is evident that at the pH range from 3.5 to 8.5, which is acceptable to eye drops taurine in the aqueous solution is present in the ionized state in both groups with almost constant values of molar fractions of ions.

Thus, at physiological pH value of the lacrimal fluid (pH = 7.4) taurine is ionized by 95.6% by the amino group and almost by 100% by the sulpho group (Table), i.e. under such circumstances taurine is almost entirely present in the form of Zwitter-ion.

To confirm the calculation data the appearance of freshly prepared aqueous solutions of taurine in the therapeutic concentration of 4.0% within the values of pH from 3.5 to 8.5 being acceptable for eye drops has been studied. The research results have shown that within the area of pH selected aqueous solutions of taurine are transparent. This confirms the solubility of taurine in the abovementioned pH range (Table).

In addition, as well as timolol maleate the pH values for taurine solution in the concentration of 4.0% are in the acidic area and are equal to 4.96. This area corresponds to stability of MS, but differs from the pH value of the lacrimal fluid and, therefore, pH of eye drops needs to be adjusted.

For preliminary estimation of compatibility timolol maleate with taurine in the aqueous solution the appear-

Table

Molar fractions (α) of ions in the aqueous solution of taurine depending on pH of the medium

pH	α (cationic form) × 10^{-2}	α (unprotonated by the amino group form) × 10^{-2}	α (anionic form) × 10^{-2}	α (protonated by the sulfonate group form) × 10^{-2}	Appearance of the solution
1	99.999	1.82×10^{-7}	24.025	75.975	-
1.5	99.999	5.75×10^{-6}	50.0	0.5	-
2	99.999	1.82×10^{-5}	75.975	24.025	-
3	99.999	1.82×10^{-4}	96.935	3.065	clear
4	99.998	1.82×10^{-3}	99.685	0.315	clear
5	99.982	0.018	99.968	0.032	clear
6	99.818	0.182	99.996	3.16×10^{-3}	clear
7	98.213	1.787	99.999	3.16×10^{-4}	clear
7.4	95.628	4.371	99.999	1.24×10^{-4}	clear
8	84.605	15.395	99.999	3.16×10^{-5}	clear
8.74	50.000	50.0	99.999	5.75×10^{-6}	clear
9	35.463	64.535	99.999	3.16×10^{-6}	-
10	5.209	94.791	99.999	3.16×10^{-7}	-
11	0.546	99.453	100	3.16×10^{-8}	-
12	0.055	99.945	100	3.16×10^{-9}	-
13	5.49×10^{-3}	99.995	100	3.16×10^{-10}	-
14	5.49×10^{-4}	99.999	100	3.16×10^{-11}	-

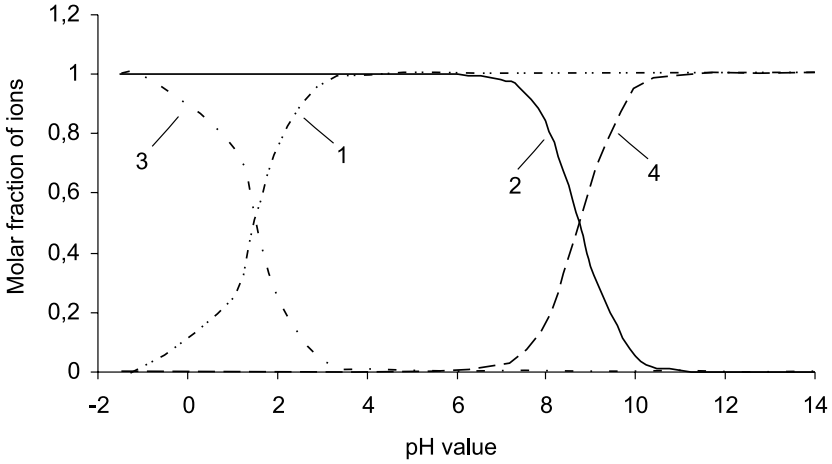

Fig. 3. Dependence of molar fractions of ions in the aqueous solution of taurine on pH of the medium. 1 – the area where anionic forms are present; 2 – the area where cationic forms are present; 3 – the area where protonated forms of sulpho groups are present; 4 – the area where unprotonated forms of amino groups are present.

ance of model MS mixtures has been studied depending on pH of the medium. For this purpose, freshly prepared aqueous solutions containing at the same time timolol maleate in the therapeutic concentration of 0.34% and taurine in the therapeutic concentration of 4.0%, were adjusted with the help of 1 M sodium hydroxide to pH values from 3.5 to 8.5. The preliminary assessment of quality at the time of preparation of the solutions was carried out visually by the indexes of transparence, colour, pH and electrical conductivity. The results of the study have shown that in the pH area selected the aqueous solutions of timolol maleate and taurine are transparent and colourless. This confirms that both MS with their simultaneous presence in the aqueous solution are in the form of water-soluble ions. The value of specific conductivity (k) of the solution containing simultaneously timolol maleate and taurine, which is equal to the sum of k values of individual solutions for each MS (295 μS/cm for timolol maleate and 2.3 μS/cm for taurine) and is 297 μS/cm, indicates the absence of interaction between the MS under study. This allows us to assume that timolol maleate and taurine maintain stability when combined in the aqueous solution in the therapeutic concentrations selected; and it makes possible the creation of combined eye drops on their basis in the pH range from 6.5 to 7.5, which is traditionally used in ophthalmic medicines of timolol [10].

