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Research Paper

Rheological characterization of a gel produced using human blood plasma and alginate mixtures

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ABSTRACT

Human blood plasma is a material used to generate tissue equivalents due to presence of fibrinogen. However, gels formed using human blood plasma has weak mechanical properties. In this study, different mixtures of sodium alginate and blood plasma were performed and evaluated. By determining ζ potential can be established the stability of the plasma–alginate mixture and by dynamic rheology can determine the most suitable parameters for the gelation of the above mixtures, when calcium chloride is used as a crosslinker. Experimental results evidence an increment in ζ potential at alginate concentrations of 0.8% and 1.6% with a resulting pseudoplastic behavior of evaluated mixtures, which described the homogenization of the mixture. On the other hand, mixtures were gelled by using aspersion of calcium chloride and characterized by dynamic rheology. Solid behavior is dominant in all range of frequency sweep test between 0.1 Hz and 100 Hz. Finally, the ultimate tensile strength of a gel reach 6.36938 ± 0.24320 kPa, which is enough for manual handling of the gel. Between the tasks of the gel would be used for cell entrapment, for controlled release of drugs or in the manufacture of wound dressings.

1. Introduction

Although many materials have been evaluated and used in generation of temporary scaffolds for cells growth in tissue engineering (Yoon and Fischer, 2007; Pachence et al., 2007; Warren et al, 2004), research community prefer the use of biocompatible and biodegradable materials (Falke and Atala, 2000). These kind of materials enhance cell adhesion and signal capabilities (Sukmana, 2012).

Natural polymers provide these properties mentioned above. They can be divided in two major groups: proteins such as collagen, gelatin, albumin, fibrinogen, and polysaccharides like chitosan, hyaluronic acid, alginate, cellulose and dextran. The mixture of polysaccharides and proteins for generating tissue scaffolds gives a promising combination of good mechanical strength provided by polysaccharides and the cell adhesion capabilities of proteins.

Particularly, Fibrinogen has gained status in tissue engineering due to its ease of acquisition and its ability to generate diverse and different scaffolds. This material can be obtained either, from a patient's own blood plasma, blood bank plasma, or from commercial sources (e.g., Tissel®, Baxter Laboratories).

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Plasma proteins can be separated into three fractions: albumin (approximately 42%), globulins (56%), and fibrinogen (1%); other proteins, such as fibronectin, lipoproteins, and others represent less than 1% of the total protein content of plasma (Moure et al., 2003; Schaller et al., 2008).

Under normal conditions, fibrinogen is present in blood plasma as a zymogen, but in case of a wound, fibrinogen is activated through a serie of reactions known as the "coagulation cascade". During these reactions, multiple enzymes and substrates are involved and lead to a formation of fibrin fibers which, along with platelets, coagulates the wound (Eyrich et al., 2007). Also, it is important to mention that some studies (Meana et al., 1998; Arvelo et al., 2004; Llames et al., 2004; Zhao et al., 2008) do not perform fibrinogen purification, instead of that, they use blood plasma directly to exploit its native fibrinogen content and other proteins presented, giving a clinical advantage that reduces significantly the cost of tissue equivalent generation. However, due to their poor mechanical properties, the gels are not able to handle by medical personal. So, in order to enhance gel mechanical properties, one alternative is mix them with calcium alginate (Bhakta et al., 2009; Shikanov et al., 2009; Zhou and Xu, 2011; Ma et al., 2012).

On the other hand, alginate is a polysaccharide water-soluble polymer derived from algae (*Macrocystis pyryfera*, *Laminaria hyyperborea*, *Laminaria digitata*, and *Ascophyllum nodosum*) or from recombinant microorganisms (*Azotobacter vinelandii*). Alginate is a polyanion composed by two monomeric units: β-D-mannuronate (*M* units) and α-L-guluronate (*G* units) and linked by β (1–4) bonds (Yoon and Fischer, 2007; Brandl et al., 2007; Eiselt et al., 2000; Coviello et al., 2007; Shapiro and Cohen, 1997). Due to its polyelectrolytic nature, alginate is cross-linked when it is exposed to divalent ions, such as calcium or barium, in order to form a polymer network known as the "egg box" model (Grant et al., 1973; Brandl et al., 2007).

Alginate is preferred in tissue engineering for their ability to gel formation by ion displacement at 37 °C in isotonic solutions, is biocompatible and does not interfere with the cellular function of the immobilized cells (Mazzitelli et al., 2011). Since their hydrophilic behavior, alginate does not provide good performance of cell adhesion (Yoon and Fischer, 2007; Pokrywcznska et al., 2008). In order to improve cell adhesion, alginate usually is mixed with protein or peptide sequences in a way such that cells can be able to recognize the proteins through existing receptors in the cell membranes (Brandl et al, 2007; Mazzitelli et al., 2011). One of these proteins mixed with alginate for scaffold generation in tissue engineering is the fibrogen (Bhakta et al, 2009; Shikanov et al, 2009).

In order to obtain a suitable material for tissue engineering purposes, human blood plasma and sodium alginate were mixed and subjected to gelation by sprayed calcium chloride. The gel obtained had workability and had the potential to be used in applications such as scaffold for trapping cells, for controlled drug release or as a wound dressing.

2. Materials and methods

2.1. Reactives

Sodium alginate (Ref: A2033, 61% manuronic acid (M), 39% guluronic acid (G); ratio *M/G* 1.56; MW: 80.000–120.000) was purchased to Sigma-Aldrich Co (Saint Louis, MO, USA); calcium chloride (Ref: 433381) and sodium chloride (Ref: 479687) were purchased to Carlo Erba (Strada Rivoltana, Italy).

