Rheological Behavior of Alginate Solutions for Biomanufacturing

Rodrigo Alvarenga Rezende,[1,2] Paulo Jorge Bártolo,[1] Ausenda Mendes,[1] Rubens Maciel Filho[2]

[1]*Centre for Rapid and Sustainable Product Development, Mechanical Engineering Department, School of Technology and Management, Polytechnic Institute of Leiria, Leiria, Portugal*
[2]*Laboratory of Optimization, Design and Advanced Control, School of Chemical Engineering, State University of Campinas, Cidade Universitária "Zeferino Vaz", Campinas, São Paulo, CP 6066, Brazil*

Received 22 June 2007; accepted 20 May 2008
DOI 10.1002/app.30170
Published online 27 May 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The rheological behavior of alginate solutions were investigated for the optimal design of a biomanufacturing system to produce alginate structures for tissue engineering. Its rheological properties were determined by a rheometer through rotational and oscillatory tests. Experimental results were used to model the alginate solutions characteristics. The findings suggest that alginate solutions undergo shear-thinning effects with increasing shear rates. It is also possible to observe that its loss modulus is higher than the storage modulus ones being both modulus dependent upon the frequency, which is a typical characteristic of dilute solutions. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 3866–3871, 2009

Key words: biomaterials; rheology; hydrogels; biopolymers; viscoelastic properties

INTRODUCTION

Tissue engineering represents a novel, emerging interdisciplinary field involving combined efforts of biologists, engineers, material scientists, and mathematicians toward the development of biological substitutes to restore, maintain, or improve tissue functions.[1] It is emerging as a rapidly expanding approach to address the organ shortage problem. In 2003, in United States alone, 87,717 patients were waiting for organs' transplantation.[2] By June 2007, this number has increased to 96,670 as shown in Table I.[3]

In tissue engineering, therapeutic strategies involve cellular implantation, where cells are derived either from an endogenous source in the patient or from a donor are injected into the damaged tissue or combined *in vitro* with a degradable scaffold and then implanted. A scaffold directly implanted into the damaged tissue stimulates the cells promoting local tissue repair.[4]

Scaffolds are support structures used in tissue engineering to provide the three-dimensional growth of cells in an organized way. They are produced from either natural materials (collagen, hydroxyapatite, alginate, etc.) or synthetic polymers (polyglycolic acid, polylactic acid, etc.), which are biocompatible and bioabsorbable, nonimmunogenic, supporting cell growth.[5–7] Scaffolds must also degrade slowly after implantation in the patient, to be replaced by new tissue.[8,9] Biodegradable scaffolds can play an important role as delivery vehicles for the sustained release of tissue growth factors.[10] Ideally, appropriate designed tissue engineering scaffolds promote natural wound healing and regeneration. Recently, a biomanufacturing system, specifically designed for soft tissue repair applications.[6,11] This system is adapted to produce alginate scaffolds by extruding, layer-by-layer, a solution of sodium alginate into a calcium chloride solution (cross-linker agent). The system illustrated in Figure 1, comprises two nozzles, one for the sodium alginate and the other for the calcium chloride deposition not shown in the Figure. In this additive technology, the designed scaffold CAD model is first converted into STL format (a tessellated model that approximates the surfaces representing the solid with a set of triangles), and then sliced into a group of two-dimensional layers, to where the scaffold material is deposited to build the final structure in a layer-by-layer way. The scaffold structure could be controlled through the material properties, process parameters such as pressure, nozzle diameter, distance between the nozzle and the fabrication platform, and the velocity of the nozzle that is controlled through a computer-controlled system.

Correspondence to: P. J. Bártolo (pbartolo@estg.ipleiria.pt).
Contract grant sponsors: The Foundation for Science and Technology (through the projects POCI/SAU-BMA/60287/2004 and POCTI/EME/60650/2004).

TABLE I
The United Network for Organ Sharing Waiting List for Organ Transplant

Type of transplant	No. of patients waiting for transplant
Kidney	72,015
Liver	16,855
Pancreas	1686
Kidney/Pancreas	2337
Heart	2702
Lung	2724
Heart/Lung	119
Intestine	231
All organs	96,670

Alginate scaffolds are usually characterized by smooth surfaces, depending on the cross-linking process, i.e., on the concentration of both alginate and cross-linker as shown in Figure 2. The fabrication of porous alginate scaffolds involves a three-step procedure:[12,13]

- Cross-linking the alginate solution with an appropriate solution.
- Freezing the cross-linked alginate structure.
- Removal of the ice crystals by sublimation.

