Ophthalmic drugs are specific medicinal forms that are subject to various quality requirements, including sterility. When formulating ophthalmic drugs, it is necessary to adjust the osmotic pressure and acidity, while also considering the stability of the drug. Other parameters that influence the effect of ophthalmic liquid medicines are viscosity and surface tension, and possibly also the size of suspended particles. The increase in viscosity is also related to the use of polymers, where we can encounter the problem of evaluating the necessary amount of tonicity-adjusting agent because osmotic as well as oncotic pressure must be considered (Bouwman-Boer et al., 2015).

As a viscosity-increasing agent, chitosan, a polymer with many benefits and having a wide application in various pharmaceutical forms, is increasingly used in ophthalmic liquid medicines (Alonso et al., 2003). Another advantage of chitosan is its antimicrobial effect (Hosseinnejad et al., 2016, Matušová et al., 2007) and nonirritating properties when applied to the eye (Felt et al., 1999). An important attribute when applying drugs to the eye is their low irritability, which is achieved through the use of nonirritating components of the dosage form and also suitable physicochemical parameters of the finished dosage form. Irritability can be evaluated in laboratory animals, but other methods are increasingly used. The European Union Reference Laboratory for Alternative Methods to Animal Testing (EURL-ECVAM) has an important role in this process (Casati et al., 2017). Alternative tests include monitoring the toxic effect in vivo on simple organisms, in vitro on tissues, cells, and in silico by modeling experiments with software tools on computers, or a combination of these approaches (Tichý, 2005). An in vitro test is performed using isolated tissues, organs, or cells in a test tube or outside a living organism. If living cells are involved, the term ex vivo is also used for these tests. Of the in silico methods, the most widespread are QSAR models – quantitative relationships between chemical structure and biological effectiveness. They are mathematical models that can be used to predict the physicochemical and biological properties of compounds and the fate of these compounds in the environment based on knowledge of their chemical structure (Vitková et al., 2016).

Although the in vivo Draize eye irritation test on albino rabbits according to OECD 405 (10) is still the standard method for evaluating the irritability of ophthalmic drugs, the use of alternative methods is increasingly being considered.
for evaluating eye irritation or assessment of eye damage potential, in vitro variants of this test are also being sought. One of the alternative methods for assessing irritation is the test on erythrocytes called the “Red Blood Cell (RBC) Hemolysis Test” using, for example, sheep erythrocytes (Scott et al., 2010, Lagarto et al., 2006).

Erythrocytes or RBCs are the most numerous blood cells, making up 45% of human blood volume. The rest is blood plasma together with white blood cells and platelets. The main role of erythrocytes is the transport of oxygen and elimination of carbon dioxide, which significantly contributes to maintaining the acid–base balance. The life cycle of red blood cells is 120 days (Dobrovolskaia et al., 2013).

Hematopoiesis takes place in the red bone marrow through the production of the cytokine erythropoietin in the kidneys. Mature erythrocytes have a biconcave shape, which gives them flexibility and allows easy penetration through capillaries. A characteristic feature is the absence of mitochondria and, in adult cells, the absence of a nucleus (Koleva et al., 2020). Due to the influence of various physical factors, the membrane of erythrocytes is destabilized and hemoglobin is subsequently released. Such hemolysis can occur, for example, even with pressure changes. It is caused by the formation of neocytes caused by hypoxia during a sudden change from hypoxia to normoxia (Song et al., 2021; Riso et al., 2014). Hemolysis is also caused by other changes in the environment, such as changes in pH, changes in osmotic pressure, changes in surface tension, and so on.

Dexamethasone is a glucocorticosteroid that is used as an antiphlogistic for noninfectious inflammation in ophthalmology. They encountered the preparation of such eye drops as part of the magistral preparation (individual compounding) in various pharmacies to replace the then temporarily unavailable mass-produced eye drops with dexamethasone in a concentration of 0.1%. In our work, we focused on the preparation of eye drops with the active substance dexamethasone for the preparation of suspension eye drops or disodium dexamethasone phosphate for the preparation of solution eye drops.

We also observed the drops’ properties after adding the gel-forming substance, chitosan. The goal was to find a suitable ratio of excipients, so that the drops meet the requirements of the pharmacopeia, and to roughly evaluate irritation of the eye drops on erythrocytes by spectrophotometric measurement of the amount of hemoglobin released by hemolysis compared to mass-produced Unidexa drops.

