



University of HUDDERSFIELD

University of Huddersfield Repository

Diryak, R., Mahdi, M.H., Kontogiorgos, Vassilis, Morris, Gordon and Smith, Alan M.

In situ rheological measurements of the external gelation of alginate

Original Citation

Diryak, R., Mahdi, M.H., Kontogiorgos, Vassilis, Morris, Gordon and Smith, Alan M. (2015) In situ rheological measurements of the external gelation of alginate. *Food Hydrocolloids*, 55. pp. 77-80. ISSN 0268-005X

This version is available at <http://eprints.hud.ac.uk/id/eprint/26484/>

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: E.mailbox@hud.ac.uk.

<http://eprints.hud.ac.uk/>

***In situ* rheological measurements of the external gelation of alginate**

M. H. Mahdi^{1†}, R. Diryak^{1†}, V. Kontogiorgos³, G. A. Morris² and A. M. Smith^{1*}

¹*Department of Pharmacy, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.*

²*Department of Chemical Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.*

³*Department of Biological Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.*

***Correspondence:**

Dr. Alan M. Smith

Tel: +44-1484-472-305

Fax: +44-1484-472-305

a.m.smith@hud.ac.uk

†These authors contributed to the work equally (co 1st authors)

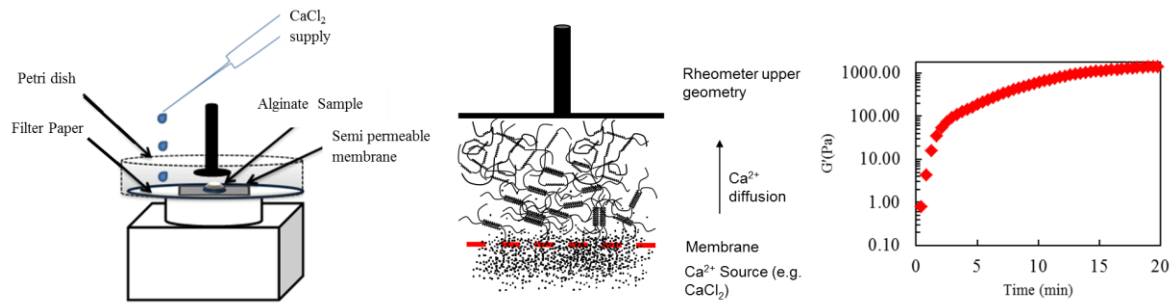
Food Hydrocolloids

Abstract

Direct mixing of alginate and divalent cations such as Ca^{2+} generally produces heterogeneous gels that form almost instantaneously. Therefore, is particularly difficult to measure the rheological properties of this gelation event due to the rapid gelation kinetics. In this study, the gelation of alginate when exposed to a solution of CaCl_2 was measured by using a modified rheometer. This modification involved attaching a petri dish to the lower plate of the rheometer into which, filter paper impregnated with CaCl_2 solution was added. A semi-permeable membrane was then placed above the filter paper as a barrier to prevent the filter paper imbibing the gel. Samples of 4% w/w alginate were loaded onto the semi-permeable membrane and measurements were taken using 55mm parallel plate geometry. Measurements of G' and G'' were determined as a function of time to monitor gelation. Once gelation was complete the filter paper was removed and replaced with filter paper impregnated with calcium chelators (EDTA, sodium citrate) to assess the degradation of the gel. The results showed that this technique was suitable for analysing the external gelation of alginate with a sharp increase in G' in the first three minutes which then plateaued over the remainder of the test. It was also shown that gel stiffness reduced to a greater extent on exposure to EDTA compared with sodium citrate. This method is not only suitable for measuring rapid gelation kinetics on exposure to cross-linkers, but has potential applications in modelling the *in situ* gelation behaviour in simulated physiological environments.

Keywords: Alginate; *in situ*; gelation; rheology; gel; degradation.