This article investigates the rheological behavior of alginate solutions, which is particularly important to design an optimized extrusion device and more stable alginate structures. Data collected are important to determine optimal design parameters for the biomanufacturing system, which uses nozzles with different geometries and dimensions and constant cross-sectional area. In the case of circular nozzles, the inner diameter ranges from 1.5 to 0.3 mm with a length of 40 mm. The effect of the type of alginate material, processing parameters, and nozzle characteristics on scaffold structural uniformity and dimensions is not considered.

ALGINATE

Alginate is an anionic copolymer of (1→4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues (Fig. 3).[14,15] The residues are arranged in a blockwise manner with G blocks and M blocks interspersed with MG alternating blocks.

The industrial manufacture of alginate is based on the extraction of a polymer from brown algae. The seaweed grows in nature mainly in temperate areas, but large amounts are also cultivated in other regions like the Far East, the coast of China or Japan. The seaweed is extracted with a dilute alkaline solution, which solubilizes the alginic acid present. Free alginic acid is obtained treating the resulting viscous material with mineral acids, being then converted to a salt. Sodium alginate is the major form currently used.

Sodium alginate is soluble in water and, when dissolved, forms a viscous solution depending on the concentration and molecular weight of the polymer. Gelation occurs when divalent ions (Ca^{2+}, Ba^{2+}, Fe^{2+}, Sr^{2+}, etc.) or trivalent ions (Al^{3+}, etc.) take part in the interchain ionic binding between G blocks in the polymer chain, giving rise to a three-dimensional network. Such binding zones between the G blocks are often referred to as "egg boxes" (Fig. 4). These ions act as cross-linkers that stabilize alginate chains forming a gel structure, which contains cross-linked chains interspersed with more freely movable chains that bind and entrap large quantities of water. The gelification process is characterized by a reorganization of the gel network accompanied by the expulsion of water.[16]

Gels made of M-rich alginate are softer and more fragile, and may also have lower porosity. This is due to the lower binding strength between the polymer chains and to higher flexibilities of the molecules. The gelification process is highly dependent upon diffusion of gelification ions into the polymer network. Transmittance, swelling, and viscoelasticity

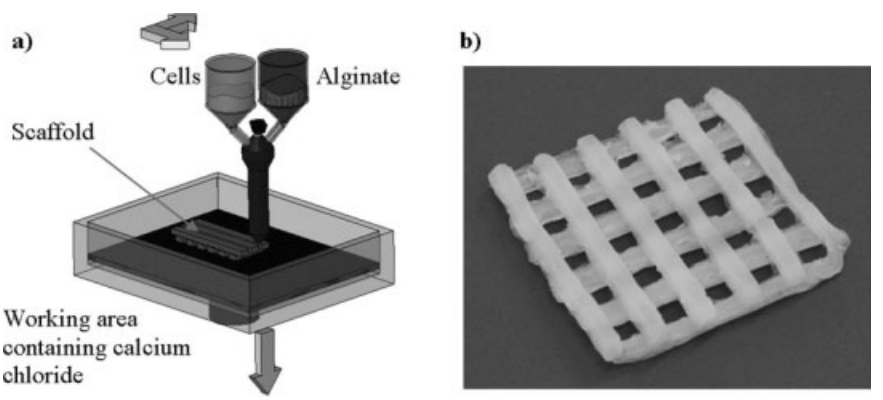

Figure 1 Alginate-based biomanufacturing system (a), and alginate scaffold structure (b).

