FORMULATION AND CHARACTERIZATION OF POLOXAMER 407®: THERMOREVERSIBLE GEL CONTAINING POLYMERIC MICROPARTICLES AND HYALURONIC ACID

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The influence of the composition and preparation method on the sol–gel transition temperature ($T_{sol-gel}$) and rheological response of poloxamer-based formulations was determined. Manual and more complex mechanical stirring were found to provide similar results. In addition, a linear dependence of $T_{sol-gel}$ on the poloxamer content was observed in the range of concentrations analyzed, and a Poloxamer 407® concentration of 18% was selected. The addition of hyaluronic acid did not lead to significant changes in the $T_{sol-gel}$ values. In contrast, the addition of microparticles caused a reduction in $T_{sol-gel}$ without a significant reduction in gel strength, and pseudoplastic characteristics were observed, indicating that a thermoreversible gel was obtained with a rheology suitable for application in the treatment of burn wounds.

Keywords: thermoreversible gel; rheology; burn wounds.

INTRODUCTION

Burns are very serious traumas. In addition to the physical problems related to skin damage (increase in fluid loss, infection, hypothermia, scarring, and compromised immunity) that can cause extreme pain and death, a large burn will leave the patient with visible physical scars and invisible psychological issues.[1]

Among the many different medicines used to treat burn wounds, some natural products, such as *Aloe vera* (*Aloe vera* Linn, synonym: *Aloe vera barbadensis* Mill.), vitamin E, hyaluronic acid, and chitosan, have been gaining increasing interest over the past few years. *Aloe vera* gel extracts have anti-inflammatory activity and promote increased levels of hyaluronic acid and dermatan sulphate in granulation tissue,[2] providing wound healing properties that have been proven to lead to faster healing of burns and the reestablishment of the vascularity of burn tissues.[3] Consequently, burn wound healing is one of the major applications of *Aloe vera* gel in many countries.[4] Vitamin E, an intermediary in arachidonic acid and prostaglandin metabolism, is also important in the treatment of inflammatory processes, including burns, due to its antioxidant capability.[5,6] Hyaluronic acid is a non-sulphated glycosaminoglycan (GAG) that is widely distributed in body tissues.[7] This acid is an extracellular matrix component of tissue that forms a pericellular coat on the surfaces of cells.[8] Hyaluronic acid has been receiving increasing attention as a material for tissue engineering because it is believed to have a supportive effect on progenitor cell development, thus facilitating tissue repair.[9] Chitosan is a natural linear biopolyaminosaccharide obtained through the alkaline deacetylation of chitin, which is the second most abundant polysaccharide after cellulose. Properties such as mucoadhesion, bioadhesion, biodegradability, low toxicity, good biocompatibility, and the ability to form films make it suitable for use in biomedicine (for the immobilization of enzymes in living cells) and pharmaceutical formulations.[10]

A potential vector for the controlled release of drugs such as those mentioned above is thermosensitive hydrogels, which undergo thermoreversible gelation, behaving as a solution at low temperatures and as a gel above a specific temperature known as the solid–gel transition temperature ($T_{sol-gel}$). These hydrogels have been used for many different pharmaceutical applications, including parenteral, ophthalmic, rectal, and topical formulations.[11,12]

Poloxamers, notably Poloxamer 407®, are substances that have recently received remarkable attention in the field of thermosensitive hydrogels.[13] Poloxamer 407® is an amphiphilic synthetic copolymer consisting of a hydrophobic poly(oxypropylene) (POP) block between two hydrophilic poly(oxyethylene) (POE) blocks[14-17] and has a molecular weight of approximately 12,600 Da (70% POE).[18] Due to their amphiphilic nature, poloxamer molecules can readily self-assemble to form micelles depending on the concentration and temperature. Dehydration of the hydrophobic POP blocks combined with hydration of the POE blocks leads to the formation of spherical micelles, and subsequent packing of the micellar structure results in a 3D cubic lattice that constitutes the main structure of poloxamer hydrogels.[19-21] These hydrogels are characterized by the ability to carry a significant amount of a drug. They are also biodegradable, non-toxic, and stable, and therefore are suitable for use as controlled-release agents.[18]

