Dexpanthenol (DXP) is the dextrorotatory form of panthenol, has the greatest biological activity. This compound is readily enzymatically oxidized to pantothenic acid (vitamin B5), a building block of coenzyme A. Pantothenic acid is essential to normal epithelial function (1-3).

The IUPAC name of DXP is (2R)-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide (Fig. 1) (4). It is soluble in water and alcohol, but insoluble in fats and oil-based substances. DXP is the most stable form of pantothenic acid in liquid products (3).

Clinical studies support the use of products with DXP in the treatment of various skin conditions. DXP at concentrations of 2.0 to 5.0% stimulates the regeneration of damaged skin. Topically applied DXP penetrates effectively into the skin and reaches high local concentrations when the water-in-oil emulsion is used as a vehicle (5). Latest in vitro studies show that novel poly lactic-co-glycolic acid (PLGA) + DXP nanofiber formulation represent a favorable choice in treating skin lesions (6). DXP has many beneficial properties for epithelial tissue, it increases fibroblast proliferation and has regene-

THE INFLUENCE OF EXCIPIENTS ON STABILITY OF VISCOUS EYE DROPS WITH DEXPANTHENOL IN PHARMACEUTICAL PRACTICE

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Abstract: Conventional eye drops exhibit weak bioavailability due to the unique physiology and anatomy of the eye. In order to increase eye drops viscosity, different concentrations of Carbopol® 940 (0.08% and 0.20%) were used. The aim of the study was to indicate the advantages and examine the influence of preservatives and the concentrations of viscosity-increasing agents on the quality of magistral viscous eye drops with dexpanthenol (DXP). The quality of the prepared formulations was tested using physico-chemical methods and biological tests. pH Value measurement was done by the potentiometric method. Viscosity measurements of the samples were performed according to Ph. Eur. 9.0. DXP content was determined by reversed-phase high-pressure liquid chromatography. Sterility testing was performed using direct sample inoculation. The results indicate that pH values of eye drops with preservatives are lower than pH values of preservative-free formulations. All formulations have recovery values that meet the requirements of the European Pharmacopoeia. The DXP content in preservative-free eye drops increased slightly during testing, unlike the DXP content in eye drops with preservatives. The formulations remained sterile during 45 days after preparation, stored at room temperature, protected from light. DXP viscous eye drops may be prepared in pharmaceutical practice using the proposed viscosity increasing agent (Carbopol® 940) and preparation procedure. All formulations express stability for 45 days after preparation. Preservative-free DXP eye drops with Carbopol® 940 concentrations of 0.08% and 0.20% show maximal stability, provide an optimal concentration of DXP (3.0%), and therefore have an advantage in pharmaceutical practice.

Keywords: dexpanthenol (DXP), eye drops, viscosity, Carbopol® 940, stability

Dexpanthenol (DXP) is the dextrorotatory form of panthenol, has the greatest biological activity. This compound is readily enzymatically oxidized to pantothenic acid (vitamin B5), a building block of coenzyme A. Pantothenic acid is essential to normal epithelial function (1-3).

The IUPAC name of DXP is (2R)-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide (Fig. 1) (4). It is soluble in water and alcohol, but insoluble in fats and oil-based substances. DXP is the most stable form of pantothenic acid in liquid products (3).

Clinical studies support the use of products with DXP in the treatment of various skin conditions. DXP at concentrations of 2.0 to 5.0% stimulates the regeneration of damaged skin. Topically applied DXP penetrates effectively into the skin and reaches high local concentrations when the water-in-oil emulsion is used as a vehicle (5). Latest in vitro studies show that novel poly lactic-co-glycolic acid (PLGA) + DXP nanofiber formulation represent a favorable choice in treating skin lesions (6). DXP has many beneficial properties for epithelial tissue, it increases fibroblast proliferation and has regene-
rative properties as well as anti-inflammatory effects (5, 7). There are a number of other indications for the use of DXP-based medicinal preparations: as an adjuvant in corneal or conjunctival lesions of the eye, or mucosal lesions of the nose; as systemic therapy in postoperative enteroparesis; in treating burning feet syndrome; it also may be included in multivitamin preparations. Positive clinical experience has been observed in ophthalmology, after the treatment of dry eyes (30 mg/mL of DXP), as well as after the therapy of corneal erosions with 5% DXP eye ointment, or DXP ophthalmic gel (3). DXP is renowned for its moisturizing properties. So, this provitamin is broadly used in cosmetic and ophthalmic industry to treat dryness. In contact lens solutions it serves as a moisturizing agent creating a barrier that prevents evaporation (8). The ocular bioavailability of drugs applied topically as eye drops is very poor. In order to lengthen the precorneal residence time of instilled dose and enhance the ophthalmic bioavailability, various ophthalmic vehicles have been used, such as suspensions, ointment, aqueous gels, and inserts. These ocular drug delivery systems have certain disadvantages such as blurred vision from ointment or low patient compliance from inserts which affected their use (9).