Graphical Abstract



Highlights:

- A novel method for the rheological measurements of the gelation of alginate from an external source of calcium ions
- Simple modification of a commercial rheometer
- Can be used to measure the degradation of alginate gel on exposure to calcium chelators
- Potential model for measurements of *in situ* gelation

1 **1. Introduction**

2 Alginates have many applications within the food, pharmaceutical and biomedical
3 industries due to their unique physicochemical properties. Of particular interest to these
4 industries is the ability for solutions of alginate to undergo a temperature independent sol-gel
5 transition in the presence of multivalent cations (*e.g.* Ca^{2+}) (Smidsrød & Draget 1996) and on
6 exposure to acidic pH (generally $< \text{pH } 3$) (Draget, Skjåk-Bræk & Smidsrød 1994; Draget,
7 Skjåk Bræk, Christensen, Gaserod & Smidsrød 1996; Draget, Stokke, Yuguchi, Urakawa, &
8 Kajiwara 2003; Draget, Skjåk Bræka, & Stokke 2006). This behaviour makes alginate
9 particularly suitable for 3D cell culture and bioresponsive drug delivery systems as these
10 environmental conditions can be found in various physiological fluids and, therefore, have
11 the potential to undergo a sol-gel transition *in situ*. Indeed the simplest and most widely used
12 method is to drop an alginate solution *via* a syringe into a solution of calcium chloride.
13 Although considerable work has been performed that exploits this sol-gel transition using
14 various techniques to introduce the alginate to the calcium chloride solution (Kierstan and
15 Bucke, 1977; Hulst, Tramper, Vantriet, & Westerbeek, 1985; Matsumoto, Kobayashi, &
16 Takashima 1986; Sugiura et al 2005; Clark et al 2008), the rapid gelation and heterogeneous
17 nature of the gels formed on direct mixture of crosslinking ions has made the rheological
18 behaviour particularly difficult to measure. Several methods have been developed to
19 overcome this to further understand the fundamental structural aspects of alginate gelation.
20 These methods include the controlled release of divalent ions from an insoluble source
21 (Draget et al 1990; Draget 2000; Draget, Moe, Skjåk-Bræk, & Smidsrød 2006) or by use of a
22 sequestering agent such as ethylenediamine tetraacetic acid (EDTA) (Toft 1982) and using
23 the slowly hydrolysing *n*-glucono delta-lactone (GDL) to lower the pH and release the
24 complexed calcium into the alginate solution. The gels produced using these methods tend to
25 be considerably more homogeneous than those produced by direct mixing of alginate to an

26 external crosslinking source as for example occurs when making alginate beads. Moreover,
27 slowly releasing crosslinking ions that are complexed and suspended within the alginate
28 solution manifests a very different mechanism compared with when alginate comes in to
29 contact with crosslinking ions in physiological environments. To replicate physiological
30 exposure, the usual method is to load sodium alginate into dialysis tubing and then immerse it
31 into a solution containing the required crosslinking ions for various periods of time before
32 removing and cutting the gel to an appropriate size for mechanical testing using a rheometer
33 (Miyazaki, Kubo & Attwood 2000; Kubo, Miyazaki, & Attwood, 2003.). Another method
34 that has been used is to pour sodium alginate into tissue culture plates containing filter paper
35 impregnated with soluble crosslinking ions (one placed beneath the alginate and one on top).
36 The alginate is then allowed to gel for a specific time before the mechanical properties are
37 measured (Hunt, Smith, Gbureck, Shelton & Grover 2010; Jahromi, Grover, Paxton & Smith
38 2011). Neither of these external gelation methods, however, offers an insight into the real
39 time gelation of alginate. To try to address this, we have used a Malvern Gemini rheometer,
40 with a modified lower plate to allow the exposure to an external source of crosslinking ions to
41 facilitate the rheological measurement of alginate gelation *in situ*.

