Simple Method to Determine Flow Characteristics of Injectable Hydrogels

Sundar Babu Nadarajan, An Nguyen, James Thomas Kolb, Leonard Stepanskiy, Elisabeth S. Papazoglou

School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania 19104

Received 2 December 2009; accepted 6 March 2010
DOI 10.1002/app.32402
Published online 21 May 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This article presents a simple inexpensive method to measure flow and viscoelastic properties of calcium alginate hydrogels or gel solutions and is particularly suitable for quality control in small laboratory settings. This method can be considered an extension of the Melt Flow Index (MFI) method, which is routinely used in the polymer industry to indirectly determine the molecular weight of polymers. Results of alginate gels at various crosslinking densities obtained with this new test are compared to complex viscosity data obtained from a standard cone and plate rheometer, and demonstrate very good correlation. A mathematical model describing the apparent viscosity of the alginate gels in this test has also been developed. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 574–579, 2010

Key words: degree of polymerization; drug delivery systems; hydrogels; modulus; viscoelastic properties

INTRODUCTION

Biocompatible and relatively inexpensive alginate hydrogels continue to be critical for a variety of applications from controlled delivery of drugs,[1–3] vaccines to functional tissue engineering scaffolds,[4–7] and alginate hydrogels are synthesized by crosslinking naturally derived alginic acid (usually its sodium salt) with various divalent cations such as calcium and strontium (Magnesium is an exception as it does not result in crosslinking). The physical properties of alginate hydrogels can be easily controlled by varying the concentration of sodium alginate and/or by varying the concentration of the crosslinking agent.[8] For applications in vaccine delivery or for injectable subcutaneous and intramuscular drug delivery systems, it is critical that the gels maintain their integrity after ejection from the needle, a high shear operation. Hydrogels with high crosslinking density thus "stronger" gels can withstand this shear force, however their drug release characteristics may be too slow for the intended application. Release of the trapped molecule from the alginate gel occurs due to both biodegradation of the alginate scaffold and diffusion through the pores of the hydrogel matrix.[9,10] The release behavior is also greatly dependent on the environment,[11] for example, the physical characteristics of hydrogels for subcutaneous delivery will be different from those used in oral delivery applications.

In a manufacturing environment, once the optimal hydrogel formulation has been selected, it is critical to maintain batch-to-batch consistency and determine the limits of storage stability for these hydrogels. The rheological behavior of a hydrogel can be used to determine its stability and assess batch to batch consistency. However, rheological characterizations are time consuming, requiring highly trained personnel and expensive instruments.[12] There are several types of rheometers, such as cone and plate or dynamic rheometers that can provide complete rheological characterization at significant cost of equipment and trained personnel.[13] The average cost of a rheometer ranges from $60,000–$150,000, and this alone renders it cost prohibitive for many small quality control laboratories. Alternatively, following the practice in the synthetic polymer industry, one can use an apparent viscosity measure as an indication of the material's behavior under extrusion. High pressure operations can be simulated with proper system design and allow fast characterization of the rheological properties of the hydrogel. In this article, we report the design, use, and validation of a simple syringe flow system that can provide inexpensive and fast characterization of a hydrogel to test its stability and its batch to batch consistency. Our method uses a minimal amount of sample, less than 1 mL of hydrogel, and does not rely on expensive instrumentation.

Correspondence to: E. S. Papazoglou (esp25@drexel.edu).

FLOW INDEX

In the polymer industry, the melt flow index (ASTM D1238) is a material property widely used for material characterization, despite its limitations.[14] Melt flow index (MFI) is defined as the rate at which a polymer flows through an extruder of fixed orifice diameter and length under a constant load.[15] Melt flow data are used for quality control, although most agree that the shear rate is too low to represent extrusion and only partially it can represent molding operations. Also, MFI is insensitive to molecular weight distribution effects in the low shear rate region.[13,16,17] The equipment used in the polymer industry requires a heated barrel (due to the melting and flow properties of these materials), where the sample is allowed to flow under a specified weight placed on top of the cylindrical barrel. To determine MFI, 5 grams of polymer is melted and allowed to flow at a certain temperature and pressure. The mass extruded is measured and the MFI is expressed as mass extruded in a given amount of time. ASTM D1238 standards determine the weight of the load to be placed on the barrel and the temperature of the test for a specific polymer.