**MATERIALS AND METHODS**

**Materials**

The materials and their manufacture information are as follows.  
*Active ingredient:*

Dexamethasone Ph.Eur. 9.0., Dr. Kulich Pharma, Hradec Králové, Czech Republic b. n. NUD181203,

<table>
<thead>
<tr>
<th>Table 1. Eye drops A composition.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Carbethopendecinium bromide</td>
</tr>
<tr>
<td>Water for injections</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Eye drops C composition.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone sodium phosphate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Carbethopendecinium bromide</td>
</tr>
<tr>
<td>Water for injections</td>
</tr>
</tbody>
</table>

Dexamethasone sodium phosphates, Fagron, Olomouc, Czech Republic, b. n. 21L03-BO6-217001

*Excipients:*

Sodium chloride (NaCl), Centralchem s.r.o., Bratislava, Slovak Republic
Chitosan low molecular weight (LMW), SIGMA-ALDRICH CHEMIE, USA
Carbetopenceminium bromide – Septonex, Supplier: PharmDr. Jozef Valúch
Lactic acid, SKLOCHEM-AGROEKOLAB, Zvolen, Slovak Republic
Sodium hydroxide (NaOH), Centralchem s.r.o., Bratislava, Slovak Republic
Reference eye drops Unidexa 0.1%, Unimed Pharma, Bratislava, Slovak Republic, b. n. 3002214

*Biological material:*

Capillary blood of a healthy volunteer

*Used devices:*

pH meter, VWR® pHenomenal®, pH 1100L, CHROMSERVIS, Czech Republic
Spectrophotometer, Helios, CHROMSPEC Slovakia
Rotary viscometer, Anton Paar, Rheolab QC, Austria
Analytical scales OHAUS PIONEER, Transfer Multisort Elektronik Sp. z o.o., Łódź, Poland
Laboratory scales RADWAG WTC 600, Radwag Slovakia

**Preparation of eye drops**

Eye drops were prepared with a composition shown in Tables 1 and 2 at room temperature. As presented in Tables 3 and 4, chitosan was dissolved in water for injection with lactic acid at 80°C, and after cooling to room temperature, the acidity was adjusted by adding sodium hydroxide.
Assessment of eye drops

We measured the pH values, density (pycnometrically), and their surface tension (stalagmometrically) of the prepared eye drops and also of the reference Unidexa eye drops. In Table 5 are shown the averages of six measurements for each parameter.

Viscosity and flow properties of all samples measured with an Anton Paar rotational viscometer are shown in Fig. 1.

To compare the irritability (due to the different tonicity of the solutions), solutions containing sodium chloride with different concentrations were prepared, in which, just as in the prepared eye drops A, B, C, D and reference eye drops Unidexa, possible hemolysis was monitored. The composition of solutions of sodium chloride prepared with different concentrations as well as a suspension of red blood cells is shown in Table 6.

After storing the mixtures of sodium chloride solutions or prepared eye drops and reference eye drops Unidexa with RBC suspension in the refrigerator for 24 h, the rate of hemolysis was monitored (see Fig. 2). The amount of released hemoglobin due to hemolysis was determined spectrophotometrically at a wavelength of \( \lambda = 550 \text{ nm} \) (see Figs 3 and 4).

<table>
<thead>
<tr>
<th>Eye drops</th>
<th>pH</th>
<th>Density (g cm(^{-3}))</th>
<th>Surface tension (N m(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.626</td>
<td>1.0088</td>
<td>70.9313</td>
</tr>
<tr>
<td>B (Ch)</td>
<td>5.878</td>
<td>0.9953</td>
<td>70.5043</td>
</tr>
<tr>
<td>C</td>
<td>7.400</td>
<td>1.0059</td>
<td>35.0392</td>
</tr>
<tr>
<td>D (Ch)</td>
<td>5.800</td>
<td>1.0030</td>
<td>63.3394</td>
</tr>
<tr>
<td>Unidexa 0.1%</td>
<td>6.757</td>
<td>1.0094</td>
<td>36.9939</td>
</tr>
</tbody>
</table>

DISCUSSION

The preparation of medicines for an individual patient (compounding) is always a certain insurance in the event of a failure of mass-produced medicines, the quality of which is usually higher than in magistral preparation. This was also confirmed in our experiment. When replacing the insufficient mass-produced product containing 0.1% dexamethasone in pharmacies, either dexamethasone itself was available for individual preparation, or in some pharmacies, they used a water-soluble salt of dexamethasone (dexamethasone sodium phosphate). Therefore, we included the preparation of suspension eye drops and solution eye drops in the experiment. After previous experiences with the use of chitosan as a viscosity-increasing substance, which at the same time affects irritation (sensitivity), we also included chitosan in the formulation of eye drops B and D at a concentration of 0.1%. When evaluating viscosity, the presence of chitosan showed an increase in viscosity, but differences were also seen when comparing suspension and solution eye drops, which had lower viscosity and other flow properties. The highest viscosity was found in the reference eye drops. Since we used only 0.1% concentration for chitosan, we can also consider a higher concentration (up to 0.2%), which would bring us closer to the viscosity of the mass-produced product. RBC Hemolysis Test is considered a method in which the excellent results obtained correspond to the results of the Draize test (Nóbrega et al., 2012). When evaluating irritability by monitoring the hemolysis of a suspension of erythrocytes obtained from a healthy volunteer, we observed both the effect of the type of preparation (suspension or solution) and the effect of viscosity, including the presence of chitosan, on irritation. The influence of pH was not manifested, apparently because the acidity was always in the euacidic region.
Figure 1. The viscosity of eye drops A, B, C, D and Unidexa depending on torque (n = 3).

Figure 2. Hemolysis in different NaCl solutions.
CONCLUSION

Based on the knowledge obtained, when preparing magistral eye drops with dexamethasone or its soluble salt, it is necessary to consider both isotonicity and viscosity.

References


