MODELLING AND BIOPHARMACEUTICAL EVALUATION OF CICLOPIROX OLAMINE GELS

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Abstract: The ciclopirox olamine (CPO) has a broad antimicrobial profile including dermatophytes, yeasts and is used in various pharmaceutical forms. The aim of this study is to evaluate the quality of the CPO gels according to biopharmaceutical tests in vitro and antifungal activity assay. Hydroxypropyl cellulose, chitosan and poloxamer 407 were selected as agents gelificants. The effects of gelling agent properties and concentration on the consistency and flow characteristics have been studied by rheometer. CPO release rates from gel were measured with Franz type diffusion cells. The antifungal activity of gels was tested using agar well diffusion technique. The results of the experimental study have shown that the rheological properties of the medications depend on the selected gelling agent and the amount of it. The higher amounts of CPO were released from the poloxamer 407 gels. Though all tested CPO gels showed great inhibition of Microsporum canis.

Keywords: ciclopirox olamine, gel, rheology, chitosan, hydroxypropyl cellulose, poloxamer 407

Fungal infections of skin are one of the common infectious diseases (1, 2). In world’s population 20-25% has skin mycoses (3). Microsporum canis (M. canis) is a pathogenic fungus that infects the skin on cats, dogs, horses and humans (4, 5). M. canis has been identified as causal agent of ringworm infection in pets, tinea capitis and tinea corporis in humans (3).

Topical medications are the primary treatment indicated for tinea corporis, tinea pedis (6). The fungus is located in keratinized tissues (the stratum corneum, hair, nails) therefore topical antifungal therapy can be used for treatment. The efficiency of the treatment is determined by penetration of drugs through the target tissue. The higher concentrations should be achieved in the skin (7). The factors that determine the bioavailability of topical medications are physiological (properties of stratum corneum, application site, condition of the skin) and formulation factors (concentration of the drug substance in the base, ability of the drug substance to transit from the carrier to the stratum corneum of the skin, penetration enhancers used, method of application) (8, 9). While producing a semi-solid drug, selecting the adequate base is the most important as the base in particular provides the proper consistency, release and resorption of a semi-solid medication (10). Proper selection of the excipients while producing a semi-solid medication ensures the stability during storage time and high distribution on the skin, a high quality release of the drug substance from the base and a desirable bioavailability of the active substance (10). Gels maintain a higher stability and a faster release of the active substances than creams or ointments. It is a non-greasy semi-solid drug form (11, 12). Various gelling agents are used in the production of gels: natural polymers, semi-synthetic cellulose derivatives and synthetic polymers (13).

Hydroxypropyl cellulose (HPC), chitosan and poloxamer 407 were selected as agents gelificants. HPC is hydroxypropylated derivative of cellulose. HPC is used as thickening agent and emulsifier (14, 15). Chitosan is deacetylated form of chitin (16). Chitosan is soluble in water, non-toxic, biodegradable and biocompatible (17, 18). Chitosan promotes wound healing and has broad antibacterial activity (19, 20). Poloxamer 407 (polyoxyethylene-polyoxypropylene-polyoxyethylene polymer) is a

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hydrophilic non-ionic surfactant. Poloxamer 407 shows thermoreversible properties: fluid state at room temperature and sol-gel transition temperature at body temperature promoting prolonged release (21). Poloxamer 407 is used in pharmaceutical formulations as surfactant, emulsifying agent, solubilizing agent, dispersing agent, and in vivo absorbance enhancer (22). As skin fungus treatment is long and complicated process, it is appropriate to produce pharmaceutical products that ensure effectiveness and has acceptable organoleptic properties. For this aim the ciclopirox olamine gels were prepared.

The ciclopirox olamine (CPO) has a broad antimicrobial profile including dermatophytes, yeasts and other nondermatophytes (23, 24). This antifungal compound is a synthetic hydroxypyridone derivative whose chemical formula is 6-cyclohexyl-1-hydroxy-4-methylpyridin-2(1H)-one (25). CPO has log P 2.3 and possesses the recommended lipophilicity (log P 1-3) from all the antifungal compounds used in dermatology (26). CPO is used in various pharmaceutical forms: cream, suspension, shampoo, gel, solution, powder, globules, lacquer and topical solution (5, 17). According to the data of scientific literature, CPO cream and lotion are active against many types of fungal infections, including tinea corporis/cruris, tinea pedis, cutaneous candidiasis, pityriasis (tinea) versicolor, and seborrheic dermatitis (27). The gel of CPO is also indicated for the treatment of seborrheic dermatitis of the scalp, interdigital tinea pedis and tinea corporis (28). Whereas the efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue, therefore it is necessary to investigate biopharmaceutical properties and influence of gelling agent. In vitro release test is used to analyze semi-solid formulations: ointments, creams, gels, because active substance penetrate to tissues when it is released from semi-solid matrices. The aim of this study is to evaluate the quality of the CPO gels according to biopharmaceutical tests in vitro and antifungal activity assay.