The increase of eye drops solution viscosity is usually made to extend the precorneal residence time thereby achieving optimal bioavailability, which is a widely used strategy in the development of carriers in ophthalmology (10). Numerous natural and synthetic viscosifying agents are added into the carrier to increase preparation viscosity, reduce the drainage rate, and finally to improve the efficacy of therapy. For example, anionic polymers such as polyacrylates are recommended as a component of artificial tears with a long-lasting effect in the treatment of dry eye syndrome and traumatic injuries (11). Polyacrylic acid has mucoadhesive properties mainly due to the existence of hydrogen bonds, whereas hydrophobic interaction with mucin is less significant. When anionic polymer reacts with mucin, maximal adhesive force occurs in the acidic area of pH, which indicates that carboxyl groups in the protonated form of the polymer are responsible for mucoadhesion. An ideal ophthalmic formulation should have the following properties: 1) to be in the form of a solution to prevent irritation and blurriness; 2) to have appropriate viscosity in order to avoid dilution and rapid elimination after administration; 3) to have appropriate mucoadhesive properties that would enable prolonged drug retention in the precorneal area, thereby increasing its bioavailability (12).

Magistral preparation of eye drops has several advantages, such as the ability to adjust the pH value of each formulation to achieve satisfactory stability and physiological tolerance, a lower degree of irritation because of the possibility to prepare a preservative-free ophthalmic composition and adjust the concentration of the active ingredients to the needs of patients. Due to the limited availability of DXP viscous eye preparations in the market, the aim of this study was to analyze available literature data and similar ophthalmic drugs and then conduct an experiment. An additional goal of this study was to emphasize the key advantages of magistral preparation of DXP viscous eye drops and its benefits in personalized therapy. The effect of CarbopolÆ 940 concentrations and the presence of a preservative solution on the quality of the prepared formulations were tested by physico-chemical methods and biological tests. Furthermore, on the basis of experimental results, the best formulation of DXP eye drops may be proposed and its expiration dates predicted.

<table>
<thead>
<tr>
<th>Table 1. Formulations of viscous eye drop with DXP.</th>
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<tbody>
<tr>
<td>Ingredients (g/10 g)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Dexpanthenolum*</td>
</tr>
<tr>
<td>Carnopol® 940†</td>
</tr>
<tr>
<td>Sol. conservans sterilisata*</td>
</tr>
<tr>
<td>Aqua ad injectabilia ad</td>
</tr>
</tbody>
</table>

* 50% DXP solution; † 1% aqueous dispersion of Carnopol® 940; Solutio conservans sterilisata (Sterile preservative solution)

Benzalkonium chloride 0.1 g
Natrii edetas 1.0 g
Aqua pro injection ad 100.0 g
EXPERIMENTAL

Chemicals and reagents
Carbopol® 940 and DXP were purchased from SB Trade d. o. o. (Serbia). Disodium edetate, sodium chloride and benzalkonium chloride were obtained from Sigma (Sigma-Aldrich GmbH, Germany) and they are official substances according to the European Pharmacopoeia 9.0 (13). All chemicals and solvents used for the chromatographic analysis are of the analytical grade of purity.

Eye drops preparation
Viscous eye drops were prepared according to the prescription shown in Table 1. Each of formulations contains the same concentration of DXP – 3.0%. A comparison of formulations was performed between that contain Carbopol® 940 in concentrations of 0.08% and 0.20%, as well as formulations with and without a preservative solution (Solutio conservans sterilisata) (14). Carbopol® polymers are completely synthetic, therefore, they are easier to manipulate with compared to semi-synthetic polymers.