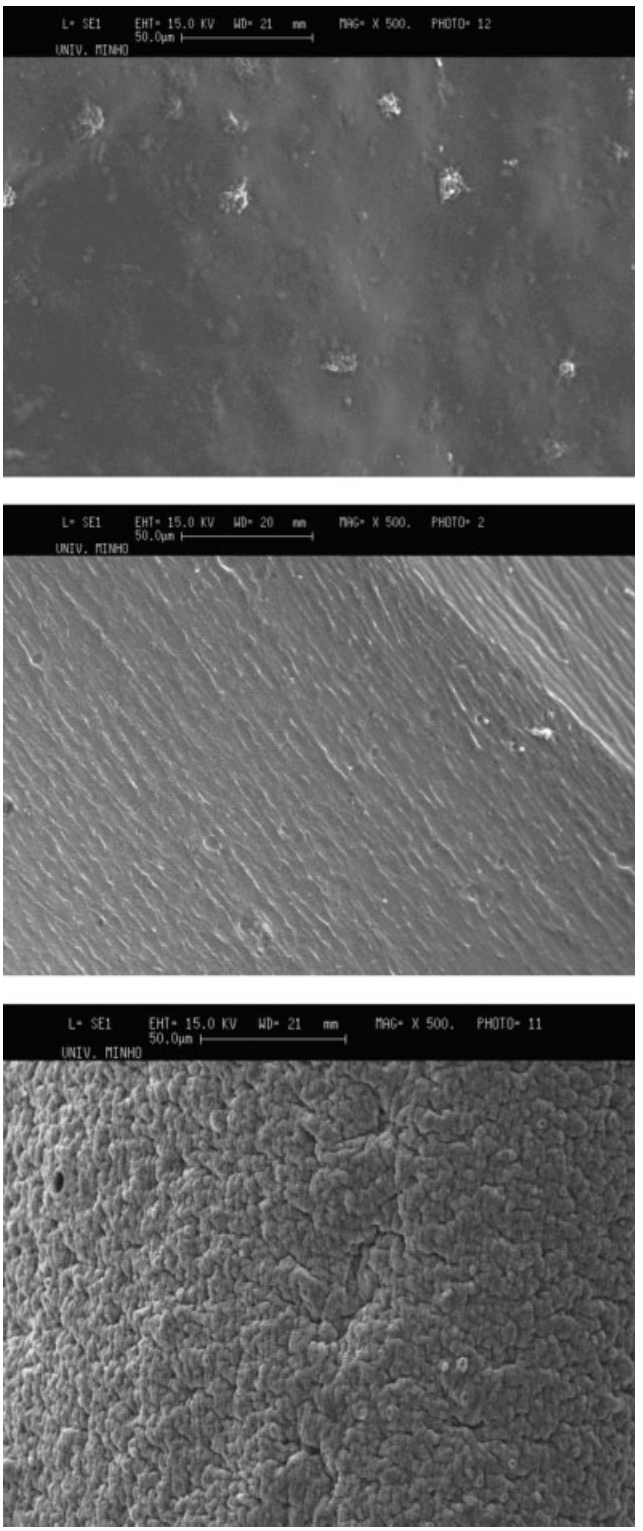

Figure 2 Surface morphology of alginate structures obtained after 48 h of gelation for two different solutions containing different concentrations of alginate and $CaCl_2$: (a) solution containing 3% of alginate mixed with a solution of 1% of $CaCl_2$; (b) solution containing 3% of alginate mixed with a solution of 3% of $CaCl_2$; (c) solution containing 2% of alginate mixed with a solution of of 1% of $CaCl_2$.

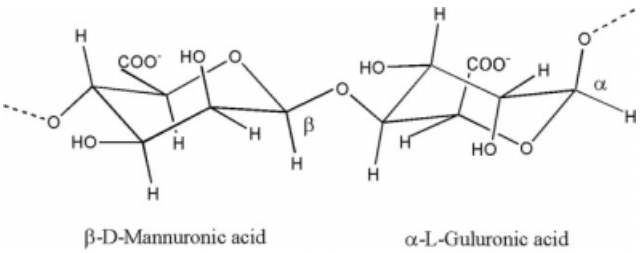

Figure 3 Structure of an alginate showing a linkage between the M and G acids.

of alginate structures are highly affected by the M/G ratio.

