Aiming to develop some dermatocosmetic products with an anti-acne action, we propose a formulation of gels based on hydroxypropyl cellulose (HPC) loaded with two antimicrobial substances: erythromycin (ER) and magnolol (MG). The characteristics of these formulations were assessed from the rheological and pharmacotechnical points of view. The viscoelastic moduli of the HPC based systems increased in the presence of the drug substance. By introducing the magnolol, the system became thermosensitive and its behaviour changed from liquid-like at 20 °C to solid-like at 37 °C. In addition, changes in the flow curves were observed. The in vitro dissolution tests showed a greatly enhanced release of ER in the presence of MG. MG presented permeation properties superior to those of ER under ex vivo test conditions. The release of both ER and MG is based on diffusion, as revealed by the fitting to the Korsmeyer–Peppas model. The HPC has proved great compatibility with both ER and MG. These results recommend further clinical investigations of the HPC dermatocosmetics formulated and characterized in this study.

Keywords: hydroxypropyl cellulose, erythromycin, magnolol, acne, viscoelasticity, thermosensitive

INTRODUCTION

Acne is a disease of the pilosebaceous system manifested by the formation of comedones, inflammatory papules, pustules, nodules, cysts and scars. Most patients and even some of the health workers consider acne a condition specific to adolescence, which evolves towards self-healing, but recent WHO reports highlight the growing incidence of acne and classify it as a chronic skin disease. The pathogenesis of acne is defined by the microcomedone theory based on follicular hyperkeratinization, sebum overproduction and microbial contamination of the pilosebaceous follicle. The anaerobic bacterium Propionibacterium acnes is the main microorganism causing acne, but other pathogens, such as Pityrosporum ovale, Staphylococcus spp., Corynebacterium spp., Malassezia spp., have recently been identified in the lesions of this dermatosis. A crucial role in the topical treatment of acne is played by antibiotics, such as tetracycline, oxytetracycline, erythromycin and clindamycin, which act on the pathogens responsible for producing pyodermitis. Erythromycin is a macrolide antibiotic with good efficacy and tolerance in topical application. However, recent data show that the microorganisms have developed a resistance to erythromycin, particularly in the case of Propionibacterium acnes and Staphylococcus spp., so that the identification of novel therapeutic agents with anti-bacterial action becomes a medical priority.1,2 In order to develop some dermatocosmetic preparations with an anti-acne action, we propose a formulation of gels loaded...
with two antimicrobial substances: erythromycin and magnolol. Magnolol is a polyphenolic extract derived from the bark of *Magnolia officinalis*, which has an antimicrobial activity on some species of fungi and Gram-positive bacteria *Helicobacter pylori*, *Propionibacterium acnes*, *Staphylococcus aureus*, etc.\textsuperscript{3-5}

Pharmaceutical and cosmetic products present complex flow behaviour, they often combine both liquid-like and solid-like properties.\textsuperscript{6-10} The dominant viscoelastic behaviour depends on the ingredient selection, formulation preparation, pH and temperature.\textsuperscript{11-13} The rheological investigations can provide useful information for optimal formulation, storage and efficient use of such products.

The objective of this study was the preparation and the rheological and pharmaco-technical characterization of two gel formulations based on sodium hydroxypropyl cellulose. We included erythromycin as active substance in the first formula, and in the second formula we associated erythromycin and magnolol. The results of this study give us essential data on the compatibility of these compounds and on their rheological, release and permeation characteristics, promoting the use of the studied formulations as antimicrobial dermo-preparations.

**EXPERIMENTAL**

**Materials**

Hydroxypropyl cellulose (HPC) 150-4000 eP was purchased from Nisso Chemical Europe GmbH, Germany. Carbopol 940 (C 940), propylene glycol and triethanolamine (TEA) were acquired from MedChim, Bucharest. Erythromycin (ER) of 99.85% purity was obtained from Zhejiang Sanmen Hengkang Pharmaceutical Co. Ltd., China, and magnolol (MG) of 98% purity was received from Sinova Inc., USA. During gel preparation and quantitative analysis, we used purified water, double distilled water and other reagents coincident with the quality requirements of the Romanian Pharmacopoeia, 10th ed. *In vitro* dissolution tests were carried out on a synthetic Nylon membrane. *Ex vivo* tests were carried out on a rat skin flap, collected from the abdomen of Wistar rats.