2.2. Blood plasma procurement

Human blood plasma was obtained from the blood bank at the District Health in Bogotá (Colombia). Type O+ plasma was used to perform all predetermined assays in this research study; plasma is not used for human patients for its high lipemic content. To validate all assays, 30 units of plasma (250 mL) were mixed under sterile conditions in a laminar flow hood after thawing and warming to 37 °C with a thermostat-controlled water bath (Cole-Parmer model 1266-02). To eliminate fibrinogen degraded during storage, the mix was centrifuged at 7100 × *g* for 20 min (Sorvall) at ambient temperature using 500 mL flasks (Nalgene, Rochester, USA). The supernatant was filtered using a Millipore filtration cartridge with an 8 μm pore size for eliminating lipemic material. Once the components were separated, the plasma was transferred into 500 mL dark amber glass containers (Schott, Mainz, Germany) and stored at −30 °C. Samples were thawed as needed for experimentation.

2.3. Determination of the ζ potential

ζ Potential was used for establishing the stability of mixtures in various proportions of alginate, to avoided precipitation in gel formation. Alginate/plasma mixtures were prepared to the following alginate concentrations: 0.2%, 0.4%, 0.8% and 1.6% (w/v) from human plasma and sodium alginate solution at 3% (w/v) in water. Then ζ Potential was determinated using an equipment ZETAMETER 3+ (Stauton, USA). The mixture was brought to a conductivity of 20 mS/cm with sodium chloride at ambient temperature using a conductimeter Oaklon 510; the last procedure was development for adjusting the ionic force to the same value in all assays.

2.4. Viscometry of blood plasma and sodium alginate

Viscometry assays were performed on human blood plasma, diverse sodium alginate solutions (0.2%, 0.4%, 0.8%, and 1.6% (w/v)), and sodium alginate/human blood plasma mixtures in the same sodium alginate proportions stated above. These assays were conducted with BOHLIN CVOR-200 Rheometer (Malvern Instruments Ltd, Worcestershire, UK) using a cone-and-plate geometry arrangement with an angle 2° and 40 mm of diameter. Assays were performed with an increasing ramp of shear rates between 0.1 s^{-1} and 150 s^{-1} for 100 s, remaining at 150 s^{-1} for 120 s, and finally descending from 150 s^{-1} to 0.1 s^{-1} for 100 s. All assays were performed in triplicate at a constant temperature of 37 °C.

2.5. Fabrication of blood plasma and alginate gels

Mixtures of plasma/alginate, 0.8% and 1.6% (w/v) in alginate, were distributed on a metallic support for producing a gel with height of 1 mm. Hollow metallic support of rectangular form was used for gellification; dimentions of support were 20 cm (length) × 20 cm (height) and 1 mm (height); on this

surface was deposited the mixture plasma/alginate. A commercial nozzle (airbrush Ranger, REF: 1140 008/W-71G 1394) was situated at a distance of 20 cm from a mixture of plasma/alginate, and a calcium chloride solution of 1%, 2%, or 3% (w/v) in water was sprayed for 5 min at a flow rate of 22 mL/min and an air flow rate of 4 L/min at pressure 20 psig (it was guaranteed excess of calcium). The gel was allowed to form for 20 min at ambient temperature and was then punched (rheology assays) or cut with scalpel blade (mechanical assays). Inmediately, dynamic rheology assays or mechanical characterization were performed. For rheology assays the film was punched with a die of 25 mm diameter puncher; for mechanical assays, the film was dissected into rectangular 2 cm × 7 cm sections using a glass lamina.

2.6. Dynamic rheology of the formed gels

Dynamic rheology testing was performed on the molded alginate/plasma films. Initially, amplitude sweep testing was conducted to determine the viscoelastic linear region (VLR). These assays were performed with a fixed shear frequency rate of 1 Hz and variable strain amplitudes between 0.0001 and 10 in a BOHLIN CVOR-200 Rheometer at a constant 37 °C temperature using the Peltier system with a 25 mm diameter parallel-plate geometry setup. All tests were performed in duplicate. Once the VLR was determined, frequency sweep tests were performed by varying the frequency between 0.1 Hz to 10 Hz, with a fixed 1% strain and 37 °C; response variables obtained from testing were the elastic modulus (G') and the viscous modulus (G'') for sodium alginate/blood plasma mixtures and calcium chloride concentrations of 1%, 2%, and 3% (w/v).

2.7. Tensile test of the formed gels

The films obtained from a mixture of blood plasma/sodium alginate 1.6% (w/v) and 3% (w/v) calcium chloride were subjected to tensile testing to failure in a TA XT Plus texture meter (TA Instruments, Delawere, USA) equipped with a data acquisition system. Tests were carried out at a speed of 0.5 mm/s in quadruplicate with a load cell of 30 kg. Young's modulus, ultimate tensile strength, and maximum percentage strains were determined based on the stress–strain curves. Tests were carried out at ambient temperature. The area of each films was determined by using a vernier caliper (Mitutoyo, Tokyo, Japan).

2.8. Evaluation of the plasma/alginate films using scanning electron microscopy (SEM)

The films from a mixture of blood plasma/sodium alginate 1.6% (w/v) and 3 % (w/v) calcium chloride were prepared as described above, and their microstructure was determined using SEM. The fabricated films were frozen at −70 °C for 24 h and then lyophilized using an LABCONCO freeze-drier (Freezone 2.5) at pressure 50 mBar and temperature −50 °C during 24 h. The films were imaged in a SEM system (FEI QUANTA 200) under 0.45 Torr vacuum conditions, 130 kV voltage potential and 130 × magnification.