In our design, we have attempted to maintain the characteristics of the MFI so that the procedure could be easily extended into an ASTM test, but we have tried to represent the conditions of interest for injectable hydrogels. No sample melting is necessary for hydrogels since these gels are able to flow at room temperature. In our study, we have demonstrated the capability of the system to characterize sodium alginate hydrogels crosslinked by $CaCl_2$ prepared by using the method described in US Patent 2,635,067.[18] We have then compared our data with rheological measurements, to assess the validity of our method. The results demonstrate that the syringe extruder system we designed can be used as a quality control tool. Further engineering design improvements can be implemented, but the principles of the methodology are demonstrated in this article.

In addition to comparing our data with rheological measurements, we also calculated an apparent viscosity based on the methodology introduced by A. Dutta.[19] Briefly, a barrel with a known radius (R_A) is connected to a capillary with known radius (R) and length (L). The gel at a constant temperature is loaded into the barrel and a load (W) drives a piston through the barrel, extruding the hydrogel through the capillary. Equation (1) was derived from the conclusions drawn in Dutta's work:

$$\eta_0 = \frac{WR^4}{8LQ_\infty R_A^2} \quad (1)$$

where η_0 is viscosity (Pas), W is the load (kg), R is the radius of the capillary (m), L is the length of the capillary (m), Q_∞ is the flow rate (m^3/s), and R_A is the barrel radius (m). Equation (1) was used in our study to report "calculated viscosity" from flow data through the setup of Figure 1.

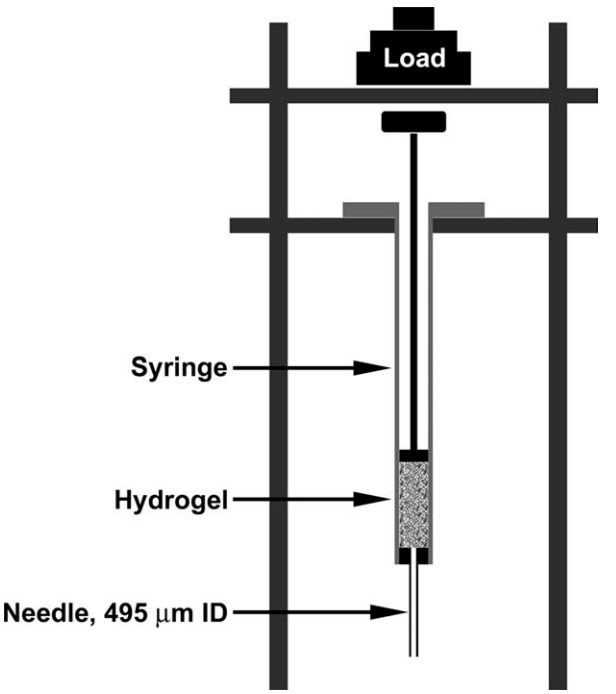

Figure 1 Syringe extruder setup.

MATERIALS AND METHODS

Alginate hydrogel preparation

All alginate hydrogels were prepared in 0.9% saline solution. Calcium chloride (97%), purchased from Sigma (St. Louis, MO) was used to prepare $CaCl_2$ solutions at 0.02%, 0.06%, and 0.10% concentrations. Low viscosity ($\sim$ 250cP) sodium alginate with high mannuronic acid content, purchased from Sigma, was subsequently added to the aqueous calcium chloride solutions taken in a 15 mL centrifuge tube at 3%, 4%, and 5% concentrations. Each of the $CaCl_2$ solutions was paired with each of the hydrogel concentrations so that a total of nine combinations were explored. Exploring such a matrix allowed us to evaluate the effect of the ratio of alginate to crosslinker concentration on the rigidity of the resulting hydrogels.

Sodium alginate in powder form was added to the $CaCl_2$ solution in a stepwise manner to prevent lumping and inhomogenous crosslinking density. Centrifuge tubes containing sodium alginate and $CaCl_2$ solution were vigorously shaken for 24 h at 25°C. Step wise addition and 24 h mixing yields more homogeneous materials.

Syringe extruder design

A syringe extruder as shown in Figure 1, consists of two platforms; the stationary lower platform is designed to hold a 1 mL disposable plastic syringe with Luer-Lok tip in place while the upper platform is able to slide vertically to apply pressure to the plunger. A 100 g weight positioned on the moveable platform, collectively weighing 194.55 g, produces the force necessary to extrude the hydrogel. A 21G needle with inner diameter (ID) of 495 μm and length cut to 1.4 mm served the purpose of an orifice. The ID of the syringe was 4.80 mm.