**Methods**

**Preparation of gels**

HPC-based formulations were prepared by dispersing the polymer in a mixture of 60 g water and 3 g of propylene glycol under stirring at 500 rpm for 15 min, at 40-50 °C. Separately, TEA was dissolved in 5 mL of water by gradually adding it to the polymer dispersion under continuous stirring. In the last stage, we added water up to the mass provided. The sample thus obtained was allowed to rest at 5 °C for 12 hours in order to restructure and then the drug substances were dispersed in the concentrations shown in Table 1.

**Rheological measurements**

Rheological investigations were carried out at 20 °C and 37 °C by using an MCR 302 Anton-Paar rheometer equipped with a Peltier device for temperature control and plane-plane geometry (diameter of 50 mm, gap of 0.5 mm). Water evaporation was limited by using an anti-evaporation device, which created a saturated atmosphere near the sample.

**Table 1**

<table>
<thead>
<tr>
<th>Substances</th>
<th>Sample formulation</th>
<th>B1</th>
<th>B1–E2%</th>
<th>B1–E-MG3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC</td>
<td></td>
<td>3.5 g</td>
<td>3.5 g</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td></td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td></td>
<td>1 g</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td>-</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Magnolol</td>
<td></td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Purified water</td>
<td></td>
<td>up to 100 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amplitude sweep tests were carried out at constant frequency ($\omega = 1$ rad/s), in order to establish the linear viscoelastic range for each sample. Oscillatory frequency sweep experiments were performed in the frequency range from 0.1 rad/s to 100 rad/s (at small amplitude of sinusoidal shear strain, within the linear viscoelastic regime) and the components of the complex modulus ($G^*$) were determined: the elastic modulus ($G'$) as a measure of the reversibly stored deformation energy and the viscous modulus ($G''$) as a measure of the irreversibly dissipated energy during one cycle. Two dimensionless parameters can be associated with the degree of viscoelasticity of the sample: the phase shift ($\delta$) or the loss tangent ($\tan \delta = G''/G'$). Flow curves and yield stress were
also determined under continuous shear conditions at 20 °C and 37 °C.

**In vitro and ex vivo dissolution drug release study**

The *in vitro* dissolution test was performed on an Enhancer cell with a diameter of 2.5 cm, employing an SR 8 Plus Series device (AB & L. Jasco), according to the following protocol: dissolution medium: phosphate buffer pH 7.4, 100 mL; mass of sample: for each formulation studied, 0.5 g; synthetic Nylon membrane with a pore diameter Ø = 45 µm (Millipore, Merck Germany); temperature: 37 °C ± 0.2 °C; harvest interval: the test was carried out over a period of 12 hours, every 60 min a sample volume of 1 mL was drawn out and was replaced with fresh medium; speed: 100 rpm. The synthetic membrane was placed in the dissolution medium for 24 h before the *in vitro* test. The *ex vivo* dissolution test was performed under similar conditions, except that we used a biological membrane made of skin detached from the abdomen of female Wistar rats with a weight of 200-250 g. After collecting, the hair and adipose tissue were removed from the patch of skin, which was then immersed into the buffer system of pH 7.4 for 24 hours prior to testing.

