RHEOLOGICAL CHARACTERISATION AND EFFECT OF ABIOTIC FACTORS ON THE ANTIMICROBIAL EFFICACY OF CHITOSAN-BASED HYDROGELS CONTAINING ALPHA-HYDROXY ACIDS

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ABSTRACT
The aim of this study was to investigate the rheological properties and antibacterial efficacy of chitosan/alpha-hydroxy acids (lactic acid and glycolic acid) and cellulose polymers, both in hydrogels, in order to produce a formulation with improved activity against Propionibacterium acnes and Staphylococcus aureus, which can potentially be used in the treatment of acne. The rheological characterisation of the hydrogels was examined using continuous shear and viscoelastic creep. The antibacterial activities of formulations were performed by the well diffusion and broth microdilution. The hydrogels formulated with only chitosan showed pseudoplastic behavior while the chitosan hydrogels with cellulose polymers presented viscoelastic properties. The antibacterial activity was proportional to AHA and chitosan concentration. It was enhanced at low pH values and with high molecular weight chitosan and did not change with the incorporation of two cellulose polymers. The antibacterial mechanism of chitosan has currently been hypothesized as being related to surface interference. The results show that chitosan - based hydrogels containing AHA and cellulose polymers are viscoelastic,
indicating good applicability onto the skin, and they present bacterial activity under various experimental conditions.

KEY WORDS: hydrogels, chitosan, alpha-hydroxy acids, rheology, antibacterial activity.

1. INTRODUCTION

Acne is a skin pathology that causes changes in the pilosebaceous follicles. This disorder is caused by the abnormal desquamation of the follicular epithelium, which results in the obstruction of the pilosebaceous canal, leading to the formation of comedones, that can become inflamed due to the overgrowth of Propionibacterium acnes[1,2].

Topical treatment act at some of the four main pathogenetic factors responsible for the development of acne, i.e. hyperseborrhea, hyperkeratosis, microbial colonization and inflammation. The agents currently available influence at least one of these factors but often have additional properties. For example, those which act in a comedolytic and anticomедogenic manner are the retinoids tretinoin, isotretinoin, adapalene and tazarotene. On the other hand, antibiotics, such as erythromycin and clindamycin may also induce bacterial resistance. Unfortunately, bacterial resistances are beginning to emerge as a significant problem, especially for erythromycin. Benzoyl peroxide as an antiseptic reduces the number of skin surface microorganisms and the treatment of acne may take several months until improvement is noticed[3].

The development of new topical anti-acne therapies reflects the need for medications that address the requirements and concerns of an increasingly mature and demanding acne affected patient population[4].

Chitosan is a polysaccharide naturally available from crustaceans which has a potential antibacterial activity against Propionibacterium acne and Staphylococcus aureus, the contributing pathogenic factors in the etiology of the acne lesions. It can breakdown the cytoplasmic membrane barrier and chelate trace metal cations which are necessary to microorganism’s metabolism. In the present study it has been used as a delivery system for anti-acne drugs as well as presenting but in antimicrobial activity[5-8]. Some of other topical agents currently used include several alpha-hydroxy acids (AHAs). These appear to exert their effect on acne by influencing the process of keratinization and/or the thickness of the stratum corneum[9-10].
The aim of this study was to develop and optimize a new chitosan /alpha-hydroxy acids hydrogels in order to improve topical treatment of acne, according to FDA recommendations. For this purpose, *in vitro* activity against *Propionibacterium acnes* and *Staphylococcus aureus* was assessed and full rheological characterisation was performed.

2. MATERIALS AND METHODS

2.1 Materials

Lactic acid and glycolic acid were purchased from Fluka Chemika. The three types of chitosan used (>75% degree of deacetylation), low molecular weight –LMW (50,000-190,000 Da), medium molecular weight-MMW (190,000-310,000 Da) and high molecular weight-HMW (~310,000 to >375,000 Da), were from crab origin and obtained from Aldrich. Hydroxypropylmethylcellulose (HPMC) and Natrosol HX 250 hydroxyethylcellulose (HEC) were supplied by Hercules-Aqualon.