Hydrogels were loaded into the syringe by removing the plunger and pouring in the hydrogel ensuring no air is entrapped. Air bubbles were removed by tapping the sides. Typically, after securing the hydrogel-loaded syringe to the stage of this setup, the platform was released so that the plunger moves downwards due to its own weight. The mass of extruded hydrogel was measured at various time intervals and the recorded values of both mass and time were used to calculate flow index. The time necessary to extrude 200 μL of the hydrogel was used to calculate a flow rate. This process was repeated five times until the 1 mL sample had been completely extruded. The flow rate represents the average of these five points and all experiments were repeated in triplicate.

Rheological characterization (cone and plate rheometer)

To assess the relevance of our approach in representing an apparent rheological measure, we compared our data to full rheological characterization using a Bohlin cone and plate rheometer. The data was collected in the frequency range of 10 Hz to 0.1 Hz. Data analysis revealed that the samples exhibited stable viscous modulus values at 1 Hz range. Later an amplitude sweep was performed between 0.3 and 100 Pa. Again, the sweep data was checked for the best range, as this will vary more than the frequency between samples. A single frequency test for the sample using the frequency and amplitude that was most appropriate was performed. After the single frequency test was complete, the spindle was locked and raised.

Mathematical model

A simple force balance on the control volume of the syringe extruder set up we used results in:

$$p\pi a_0^2 = (8\mu L_0 V_0/a_0^2)\pi a_0^2 + (8\mu L V_0 a_0^2/a^2)\pi a_o^2 + 1,5\mu\frac{V_0}{a_0}[1 + 2.8\ln(a_0/a)]\pi a_0^2 \quad (2)$$

where p is the pressure acting on the plunger; μ is the dynamic viscosity of the hydrogel; and a_0, L_0, a, L are the dimensions shown in Figure 2. The first term of the right-hand side of eq. (2) represents the shear stresses on the walls of the syringe extruder; the second term represents the shear stresses acting on the surface of the capillary needle, and the third term corresponds to the pressure in the entrance of the capillary (needle).

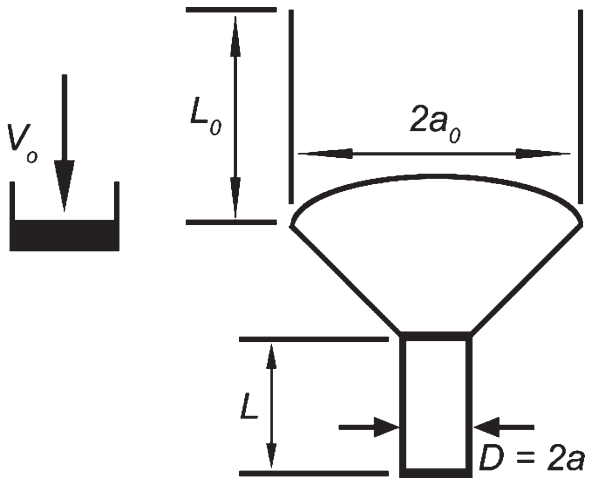

Figure 2 Schematic of the syringe extruder showing the dimensions used in eq. (2).

The above equation can be simplified as:

$$p = \mu V_0\{8L_0/a_0^2 + 8La_0^2/a^4 + 1,5\frac{1}{a_0}[1 + 2.8\ln(a_0/a)]\} \quad (3)$$

which allows the flow index F_i to be represented by:

$$F_i = \rho\pi a_0^2 V_0 = \frac{\rho\pi a_0^2 p}{\mu[8L_0/a_0^2 + 8La_0^2/a^4 + 1.5/a_0 + (4.2/a_0)\ln(a_0/a)]} = \frac{\rho W}{\mu[8L_0/a_0^2 + 8La_0^2/a^4 + 1.5/a_0 + (4.2/a_0)\ln(a_0/a)]} \quad (4)$$

where ρ is the density of the hydrogel and W is the weight acting on the plunger;

$$W = \pi a_0^2 p \quad (5)$$

Let us assume that the dynamic viscosity of the extruded hydrogel can be represented as a function of the alginate and calcium chloride concentrations in the following manner:

$$\mu = \mu_0 \exp(\mu_1 C_1 + \mu_2 C_2) \quad (6)$$

where C_1 is the alginate concentration (%); C_2 is the calcium chloride concentration (%); and the terms μ_0, μ_1, μ_2 are characteristic constants defined as the regression coefficients of the linear regression

$$Z = b + \mu_1 X + \mu_2 Y \tag{7}$$

Here

$$Z = \ln\left\{\frac{\rho\pi a_0^2 p}{[8L_0/a_0^2 + 8La_0^2/a^4 + 1.5/a_0 + (4.2/a_0)\ln(a_0/a)]F_i}\right\}$$

$$Z = \ln\left\{\frac{W\rho}{[8L_0/a_0^2 + 8La_0^2/a^4 + 1.5/a_0 + (4.2/a_0)\ln(a_0/a)]F_i}\right\}$$

$$b = \ln \mu_0 \qquad X = C_1 \qquad Y = C_2$$

If N were the total number of experiments and Z_i, X_i, Y_i are values of Z, X, Y in the ith experiment. Then the constants μ_0, μ_1, μ_2 are:

$$\mu_1 = \frac{\sigma_z}{\sigma_x}\frac{r_{zx} - r_{xy}r_{zy}}{1 - r_{xy}^2} \qquad \mu_2 = \frac{\sigma_z}{\sigma_y}\frac{r_{zy} - r_{xy}r_{zx}}{1 - r_{xy}^2}$$

$$b = \bar{Z} - \mu_1\bar{X} - \mu_2\bar{Y}$$

Here

$$\sigma_z = \sqrt{\frac{\sum_{i=1}^{N}(\bar{Z} - Z_i)^2}{N-1}} \qquad \sigma_y = \sqrt{\frac{\sum_{i=1}^{N}(\bar{Y} - Y_i)^2}{N-1}}$$

$$\sigma_x = \sqrt{\frac{\sum_{i=1}^{N}(\bar{X} - X_i)^2}{N-1}}$$

$$\bar{Z} = \frac{1}{N}\sum_{i=1}^{N} Z_i \qquad \bar{Y} = \frac{1}{N}\sum_{i=1}^{N} Y_i \qquad \bar{X} = \frac{1}{N}\sum_{i=1}^{N} X_i$$

$$r_{xy} = \frac{\sum_{i=1}^{N}(\bar{Y} - Y_i)(\bar{X} - X_i)}{(N-1)\sigma_y\sigma_x}$$

$$r_{zz} = \frac{\sum_{i=1}^{N}(\bar{Z} - Z_i)(\bar{X} - X_i)}{(N-1)\sigma_z\sigma_x}$$

$$r_{zy} = \frac{\sum_{i=1}^{N}(\bar{Z} - Z_i)(\bar{Y} - Y_i)}{(N-1)\sigma_z\sigma_y}$$

RESULTS AND DISCUSSION

The optimal length of the orifice used in this study was found to be 1.4 cm at a load of 194.5 g since longer or shorter lengths resulted in impractical flow rates.

In this discussion, flow rate was defined as volume extruded per second whereas flow index was calculated as grams extruded per minute. As a note, in the polymer literature MFI is reported as mass of flow per 10 min. As expected due to increased crosslinking density, for all $CaCl_2$ concentrations, increasing alginate content decreases flow rate. The flow rate is inversely related to the concentration of alginate or $CaCl_2$ [see Fig. 3(a)], and the concentration of $CaCl_2$ seems to have a non-linear (especially at higher concentrations of alginate) effect on the flow rate at various concentrations of alginate. The flow index (FI) is defined as grams extruded per minute. As shown in Figure 3(b), the FI decreases with increased crosslinking ($CaCl_2$ concentration) and the rate of decrease is higher at low alginate concentrations. It appears that at lower alginate concentrations, the rigidity of the hydrogel is significantly affected by calcium chloride concentration. It is worth noting that the flow index of hydrogels with higher alginate concentrations show reduced variation between samples with increasing calcium chloride concentration, indicating a tighter and more homogenous network.