**HPLC assay**

HPLC analyses were carried out on a Thermo Fisher Scientific Ultimate 3000 Liquid Chromatograph, equipped with a UV-Vis with Diode Array Detector. The methods are based on the use of a chromatography column type Teknokroma Tracer Excel 120 OSDB C18, 150 mm x 4.6 mm, particle size 5 µm. For the analysis of ER, the mobile phase consisted of a mixture of 0.001 M disodium phosphate solution: acetonitrile in the ratio 20:80; the volume of sample injected was 20 µL and the injection rate was 1 mL/min. The calibration curve was linear (regression coefficient, \( r^2 \), of 0.9997) in the concentration range 0.025 to 1.5 mg/mL. The ER spectrum was recorded at \( \lambda = 200 \) nm. In the MG determination method, the mobile phase consisted of a mixture of an aqueous solution of 0.1% trifluoroacetic acid and methanol in a ratio of 20:80 (v/v). A sample volume of 20 mL was also injected at a rate of 1 mL/min. The method was linear over the concentration range of 0.5-1 mg/mL (\( r^2 = 0.9985 \)) and the MG chromatogram was recorded at 290 nm wavelength.

**Determination of permeation coefficient for erythromycin and magnolol**

The permeability coefficient of active substances was calculated by the following equation:

\[
K_p = \frac{J}{C \times A}
\]

where: \( K_p \) – coefficient of permeability (cm/h); \( J \) – rate of drug substance permeation or stationary phase flow (µg/h); \( C \) – concentration in the donor compartment (µg/mL); \( A \) – contact surface area (cm²).

**Evaluation of release kinetics for erythromycin and magnolol**

The experimental data obtained from the ER release studies carried out on synthetic or biological membranes were analyzed by fitting to the Higuchi (Eq. (2)) and Korsmeyer–Peppas (Eq. (3)) models, according to the following relations:

\[
M = K_\mu t^{1/2}
\]

where: \( M \) – the amount of drug released at time \( t \); \( K_H \) – Higuchi release constant.

\[
\frac{M}{M_\infty} = \frac{t}{M_\infty} = K_p t^n
\]

where: \( M/M_\infty \) – the ratio between the amount of drug released at time \( t \); \( K_p \) – Peppas–Korsmeyer constant of release rate; \( n \) – diffusion coefficient.

Data fitting was performed by linear and nonlinear regression using Matlab 7.1. The data were presented as mean ± standard deviation and were considered statistically significant at \( p < 0.05 \).

**RESULTS AND DISCUSSION**

**Viscoelastic behaviour**

The oscillatory shear experiments were carried out within the linear viscoelastic regime, which was firstly established for each sample at 20 °C and 37 °C. According to Fig. 1, which shows for example the curves obtained at 37 °C in amplitude sweep tests for sample B1, the linear viscoelasticity is reached for \( 0.2% < \gamma < 30% \), when the viscoelastic moduli (\( G' \) and \( G'' \)) are independent of the strain amplitude (\( \gamma \)). Samples B1–E2% and B1–E-MG3% present an extended linear viscoelastic regime, suggesting that these samples are structured. In addition, a solid-like behaviour, typical to gels, is observed for sample B1–E-MG3%: \( G' > G'' \) and \( \tan \delta < 1 \). Based on the viscoelastic parameters obtained in the amplitude sweep tests, for the present study a strain of 1% was selected for all samples.

Fig. 2 presents the dependences of the viscoelastic parameters (\( G' \), \( G'' \), \( \tan \delta \)) obtained for samples B1, B1–E2% and B1–E-MG3% in frequency sweep tests at 20 °C and 37 °C. According to these plots, the samples present liquid-like behaviour, excepting sample B1–E-MG3%, which shows a solid-like behaviour at 37 °C (better evidenced in Fig. 3). From the crossover frequency (\( \omega_c \)) at which \( G' = G'' \), the longest relaxation time, \( \theta_l \), was determined as \( 1/\omega_c \). The values of \( \theta_l \) are given in Table 2.

For sample B1–E-MG3%, a liquid-like behaviour can be remarked at 20 °C, whereas at 37 °C the viscoelastic moduli shows a solid-like behaviour: \( G' \) becomes higher than \( G'' \) and \( \tan \delta < 1 \) (Fig. 3).
3). The gel structure formed at 37 °C can be due to the bioactive compound (magnolol), which interacts with HPC and forms a network structure.