2.2 Production of hydrogels

The hydrogels were prepared in deionized water containing lactic acid (2.5 to 5% w/v), glycolic acid (2.5 to 5% w/v), chitosan (0.5, 1 and 1.5% w/v) and 1.5% of hydroxypropylmethylcellulose (HPMC) or hydroxyethylcellulose (HEC). The composition of each gel is as follows (Table 3): Hydrogel A: 0.5% MMW chitosan and 10% AHA; Hydrogel B: 1% MMW Chitosan and 10% AHA; Hydrogel C: 1.5% MMW chitosan and 10% AHA and Hydrogel D: 1.5% MMW Chitosan and 5% AHA. The cellulose chitosan hydrogels were composed of: 1.5% HPMC, 1.5% chitosan (HPMC – HMW chitosan hydrogel) and 10% AHA and 1.5% HEC 1.5% HMW chitosan and 10% AHA ((HEC – chitosan hydrogel).

Briefly, the polymers (chitosan and cellulose) were dispersed in an aqueous solution of acids. The resulting mixture was stirred without heating, at room temperature for 60 min until chitosan was dissolved and HPMC and HEC were hydrated. A hydrogel with no cellulose polymers was used in several assays. Aqueous solutions of lactic acid and glycolic acid 5.0 and 10.0% were also prepared and used as controls.

2.3 Hydrogels Characterisation

Organoleptic characteristics (color, odor, fluidity, aspect and consistency) were evaluated for all the prepared formulations. For pH assessment: a potentiometric method (Methrom, Herisau, pH meter E 516) was used on native hydrogels. Results were expressed as the average of three determinations (mean ± SD: n=3)
2.4 Rheological Characterisation
The rheological characteristics of the hydrogels were examined at high shear rates using continuous shear techniques (Brookfielfd viscometer (Model DVII, small sample adapter, spindle 27, at 25°C) and viscoelastic region techniques (Haake model Rheostress RS-1, plate/plate, at 25°C). Rheograms and creep curves of the different formulations were then determined. Results were expressed as the average of three determinations (mean ± SD: n=3).

2.5 Antibacterial activity
The antibacterial activities of hydrogel formulations were determined against the reference strains *Propionibacterium acnes* ATCC 6919 (origin from Facial acne, London, UK, 1920) and *Staphylococcus aureus* ATCC 6538. Two methodologies were performed: the well diffusion and broth microdilution methods.

The well diffusion method was used for preliminary studies and allowed to evaluate the different modifications in hydrogels on the antibacterial activity, namely the effect of AHAs (glycolic and lactic acids) and chitosan concentrations, the effect of molecular weight of chitosan and the effect of pH. Fifteen μL of each sample (n=5) and controls were applied in a 6 mm diameter well, after inoculation of reference strains on surface of the culture media, Brain Heart Infusion agar (Oxoid, pH=7.0). The plates were incubated at 37°C during 24-48 h. Inhibitory zones were measured after the incubation.

Two cellulose polymers, HPMC and HEC, were added to increase the viscosity of the hydrogels that previously showed antibacterial activity. To evaluate the effect of viscosity in antibacterial efficacy of chitosan/alpha-hydroxy acids hydrogels against *P. acnes* and *S. aureus* were performed by the well diffusion method (previously described) and the Minimum Inhibitory Concentration (MIC) that was determined by broth microdilution with Mueller-Hinton broth, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (n=3). The colony forming units (CFU) were counted with an Absorvance Microplate Reader set to 630 nm (ELX808™ - BioteK) in a concentration ranging from 100 mg/ml to 0.000097 mg/ml. Erythromycin solution (1mg/ml) and clindamycin solution (1mg/ml) were obtained from Aldrich, Germany and Cipan, Portugal, respectively and were used as controls. Aqueous solutions of lactic acid and glycolic acid 5% and 10% were also prepared and studied as mentioned above.
2.5.1 Effect of AHA and chitosan concentration on the antibacterial activity of hydrogels:
The hydrogels were prepared with two different amounts of lactic and glycolic acids, 5 and 10%, and with 0.5, 1 and 1.5% of MMW chitosan.

2.5.2 Effect of the molecular weight of chitosan on the antibacterial activity of hydrogels:
Hydrogel formulations were prepared with 1.5% of the three different molecular weight chitosans-LMW, MMW and HMW. Three hydrogels were prepared using 5% of glycolic and 5% of lactic acid.