**EXPERIMENTAL**

**Materials**

Ciclopirox olamine was purchased from Chemical Point (Deisenhofen, Germany). Hydroxypropyl cellulose, M.W. 100.000 was obtained from Alfa Aesar (Karlsruhe, Germany). Chitosan middle-viscous was supplied from Sigma Aldrich (Japan). Poloxamer 407 was acquired from Fagron (Rotterdam, The Netherlands). Lactic acid was sourced from Sigma Aldrich (Saint Louis, Missouri, USA). Ethanol > 96% was from Spiritus Vilnensis (Vilnius, Lithuania).

**Preparation of gels**

CPO solubility in water is limited therefore it is improved by cosolvent - ethanol. Poloxamer 407 improves water solubility of the substances. Ethanol reduces the strength of poloxamer 407 gel and increases gelation temperature (21). Chitosan and HPC gels prepared in this study were hydroalcoholic whereas poloxamer 407 gels were prepared without ethanol.

Experimental CPO gels were prepared according to the general technological principles of semi-solid preparation (9).

**Hydroxypropyl cellulose gels (C-1, C-2)**

CPO was dissolved in ethanol solution (40% w/w). HPC was poured into the solution and stirred at room temperature until a clear gel formed (29).

**Chitosan gels (C-3, C-4)**

CPO was dissolved in ethanol solution (40% w/w) with lactic acid. The solution was stirred with chitosan until a clear gel formed (17).

<table>
<thead>
<tr>
<th>Table 1. Composition of different gel samples.</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>CPO, %</td>
</tr>
<tr>
<td>Distilled water, %</td>
</tr>
<tr>
<td>Ethanol, %</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose, %</td>
</tr>
<tr>
<td>Chitosan, %</td>
</tr>
<tr>
<td>Lactic acid, %</td>
</tr>
<tr>
<td>Poloxamer 407, %</td>
</tr>
</tbody>
</table>
Modelling and biopharmaceutical evaluation of ciclopirox olamine gels

Poloxamer 407 gels (C-5, C-6)
CPO was dissolved in distilled water. Then, poloxamer 407 was added and slowly mixing. Formulation was kept in refrigerator at +4°C until dissolved. Hydrogels were formed in room temperature (21).

Before the measurement of rheological properties and value of pH, the gels were held at room temperature for 24 h. The compositions of gels are given in Table 1.

PH measurement
The pH of samples was measured by pH-meter 766 Calimatic (Knick, Germany) at temperature 20 ± 1°C.

Flow curves
Rheological measurements were done by MCR102 modular compact rheometer (Anton Paar, Austria) with a cone and plate fixture with 2° cone angle. The rheological properties were assessed in 20, 32 and 37°C. The test of flow curve was done in 1-50 1/s interval. After adapting the model of Power law, flow behavior index n and consistency index K were calculated.

In vitro release study
In vitro release experiments were performed using the modified Franz type diffusion cells and the dialysis membranes of natural cellulose Cuprophan (Medicell International Ltd., London, UK) (9). A diffusion area was 1.77 cm². The infinite dose of the donor phase was placed into the diffusion cell. The aqueous receptor medium was stirred using the hot-plate magnetic stirrer IKAMAG C-MAG HS7 (IKA-Werke GmbH & Co.KG, Staufen, Germany) maintaining the temperature of 37°C. The samples from the receptor solution were removed at 1, 2, 3, 4, 5, 6 h and replaced with the same volume of fresh receptor solution. All samples were analyzed by spectrophotometer (Agilent 8453, Australia) at the wavelength of maximum absorption of CPO (303 nm).

Antifungal activity
The antifungal activity of tested gels was tested using agar well diffusion technique. According to the standard approved by the Clinical and Laboratory Standards Institute (CLSI), 35 mL of liquid sterile Sabouraud dextrose agar (SDA) with chloramphenicol was poured into each Petri plate (10 cm in diameter) and left in a horizontal position to clot.