Recently, various hydrophilic polymers, for instance water-soluble cellulose derivatives, polyacrylic acid,[22] and chitosan,[23] have been incorporated into poloxamer gels as auxiliary agents for controlled drug release and/or improvement of their mucoadhesive capability. In these systems, the poloxamer acts as a gelling agent, and chitosan as a carrier and bactericidal agent.[24] When these hydrogels are used for wound treatment applications, the $T_{sol-gel}$ is recognized as one of the most important parameters for *in situ* gel-forming systems, because they must satisfy two basic requirements: *i)* be free-flowing liquids at room temperature to allow spraying onto the injured tissue; and *ii)* easily form *in situ* gels at skin temperature. These characteristics are required to avoid friction of the product with the injured tissue, thus reducing the suffering of patients with skin burns. For this reason, the ideal $T_{sol-gel}$ values are in the range from 30-32 °C.[25]

Literature data indicates that the poloxamer concentrations in hydrogel formulations based on binary poloxamer/water mixtures are

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generally in the range from 16-20%, with a value of approximately 18% most frequently used. The techniques most commonly employed for the determination of the $T_{sol-gel}$ are rheometry and visual analysis.[23,26-29] For formulations with 18% Poloxamer 407®, $T_{sol-gel}$ values between 25 and 32 °C have been obtained using rheological data [23,27,29-31] and 28 and 32 °C using visual analysis.[27,29,30]

For ternary formulations of poloxamer-based hydrogels, studies show that $T_{sol-gel}$ is dependent on both the nature of the third component and its percentage. For poloxamer/water/polyethylene glycol (PEG) hydrogels, Edsman and co-workers (1998) found that, compared to a binary poloxamer/water mixture, the $T_{sol-gel}$ increased with 2% PEG and decreased with 5% and 7% PEG. In contrast, for poloxamer/water/vancomicyn hydrogels, no change in the $T_{sol-gel}$ was observed with the addition of the third component,[32] while for poloxamer/water/Carbopol 934, the $T_{sol-gel}$ decreased monotonically with increasing Carbopol 940 concentration.[27] For poloxamer-based formulations containing chitosan, Ur-rehman *et al.* reported that the use of chitosan caused an increase in the $T_{sol-gel}$.[31] With respect to the use of polymeric microparticles, no data was found in the literature regarding the influence of this type of component on the $T_{sol-gel}$ of poloxamer-based formulations.

In this context, the aim of this study was to determine the influence of the formulation composition on the $T_{sol-gel}$ and rheological response of poloxamer-based formulations for burn wound treatment applications containing hyaluronic acid and polymeric microparticles composed of *Aloe vera*/chitosan/vitamin E/polysorbate 80. In addition, to enable the large-scale production of the optimized formulation, the influence of the preparation method on the $T_{sol-gel}$ was also analyzed.

MATERIALS AND METHODS

Chemicals

Poloxamer 407® (Pluronic® F127) was purchased from Sigma Aldrich (Steinheim, Germany), and hyaluronic acid was obtained from Deg (São Paulo, Brazil). *Aloe vera* in the form of a spray-dried powder, 200:1 in maltodextrin, was obtained from Brasquim (Porto Alegre, Brazil). Vitamin E oil was supplied by Pharma Nostra (Rio de Janeiro, Brazil), polysorbate 80 by Vetec (Rio de Janeiro, Brazil), low molecular weight chitosan by Sigma Aldrich (Steinheim, Germany), and acetic acid by F Maia (Cotia, Brazil). Ultrapure water (Milli-Q Millipore Simplicity 185, Bedford, MA, USA) was used to prepare all of the solutions.

Microparticle preparation

The *Aloe vera*/chitosan/vitamin E/polysorbate 80 (4/50/36/10 w/w/w/w) microparticles were prepared as follows: *i)* chitosan was dissolved in acetic acid (1%); *ii)* *Aloe vera*, vitamin E, and polysorbate 80 were added with stirring until total dispersion was achieved; and *iii)* the mixture was dried in a spray drier (Model 190 Buchi) under controlled conditions (inlet air temperature: 120 ± 2 °C, outlet air temperature: 78 ± 3 °C, aspirator setting: 10, suspension feed flow rate: 0.30 L/h, and airflow rate: 500 L/h).