Unlike flow rate and flow index, viscosity has, as expected, a positive correlation with hydrogel

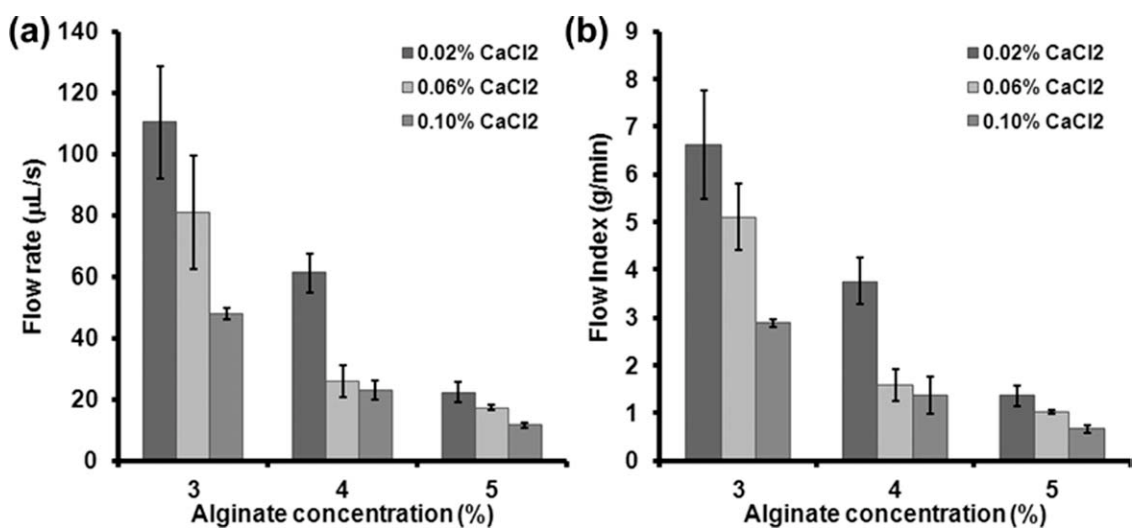

Figure 3 Effect of alginate and calcium chloride concentration on (a) flow rate and (b) flow index.

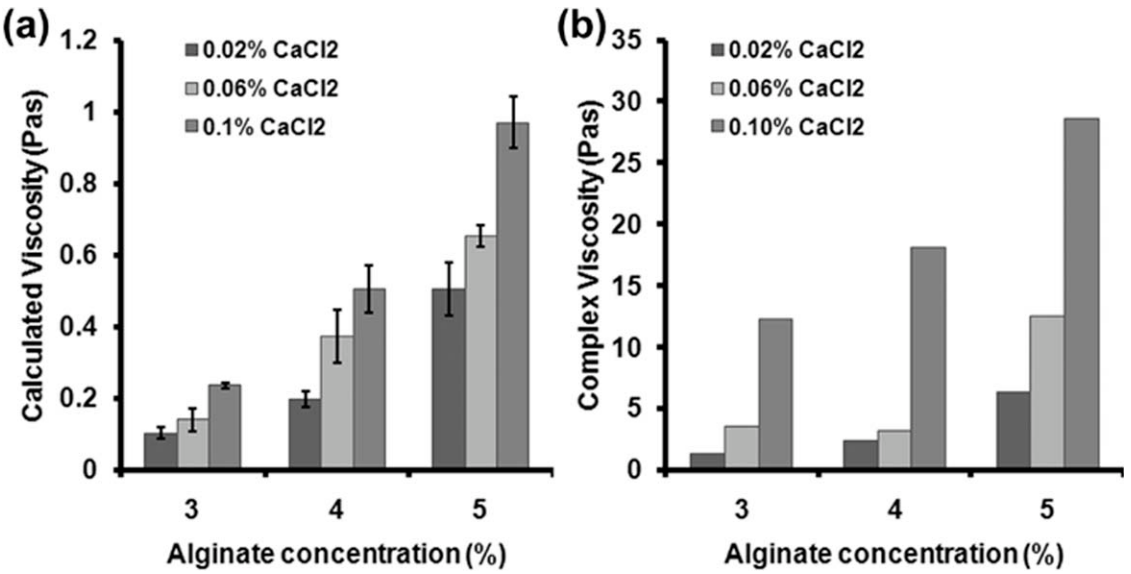

Figure 4 Effect of sodium alginate concentration on (a) calculated viscosity as determined using eq. (1) and (b) complex viscosity as measured by a rheometer.