3. Results

3.1. Procurement of blood plasma lots

The characterization of the plasma was performed using kits distributed by Wiener Lab Group (Rosario, Argentine) setting and is summarized in Table 1. All values in Table 1 are within the range for patient use except for the glycemic levels, which are above normal baseline for patients because the source plasma donated by the District Health Ministry were originally discarded due to their elevated sugar content. For gel formation, fibrin content and thrombin time are both important. Thrombin time is inversely related to the concentration of thrombin that results in longer thrombin clotting times for low thrombin content. Fibrinogen concentration is within the normal patient range, which is between 250 mg/dL to 300 mg/dL (Standeven et al., 2005). The pH value (7.81±0.00) is slightly higher than physiological levels (pH 7.4) likely because the samples were cryopreserved and carbon dioxide solubilized and bound to hemoglobin, thus leading to a rise in pH level.

3.2. Determining ζ potential

The Fig. 1 shows ζ potential for sodium alginate and human blood plasma mixtures. According to this figure, ζ potential of the mixtures rose with increasing sodium alginate content and reached a maximum value at 1.6% (w/v) (Fig. 1). The ζ potential for 3% (w/v) sodium alginate was 25.3 mV, and for human blood plasma, it was 42.20 mV; however, all values observed in the alginate/plasma mixtures were higher.

Table 1 – Characterization of the stock of human blood plasma.

Parameter	Value	Basis of method	Kit
Fibrinogen	273.5±2.12 mg/dL	Clauss method (Clauss)	Fibrinogen
Thrombin Time	21.55±0.78 s	Measurement of coagulation time of plasma	Thrombin time
Basal Glicemia	300±12.73 mg/dL	Determination of red quinonimine	Glicemia enzymatic AA
Cholesterol, Total	132.9±3.39 mg/dL	Determination of red quinonimine	Colestat enzymatic AA
Cholesterol, HDL	38±1.41 mg/dL	Development of color	HDL Cholesterol monofase AA plus
Cholesterol, LDL	78.35±2.33 mg/dL	Development of color	LDL cholesterol monofase AA
Triglycerides	83.00±1.41 mg/dL	Determination of red quinonimine	TG Color GPO/PAP AA
Density	0.9965±0.00288 g/mL	Pycnometer	
pH	7.81±0.00	pH-meter	

3.3. *Blood plasma and sodium alginate viscometry*

The flow curve for blood plasma is depicted in Fig. 2. Hysteresis was observed, implying that the stress curve differs between increasing and decreasing strain rates. The flow curve in Fig. 2 displays linear behavior during the increasing strain ramp, which corresponds to Newtonian behavior with a mean correlation coefficient of 0.9446 across the three assays performed (see Table 2). The dynamic viscosity for the increasing strain ramp of blood plasma is 6.8 mPa s. Because water content in plasma is 90–91% (Moure et al., 2003; Schaller et al., 2008), we expected to observe a predominant Newtonian rheological behavior; however, protein presence in suspension (7%) leads to a rise in viscosity with respect to water.

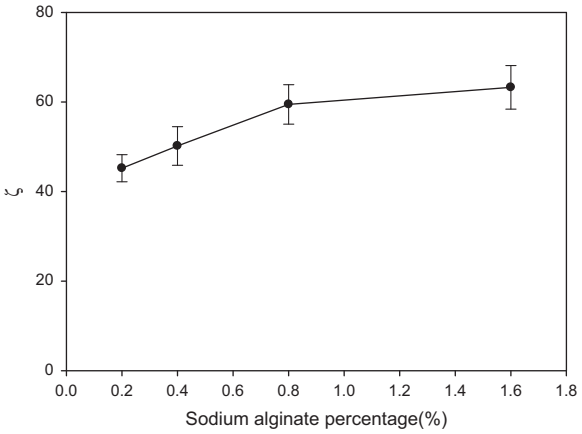

Fig. 1 – ζ potential for sodium alginate and human blood plasma mixtures adjusted with NaCl to 20 mS/cm electric conductivity.

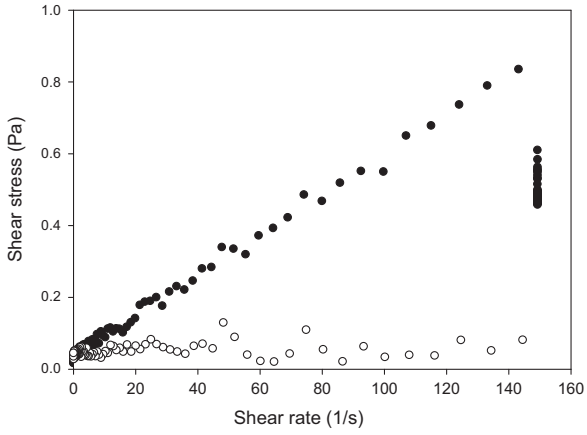

Fig. 2 – Flow curve for human blood plasma between shear rates of 0.1 s^{-1} and 150 s^{-1} and 37 °C. (•: increasing ramp; ○: descending ramp).

Fig. 3 (upper) displays the rheological behavior of sodium alginate, which corresponds to a pseudoplastic fluid (Mancini et al., 1995; Yang et al., 2013). Based on this figure, it was determined that low alginate contents (0.2% and 0.4% (w/v)) display higher hysteresis wich reduce at higher concentrations (0.8% and 1.6% (w/v)). Flow curves for mixtures of alginate and human blood plasma using diverse sodium alginate contents are displayed in Fig. 3 (lower).