Alginic acid and its sodium and calcium salts are nontoxic and biocompatible, being widely used in the medical, pharmaceutical, cosmetic, and food industry.[17] In tissue engineering, alginate gels are currently explored for cell encapsulation and drug delivery.[18,19] For instance, the encapsulation of islets of Langerhans and parathyroid tissue for the treatment of diabetes mellitus and hypoparathyroidism, or the encapsulation of human chondrocytes and mesenchymal stem cells for cartilage repair.[20–23] Alginate materials are also important for wound healing, as they can be converted into a hydrophilic gel by an ion-exchange interaction between calcium in alginate and sodium in the blood and wound fluid.[24]

EXPERIMENTAL

Materials

Sodium alginate was purchased at Panreac (Barcelona, Spain). Calcium chloride was supplied by Carlo Erba (Milano, Italy). All solutions were prepared with pure water, with a conductivity of 0.054 µS/cm. Alginate solutions were prepared by the addition of weighted portions of sodium alginate to measured water volumes. These solutions were agitated by orbital shaking for 3 h at 50°C to ensure good homogeneity, due to their high viscosity. Calcium chloride solution 5% (w/v) was obtained dissolving the salt into water. This solution was diluted to obtain solutions with different concentrations of calcium chloride.

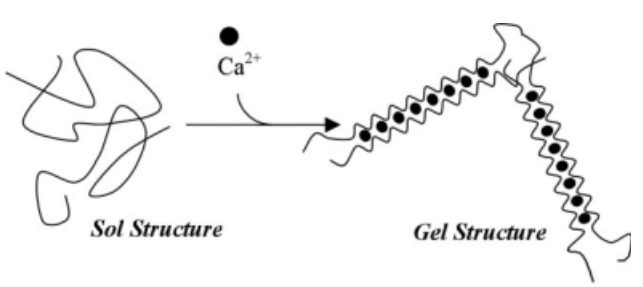

Figure 4 Alginate gelling obtained from reticulation of the chains by calcium ions.

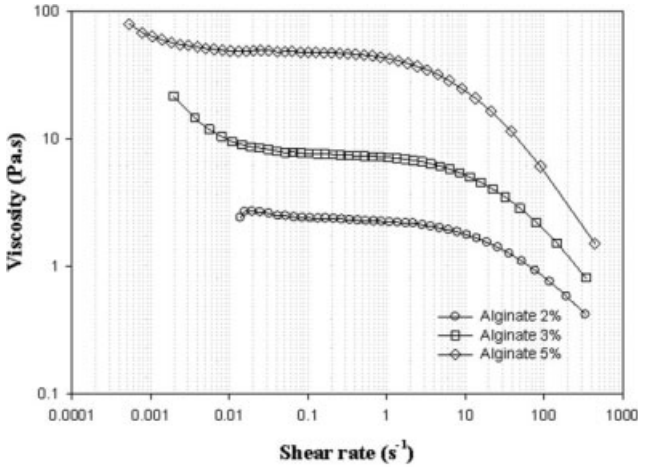

Figure 5 Viscosity variation as a function of shear rate for different alginate solutions.

Rheological tests

The rheological analysis of alginate solutions was carried out using a Reologica StressTech HR rotational rheometer fitted with plate-plate geometry. Two different rheological measurements were made to characterize the alginate solutions. First, rotational tests were considered. It is assumed that the material flows by applying a stress being the response measured alongside time or temperature (not considered in this work). A controlled stress is applied and the resulting movement is measured. The rotational speed depends on sample viscosity, computed by means of stress and shear rate. Second, oscillatory rheological measurements were made over a frequency range from 0.01 to 10 Hz. Sample deformation depends on both frequency and stress. All measurements were carried out at room temperature.

RESULTS AND DISCUSSION

Rotational analysis

Figures 5 shows that viscosity decreases with shear rate indicating a shear-thinning behavior. Increasing the alginate content increases both the viscosity and stress for a specific value of shear rate. The power-law model was used to fit the experimental data. According to this model, the rheological behavior of a material is described by:

$$\tau = k\dot{\gamma}^{n} \tag{1}$$

where τ is the shear stress (Pa), γ the shear rate (s^{-1}), k the consistency index (Pa s^n) and n is the power law index (dimensionless). Three range values can be identified for n:

$n < 1$: shear-thinning system
$n = 1$: Newtonian system
$n > 1$: shear-thickening system.