Following the general pharmacopoeial regulation for making eye preparations, four different ophthalmic formulations were prepared, at room temperature, in aseptic conditions (13). After the optimal process of sterilization of each compound, final formulations were prepared by mixing all components. DXP aqueous solution was prepared in a concentration of 50% w/w due to its dense consistency. DXP solutions were sterilized using saturated aqueous steam under pressure at 100°C for 30 min. High temperatures need to be avoided when preparing formulations with DXP as an active principle, to prevent the racemization and conversion of D-panthenol into L-panthenol. An additional difficulty in preparation of DXP solution may be the stickiness of the product when high concentrations are used. Carbopol® 940 dispersion (1%) was prepared in aseptic conditions. This polymer was dispersed in a certain amount of distilled water by mixing using a laboratory mixer RV 16 basic (IKA® – Werke, Germany) until the dispersion became homogenous. The dispersion was then transferred to an infusion bottle and sterilized using saturated steam under pressure at 120°C for 20 min. After that, 10% sodium hydroxide solution, as a neutralizing agent for the acid, was added to the dispersion and mixed to form a homogenous gel. The resulting super absorbent polymers sodium polyacrylate formed a gel network by the cross-linking reaction. A sterile DXP solution, as well as a preservative solution (only in D1 and D2 formulations), was added to the previously neutralized Carbopol® 940 dispersion, with different polymer concentrations (0.08% and 0.20%). During the preparation of formulations was applied constant stirring until a homogenous viscous solution was created. The mass of the preparation was corrected with water of Aqua ad injectabilia quality. Solutio conservans sterilisata consists of 0.1% benzalkonium chloride, 1.0% disodium edetate and water for injection. It was sterilized using saturated steam under pressure (at 120°C for 20 min) (14). Benzalkonium chloride (BAC) is a quaternary ammonium compound and a preservative most commonly used in ophthalmic preparations on the EU market. Over 65% of commercial ophthalmic preparations have BAC as a preservative. It features great chemical stability and very good antimicrobial properties. It is usually used in ophthalmic preparations in the concentration range from 0.004 to 0.02% (most commonly 0.01%) (15). The Pharmacopoeia prescribes that ophthalmic preparations are prepared and packed in a way that sterility is ensured at the time of their first use and maintained during later use. Preservatives aim to prevent microbiological contamination of preparations packed in different containers. However, since they can irritate eye tissues or result in adverse toxic reactions, the use of preservatives in eye preparations is not recommended or is even excluded in certain cases: when used after surgical interventions, when prescribed explicitly without preservatives or when the active substance already demonstrates appropriate antimicrobial activity (16). In preparations with a preservative solution (D2 and D4), the concentration of BAC was 0.01%. A stabilizing and chelating agent disodium edetate was added to increase the activity of benzalkonium chloride. Samples were placed in plastic, sterile, tightly closed bottles with a dropper. They were marked adequately and kept in the dark.

Physico-chemical testing of preparations

pH Values measurements
pH Values of the prepared samples and the content of DXP in the formulations were tested. The European Pharmacopoeia 9.0 provides a description of the potentiometric method for determining pH values in General Chapters, Methods of the Analysis (physical and physicochemical methods) (13), pH value measurements were performed by direct immersion of the pH meter probe (Microprocessor 211, HANNA instruments, Italy) into the samples, at a temperature of 20 ± 5°C, with the prior calibration of the device with standard buffer solutions (pH 7.01 and pH 4.01).
Table 2. pH Values of sample formulations 24 h, 15, 30 and 45 days after preparation.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Time (days)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>D1</td>
<td>7.83 ± 0.08a</td>
<td>7.71 ± 0.17a</td>
<td>7.72 ± 0.25a</td>
<td>7.47 ± 0.16a</td>
</tr>
<tr>
<td>D2</td>
<td>6.12 ± 0.19c</td>
<td>6.32 ± 0.16b</td>
<td>6.60 ± 0.13b</td>
<td>6.81 ± 0.29b</td>
</tr>
<tr>
<td>D3</td>
<td>7.32 ± 0.19b</td>
<td>7.41 ± 0.14c</td>
<td>7.56 ± 0.03a</td>
<td>7.41 ± 0.29a</td>
</tr>
<tr>
<td>D4</td>
<td>6.09 ± 0.20c</td>
<td>6.21 ± 0.09b</td>
<td>6.43 ± 0.29a</td>
<td>6.52 ± 0.16b</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD (n = 5). Different superscript letters in columns denote significant differences at p < 0.05 – Tukey’s test