3.4. *Dynamic rheology of the prepared gels*

Based on the Zetametry results, it was determined that the testing conditions for dynamic rheology would be at 0.8% and 1.6% (w/v) alginate conditions; in addition, three concentrations, 1%, 2%, and 3% (w/v) of calcium chloride were chosen for gelation.

Initially, amplitude sweep tests were performed to determine the viscoelastic linear region (VLR). This assays determined the presence of two domains: the first one in which G' and G'' were nearly constant, and a nonlinear region, in which G' and G'' decreased with increasing strain (data not shown). Accordingly to results, we decided that the conditions for the frequency sweep testing should be limited to the 1% frequency to avoid any potential material instability. These results are similar to those published by Moresi et al. (2001), who evaluated the rheological behavior of commercially available, medium-range viscosity sodium alginate and reported a maximum critical strain value of 3.3% for 1.50% and 1.75% (w/v) alginate concentrations.

The results from the frequency sweep testing are depicted in Fig. 4(a and b). It was observed across all of these assays that the elastic modulus, G', predominates over the viscous modulus, G'', indicating that the phase transition from liquid-to-solid behavior occurred. Indeed, there is an order of magnitude separating G' and G'' once gelation occurs that confirms this hypothesis, this phenomenon is characteristic of alginates (Moresi et al., 2004, 2001; Mancini et al., 1995) and, moreover, given the behavior in which the moduli is plateau

Table 2 – Adjusting Ostwald de Waele Model to human blood plasma, sodium alginate and mixtures of sodium alginate/human blood plasma.

Assay	K (Pa s)	Confidence intervale for K	n	Confidence intervale for n	r^2
Blood plasma	0.0068	0.0063–0.0072	1.000	0.973–1.000	0.9446
Alginate 0.2	0.0161	0.0135–0.019	1.000	0.932–1.000	0.9015
Alginate 0.4	0.0322	0.0286–0.0362	1.000	0.953–1.000	0.9533
Alginate 0.8	0.1005	0.0934–0.1081	1.000	0.967–1.000	0.9732
Alginate 1.6	1.0020	0.9704–1.0346	0.897	0.884–0.910	0.9949
Alginate-plasma 0.2	0.0084	0.3000–0.033	1.000	0.988–1.000	0.9703
Alginate-plasma 0.4	0.0301	0.078–0.0835	1.000	0.988–1.000	0.9941
Alginate-plasma 0.8	0.1208	0.115–0.124	0.925	0.910–0.939	0.9936
Alginate-plasma 1.6	0.9280	0.9000–0.9560	0.884	0.870–0.889	0.9954

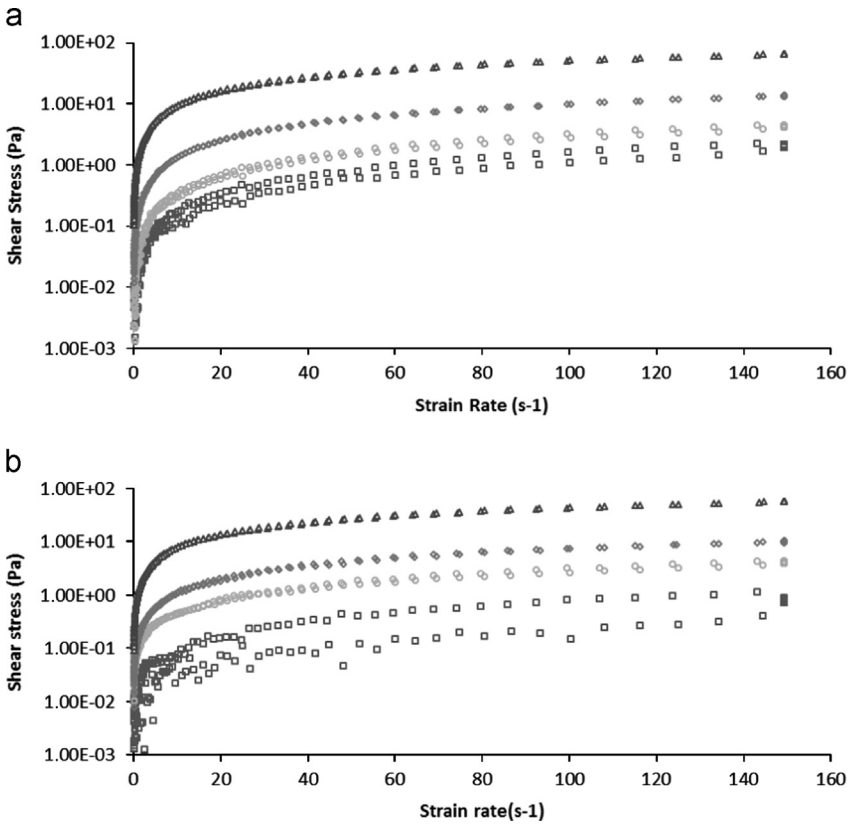

Fig. 3 – Flow curves for different alginate concentrations: □ 0.2%, ○ 0.4%, ◇ 0.8%, △1.6% between shear rates of 0.1 s^{-1} and 150 s^{-1} for: (a) sodium alginate solutions; (b) sodium alginate and human blood plasma mixtures.

with frequency sweep, high gel stability can be predicted, which is desirable to allow handling by medical personnel.