2.5.3 Effect of pH on the antibacterial activity of hydrogels:
Hydrogel formulations were prepared with 1.5% of HMW chitosan in water with 10% of lactic and glycolic acids. The pH was set to 3.5, 5 and 7 with 0.1M of sodium hydroxide solution.

2.5.4 Effect of different cellulose polymers on the antibacterial activity of hydrogels:
The hydrogels were prepared in deionized water containing lactic acid (5%), glycolic acid (5%), chitosan HMW (1.5%) and HEC or HPMC (1.5%). A hydrogel with no cellulose polymers was used as a control. The pH was set at 3.5 for all the formulations using 0.1M of sodium hydroxide solution.

3. RESULTS AND DISCUSSION
3.1 Characterisation of hydrogels
All the gels appeared transparent, colorless and homogeneous. The pH values obtained for the different native chitosan hydrogels were 2 and 2.5 (± 0.2) for gels containing 10% and 5% of AHA respectively. These pH values were suitable for chitosan stability (Table 1).

Table 1. Hydrogels: pH and apparent viscosity values calculated at 24.47 s⁻¹

<table>
<thead>
<tr>
<th></th>
<th>Hydrogel A</th>
<th>Hydrogel B</th>
<th>Hydrogel C</th>
<th>Hydrogel D</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH values</td>
<td>2.15</td>
<td>2.17</td>
<td>2.23</td>
<td>2.58</td>
</tr>
<tr>
<td>Apparent Viscosity (Pa.s)</td>
<td>3.05</td>
<td>15.3</td>
<td>230</td>
<td>71.4</td>
</tr>
</tbody>
</table>

3.2 Rheological Characterisation
The viscosity of chitosan hydrogel increased with an increasing amount of the acids (lactic acid and glycolic acid) and chitosan. Apparent viscosity was higher (230 Pa.s) for the
formulation containing 1.5% chitosan and 10% of AHAs – Hydrogel C - and was lower (3.05 Pa.s) for the Hydrogel A with 0.5% chitosan and 10% of lactic and glycolic acids (Table 1). Representative plots are shown in Figure 1.

![Figure 1: Rheograms of hydrogels A (-), B (▲), C (■) and D (♦).](image)

The hydrogels formulated with only chitosan showed pseudoplastic behavior, indicating good applicability onto the skin, but not viscous enough, while the chitosan hydrogels with cellulose polymers showed viscoelastic properties.

Analysis of the data (Table 2) indicated that each hydrogel was a viscoelastic liquid that can be represented by a Maxwell unit in series with one Voigt unit model.

<table>
<thead>
<tr>
<th>Cellulose Hydrogel</th>
<th>η₀ (Pa.s)</th>
<th>J₀ (1/Pa)</th>
<th>J₁ (1/Pa)</th>
<th>τ₁ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC-chitosan</td>
<td>998.7</td>
<td>0.0037</td>
<td>0.1587</td>
<td>112.3</td>
</tr>
<tr>
<td>HEC-chitosan</td>
<td>733.1</td>
<td>0.0012</td>
<td>0.1084</td>
<td>69.17</td>
</tr>
</tbody>
</table>

To formulate a hydrogel with chitosan the configuration and structure of the polymer had to be modified. Chitosan is a cationic polysaccharide polymer, naturally available and a product/derivative obtained by N-deacetylation of chitin in the presence of alkali, which is the structural element in the cuticles of insects and exoskeleton of crustaceans[^11^-^13]. It is not water soluble unless the pH is below 7.0. Lactic and glycolic acid are weak acids, and they were used to be sequentially grafted onto the chitosan chains via the arrangement between the...
carboxylic groups and the amino and/or hydroxyl groups. The viscosity of the gels is caused by gelating agents, which consist mainly of polymers that build up a three dimensional network [11, 14].

The percentage of alpha-hydroxy acids and chitosan influenced the viscosity. The flow curve for the Hydrogel C was complex and different from the others hydrogels. The presence of a hysteresis loop implies that the shearing cycle itself might have caused structural build-up (rather than destruction, as observed in the other hydrogels), and the high apparent viscosity value indicated that this hydrogel was thicker and more resistant to structure breakdown than the others.