Table 3. Absolute viscosity for examined formulations (mPa·s).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Time (days)</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>D1</td>
<td>6.1 ± 0.11c</td>
<td>6.4 ± 0.21d</td>
<td>6.1 ± 0.16d</td>
<td>6.2 ± 0.04d</td>
</tr>
<tr>
<td>D2</td>
<td>7.4 ± 0.18b</td>
<td>7.5 ± 0.13c</td>
<td>7.3 ± 0.21c</td>
<td>7.7 ± 0.27c</td>
</tr>
<tr>
<td>D3</td>
<td>8.4 ± 0.24a</td>
<td>8.6 ± 0.16b</td>
<td>8.9 ± 0.16c</td>
<td>8.3 ± 0.15c</td>
</tr>
<tr>
<td>D4</td>
<td>8.6 ± 0.27a</td>
<td>9.5 ± 0.31c</td>
<td>9.8 ± 0.31c</td>
<td>9.7 ± 0.34c</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD (n = 5). Different superscript letters in columns denote significant differences at p < 0.05 – Tukey’s test

Table 4. DXP content in examined formulations (g/100 g).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Time (days)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>D1</td>
<td>2.996 ± 0.011a</td>
<td>2.980 ± 0.023a</td>
<td>3.010 ± 0.016a</td>
<td>3.000 ± 0.019a</td>
</tr>
<tr>
<td>D2</td>
<td>2.999 ± 0.035a</td>
<td>3.020 ± 0.022b</td>
<td>3.010 ± 0.017a</td>
<td>2.980 ± 0.030a</td>
</tr>
<tr>
<td>D3</td>
<td>2.996 ± 0.037a</td>
<td>2.990 ± 0.016b</td>
<td>3.000 ± 0.019a</td>
<td>3.010 ± 0.019a</td>
</tr>
<tr>
<td>D4</td>
<td>2.998 ± 0.015a</td>
<td>3.020 ± 0.021b</td>
<td>2.980 ± 0.019a</td>
<td>2.990 ± 0.021a</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD (n = 5). Different superscript letters in columns denote significant differences at p < 0.05 – Tukey’s test

Table 5. Recovery values for examined formulations (%).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Time (days)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>D1</td>
<td>99.87 ± 0.04a</td>
<td>99.33 ± 0.05a</td>
<td>100.33 ± 0.03a</td>
<td>100.0 ± 0.05a</td>
</tr>
<tr>
<td>D2</td>
<td>99.97 ± 0.02a</td>
<td>100.67 ± 0.04a</td>
<td>100.33 ± 0.05a</td>
<td>99.33 ± 0.02a</td>
</tr>
<tr>
<td>D3</td>
<td>99.87 ± 0.04a</td>
<td>99.67 ± 0.04b</td>
<td>100.00 ± 0.04a</td>
<td>100.33 ± 0.06a</td>
</tr>
<tr>
<td>D4</td>
<td>99.93 ± 0.02a</td>
<td>100.67 ± 0.03a</td>
<td>99.33 ± 0.05a</td>
<td>99.67 ± 0.04a</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD (n = 5). Different superscript letters in columns denote significant differences at p < 0.05 – Tukey’s test
Viscosity measurement

The viscosity of samples was determined by rotating viscometer Visco Basic Plus (Fungilab, USA). Ph. Eur. 9.0 in the General chapters, Methods of analysis, describes the method for determining the viscosity using the rotating viscometer at a temperature of 20 ± 5°C. The principle of the method is to measure the force acting on a rotor when it rotates at a constant rotational speed in the formulation. The absolute (dynamic) viscosity is measured. The unit of absolute viscosity is the Pascal second (Pa s). The most commonly used submultiples are the millipascal second (mPa s).

HPLC analysis

The qualitative and quantitative determination of DXP in different eye formulations was achieved using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with a diode-array detector (DAD) (17). D-Pantothenic acid hemicalcium salt (Sigma-Aldrich GmbH, Germany) was separated in the reversed-phase LiChroCART 125-4 Purospher RP-18 column (125 mm × 4 mm I.D., 5 µm) (Merck Millipore, USA), using the isocratic elution program, with a mobile phase containing filtered and degassed 25 mM phosphate buffer (pH 2.0) and acetonitrile (67 : 33, v/v). The flow rate of the mobile phase was 1.0 mL/min, and the column temperature was maintained at 35OC. Peaks were detected with Shimadzu SPD-M10Avp DAD at 204 nm. The identification of DXP was done by comparing the retention time and absorption spectra of each sample with a standard compound. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using Shimadzu CLASS-VP software version 6.12 SP4 (Shimadzu Co. Ltd., Japan).