3.5. *Mechanical characterization of the prepared gels*

Characterization of the mechanical behavior of the gels was performed using films obtained by mixing human blood plasma with 1.6% sodium alginate and 3% calcium chloride. The measured mean ultimate tensile strength (UTS), Young's modulus and maximum deformation were 6.369±0.243 kPa, 22.486±1.281 kPa and 0.2425±0.0096 kPa, respectively. For skin, the reported values for the Young's Modulus is 0.1–2.0 MPa and UTS is 1–20 MPa (Saltzman, 2004), these values are fairly high with respect to that obtained for gels in this paper, however, these ones are in similar order, for the Young's Modulus, reported for collagen sponge (0.017 MPa to 0.029 MPa) and fibroblast-populated matrix (0.08 MPa to 0.8 MPa). The values reported for fibrin gel is 50.85–133 kPa for UTS (Ahlfors and Billiar, 2007; Linnes et al., 2007) and 28.56–26.96 kPa for Young's modulus (Huang et al., 2010), the values reported are greater because the production process uses thrombin and fibrin purified or concentrated, additionally, concentration of this components is greater than used for us.

3.6. *Evaluation of the films using scanning electron microscopy (SEM)*

Fig. 5 displays SEM microphotographs of gels form calcium alginate/human blood plasma mixtures at 0.8% Fig. 5(A) and 1.6% Fig. 5(B), in contrast with calcium alginate Fig. 5(C) and human blood plasma Fig. 5(D) samples alone. The mixture's microstructure displays an alginate skeleton upon which plasma proteins anchor. Nonetheless, the protein structure (hairy) seems to differ in the mixture from that in the plasma when the latter is cross-linked alone in the presence of calcium but without alginate Fig. 5(D). The structure in panel A is highly porous, which ensures an elevated surface area to enable attachment in human cell cultures.

4. Discussion

4.1. *ζ Potential and viscometry of sodium alginate and human plasma*

ζ Potential obtained for sodium alginate and human plasma could be explained by electrostatic interactions between alginate and plasma. The chemical structure of alginate is negatively charged due to abundant hydroxyl groups, while the surface of plasma proteins surpass the isoelectric points of human albumin (pI 5.8) and fibrinogen (pI 4.8) yielding a negative charge, thus generating electrostatic repulsion that confers mixtures with higher stability and delays protein precipitation from suspensions and prevents non-homogeneities formation. The pH value of mixtures was 7.53±0.00 and 7.68±0.00 for 1.6% (w/v) and 0.8% (w/v), respectively. Through ζ potential assays, high potentials were observed, thus suggesting that protein precipitation might

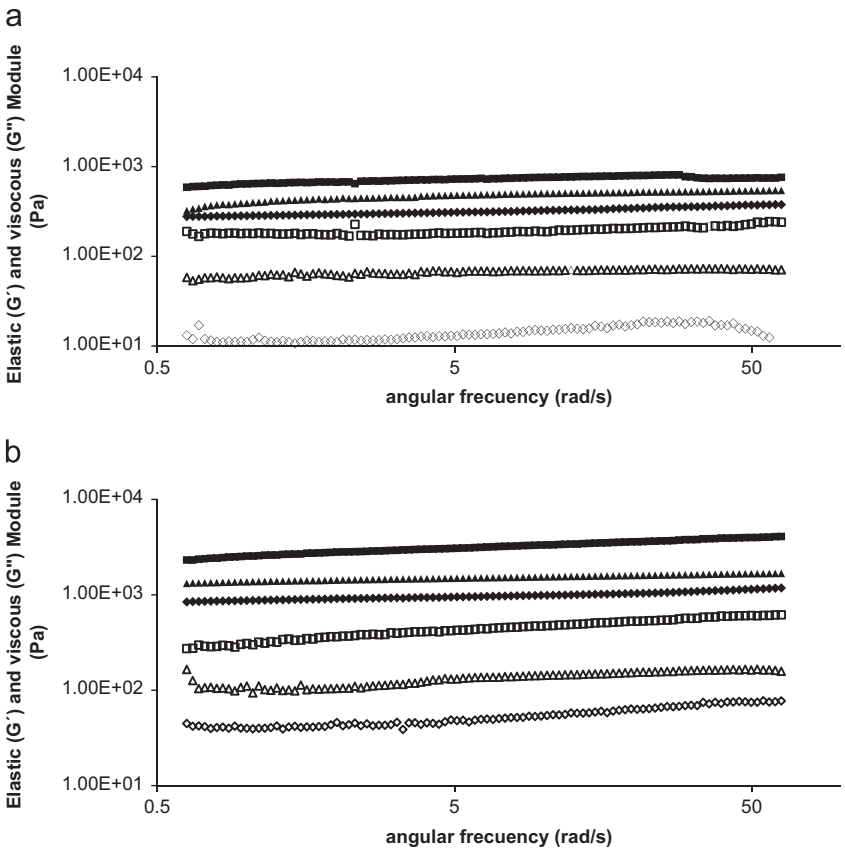

Fig. 4 – Frequency sweep testing of alginate/plasma films for: (a) concentrations of 0.8% in alginate and (b) concentrations of 1.6% in alginate for calcium concentrations: ♦,◇ 1% w/v; ▲, △ 2% w/v; ■, □ 3% (w/v). Storage modulus G': closed symbols and loss modulus G'': open symbols.

not occur, while at potential levels close to the pI level (i.e., ζ potential=0), aggregates could form. Therefore, 0.8% and 1.6% (w/v) alginate concentrations were chosen for evaluation by rheology due to their higher stability relative to the other alginate contents tested.