As expected, the viscosity of all the hydrogels increased with an increasing concentration of chitosan (Hydrogel A< Hydrogel B< Hydrogel C and Hydrogel D). Nevertheless, all formulations presented a pseudoplastic behavior (as shear rate decreases the material will gradually recover the original internal structure before shear) indicating good applicability in the skin.

The HPMC - chitosan and the HEC - chitosan hydrogels were viscoelastic when they were tested in creep mode exhibiting the same general shape. Figure 2 shows typical reproducibility data for the two cellulose hydrogels.

![Figure 2. Creep curves of different measurements of HEC Hydrogel (A) and HPMC Hydrogel (B) to illustrate reproducibility. (Creep time: 300 seconds)](image)

Analysis of the data (Table 2) indicated that each hydrogel was a viscoelastic liquid that could be represented by a Maxwell unit in series with a number of Voigt units model [15]. The rheological equation of state for this model is the following:
\[
J(t) = J_0 + \sum_{i=1}^{n} J_i \left(1 - e^{-t/\tau_i}\right) + t/\eta_0
\]

where \( J \) is the ratio of shear strain to shear stress, \( J(t) \) is the total creep compliance at time \( t \), \( J_0 \), the residual shear compliance, \( J_i \) the compliance of the Voigt unit, \( t \) the retardation time of the Voigt unit and \( \eta_0 \) the residual shear viscosity.

The initial elastic component of the creep curve is associated with the residual (uncoupled) Hookean spring, which represents bonds being stretched elastically and simulates the elasticity of the gel network structure. Table 2 shows that the instantaneous compliances \( J_0 \) (which are the reciprocal of the moduli of elasticity) of the cellulose hydrogels are the same for both, implying that they possessed a delicate network. The curved portion of the creep curve is the region where the flow is viscoelastic, and the model representation consisted of Voigt units connected in series. They represent that part of the structure in the gel network in which secondary bonds were breaking and reforming during the test. In theory, the number of Voigt units may be infinite; but in the present work one Voigt unit was required to reproduce the creep curves. The retardation time \( \tau \) represents the time during which bonds break and reform and as all bonds did not do this at the same rate (the weaker bonds break at smaller values of \( t \) than the stronger ones) a spectrum of retardation times exists.

When the stress had been applied for sufficient time to ensure that all the Voigt units were essentially fully further extension, the nature of the flow was viscous. This strain was represented by the residual dashpot. In this region the cellulose hydrogels were flowing as dispersions and the viscosities were high. However, the residual Newtonian viscosities of HPMC chitosan hydrogel and the HEC chitosan hydrogel were again similar\(^{16,17}\).

### 3.3 Effect of AHA and chitosan concentration on the antibacterial activity of hydrogels

It was observed that with an increasing MMW chitosan concentration (Hydrogel A-0.5%; Hydrogel B- 1% and Hydrogel C- 1.5%), the diameter of the inhibition zones was higher: from 13 mm to 21 mm for \( P. \) acnes and 11.5 mm to 17 mm for \( S. \) aureus. The results are shown on Table 3. Despite chitosan concentration (Hydrogel C and D – 1.5%), hydrogels with different percentage of alpha-hydroxy acids (Hydrogel C-10% and Hydrogel D-5%) showed a decrease on the bacterial activity with a lower concentration of AHA: from 21 mm to 14 mm for \( P. \) acnes and 12 mm to 17 mm for \( S. \) aureus. The aqueous solutions of lactic
and glycolic acids showed no antibacterial activity. As expected, the inhibition zones were larger for the antibiotics (erythromycin and clindamycin) than for the hydrogels formulations.