**Flow curves**

The behaviour of the samples in continuous shear flow depends on their composition and temperature (Fig. 4). All flow curves present a Newtonian domain at low shear rates, followed by a pseudoplastic behaviour under high shear rate conditions. The values of Newtonian viscosity are given in Table 2. Sample B1 shows a Newtonian behaviour up to shear rates, \( \dot{\gamma} \), of approx. 1 s\(^{-1}\) and for the non-Newtonian region \( \eta \sim \dot{\gamma}^{-0.4} \). The shear viscosity of sample B1–E2% increases considerably, as compared with sample B1 and is not sensitive to temperature increases. The non-Newtonian region starts at lower shear rates (approx. 0.2 s\(^{-1}\)) and the viscosity scales as \( \dot{\gamma}^{-0.66} \). The viscosity increases in the presence of magnolol, especially at 37 °C, when the Newtonian viscosity is of 1120 Pa·s. The Newtonian plateau for sample B1–E-MG3% is registered below 0.03 s\(^{-1}\) and in the non-Newtonian domain \( \eta \sim \dot{\gamma}^{-0.7} \).

For pharmaceutical products and cosmetics, the yield stress, defined as minimum shear stress required to determine the flow of the sample, is an important parameter in their storage and use. The values of yield stress were found to be independent of the formulation, but they depended on temperature: at 20 °C the yield stress was approx. 20 Pa, whereas at 37 °C its value was around 37 Pa.
Hydroxypropyl cellulose

Figure 3: Evolution of viscoelastic parameters for sample B1–E-MG3% in frequency sweep tests at 20 °C and 37 °C

Table 2
Rheological characteristics of the samples determined at 20 °C and 37 °C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relaxation time, θ (s)</th>
<th>Newtonian viscosity, η₀ (Pa·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>B1</td>
<td>0.039</td>
<td>0.01</td>
</tr>
<tr>
<td>B1–E2%</td>
<td>0.1585</td>
<td>0.1</td>
</tr>
<tr>
<td>B1–E-MG3%</td>
<td>0.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 4: Flow curves for the investigated samples obtained at 20 °C and 37 °C

In vitro and ex vivo drug delivery of HPC based formulations

The results of the in vitro dissolution tests showed a greatly enhanced release of ER in the presence of MG in the B1–E-MG3% gel, as compared with sample B1–E2% (Fig. 5). After the first hour of the test, the amount of ER released from the B1–E-MG3% gel was nearly 7 times higher than the amount of ER released from sample B1–E2% (25.34 µg/cm² versus 3.72 µg/cm²). This difference was recorded for the whole duration of the dissolution test and in the end the cumulative amount of ER released from sample B1–E-MG3% was of 376.29 µg/cm², compared to 112.61 µg/cm² ER released from formula B1–E2%. These results are correlated with the permeation coefficients of ER through the synthetic membrane, which showed a flow of ER from the B1–E-MG3% gel of 34.558 µg/cm²/h (Table 3).

The results of the ex vivo test were surprising, because the cumulative amount of ER released from formula B1–E-MG3% presented a very close value to the cumulative amount of ER released from sample B1–E2% (58.84 µg/cm² versus 57.47 µg/cm²) (Fig. 6). These results are consistent with literature data showing that under in vitro conditions, ER permeability may be
influenced by certain formulation factors (presence of hydrotropic substances, permeation agents, etc.). However, during \textit{in vivo} or \textit{ex vivo} tests ER does not preserve its release rate. \cite{19,20}

![Figure 5: Cumulative release of ER and MG through a Nylon membrane](image1)

![Figure 6: Cumulative release of ER and MG through a biological membrane](image2)