Stock inoculums of M. canis strains isolated from cats affected with dermatophytosis were prepared from 7 to 14 day cultures grown on Sabouraud dextrose agar. Five mL sterile normal saline was added and the suspensions were made by gently scraping the colony with the tip of a sterile Pasteur pipette. The resulting suspended mixture was withdrawn and transferred to a sterile tube. Heavy particles of the suspension present were allowed to settle for 15 min at room temperature and the upper homogenous suspensions were mixed using vortex mixer for 15 s and adjusted with sterile normal saline to match an opacity of 0.5 McFarland’s standard. The inoculums were adjusted to between 1.0 ◊ 10⁶ and 5.0 ◊ 10⁶ spores per mL by microscopic enumeration with a cell counting hemocytometer (Neubauer counting chamber). The prepared dermatophytes inoculums were seeded over the surface of the SDA plates and allowed to dry.

Six wells with a diameter of 7 mm were made with the help of a template on the surface of the agar plate. About 0.1 mL of the gel was delivered into the well using a micropipette. CPO free gels were used as negative control.

The plates were incubated at 28°C for 7 days. Antimicrobial activity was evaluated by measuring the zone of inhibition in millimeters. If zone of inhibition was not present around the well, it was considered that the investigated material does not have

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Spreadability</th>
<th>Washability</th>
<th>Color</th>
<th>Odor</th>
<th>Phase separation</th>
<th>pH</th>
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<tbody>
<tr>
<td>C-1</td>
<td>Easy</td>
<td>Washable</td>
<td>White transparent</td>
<td>Weak ethanol</td>
<td>No</td>
<td>7.93</td>
</tr>
<tr>
<td>C-2</td>
<td>Easy</td>
<td>Washable</td>
<td>White transparent</td>
<td>Weak ethanol</td>
<td>No</td>
<td>7.71</td>
</tr>
<tr>
<td>C-3</td>
<td>Easy</td>
<td>Washable</td>
<td>Yellowish transparent</td>
<td>Weak ethanol</td>
<td>No</td>
<td>5.49</td>
</tr>
<tr>
<td>C-4</td>
<td>Easy</td>
<td>Washable</td>
<td>Yellowish transparent</td>
<td>Weak ethanol</td>
<td>No</td>
<td>6.02</td>
</tr>
<tr>
<td>C-5</td>
<td>Easy</td>
<td>Washable</td>
<td>White transparent</td>
<td>No</td>
<td>No</td>
<td>8.03</td>
</tr>
<tr>
<td>C-6</td>
<td>Easy</td>
<td>Washable</td>
<td>White transparent</td>
<td>No</td>
<td>No</td>
<td>8.09</td>
</tr>
</tbody>
</table>

Table 2. Physical evaluation of formulations.
Table 3. Flow behavior index (n) and consistency index (K).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature</th>
<th>n (20°C)</th>
<th>K (Pa s^n)</th>
<th>n (32°C)</th>
<th>K (Pa s^n)</th>
<th>n (37°C)</th>
<th>K (Pa s^n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>20°C</td>
<td>0.497</td>
<td>79.25</td>
<td>0.564</td>
<td>48.12</td>
<td>0.597</td>
<td>37.13</td>
</tr>
<tr>
<td>C-2</td>
<td>20°C</td>
<td>0.387</td>
<td>253.93</td>
<td>0.503</td>
<td>143.95</td>
<td>0.542</td>
<td>114.02</td>
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<tr>
<td>C-3</td>
<td>20°C</td>
<td>0.432</td>
<td>57.57</td>
<td>0.361</td>
<td>50.81</td>
<td>0.366</td>
<td>42.75</td>
</tr>
<tr>
<td>C-4</td>
<td>20°C</td>
<td>0.269</td>
<td>437.45</td>
<td>0.271</td>
<td>339.7</td>
<td>0.292</td>
<td>304.13</td>
</tr>
<tr>
<td>C-5</td>
<td>20°C</td>
<td>0.413</td>
<td>10.371</td>
<td>0.071</td>
<td>324.56</td>
<td>0.056</td>
<td>385.54</td>
</tr>
<tr>
<td>C-6</td>
<td>20°C</td>
<td>0.121</td>
<td>319.96</td>
<td>0.026</td>
<td>437.91</td>
<td>0.002</td>
<td>629.02</td>
</tr>
</tbody>
</table>

Figure 1. Flow curves of gels: a) C-1, b) C-2, c) C-3, d) C-4, e) C-5, f) C-6
a fungicidal effect on the tested strains. All the experiments were repeated thrice and results were recorded.

**Statistical analysis**

All tests were repeated three times. The mean values and standard deviations of the results were calculated using IBM SPSS statistics 20 and Microsoft Office Excel 2016 programs. The significance of differences was evaluated using Student’s *t*-test. The differences were statistically significant at *p* < 0.05.