Table 3. Hydrogel composition and antibacterial activity: inhibition zones diameter (n=5, ± SD)

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Chit%</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHA%</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>P. acnes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>0</td>
<td>0</td>
<td>13 (± 0.1)</td>
<td>15.5 (± 0.1)</td>
<td>21 (± 0.1)</td>
<td>14 (± 0.1)</td>
<td>40 (± 0.1)</td>
<td>35 (± 0.1)</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>0</td>
<td>0</td>
<td>11.5 (± 0.1)</td>
<td>14 (± 0.1)</td>
<td>17 (± 0.1)</td>
<td>12 (± 0.1)</td>
<td>30 (± 0.1)</td>
<td>26 (± 0.1)</td>
</tr>
</tbody>
</table>

3.4 Effect of the molecular weight of chitosan on the antibacterial activity of hydrogels

The results so far obtained (Table 4) show that the alpha-hydroxy acids (AHA) - chitosan formulations with high molecular weight had a better inhibition in the growth of *P. acnes* and *S. aureus in vitro* thus suggesting a potential for their use in the acne therapy.

Table 4. Antibacterial activity of hydrogels with different molecular weight chitosans (n=5, ± SD)

<table>
<thead>
<tr>
<th></th>
<th>LMW Chitosan hydrogel</th>
<th>MMW Chitosan hydrogel</th>
<th>HMW Chitosan hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. acnes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>20.5(± 0.1)</td>
<td>24.5(± 0.1)</td>
<td>26.5(± 0.1)</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>18.5(± 0.1)</td>
<td>21.0(± 0.1)</td>
<td>22.0(± 0.1)</td>
</tr>
</tbody>
</table>

3.5 Effect of pH on the antibacterial activity of hydrogels

The effects of pH on the inhibition of *P. Acnes* and *S. aureus* are shown on Table 5. Aqueous solutions of alpha-hydroxy acids with low pH, had no inhibitory effects on the growth of bacterial strains. In addition no hydrogel was formed at pH 7. At pH 5 no inhibition zones
were observed and at pH 3.5 the diameter of the inhibition zones was similar to those previously obtained.

Table 5. Antibacterial activity of hydrogel with 1.5% HMW chitosan, 10%AHA with different pHs (n=5, ± SD)

<table>
<thead>
<tr>
<th></th>
<th>pH 3.5</th>
<th>pH 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. acnes</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>21.5 (± 0.1)</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>18.0 (± 0.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

3.6 Effect of different cellulose polymers on the antibacterial activity of hydrogels

The hydrogels associated to cellulose polymers maintained the antibacterial activity. The results obtained by the well diffusion method showed similar inhibition zones when compared with control, as well the MIC values were also similar. All hydrogels had similar MIC values for *S. aureus* and for *P. acnes* in a concentration ranging from 100 mg/ml to 0.000097 mg/ml (Table 6).

Table 6. Effect of cellulose polymers in antibacterial activity of hydrogel with 1.5% HMW chitosan, 10% AHA, pH= 3.5 (n=3, ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HPMC-Chitosan hydrogel</th>
<th>HEC-Chitosan hydrogel</th>
<th>Erythromycin solution</th>
<th>Clindamicin solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. acnes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>21 (± 0.1)</td>
<td>20(± 0.1)</td>
<td>20.5(± 0.1)</td>
<td>35(± 0.1)</td>
<td>29(± 0.1)</td>
</tr>
<tr>
<td>MIC (mg/ml)</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>0.0048</td>
<td>0.000097</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition zones (mm)</td>
<td>18(± 0.1)</td>
<td>17.5(± 0.1)</td>
<td>17(± 0.1)</td>
<td>28(± 0.1)</td>
<td>25(± 0.1)</td>
</tr>
<tr>
<td>MIC (mg/ml)</td>
<td>0.625</td>
<td>0.625-0.781</td>
<td>0.625-0.781</td>
<td>0.0048</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

Chitosan needs to be dissolved in an acid solution in order to activate its antibacterial activity. The results obtained confirm the literature data [18]. The results suggest that increasing the chitosan and AHA concentration as well as the molecular weight of chitosan the antibacterial activity is higher. These are probably due to an increase of the polymerizable chain and that
increases the antibacterial activity. Chitosan contains abundant amino groups, through which both polymerizable and water-soluble groups can be conveniently introduced. Lactic and glycolic acid react with amino groups. The chains of chitosan become water soluble. However, the pH of these formulations (pH=2.0 ± 0.2) are not in accordance with FDA guidance for industry. In order to develop a formulation with improved activity and with pH values advised by the FDA, was investigate the influence of the pH on the antibacterial efficacy of chitosan/alpha-hydroxy acids hydrogels.