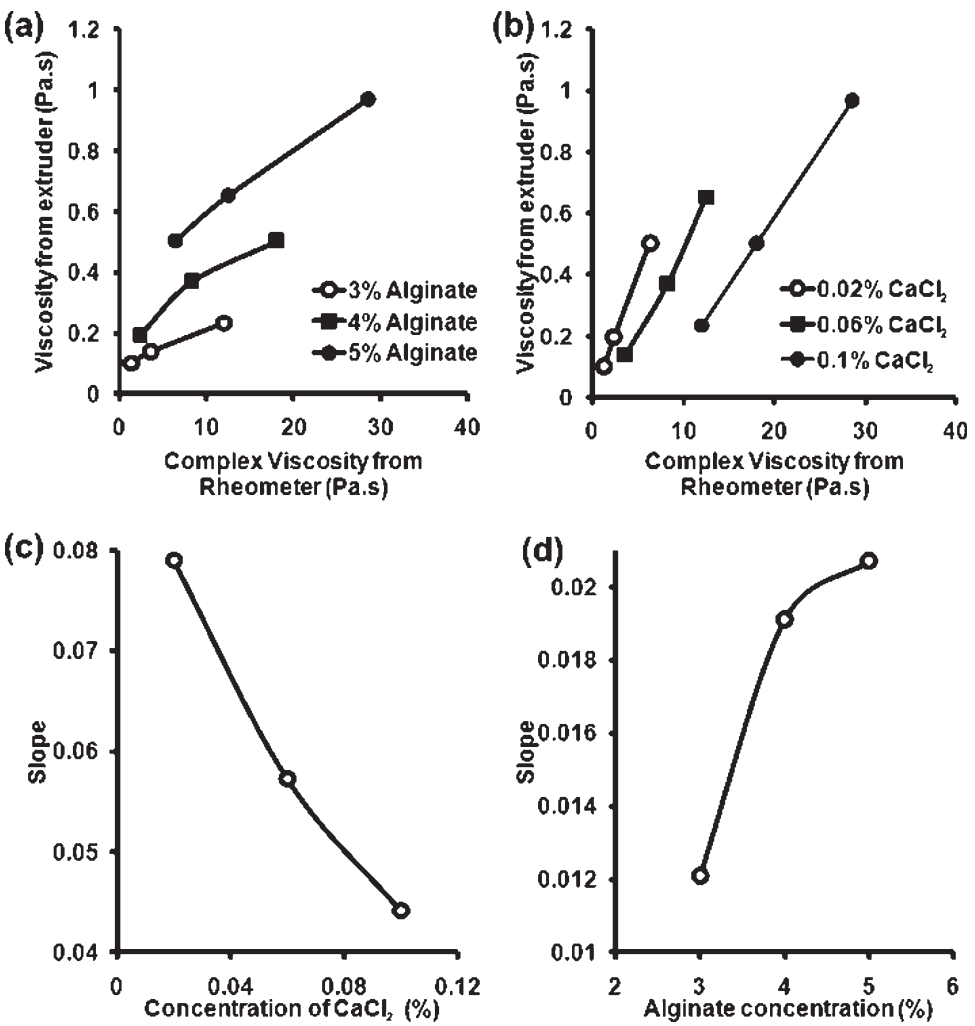

Figure 5 Comparing viscosity according to our model and calculated viscosity according to Dutta's equation.

rigidity. As shown in Figure 4, viscosity increases with increasing alginate and calcium chloride concentration due to the increased number of crosslinks. The increasingly viscous behavior for hydrogels of higher alginate and calcium content is evident in both calculated viscosity using eq. (1) [Fig. 4(a)] and complex viscosity [Fig. 4(b)-rheometer data]. However, the relationship between the viscosity values obtained by the method under investigation and the rheometer values appears to be linear at the concentration intervals tested, while the magnitude differs by an order. We have also constructed a simple mathematical model to represent the flow system and be able to predict flow rates of different alginate hydrogels. Fitting the model to our data using $W = 194.5$ g; $2a = 0.0495$ cm; $2a_0 = 0.477$ cm; $L = 1.5$ cm; $L_0 = 5$L and $\rho = 1000$ kg/m^3.