Table II indicates the values of the power law coefficients for different alginate solutions. A good approximation ($R^2 \approx 0.99$) between the power law and experimental data was obtained. The results confirm that the alginate solution is a shear-thinning system and that the consistency index increases with the alginate content. This observation is particularly important as the nozzles used in the biomanufacturing system can be small in diameter to control macroporosity, reducing the effective flow rate and requiring higher pressures. The shear-thinning behavior of alginate solution facilitates the flow compared with a Newtonian fluid allowing high flow rates for equal deposition pressures. In the case of circular nozzles, the length is 40 mm providing a maximum length-to-diameter ratio (L/D) of 133.3. For solutions containing 3% of alginate the extrusion velocity is 50 mm/s, which allows viscous flows with Reynolds numbers ranging between 0.003 and 0.015, far below the turbulent flow transition boundary. Similar results are observed for the other solutions, with Reynolds numbers ranging from 0.0004 to 0.045. The density of each alginate solution considered in this study varies from 1.11 g/cm^3 and 1.4 g/cm^3.

For a viscous Newtonian fluid, the flow rate is given by the Hagen-Poiseuille equation:[25]

$$Q = \frac{\pi \Delta P}{128\ \eta L} d^4 \tag{2}$$

that establishes a direct proportionality among the flow rate (Q), the pressure differential (ΔP), and nozzle diameter (d), and an inverse proportionality between the flow rate and the viscosity (η), and nozzle length (L). For shear-thinning materials, viscosity decreases with shear rate and the flow rate is given by the following equation:[25]

$$Q = \frac{n(n+1)}{(3n+1)(n+1)} \frac{\pi(\dot{\gamma}_0)^{(n-1)/n}}{(2\eta_0)^{1/n}} \left(\frac{\Delta P}{L}\right)^{1/n} R^{(3n+1)/n} \tag{3}$$

where n is the power-law index, R is the nozzle radius.

TABLE II
Coefficients of the Power Law for Different Alginate Solutions

Alginate content (%)	k (Pa s^n)	n
2	2	0.87
3	6	0.84
5	28	0.84

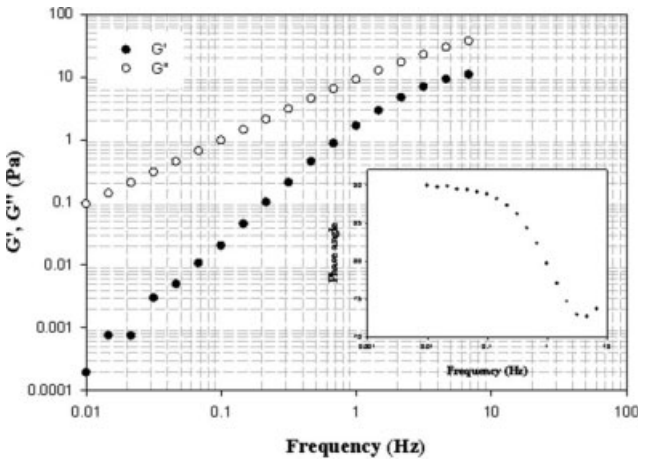

Figure 6 Log-log plot of G'', G' vs. frequency and phase angle vs. frequency for an alginate solution containing 2 wt % of alginate.

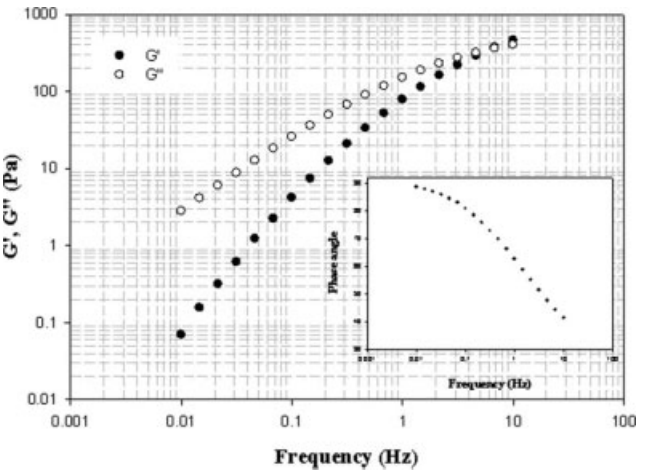

Figure 8 Log-log plot of G'', G' vs. frequency and phase angle vs. frequency for an alginate solution containing 5 wt % of alginate.