Plasma viscometry assay show behavior which implies that proteins in suspension are reorganize in different manners under high strain rates, leading to decreased viscosity. A similar trend has been reported (Rampling, 2007) for the rheological behavior of blood, in which red blood cells loosely cluster into aggregates known as "rouleaux," which make blood viscosity highly dependent on strain rate. Thus, low deformation rates lead to higher viscosities in blood that drop as strain rates rise (by rouleaux destruction). One of the contributing proteins to this phenomenon is fibrinogen, which is present in blood plasma.

In contrast, mixtures of plasma and alginate viscometry behavior obtained could be explained due to intermolecular hydrogen bonds or Van der Waals interactions that lead to entanglement among alginate chains that increase as alginate content is enriched. Thus, a lower frequency of entanglement events can explain hysteresis at low alginate concentrations, with decreased hysteresis at higher alginate concentrations because intermolecular interactions can better preserve alginate structure under high strain rates (see Fig. 3). As can be seen in Fig. 3, there is an area between increasing and decreasing strain rates which is called hysteresis; these area decreases to the extent that alginate concentration increases. The behavior displayed by these mixtures is similar to that of sodium alginate (pseudoplastic), with higher hysteretic effects at low alginate concentrations and concomitant decreasing effects with increasing alginate concentrations. This result indicates that the molecular associations among alginate molecules increase at higher alginate concentrations and that the effects of blood plasma on the mixture are not as drastic as those from alginate molecules.

It can be observed that plasma proteins do not affect the rheological behavior of the mixture, whose qualitative characteristics are similar to those found in alginate solutions in equivalent concentrations. This finding suggest that the lack of attractive forces among plasma proteins and alginate polysaccharides, as determined by the ζ potential measurements, allows the plasma proteins to self-aggregate, while the alginate polysaccharides extend in solution and ultimately govern the rheological behavior of the overall mixture.

Based on the results obtained (Fig. 3) adjustment was performed, using software available in BOHLIN CVOR-200 Rheometer, by a model Ostwald-de Waele Power (Mancini et al., 1995) and are showed in Table 2

$$\sigma = K(d\gamma/dt)^n \quad (1)$$

where

σ is shear stress, Pa.

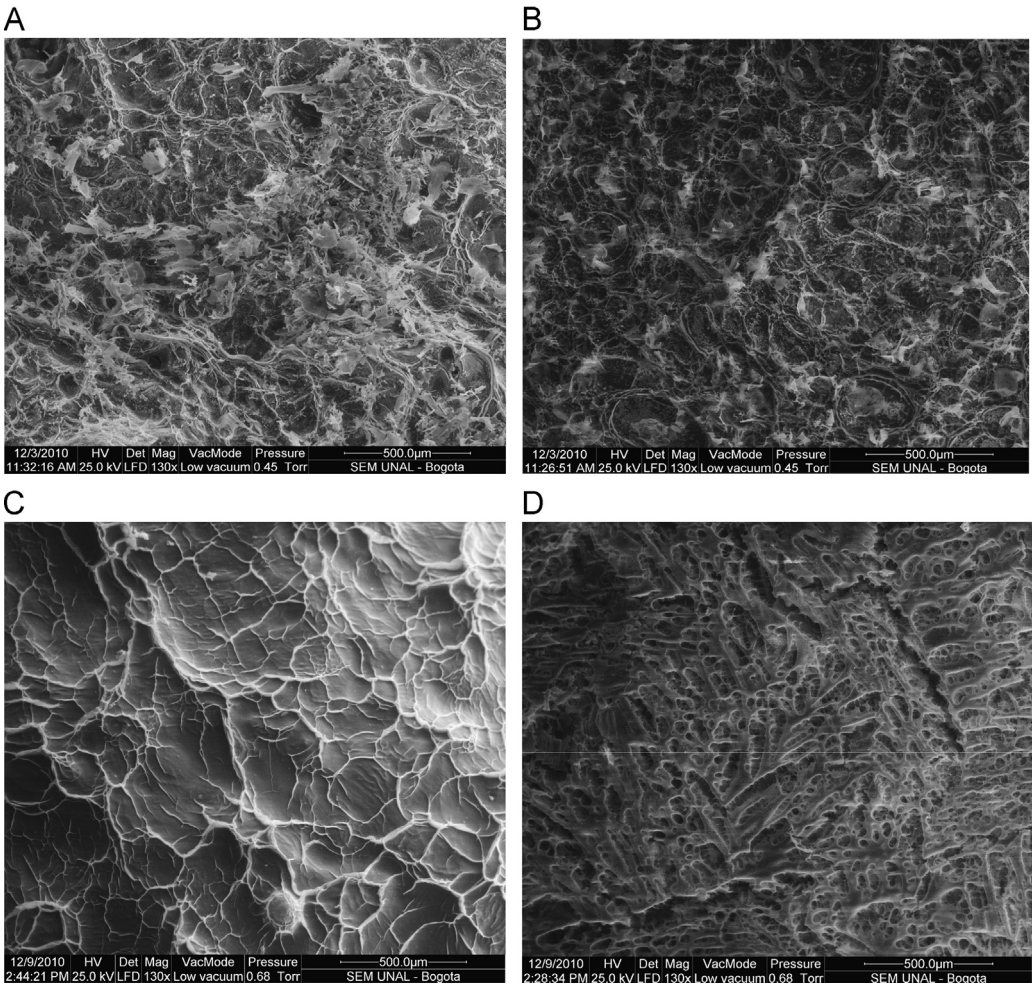

Fig. 5 – SEM microphotographs of (A) 0.8% calcium alginate with 3% calcium chloride. (B) 1.6% Calcium alginate with 3% calcium chloride. (C) 1.6% Sodium alginate cross-linked with 3% calcium chloride. (D) Human blood plasma cross-linked with calcium chloride in the same conditions reported by Meana et al. (1998).