Microparticle characterization

The final chemical composition of the particles was evaluated using infrared (IR) spectroscopy. To obtain spectra in the IR region, a sample (approximately a 1.5 mg) was triturated together with 150 mg potassium bromide, and then the mixture was subjected to compression in a hydraulic press using a pressure of 6 X 10^4 N/cm² for the preparation of the pastilles. All of the spectra were obtained in the transmission mode (32 accumulations) with a resolution of 4 cm⁻¹ over the frequency range from 4000 to 400 cm⁻¹

Gel preparation

All of the poloxamer solutions used in this study (17.0; 17.5; 18.0; 18.5; 19.0; 19.5; 20.0; 20.5; and 21.0% w/w) were prepared by dispersing the polymer in ultrapure water. Three different methods were used for the dispersion of the poloxamer in water: manual stirring, mechanical stirring (400 rpm), and high-performance stirring (13,000 rpm) (T25 Ultra-Turrax®, Ika, Wilmington, USA).The solutions were then stored in a refrigerator at 4 ºC for at least 24 h to ensure complete dissolution. Microparticles (1.0% w/w) and hyaluronic acid (1.0% w/w) were added to the gel with manual stirring until a homogeneous dispersion was obtained.

Gelation temperature measurement

The sol–gel transition temperature ($T_{sol-gel}$) for each formulation was measured in a rotational rheometer (ARES, T.A. Instruments®, New Castle, USA) using a concentric cylinder geometry with a cup diameter of 27 mm and a diameter/height ratio of 25 mm/32 mm. As proposed by Bonacucina and co-workers,[33] the $T_{sol-gel}$ was defined using temperature sweep tests as the temperature at which the loss modulus (G'') was half way between the values of this parameter for the solution and the gel. Based on the results of preliminary strain sweep tests, a strain amplitude of 0.1% was used for all of temperature sweep tests to provide adequate torque and linear viscoelastic behavior. The temperature sweep tests were performed in the range from 5–45 °C at a frequency of 1.0 Hz and a heating rate of 5 °C/min. Approximately 8 g of sample was used for each test, and at least three replicates were measured for each formulation. Frequency sweep tests in the range from 0.1–100.0 rad s⁻¹ were also performed at 30 and 35 °C for selected samples.

Statistical analysis

Analysis of variance (ANOVA) was used for the experimental data analysis using the software Statistica 7.0 for Windows (Statistica 7.0, Statsoft®, Tulsa, USA) and a degree of confidence of 95%. Post-hoc comparisons were made using the Tukey HSD test with a degree of confidence of 95% ($P<0.05$).

RESULTS AND DISCUSSION

Characterization of the gels containing microparticles

Note that it was possible to detect all of the components in the IR spectrum of the gel containing the microparticles (see the Supplementary Material).

I) Poloxamer gels: influence of different preparation methods

Table 1 presents the $T_{sol-gel}$ values for water/poloxamer gels containing 18% (P18) poloxamer that were obtained using different preparation methods. It can be seen in the table that the values obtained using manual and mechanical stirring were not significantly different ($p<0.05$), while the use of the Ultraturrax® reduced the $T_{sol-gel}$. This result can be attributed to the higher shear stresses in the Ultraturrax®.

Based on these results, manual stirring was the method selected for the preparation of the remaining formulations evaluated in the study because of its ease of operation and the fact that it provided results similar to those obtained with mechanical stirring, which is one of the methods employed in industry. Thus, it should be possible to be

Table 1. Sol-gel transition temperature ($T_{sol-gel}$) of different gel preparations using poloxamer at 18% (w/w)

| Preparations	$T_{sol-gel}$ (°C)
Manual Stirring	32.62 ± 0.06 [a]
Mechanical Stirring	33.45 ± 1.25 [a]
Ultraturrax®	30.84 ± 1.16 [b]

Each value is expressed as mean ±1 S.D (n=3). Means within a column followed by different letters are significantly different (p≤0.05).

readily scale up the optimal formulation for industrial applications.

II) Influence of the composition on the $T_{sol-gel}$
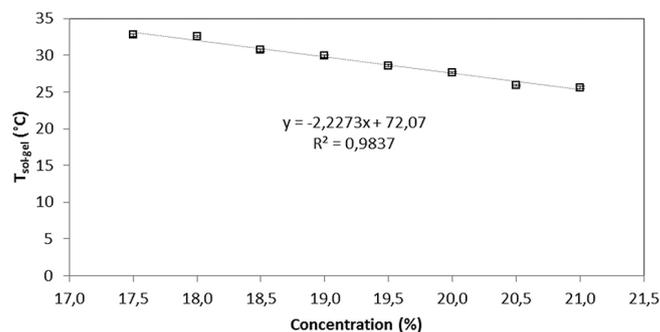
a) Water/poloxamer formulations