In previous studies chitosan hydrogel exhibited antimicrobial activity for the treatment of P.acnes at very low pH. It was shown that the antibacterial activity of chitosan is affected by the pH value. In the literature the highest efficacy of chitosan against S. aureus was observed at pH 5.3 from a range 5.0 to 5.5. These results are in contrast to the data obtained with the strains used in this work. The highest inhibition was at pH 3.5. Since the pK_a of chitosan is about 6, the amino groups on the chitosan carry positive charges when the pH is below 6. The inhibiting mechanism of this activity is probably due to the interaction between polycationic chitosan and electronegative residues at the cell surface, changing the cell permeability and causing the leakage of intracellular electrolytes and proteins.

Considering the FDA guidance for industry, which states: "Based on the available information included in this report, the CIR Expert Panel concludes that Glycolic and Lactic Acid, their common salts and their simple esters, are safe for use in cosmetic products at concentrations ≤10%, at final formulation pH ≥3.5, when formulated to avoid increasing sun sensitivity or when directions for use include the daily use of sun protection", it can be concluded that the hydrogel formulated with 1.5% HMW chitosan, 10%AHA and pH ≥ 3.5 can be effective in vivo against P. acnes and S. aureus and is in accordance with FDA guidance. Nevertheless, these products were not viscous enough to allow a suitable application onto the skin.

In order to optimize the viscosity of the hydrogels that previously showed antibacterial activity, the effect of two cellulose polymers, hydroxypropylmethylcellulose (HPMC) and hydroxyethylcellulose (HEC) on the antibacterial efficacy of chitosan AHA hydrogels was assessed. The incorporation of these two cellulose polymers did not change the hydrogels activity against P. acnes and S. aureus.
The results so far obtained suggest that chitosan alpha-hydroxy acids hydrogels with HMW chitosan and cellulose polymers (either HPMC or HEC) inhibited the growth of *Propionibacterium acnes* and *Staphylococcus aureus in vitro* which is due to, not only chitosan itself, but also lactic and glycolic acids, or to a synergistic effect. They are viscoelastic, indicating good applicability onto the skin. These data are in accordance with the FDA guidance (pH ≥ 3.5 and 10% AHA) and can contribute to the development of a pharmaceutical formulation for the treatment of acne.

5. CONCLUSIONS

Acne is a skin disease that causes changes in the pilosebaceous follicles which can become inflamed because of the overgrowth of *Propionibacterium acnes* and *Staphylococcus aureus*. Bacterial resistances are a significant problem. The development of new topical anti-acne therapies reflects the need for medications that address the requirements and concerns of increasingly mature and demanding acne suffering patient population. Chitosan and chitine are natural biopolymers that are the second-most abundant biopolysaccharides after cellulose and have potential applications in medicine as a result of their biocompatibility and useful biological reactivity. On the other hand, the alpha hydroxy acids (AHA) are also used in cosmetic and dermatologic products to induce exfoliation of the outermost stratum corneum. The viscosity of all the hydrogels increased with an increasing concentration of chitosan. All formulations presented a pseudoplastic behavior. Nevertheless, these products were not viscous enough to allow a suitable application onto the skin. The incorporation of cellulose polymers in chitosan hydrogels (HPMC - chitosan and the HEC - chitosan hydrogels) were viscoelastic liquid that can be represented by a Maxwell unit in serie with a Voigt units model.

The results so far obtained suggest that chitosan alpha-hydroxy acids hydrogels with HMW chitosan and cellulose polymers (either HPMC or HEC) inhibited the growth of *Propionibacterium acnes* and *Staphylococcus aureus in vitro* which is due to, not only chitosan itself, but also lactic and glycolic acids, or to a synergistic effect. These data are in accordance with the FDA guidance (pH ≥ 3.5 and 10% AHA) and can contribute to the development of a pharmaceutical formulation for the treatment of acne.

6. ACKNOWLEDGMENTS

This work was supported by *Associação para o Desenvolvimento do Ensino e Investigação*
da Microbiologia – ADEIM (Portugal), and FINEP (Financiadora de Estudos e Projetos, Brazil).

7. REFERENCES