**RESULTS AND DISCUSSION**

The evaluation parameters (spreadability, washability, color, odor, phase separation and pH) were determined (Table 2). The results of the experimental study have shown that the pH value of the produced gels depends on the base that was chosen. The semi-solid C-3 system had lower pH value compared with other systems and it is close to the physiological pH of the skin. It can be stated that the pH value of the produced gels ensures effectiveness of CPO on the basis of published data of scientific research (30). Therefore, the produced systems are suitable for use on the skin.

A flow curve can provide important information about the storage stability, optimal conditions for producing and end-user applications. Flow behavior index (*n*) can be described as the rate of change of structure with shear rate. The values of stronger gels are lower due to increased noncovalent forces between particles (31). After the power law slope analysis consistency index and flow behavior index were found (Table 3).

Chitosan and hydroxypropyl cellulose gels match the Oswald de Waele model (Fig. 1 a-d) and act like typical thinning substances – as the temperature gets higher, flow behavior index increases (gel structures get weaker) whereas consistency index decreases (Table 3). The results of this study and the data presented in scientific literature confirm that poloxamer 407 gels are characterized by thermoreversible properties: at room temperature C-5 is a liquid while at 32°C - a gel (Fig. 1 e, f) (2). The results of the study have confirmed that as the temperature rises their viscosity increases: flow behavior index decreases while consistency index increases (21).

Based on the study results it can be stated that temperature least affects the structure of chitosan gels. No statistically significant difference (*p* < 0.05) was determined between the consistency index and flow behavior index of the examined chitosan gels in different temperatures. It was found that temperature has the most effect on gels produced on the base of poloxamer 407. There was a statistically significant difference (*p* < 0.05) between the flow behavior indexes of poloxamer 407 gels at different temperatures. The consistency index of these gels varied statistically significantly (*p* < 0.05) as well. The obtained results correspond with other researchers published results (18).

Experimental study found that the rheological properties of the medications depend on the selected gelling agent and the amount of it. A statistically significant difference (*p* < 0.05) between the consistency indexes and flow behavior index of gels having a different concentration of a gelling agents was found (C-1 and C-2, C-3 and C-4, C-5 and C-6).

The results showed that the selected gelling material affected the release of CPO content from semi-solid systems (Fig. 2). Formulations C-2 and C-4 released the lowest amounts of CPO within 6 h of testing. The higher amounts of CPO were released from the gels (C-5, C-6) in which poloxamer 407 was used as the gelling material. The results confirm the data from the scientific literature that poloxamer 407 gels relatively fast dissolve under physiological conditions (18). The highest CPO content was released after 4 h, after which time a slowdown of the release was observed. The results of the analysis supported published data confirming that chitosan and HPC could be using for sustained release of CPO (32, 33).

The analysis of the CPO release study results demonstrate that the release of CPO from semi-solid vehicles is influenced not only by the properties of gelling substance but also by concentration of gelling agent in the preparation.

The released CPO amount is decreasing when the concentration of hydroxypropyl cellulose and chitosan as the gelling agents in semisolid dosage forms is increasing. Data of the statistical analysis showed a statistically significant difference (*p* < 0.05) between CPO amounts released from C-1 and C-2, also between C-3 and C-4. Although poloxamer 407 addition does not change the amount of CPO released, but the C-6 kinetic is slower than C-5.

Experimental study found that the released amount of CPO from gels depend on the viscosity. Data analysis showed that statistically significant difference (*p* < 0.05) existed among amounts of CPO released from HPC gels C-1 and C-2, also from chitosan gels C-3 and C-4. The observed differences of released amount of CPO could be explained by respective differences in concentration of gelling agent.
Linearity of Higuchi-plots was confirmed by coefficients of determination ($R^2$) ranging from 0.931 to 0.989 for the gels and indicated that diffusion of CPO from tested gels was a rate limiting step in the release of active compounds. Also it confirmed that applied membrane was not affecting diffusion of CPO from formulated gels. All formulations of CPO gels showed great inhibition of pathogenic fungi (Table 4). Although a different CPO diffusion from the tested gels was found, the studies of the antifungal effect showed that all studied medications have antifungal activity. It can be stated that selected gelation substances are suitable for CPO introduction. The obtained results confirm scientific literature data that CPO is active against many fungi including dermatophytes and yeast (34).

### CONCLUSIONS

Based on the results of rheological studies it can be stated that the changes in modelled gel structures are influenced by gelling agents – the viscosity of HPC and chitosan gels decreases at a higher temperature whereas the viscosity of poloxamer 407 gels increases. It is determined that a greater amount of gelling agent determines a higher viscosity and a slower CPO release from the gel \textit{in vitro}. Tested gels release the required amount of active substance which ensures the effectiveness of the medication and is characterized by a strong effect against \textit{M. canis} fungus.

### Conflict of interests

The authors declare they have no conflict of interests.

### REFERENCES


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