Biological tests

Formulation sterility testing was carried out following pharmacopoeial regulations, under aseptic conditions, by direct inoculation method which involves the aseptic transfer of test preparation into the sterility test growth medium (at the surface of agar-gel). A prescribed amount of sterility test growth medium C, (15 mL) and a prescribed amount sterility test growth medium C, (15 mL) are simultaneously inoculated with a prescribed amount of preparation (1 mL). Medium C, is a medium for bacterial growth whose pH value after sterilization is from 7.0 to 7.2. Medium C, is a growth medium for fungi and molds, and its pH value after sterilization is from 5.6 to 5.8. During incubation, the media with introduced samples should be observed at specified time intervals. The inoculated medium C, is incubated for 10 days at a temperature of 33-35°C, whereas the inoculated medium C, is incubated for 14 days at a temperature of 20-25°C. If there is no evidence of microbial growth, the tested product is considered sterile. Otherwise, if there is evidence of microbial growth, the tested product does not comply with the sterility test. If the test is declared to be invalid, it is repeated with the same number of units as in the original test. If there is no evidence of microbial growth in the repeated test, the examined product is in accordance with the sterility test. If there is evidence on microbial growth in the repeated test, the examined product is not consistent with the sterility test (13, 18).

Statistical analysis

Statistical differences represented by letters (Tables 2, 3, 4 and 5) were obtained through one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post hoc test with α = 0.05, coupled with Welch’s statistic. The statistical package SPSS (IBM Corp, released 2013, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for the statistical analysis.

RESULTS AND DISCUSSION

The quality of the prepared formulations was tested for 45 days (24 h, 15, 30 and 45 days after preparation). The results of pH value measurements (mean value of five measurements) are shown in Table 2.

The results for viscosity measurements are given in Table 3.

The chromatogram obtained from the DXP standard solution is shown in Figure 2. The overlapped chromatograms of sample DXP drops and DXP standard solution are shown in Figure 3. The figures show that despite the existence of peaks of the other compounds in the formulations on the obtained chromatograms, the peak of DXP statistically separated from them.

Table 4 shows the results of determining DXP content in the formulations of samples.

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of analyte recovered (13). Recovery values for DXP are presented in Table 5. The results of biological testing show that all formulations meet the quality requirements for eye preparations (no contaminants were detected in the samples during officinal pharmacopoeial testing).
Specific anatomy and physiology of the eye make it difficult to achieve effective drug concentrations at a target location. When applying to the eye, the aim is to get the active substance bypassing protective barriers of the eye without causing permanent tissue damage or an increase of the risk of systemic side effects (10). The key disadvantages of conventional dosage forms are poor ocular bioavailability of the active substance, possible resorption in the systemic bloodstream due to the nasolacrimal duct drainage, a lack of effective systems for drug delivery to the posterior part of the eye tissue, relative impermeability of the epithelial membrane of the cornea, tear dynamics and high efficacy of the blood-ocular barriers. It is believed that only 1.0% or less of the topically administered dose is absorbed via the cornea and therefore reaches the anterior segment of the eye (19, 20).

Nowadays, there is a tendency in the field of eye preparations to find systems for drug delivery which patients comply well with and which are able to double the time of precorneal contact and slow down the release of drugs from the medium (15). Rather simple content and the technology of the preparation process imply that many correctors of viscosity may be incorporated into eye formulations. Viscosity correctors are often hydrophilic polymers, such as polyacrylic acid (19). Polyacrylic polymers have an excellent role in ophthalmology since they are able to increase viscosity when they are in the physiological range of pH (21).