K is the flow consistency index, Pa s.
$(d\gamma/dt)$ is shear rate, s^{-1}.
n is the flow behaviour index.

The estimated parameters were adjusted to 97% of response variability with a confidence interval of 95%. From the above results it can be concluded that at low concentrations of sodium alginate,(from 0.2% to 0.8% alginate alone and from 0.2% to 0.4% for the mixture alginate/human blood plasma, behavior is Newtonian ($n=1$) and for 1.6% alginate alone and 0.8% and 1.6% for alginate/human blood plasma becomes pseudoplastic behavior ($n<1$). This corresponds with that reported in the literature consulted (Mancini et al., 1995), where reported a pseudoplastic behavior for different types of sodium alginate to evaluate strain rate until 10,000 s^{-1}, which is much higher with respect to the speed used in this work (150 s^{-1}). Thus, one would expect that if the test is performed until a strain close to 1000 s^{-1} obtained behavior would be Newtonian and pseudoplastic again, which is reached at the concentration noted in 1.6% sodium alginate and 0.8% and 1.6% for mixtures with blood plasma. This pseudoplastic behavior is characteristic of polymeric materials solutions such as sodium alginate, which high molecular weight molecules are extended and interwoven in a solution reaching in this way a very stable configuration, if the concentration is increased, tends to raise the consistency index value (K) of the solution and decrease the value of the flow behavior index (n) and said solution behavior will move away from the solvent (water) which is a Newtonian fluid.

Yang et al. (2013) performed rheological tests for alginate solutions using the same reference as used in this work and reported K values of 2.75 Pa s and 0.25 Pa s for concentrations of 1.6% and 0.8% w/v sodium alginate, evaluated at a temperature of 25 °C versus temperature controlled in this study of 37 °C, which shows that at a lower temperature is a greater interaction between molecules and therefore requires more effort to slide the alginate solution; as an reported values of 0.70 and 0.85 for concentrations of 1.6% and 0.8% w/v, which are typical of pseudoplastic fluids, however with increasing temperature (this work) there is a tendency to get a Newtonian behavior (for concentration of 0.8%) or

approaching such behavior by increasing the value of n for the concentration of 1.6%.

4.2. Dynamic rheology of human plasma and calcium alginate

An important criterion in gelation studies is the gelation point, which is the point of separation between two distinct phases: one dominated by viscous behavior ($G'' > G'$) and another dominated by elastic behavior ($G' > G''$) (Winter and Chambon, 1986; Chambon and Winter, 1987). Moresi et al. (2004) described the phase transition of cross-linking of alginate near the gelation point or slightly after the sol–gel transition as a function of both the storage or elastic modulus (G') and the loss or viscous modulus (G'') in terms of the shear rate

$$G'(\omega) = G\infty, \alpha + \sqrt{\frac{2}{\pi}} s^*_\alpha \cos\left(\frac{\pi}{2}\alpha\right)\omega^\alpha \quad (2)$$

$$G''(\omega) = \sqrt{\frac{2}{\pi}} s^*_\alpha \sin\left(\frac{\pi}{2}\alpha\right)\omega^\alpha \quad (3)$$

In Eqs. (2) and (3) $\omega = 2\pi f$ is the angular frequency, f is the frequency, α is the order of the relaxation function, and $G\infty,\alpha$ and s^* are the equilibrium modulus and material parameter for the value of α, respectively. Assuming that the equilibrium modulus ($G\infty,\alpha$) is either zero (which is expected for the sol state and the gelation point) or negligible (only for a small limited frequency range within the gel state), the tangent of phase angle (δ) depends on α only, while the complex dynamic shear modulus (G^*) is a function of α and S^*_α

$$\tan\delta = \frac{G''}{G'} = \tan\left(\frac{\pi}{2}\alpha\right) \quad (4)$$

$$G^*(\omega) = \sqrt{(G')^2 + G''^2} = \sqrt{\frac{2}{\pi}} s^*_\alpha \omega^\alpha \quad (5)$$

$$G^* \approx A_\alpha \omega^\alpha \quad (6)$$

From the perspective of three-dimensional structure, the parameter α relates to the order of the relaxation function, and the term A_α is a measure of strength by the polymer network cross-linking. By minimizing the sums of squares between experimental and adjusting data for the complex modulux G^*, it was obtained a mathematical fit as a function of the angular frequency, ω. This fit (Table 3) was performed using the built-in function of the BOHLIN CVOR-200 system software for the duplicate assays; estimated parameters were adjusted between a confidence interval of 95%.

Moresi et al. (2001) reported values of $A_\alpha = 8.38 \pm 0.19$ kPa rad$^{-\alpha}$ s$^\alpha$ and 10.94 ± 0.22 kPa rad$^{-\alpha}$ s$^\alpha$ for alginate gels with a concentration of 1.5% and 1.75%, for medium-range viscosity sodium alginate (Sigma-Chem) with a concentration of guluronic acid of 35% and mannuronic acid of 65%. The values of A_α reported are larger to those observed in our studies; this difference could be explained by the presence of proteins in blood plasma causing the "egg-box" structure, reported by Grant et al. (1973) for polysaccharides such as alginate, not being formed because of protein interference with guluronic acid binding, leading to lower gel strength.