Figure 1 shows the behavior of G'' as a function of temperature for the sample with a poloxamer concentration of 17.5 % w/w, where the abrupt change in the modulus was used to identify the $T_{sol-gel}$, as described above. Similar behavior was observed for all of the poloxamer concentrations tested, except the formulation with 17% poloxamer, for which no sol–gel transition was observed in the range of tested temperatures (5 to 45 °C).
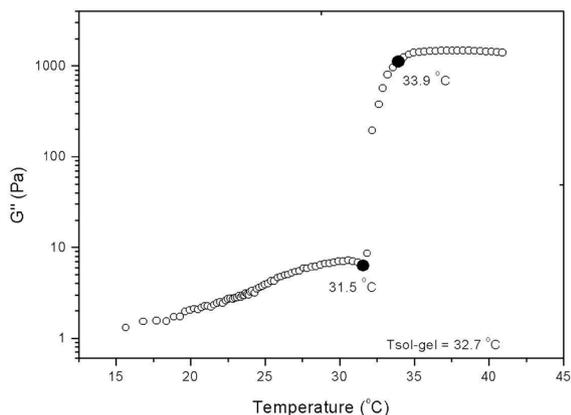
Figure 1. Sol-gel transition temperature ($T_{sol-gel}$) using 17.5% Poloxamer 407®

The $T_{sol-gel}$ values obtained for poloxamer concentrations of 17.5, 18.0, 18.5, 19.0, 19.5, 20.0, 20.5, and 21.0 % w/w (Figure 2) are in agreement with previous results reported in the literature[29] and confirm that the $T_{sol-gel}$ is dependent on the polymer concentration. In the range of concentrations studied, the $T_{sol-gel}$ of the binary water/poloxamer formulations was inversely proportional to the poloxamer concentration, with the appropriate $T_{sol-gel}$ value (32.55 ± 0.09 °C) corresponding to the formulation containing 18% Poloxamer 407®. This dependence of $T_{sol-gel}$ on the poloxamer concentration is in agreement with the mechanism of gel formation described by Cabana *et al.*,[13] which involves the packing of micelles and micelle entanglements. In this regard, the absence of the sol–gel transition for a poloxamer concentration of 17.0% can probably be attributed to the low concentration of the polymer, which prevents micelle formation.

b) Formulations containing hyaluronic acid and polymeric microparticles

Table 2 shows the values of $T_{sol-gel}$ for the binary water/poloxamer formulation (P18) alone, with added hyaluronic acid, and with added hyaluronic acid and polymeric microparticles (P18/HA and P18/HA/MP). The poloxamer concentration in all three formulations was 18%. Both the P18/HA and P18/HA/MP formulations contained 1% w/w hyaluronic acid, with P18/HA/MP also containing 1% polymeric microparticles. Statistical analyses revealed that the addition of

Figure 2. Sol-gel transition temperatures ($T_{sol-gel}$) for different Poloxamer 407® concentrations

hyaluronic acid led to no significant change in the $T_{sol-gel}$ in relation to the binary water/poloxamer formulation, even though it altered the pH from 8.0 to 7.5.[32] With the addition of the polymeric microparticles, however, the pH remained at approximately 7.5, while the $T_{sol-gel}$ of the system was reduced, although it did remain in the acceptable range for burn wound treatment. This reduction in the $T_{sol-gel}$ indicates that for the components and concentrations under study, the effect of the addition of the polymeric particles is more intense than that of the medium acidification. The decrease in $T_{sol-gel}$ promoted by the addition of the polymeric microparticles is possibly related to higher packing of the poloxamer micelles caused by the presence of the particles and to some level of interaction with the chitosan present in the microparticles.[13,29,34] Previous studies have shown that due to the size of the chains and the high charge density of –NH_2, chitosan affects poloxamer gelation through its effect on the formation of water channels around the PPO block of the poloxamer.[31]

Another possibility relates to the fact that chitosan forms complexes with natural polyelectrolyte polyanions, such as carboxymethylcellulose, polyvinyl alcohol, dextran sulfate, hyaluronic acid, carboxymethyldextrane, collagen, and xanthan. When hyaluronic acid forms a complex with chitosan, it exhibits the same behavior as observed during the formation of other complexes based on chitosan. Two oppositely charged polyelectrolytes, chitosan (polycation) and hyaluronic acid (polyanion), interact to form an insoluble complex. Swelling of the hydrogel is influenced mainly by the ionic interactions that occur during complex formation. An increase in the density of the interactions between the chains induces a decrease in the swelling of the hydrogel and also its pH sensitivity, improving the stability of the three-dimensional network. In addition, hydrogels formed by ionic forces can swell under basic or acidic conditions, which enables biomedical applications in various areas, e.g., adhesives and dental restorations, controlled-release devices, and biocompatible materials.