The degree of interaction of polyacrylic acid/mucin depends to a great extent on the concentration of the polymer. The applied concentration varies from 0.05 to 0.20% (v/v), which is a range between the concentration at which preparation is viscous enough to be comfortable to use and the maximal concentration which is applied in commercial preparations (22). For the purposes of this study, four different formulations were prepared with Carbopol® 940, which was in different concentrations in the samples, i.e. 0.08% and 0.20%. Two formulations were prepared with 1.0% preservative solution (Table 1). The study tested the effects of the preservative solution and the concentration of the viscosity correction agent to the stability of the prepared formulations. The selected formulations in the treatment of eye injuries were made with Carbopol® 940, a polymer which provides viscosity reduces the hydrophobic film and extends the contact time with the cornea (by reducing the drainage speed). The use of a bioadhesive polymer (e.g. polyacrylic acid) extends the retention of DXP in the precorneal space.

Viscosity enhancers are used in ophthalmic preparations to extend the contact time of the active substance with the cornea, as well as to increase their bioavailability. However, the preservative action time also extends which may be a disadvantage since it may lead to eye damage. The use of viscosity correction agents needs to be in balance with possible adverse effects of preservatives which may be manifested in different parts of the eye (16).
Literature data show that preservatives used in eye drops increase the permeability of the cornea, especially benzalkonium chloride, which is a surfactant and as such dissolves membranes of not only the cells of microorganisms but of epithelial cells as well (16).

The obtained results reveal that pH values of the prepared formulations of DXP eye drop with a preservative solution (D2 and D4) are lower than pH values of the preservative-free formulations (D1 and D3) (Table 2).

DXP dispersion has a neutral pH value (23). The optimal pH value for ophthalmic preparations ranges from 5.50 to 7.50, whereas the eye tolerates solutions with pH values ranging from 7.0 to 9.0 (18). Ideally, ophthalmic preparations should be formulated to the pH value of the tear fluid which is 7.40. The most common are drops with stability achieved at the euhydric pH. It is the pH value of a solution at which the active substance is stable with a maximal approximation to the physiological value. Given that pH values of the prepared preservative-free viscous eye drops range from 7.32 to 7.82, i.e. 6.09 to 6.81 in solutions with preservative, all formulations have satisfactory pH values which did not change significantly during storage and testing. The advantage in developing DXP eye drops should be given to preservative-free ones (D1 and D3) since their pH values are closer to the pH value of the tear fluid – 7.40.

The stability tests include viscosity measurement. On the basis of obtained results (Table 3), the viscosity of the formulations D4 and D3 are greater compared with samples D1 and D2, which has the same content of viscosity correction agent (Carbopol® 940). This is probably the results of the interaction of the surface-active benzalkonium chloride and polyacrylate (23). The viscosity of D3 formulation significantly increased after 15, 30 and 45 days. Generally, the viscosity of preservative-free samples (D1 and D3) shows higher stability.
The DXP content in eye drops without a preservative solution increases during the time, contrary to the content in eye drops with a preservative solution (the DXP content decreases) (Table 4). All formulations have recovery values that meet the criteria from the European Pharmacopoeia (uniformity of mass for single-dose preparations – test B) (Table 5) (13).

Final sterilization is not possible in most ophthalmic preparations, therefore, they are prepared in aseptic conditions, and the sterility check is done in all stages of production. Sterility testing, validation of aseptic conditions, monitoring of production environment, and the use of sterilized equipment and agents ensure that products meet sterility requirements. According to the literature, magistral preservative-free eye drops have a shelf life of 24 h, whereas the shelf life of eye drops with preservatives is four weeks (24). DXP eye formulations remain sterile 45 days after preparation if stored at room temperature and protected from light.

CONCLUSION

The obtained results reveal that all prepared ophthalmic formulations, viscous eye drops with DXP in prescribed conditions meet the pharmacopoeial requirement for the quality of ophthalmic preparations. In pharmaceutical practice, optimal viscous eye drops formulations with DXP may be prepared with a prescribed viscosity correction agent (Carbopol® 940) and using a validated preparation process. All samples show stability during 45 days after the preparation. There are no statistically significant differences in pH values and the DXP content between D and D formulations (preservative-free formulations). Therefore, different concentrations of Carbopol® 450 as a viscosity correction agent do not have a significant effect on the quality of the prepared viscous eye drops. Formulations of DXP eye drops without preservatives and with Carbopol® 450 (in concentrations of 0.08% and 0.20%) show maximal stability and an optimal concentration of DXP (3.0%). Those formulations are recommended for magistral preparation, and their galenic preparation may also be taken into consideration.

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Conflict of interest

The authors declare no conflicts of interest.

REFERENCES


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