Table 3

<table>
<thead>
<tr>
<th>Gel formula</th>
<th>( J_{50} ) (µg/cm(^2)/h)</th>
<th>( K_P \times 10^{-6} ) (cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic membrane (\textit{in vitro})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1–E2%</td>
<td>10.9329 ± 0.4619</td>
<td>1231.18</td>
</tr>
<tr>
<td>B1–E-MG3%</td>
<td>34.5589 ± 1.5705</td>
<td>3822.43</td>
</tr>
<tr>
<td>Biological membrane (\textit{ex vivo})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1–E2%</td>
<td>5.5073 ± 0.8777</td>
<td>620.78</td>
</tr>
<tr>
<td>B1–E-MG3%</td>
<td>6.2228 ± 1.2436</td>
<td>437.47</td>
</tr>
</tbody>
</table>

MG permeation parameters

<table>
<thead>
<tr>
<th>Gel formula</th>
<th>( J_{50} ) (µg/cm(^2)/h)</th>
<th>( K_P \times 10^{-6} ) (cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic membrane (\textit{in vitro})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1–E-MG3%</td>
<td>8.9053 ± 4.1192</td>
<td>89.05</td>
</tr>
<tr>
<td>Biological membrane (\textit{ex vivo})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1–E-MG3%</td>
<td>14.6179 ± 7.4862</td>
<td>108.3884</td>
</tr>
</tbody>
</table>

Table 4

Parameters of release kinetics of ER from HPC-based formulations

<table>
<thead>
<tr>
<th>Substance</th>
<th>Sample</th>
<th>( K_H ) (h(^{-0.5}))</th>
<th>( R^2 )</th>
<th>( K_P ) (h(^n))</th>
<th>( n )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic membrane (\textit{in vitro})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>B1–E2%</td>
<td>21.5391</td>
<td>0.9434</td>
<td>10.4687</td>
<td>0.85</td>
<td>0.9962</td>
</tr>
<tr>
<td>ER</td>
<td>B1–E-MG3%</td>
<td>16.4834</td>
<td>0.8902</td>
<td>6.5517</td>
<td>0.95</td>
<td>0.9937</td>
</tr>
<tr>
<td>MG</td>
<td>B1–E-MG3%</td>
<td>4.3375</td>
<td>0.8602</td>
<td>1.5953</td>
<td>0.99</td>
<td>0.9804</td>
</tr>
<tr>
<td>Biological membrane (\textit{ex vivo})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>B1–E2%</td>
<td>19.1707</td>
<td>0.9512</td>
<td>9.2947</td>
<td>0.85</td>
<td>0.9947</td>
</tr>
<tr>
<td>ER</td>
<td>B1–E-MG3%</td>
<td>2.3403</td>
<td>0.8379</td>
<td>0.8628</td>
<td>0.99</td>
<td>0.9751</td>
</tr>
<tr>
<td>MG</td>
<td>B1–E-MG3%</td>
<td>5.2642</td>
<td>0.7865</td>
<td>1.9676</td>
<td>0.99</td>
<td>0.9478</td>
</tr>
</tbody>
</table>

MG presented permeation properties superior to ER under \textit{ex vivo} test conditions (Fig. 6), which generated a cumulative amount of 128.75 µg/cm\(^2\) MG released after 12 hours.

The analysis of the release kinetics of the drug substances in the gels investigated revealed a fitting to the Korsmeyer–Peppas model, which confirms the fact that both ER and MG are
released through a diffusion process from the polysaccharides based gels (Table 4). \(^{21-24}\)

**CONCLUSION**

The *in vitro* dissolution tests showed a greatly enhanced release of ER in the presence of MG. The permeation properties of ER formulated with MG, recorded during the *in vitro* tests, were not found in the *ex vivo* assay. MG presented permeation properties superior to those of ER under *ex vivo* test conditions. The release of both ER and MG is based on diffusion, as concluded from the fitting to the Korsmeyer–Peppas model. The viscoelastic moduli of HPC-based formulations increase in the presence of a drug substance. Our results highlight that MG induces a temperature-responsive behaviour, the sample forms a strong structured gel at physiological temperature (37 °C), influencing the viscoelastic and drug delivery behaviour.

The HPC has proved great compatibility with both ER and MG. These results recommend further clinical investigations of the HPC dermatocosmetics formulated and characterized in this study.

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