We obtain that:

$$\mu = 0.0055 \exp(0.887\, C_1\% + 8.6\, C_2\%) \text{ Pas}$$

Figure 5 clearly shows that the apparent viscositiy calculated according to the model of Dutta matches the prediction of our model at varying crosslinking densities. Figure 5(a) shows the relationship between the calculated viscosity and obtained viscosity at various alginate concentrations, while Figure 5(b) compares the viscosities at varying concentrations of calcium chloride. The relationships, irrespective of mode of analysis, seem to remain linear between the two systems, indicating the possibility of using the model under investigation for rapid analysis of samples. Therefore, we would propose using our model if the system were to be used as a quality control methodology for hydrogels of varying gel strengths. The constants of eq. (2) will be depending on the chemistry of the gel. One expects they would be different for $CaSO_4$ crosslinked alginates. Further analysis of the data, by comparing the slope values obtained from Figure 5(a,b) is shown in Figure 5(c,d). It is interesting to note that the rate of change (slope) in the deviation of our model decreases with increasing concentration of alginate [Fig. 5(c)] while remaining almost linear with respect to crosslinker concentration [Fig. 5(d)]. This is anticipated as low viscosity systems exhibit higher flow rates and requires higher precision in measurements.

CONCLUSIONS

In this study, we constructed a simple system that allows measurement of hydrogel flow inexpensively. The syringe extruder worked well with calcium alginate hydrogels between concentrations as low as 0.04% of calcium chloride and 1% of alginate. Our results were compared to rheological characterization using a Bohlin cone and plate rheometer and expected correlations were observed. We conclude that the syringe extruder embodied the characteristics of the traditional melt flow test as used in plastic industries and that the system was able to measure apparent viscosity for injectable hydrogels. We believe altering the dimension of the tube through which alginate is extruded and/or increasing the load would allow us to successfully characterize systems of higher rigidity. Extensive studies with smaller increments in concentrations of alginate and crosslinker along with precise measurements of the flow rate will shed more light on the full capability of the model as well as the device.

References

1. Lee, K. Y.; Bouhadir, K. H.; Mooney, D. J. Biomaterials 2004, 25, 2461.
2. Tu, J.; Bolla, S.; Barr, J.; Miedema, J.; Li, X.; Jasti, B. Int J Pharm 2005, 303, 171.
3. Williams, P. A. In Handbook of Hydrocolloids; Phillips, G. O., Ed; Woodhead Publishing, 2000; Chapter 22.
4. Leone, G.; Torricelli, P.; Chiumiento, A.; Facchini, A.; Barbucci, R. J Biomed Mater Res A 2008, 84, 391.
5. Meena, R.; Chhatbar, M.; Prasad, K.; Siddhanta, A. K. Polym Int 2008, 57, 329.
6. Vallee, F.; Muller, C.; Durand, A.; Schimchowitsch, S.; Dellacherie, E.; Kelche, C.; Cassel, J. C.; Leonard, M. Carbohydr Res 2009, 344, 223.
7. Manojlovic, V.; Djonlagic, J.; Obradovic, B.; Nedovic, V.; Bugarski, B. Int J Nanomed 2006, 1, 163.
8. Al-Musa, S.; Abu Fara, D.; Badwan, A. A. J Control Release 1999, 57, 223.
9. Rousseau, I.; Le Cerf, D.; Picton, L.; Argillier, J. F.; Muller, G. Eur Polym J 2004, 40, 2709.
10. Aminabhavi, T. M.; Agnihotri, S. A.; Naidu, B. V. K. J Appl Polym Sci 2004, 94, 2057.
11. Pavli, M.; Kristl, J.; Dolenc, A.; Zvonar, A.; Vrecer, F.; Kosel, F.; Baumgartner, S. E-Polymers No. 017, 2009.
12. Haidekker, M. A.; Theodorakis, E. A. Org Biomol Chem 2007, 5, 1669.
13. Shenoy, A. V.; Saini, D. R. Rheologica Acta 1984, 23, 608.
14. Johnson, K. C. Plastics—Racing into the Future: ANTEC '96; Society of Plastics Engineers: Indianapolis, 1996; p 3545.
15. Shenoy, A. Rheology of Filled Polymer Systems; Kluwer Academic Publishers: Dordrecht, 1999.
16. Barrera, M. A.; Vega, J. F.; Aguilar, M.; Martinez-Salazar, J. J Mater Process Techol 2006, 174, 171.
17. Shenoy, A. V.; Saini, D. R. J Appl Polym Sci 1984, 29, 1581.
18. Steiner, A. B.; Rothe, B. U.S. Patent 2,635,067, April 1953.
19. Dutta, A. Rheologica Acta 1984, 23, 565.