Oscillatory analysis

The viscous and elastic responses of viscoelastic systems can be quantified by undertaking dynamic oscillatory measurements. The basis of these measurements is the application of a sinusoidal strain of frequency ω to the system and the measurement of its corresponding stress. For viscoelastic systems, the stress and the strain are out of phase. Oscillatory analysis enables to determine several viscoelastic parameters, such as the complex modulus (G^*), the in-phase elastic component or storage modulus (G') and the out-of-phase viscous component or loss modulus (G'') of the complex modulus and tan δ. The relationships among these parameters are given by the following equations:

$$G^* = \frac{\tau}{\gamma} \tag{4}$$

$$G^* = \sqrt{G'^2 + G''^2} \tag{5}$$

$$G' = G^* \cos\delta \tag{6}$$

$$G'' = G^* \sin\delta \tag{7}$$

Figures 6 to 8 show how the store modulus and loss modulus change as a function of frequency. These modulus increases with frequency as demonstrated by findings. It is also possible to observe that the loss modulus is always higher than the storage one, being the viscous behavior the dominant effect. However, for high alginate content solutions and

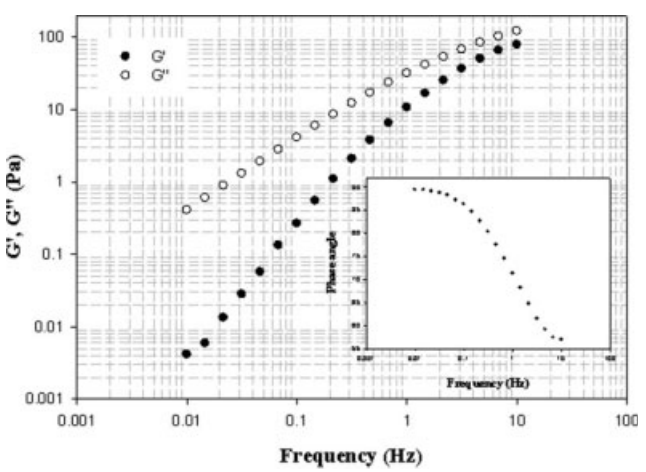

Figure 7 Log-log plot of G'', G' vs. frequency and phase angle vs. frequency for an alginate solution containing 3 wt % of alginate.

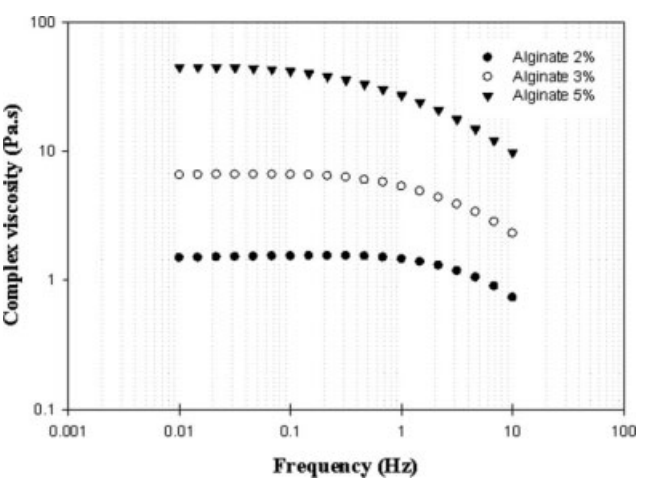

Figure 9 Variation of complex viscosity as a function of frequency for different alginate solutions.

high frequency a shift in this trend can be observed showing a more elastic behavior. Figures also show how the phase angle varies with frequency. At low frequencies (long time scales), the response of alginate solutions is viscous. The phase angle decreases as the frequency increases. For alginate solutions with high alginate content, the system becomes more elastic as the frequency increases.

The complex viscosity variation as a function of frequency is shown in Figure 9. For solutions containing low alginate contents (lower than 5%), the complex viscosity is almost constant. Comparing Figures 9 and 1, it is possible to observe that the Cox-Merz rule hold for these alginates.