Moresi et al. (2001) also found a single value of $\alpha = 0.050$ for concentrations of alginate between 1.0% and 1.75% in stoichiometric amount of calcium. The wide range of α values shown in Table 3 might be related to the excess of calcium used in the preparation of the gel, this is consistent with our results as for trials with 0.8% alginate and calcium chloride 1% the value of α is closer to 0.05 while at concentrations of calcium of 2% and 3% the value of α is much larger; also, at values of calcium of 2% but with a concentration of 1.6% alginate the value of α is again closer to 0.050 while at a concentration of 3% of calcium the value of α rises again. In a similar fashion, gel strength rises with increasing calcium chloride concentrations (Table 3) probably because it results in more cross-linked sites among guluronic acid units.

Since the magnitude of G' is much larger than that of G'' (see Fig. 4), the contribution of the viscous modulus, G'', to the magnitude of the complex modulus, G^*, is close to negligible, and therefore the complex modulus, G^*, is nearly equal to the elastic modulus, G'. This result implies that the physical behavior of these films is much closer to that of a solid (elastic modulus). Thus, the complex modulus, G^*, can be expressed by analogy as the elastic modulus, G', as shown in Eq. (7).

$$G^*(\omega) \cong G'(\omega) = \sqrt{\frac{2}{\pi}} s^*_\alpha \cos\left(\frac{\pi}{2}\alpha\right)\omega^\alpha \quad (7)$$

According to Table 3, the mixture of 1.6% sodium alginate with 3% calcium chloride (w/v) offers a higher complex modulus, which in turn will be reflected into a higher strength; therefore, this mixture was chosen for mechanical tensile strength testing.

4.3. Fabrication of sodium alginate/human blood plasma gels

The cross-linking rate of sodium alginate is fast enough to induce instantaneous gelation upon initial contact with calcium chloride. This result is similar with some reports which conclude that calcium alginate gelation is instantaneous, irreversible, and governed by the relative diffusion rate of calcium ions and polymer molecules within the gelation zone (Blandino et al., 1999). Some authors have

Table 3 – Mathematical model fit for frequency sweep assays to alginate/ blood plasma mixtures.

Assay [%alginate/ – %calcium chloride]	A_α (Pa rad$^{-\alpha}$ s$^\alpha$)	Confidence intervale for A_α,	α	Confidence intervale for α	r^2
0.8–1	279.5	277.95–281.05	0.0607	0.058–0.063	0.9550
0.8–2	386.6	382.42–390.81	0.1646	0.1598–0.1693	0.9404
0.8–3	431.05	427.54–434.58	0.1303	0.0094–0.016	0.9955
1.6–1	933.9	929.64–938.16	0.0650	0.063–0.067	0.9766
1.6–2	1405	1400.98–1409.02	0.0488	0.047–0.050	0.9829
1.6–3	2490	2420.47–2561.52	0.1040	0.0974–0.1105	0.0067

attempted to evaluate gelation kinetics which used glucono-δ-lactone, which acts as a calcium chelator, and then released the calcium to the extent that the pH decreases, thereby inducing progressive gelation (Moresi et al., 2004).

In this study, technology was implemented to produce thin films (1 mm thick) of the alginate/blood plasma mixture and then cross-link the films by adding sprayed calcium chloride, thus forming a uniform, cross-linked film. Using this method, it is possible to obtain films because the calcium solution is homogeneously distributed and begins diffusing through the mixture until it covers the entire film. Blandino et al. (1999) report that because the metallic cation is much smaller than the polymer molecules it will be the calcium that diffuses through alginate molecules and cross-links in sites unoccupied by the polymer. Once the cationic solution is added into the alginate, a membrane is instantaneously formed that grows in the flow direction of calcium ions (Ca^{2+}) until they reach the bottom of the film and complete the gelation process. This procedure is relatively easy and does not require a sophisticated setup. In addition, the procedure can be performed under sterile conditions in a laminar flow hood, and the commercial nozzle can be sterilized with humid water vapor (if made of stainless steel) or ethylene oxide (aluminum). Furthermore, because temperature changes are not required for gelation, cells can be added to the mixture.

This paper shows that it is not necessary to perform the purification of fibrinogen from human blood plasma for mixture with alginate for obtaining a gel. Using human plasma is possible to obtain directly to gel, which is closest to the medical procedure. The alginate-human plasma gel could be used for cells entrapment, release of drugs or simply as a wound dressing.

5. Conclusions

The aim of this work was the process standardization and rheological characterization of a gel based on a mixture of human blood plasma and sodium alginate solution. Mixtures of alginate/plasma at concentrations of 0.2%, 0.4%, 0.8% and 1.6% of sodium alginate were characterized by determination of its rheological behavior and ξ potential. As expected, the presence of alginate increased the viscosity of the mixture. Also, the ξ potential characterization gave that mixtures with 0.8% and 1.6% of sodium alginate were the more stable ones. After that, these mixtures were gelled using calcium chloride as cross-linking agent by spraying. On the other hand, by increasing the concentration of alginate, shear rate hysteresis decreases probably due to increased stability of the formed structure by the union or association between biomolecules. In addition, and according to dynamic rheology, the Viscoelastic Linear Region of the gels (VLR) was determined in a range up to 1% strain. Also, frequency sweep test between 0.1 Hz and 100 Hz showed that solid behavior is dominant along the whole range. Finally gels with 1.6% of alginate reached an ultimate tensile strength of 6.369 ± 0.243 kPa and showed a uniform superficial protein distribution.

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