Table 2. Sol-gel transition temperature ($T_{sol-gel}$) for poloxamer formulations

Formulation	$T_{sol-gel}$ (°C)
P18	32.62 ± 0.06 [a]
P18/HA	32.90 ± 0.31 [a]
P18/HA/MP	30.25 ± 0.84 [b]

Each value is expressed as mean ±1 S.D (n=3). Means followed by different letters differ significantly according to the Tukey test (p≤0.05).

III) Rheological behavior of the poloxamer gels containing polymeric microparticles

Figure 3 shows the behavior of the dynamic moduli of the three formulations listed in Table 2 at 35 ºC. Although the behavior of the G' and G'' curves is qualitatively similar for the three formulations, the crossover between the G' and G'' curves is clearly displaced toward

higher frequencies for the gel containing microparticles (Figure 3c), which is in agreement with the reduction in the $T_{sol-gel}$ of this formulation, as discussed above. Additionally, it can be seen in the figure that the final G′ values for P18/HA/MP are of the same order of magnitude as those for P18/HA, indicating that the addition of the microparticles does not significantly affect the strength of the gel.

Viscosity *vs.* temperature curves for the three formulations listed in Table 2 are shown in Figure 4. It can be seen in the figure that at temperatures below the $T_{sol-gel}$, the viscosity of the P18/HA/MP sample was higher than that of the P18/HA and P18 samples, but remained within the acceptable range for spray applications. At temperatures above $T_{sol-gel}$, the viscosities were quite similar for all three formulations, indicating a low dependence on the temperature, and were two

orders of magnitude greater than the viscosity values obtained below the $T_{sol-gel}$. The dependence of the viscosity on the frequency was also investigated, because this characteristic is also important with respect to the application of these materials. The results are shown in Figure 5. It can be seen in the figure that all of the formulations exhibited pseudoplastic behavior; the viscosity decreased as the frequency increased. Notably, the P18/HA/MP and P18/HA formulations exhibited slightly higher pseudoplasticity than the binary formulation, and thus the viscosities of the three formulations become similar at high frequencies despite the fact that P18/HA/MP and P18/HA were much more viscous at low frequencies. These characteristics are also desirable from the point of view of the application of the formulations in burn wound treatment. A high viscosity at low frequencies is important for avoiding sag after application on the skin. On the other hand, a rapid decrease in the viscosity with deformation is advantageous for debridement intervention, for which a low viscosity of the gel layer is required to lower the suffering of patients during the application of a force to remove dead skin.
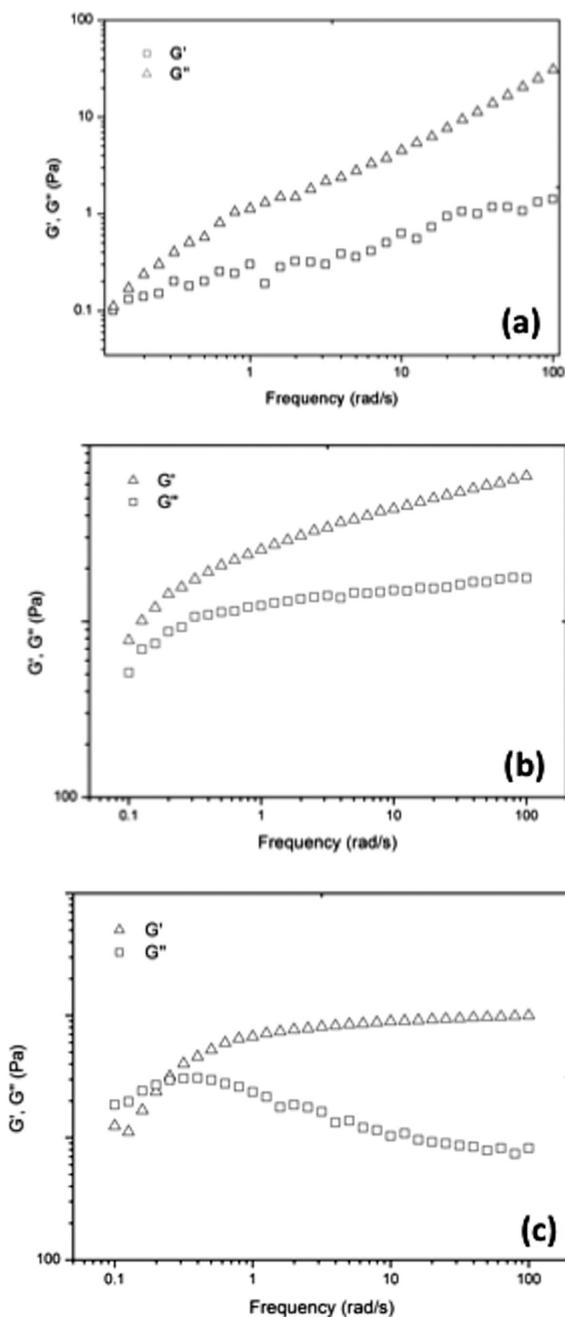