42

43 **2. Materials and Methods**

44 **2.1 Materials**

45 Dialysis tubing (14000 MWCO) was from Thermo Scientific, UK, the filter paper used was
46 Whatman Grade 1 supplied by Fisher scientific UK, sodium alginate was from Sigma Aldrich
47 (UK) and was described as medium molecular weight (80,000 - 120,000) with a M:G ratio of
48 0.39:0.61. All the other chemicals were obtained from Sigma Aldrich (UK) and where of
49 analytical grade and were used without any further purification.

50 2.2 Methods

51 2.2.1 Preparation of alginate solutions

52 Solutions of 4% w/w alginate were made by dispersing weighed amount of alginate in 100 ml
53 distilled water and stirring at 60 °C for 30 min. Any evaporated water was replaced and the
54 sample was stored in a sealed vial prior to use.

55 2.2.2 Preparation calcium chloride solution

56 Three different concentrations of CaCl₂ (50,100 and 200 mM) were prepared by dissolving
57 the correct weight of calcium chloride dihydrate powder in 100 ml deionized water.

58 2.2.3 Preparation of EDTA solution

59 500 mM of EDTA was prepared by dissolving the weighted amount of EDTA powder in 100
60 ml warm deionized water with continuous stirring for 30 min. The pH was then adjusted to
61 pH 7.0 using 1 M NaOH.

62 2.2.4 Preparation of sodium citrate solution

63 Sodium citrate was prepared at a concentration of 500 mM in the same manner as the EDTA,
64 by dissolving the correct amount sodium citrate powder in 100 ml warm deionized water with
65 continuous stirring for 30 min and the pH adjusted to pH to 7.0 using 1 M NaOH.

66 2.2.5 In situ gelation

67 The experimental setup used a Malvern Gemini Nano HR rheometer with a modified lower
68 plate as shown in **Figure 1**. Briefly, a petri dish containing a filter paper soaked with CaCl₂
69 solution was securely attached to the lower plate of the rheometer. The theoretical amount of
70 total calcium added was estimated by weighing the filter paper before and after soaking. This
71 was calculated as 2.5, 5 and 10 mg of calcium for 50, 100 and 200 mM CaCl₂ solutions
72 respectively. A dialysis membrane (MWCO 14000 Da) which had previously been hydrated

73 in deionised water was placed on top of the filter paper to prevent the sample being imbibed
74 by the filter paper. The gap was then zeroed, the samples of alginate were loaded onto the
75 dialysis tubing and light silicone oil was used around the periphery of the geometry to
76 prevent evaporation. Small deformation oscillatory measurements of storage and loss moduli
77 (G' and G'') were then performed as a function of time at 0.5% strain and a frequency of 10
78 rad s^{-1} using a 55 mm diameter parallel plate geometry with a 1 mm gap. All measurements
79 were performed within the linear viscoelastic region previously determined using amplitude
80 sweeps. Alginate solutions measured in the same way but using filter paper impregnated with
81 deionized water served as control.

82 *2.2.6 In situ gel degradation*

83 Following a 20 min exposure to CaCl_2 solution the geometry was raised and the filter paper
84 was carefully removed from the petri dish and replaced with a filter paper impregnated with a
85 calcium chelator (either 500 mM EDTA or 500 mM sodium citrate). The rheological
86 measurements of G' and G'' as a function of time were then resumed using the same
87 conditions as used in the gelation measurements. During the procedure of changing the filter
88 paper the crosslinked alginate gel remained adhered to the upper geometry which facilitated
89 the change without significantly disturbing the gel. Moreover, no significant changes in
90 normal force were apparent following the change of filter paper.