CONCLUSIONS

1. At the stage of pharmaceutical development of combined eye drops containing timolol maleate and taurine the behaviour of medicinal substances in the aqueous solution at different pH values has been analyzed.

2. The optimal pH limits, under which the medicinal substances are in the form of water-soluble ions in the solution, have been substantiated based on the molar fractions of ions of the medicinal substances of timolol maleate and taurine calculated depending on pH of the medium, as well as the study of appearance of their aqueous solutions.

3. The complex of studies has grounded the possibility of creating combined eye drops with such medicinal substances as timolol maleate and taurine in the pH range from 6.5 to 7.5 being physiologically suitable for eyes.

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ОБҐРУНТУВАННЯ ОБЛАСТІ pH ДЛЯ СТАБІЛЬНОСТІ ЛІКАРСЬКИХ РЕЧОВИН ТИМОЛОЛУ МАЛЕАТУ І ТАУРИНУ У ВОДНОМУ РОЗЧИНІ

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***Ключові слова**: комбіновані очні краплі; тимололу малеат; таурин; водний розчин; молярні частки іонів; область pH; стабільність*

На етапі фармацевтичної розробки комбінованих очних крапель на основі тимололу малеату і таурину проведено аналіз поведінки лікарських речовин у водному розчині в залежності від їх хімічної природи та pH середовища. На основі розрахованих молярних часток іонів обґрунтовані оптимальні межі pH, при яких лікарські речовини присутні у формі іонів. Результати дослідження зовнішнього вигляду свіжоприготованих водних розчинів як тимололу малеату в концентрації 0,34%, так і таурину в концентрації 4,0% показали, що в допустимій для очних крапель області pH від 3,5 до 8,5 досліджувані розчини прозорі. Це дало можливість обґрунтувати оптимальні межі pH, при яких зберігається стабільність лікарських речовин у вигляді водних розчинів. Проведено попередню оцінку сумісності тимололу малеату в концентрації 0,34% і таурину в концентрації 4,0% при їх сумісній присутності у водному розчині в залежності від pH середовища за показниками: прозорість, кольоровість, pH і питома електропровідність. В області pH від 3,5 до 8,5 водні розчини тимололу малеату і таурину при їх сумісній присутності прозорі та безбарвні, що підтверджує присутність обох лікарських речовин у формі водорозчинних іонів. Значення питомої електропровідності розчину тимололу малеату і таурину при їх сумісній присутності, яке дорівнює сумі значень питомої електропровідності індивідуальних розчинів лікарських речовин, свідчить про відсутність взаємодії між досліджуваними лікарськими речовинами. Проведені дослідження дозволили науково обґрунтувати область pH, що забезпечує стабільність і комфортність у застосуванні комбінованих очних крапель із лікарськими речовинами різної хімічної природи.

ОБОСНОВАНИЕ ОБЛАСТИ pH ДЛЯ СТАБИЛЬНОСТИ ЛЕКАРСТВЕННЫХ ВЕЩЕСТВ ТИМОЛОЛА МАЛЕАТА И ТАУРИНА В ВОДНОМ РАСТВОРЕ

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***Ключевые слова**: комбинированные глазные капли; тимолола малеат; таурин; водный раствор; молярные доли ионов; область pH; стабильность*

На этапе фармацевтической разработки комбинированных глазных капель на основе тимолола малеата и таурина проведен анализ поведения лекарственных веществ в водном растворе в зависимости от их химической природы и pH среды. На основе рассчитанных молярных долей ионов обоснована оптимальная область pH, при которой лекарственные вещества присутствуют в форме ионов. Результаты исследования внешнего вида свежеприготовленных растворов как тимолола малеата в концентрации 0,34%, так и таурина в концентрации 4,0% показали, что в допустимой для глазных капель области pH от 3,5 до 8,5 исследуемые растворы прозрачные. Это позволило обосновать оптимальные границы pH, при которых сохраняется стабильность лекарственных веществ в виде водных растворов. Проведена предварительная оценка совместимости тимолола малеата в концентрации 0,34% и таурина в концентрации 4,0% при их совместном присутствии в водном растворе в зависимости от pH среды по показателям: прозрачность, цветность, pH и удельная электропроводность. В области pH от 3,5 до 8,5 водные растворы тимолола малеата и таурина при их совместном присутствии прозрачные и бесцветные, что свидетельствует о наличии обоих лекарственных веществ в форме водорастворимых ионов. Значение удельной электропроводности раствора тимолола малеата и таурина при их совместном присутствии, равное сумме значений удельной электропроводности индивидуальных растворов лекарственных веществ, свидетельствует об отсутствии взаимодействия между исследуемыми лекарственными веществами. Проведенные исследования позволили научно обосновать область pH, обеспечивающую стабильность и комфортность в применении комбинированных глазных капель с лекарственными веществами различной химической природы.