CONCLUSIONS

The alginate-based biomanufacturing system is a fabrication technique developed to produce alginate solid structures, by extruding a solution of sodium alginate, mixed with a solution of calcium chloride, providing a temporary support for the seeded cells in culture. The understanding of the rheological behavior of alginate solutions is fundamental either to design optimized extrusion devices or to ensure stable flows. Rotational and oscillatory dynamic tests were carried out to characterize the rheological behavior of different alginate solutions. Experimental results were used to model the alginate solutions characteristics. Stresses and shear rates were correlated using the power-law model. A good correlation was achieved. The results suggest that alginate solutions undergo shear-thinning effects with increasing shear. These findings are particularly important as the shear-thinning effects can be used to maximize flow rates and minimize viscosity.

Results also show that loss modulus is higher than the storage one, being both modulus dependent upon the frequency, which is a typical characteristic of a dilute solution. Increasing the alginate content and frequency enables both modulus to become closer in value.

References

1. Langer, R.; Vacanti, J. P. Science 1993, 260, 920.
2. Mendes, A.; Lagoa, R.; Bártolo, P. J. Proceedings of the 1st International Conference on Advanced Research in Virtual and Rapid Prototyping; Bártolo, P. J., Ed.; Leiria, Portugal, 2003.
3. UNOS website: http://www.unos.org, accessed on July 2007.
4. Bártolo, P. J.; Amorim, H.; Laoui, T. Int J Mater Prod Technol, to appear.
5. Desai, S.; Bártolo, P. J.; Bidanda, B. In Bio-materials & Prototyping Applications in Medicine; Bidanda, B.; Bártolo, P. J., Eds.; Springer: New York, 2007; Chapter 1.
6. Bártolo, P. J. In Design and Nature III: Comparing Design in Nature with Science and Engineering; Brebbia, C. A., Ed.; WIT Press: Southampton, 2006.
7. Gomes, M. E.; Reis, R. L. Int Mater Rev 2004, 49, 274.
8. Lee, J. J.; Lee, S. G.; Park, J. C.; Yang, Y. I.; Kim, J. K. Curr Appl Phys 2007, 7, 37.
9. Sung, H. J.; Meredith, C.; Johnson, C.; Galis, Z. S. Biomaterials 2004, 25, 5735.
10. He, H.; Cao, X.; Lee, L. J. J Control Release 2004, 95, 391.
11. Bártolo, P. J.; Mendes, A.; Jardini, A. In Design and Nature II; Collins, M. W.; Brebbia, C. A., Eds.; WIT Press: Southampton, UK, 2004.
12. Zmora, S.; Glicklis, R.; Cohen, S. Biomaterials 2002, 23, 4087.
13. Shapiro, L.; Cohen, S. Biomaterials 1997, 18, 581.
14. Draget, K. I.; Braek, G. S.; Stokke, B. T. Food Hydrocolloids 2006, 20, 170.
15. Eiselt, P.; Yeh, J.; Latvala, R. K.; Shea, L. D.; Mooney, D. J. Biomaterials 2000, 21, 1921.
16. Serp, D.; Mueller, M.; Stockar, U.; Marison, I. W. Biotechnol Bioeng 2002, 79, 253.
17. Gombotz, W. R.; Wee, S. W. Adv Drug Deliv Rev 1998, 31, 267.
18. Wandrey, C.; Espinosa, D.; Rehor, A.; Hunkeler, D. J. Microencapsulation 2003, 20, 597.
19. Tønnesen, H. H.; Karlsen, J. Drug Dev Ind Pharm 2002, 28, 621.
20. Schneider, S.; Feilen, P. J.; Brunnenmeier, F.; Minnemaan, T.; Zimmermann, H.; Zimmermann, U. Diabetes 2005, 54, 687.
21. Hasse, C.; Klöck, G.; Schlosser, A.; Zimmermann, U.; Rothmund, M. Lancet 1997, 350, 1926.
22. Weber, M.; Steinert, A.; Jork, A.; Dimmler, A.; Thürmer, F.; Schütze, N. Biomaterials 2002, 23, 2003.
23. Chung, T. W.; Yang, J.; Akaike, T.; Cho, K. Y.; Nah, J. W.; Kim, S.; Cho, C. S. Biomaterials 2002, 23, 2827.
24. Ichioka, S.; Harii, K.; Nakahara, M.; Sato, Y. Scand J Plast Reconstr Hand Surg 1998, 32, 311.
25. Crawford, R. J. Plastics Engineering; Butterworth-Heinemann: Oxford, 1998.