Figure 4. *Viscosity vs. temperature curves for the three formulations listed in Table 2 at 1 rad/s*
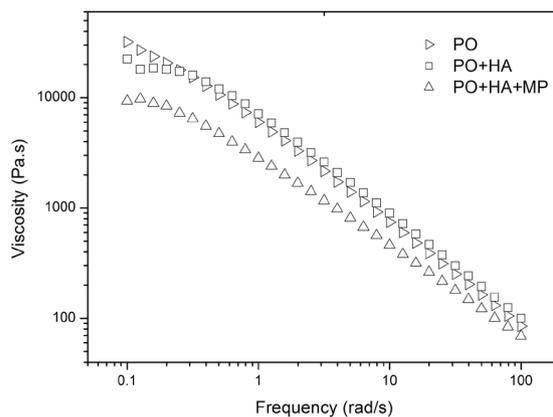
Figure 5. *Viscosity vs. frequency curves at 35 °C for the three formulations listed in Table 2*

CONCLUSIONS

The influence of the composition and preparation method on the $T_{sol-gel}$ and rheological response of water/poloxamer-based formulations was evaluated. Manual stirring was the method selected due to its ease of operation and the fact that it provides results similar to those obtained with mechanical stirring. In the range of concentrations analyzed, a linear dependence of the $T_{sol-gel}$ on the poloxamer content was observed, from which an 18% Poloxamer 407® concentration was selected considering the required $T_{sol-gel}$ range for formulations for

Figure 3. *Elastic (G') and viscous (G") modulus as a function of the frequency (rad/s) at 35°C for formulations containing: (a) 18% poloxamer; (b) 18% poloxamer + 1% hyaluronic acid, and;(c) 18% poloxamer + 1% hyaluronic acid + 1% microparticles*
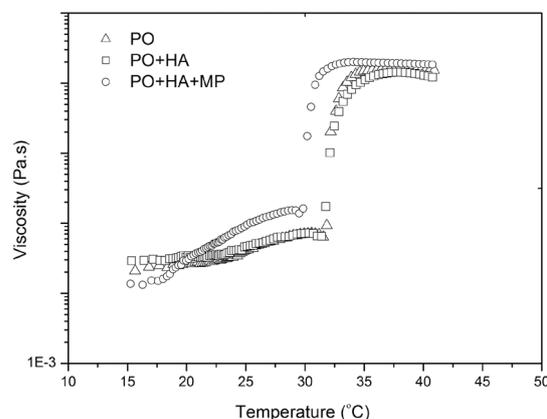
skin burn wound treatment. The addition of hyaluronic acid led to no significant change in the $T_{sol-gel}$ in relation to the binary water/poloxamer formulation. However, the addition of polymeric microparticles caused a slight reduction in the $T_{sol-gel}$ without a significant reduction in the gel strength and providing a $T_{sol-gel}$ value that still lies within the range of interest. The behavior of the P18/HA/MP formulation with respect to the dynamic moduli, pseudoplastic characteristics, and viscosity values at low and high frequency limits indicates that this system is rheologically suitable for application as a thermosensitive hydrogel for the treatment of burn wounds.

SUPPLEMENTARY MATERIAL

The IR spectra are located at http://quimicanova.sbq.org.br in a pdf file, with free access.

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