91 **3. Results and Discussion**

92 *3.1 In situ gelation*

93 The changes in G' and G'' showing the gelation behaviour of alginate when exposed to an
94 external source of calcium chloride was measured by using a modified Malvern Gemini
95 rheometer. The concentration of the alginate was chosen at 4% to ensure a good signal to
96 noise ratio from the non-crosslinked sample and to facilitate a strong and rapid gelling

97 reaction to emphasize the ability to measure the rapid changes in moduli. **Figure 2A-C** show
98 a rapid increase in G' and G'' over the first 3 min of exposure with G' overtaking G'' within 2
99 min in all the concentrations of CaCl_2 tested. The gelation reaction was allowed to proceed
100 for 20 min and the values for G' were recorded and showed an increase that was proportional
101 to the concentration of CaCl_2 (**Figure 2D**). This proportional increase in G' has been shown
102 previously with alginate crosslinked by internal gelation mechanisms (Draget et al 2006).

103

104 3.1 *In situ gel degradation*

105 To highlight the potential of this method to analyse changes in rheological properties of gels
106 on exposure to external sources of salts, the effect of commonly used calcium chelators on
107 4% alginate crosslinked for 20 min by an external source of 200 mM CaCl_2 was studied
108 (**Figure 2E**). EDTA was shown clearly to be a more potent calcium chelator than sodium
109 citrate, causing G' to return to a similar modulus to that of the original sodium alginate, prior
110 to crosslinking, after only 35 min of exposure. In contrast, sodium citrate only reduced G' by
111 one order of magnitude in comparison with the two orders of magnitude achieved when using
112 EDTA. This can be explained by EDTA having a higher calcium ion binding constant than
113 sodium citrate as previously demonstrated by Keowmaneechai & McClements (2002).

114

115 **4. Conclusion Limitations and Future Perspectives**

116 This study has demonstrated a novel method to measure the rapid changes in rheological
117 properties of alginate during external gelation on exposure to CaCl_2 . Differences in gel
118 strength could also be measured when changing the source concentrations of CaCl_2 .
119 Moreover, the degradation of calcium cross-linked alginate gels can also be monitored in real
120 time by replacing a crosslinking ion source for a calcium chelator. Indeed, results obtained
121 using this method showed that EDTA was a more effective chelator than sodium citrate. It

122 should be mentioned however, that for a suitable comparisons between samples it is crucial to
123 begin the measurements at a consistent time following loading of the sample as this is
124 particularly important with rapid gelling systems such as alginate. Furthermore,
125 quantification of the concentrations of ions diffused into the sample is unknown and could
126 result in the possibility of an inhomogeneous gel with the sample being more crosslinked
127 close to the filter paper. This effect would have greater significance, however, on thicker gels
128 i.e those measured with a larger gap size. It is proposed that this technique could be applied
129 to studying gelation of pectins, carrageenans and other biopolymers that gel in the presence
130 of metal ions, small molecule crosslinkers or by changes in pH. The wider implication of this
131 is an ability to choose isolated biopolymers for many different industry applications where
132 there may be a need for rapid or slow gelation. For example, this system could be used as a
133 model for understanding changes in rheological behaviour when biopolymers are exposed to
134 various physiological fluids. This could therefore, have particular applications in designing
135 bioresponsive delivery systems in the food, pharmaceutical and biomedical industries.

136

137 **5. Acknowledgement**

138 The authors would like to thank Dr. Adrian Hill from Malvern Instruments for his helpful
139 discussions.

140

141 **6. References**

142 Clark S, Cross M.L., Smith A.M., Court P., Vipond J., Nadian A., Hewinson R.G., Batchelor
143 H.K., Perrie Y., Williams A., Aldwell F.E., Chambers M.A. (2008) Assessment of different
144 formulations of oral Mycobacterium bovis bacille Calmette-Guerin (BCG) vaccine in rodent
145 models for immunogenicity and protection against aerosol challenge with M. bovis. *Vaccine*.
146 26(46), 5791–5797.

147 Draget, K. I., Ostgaard, K., and Smidsrød, O. (1990) Homogeneous Alginate Gels - A
148 Technical Approach. *Carbohydrate Polymers*, 14, 159-178.

149 Draget, K.I., Skjåk Bræka, G., and Smidsrød, O. (1994) Alginic acid gels: the effect of
150 alginate chemical composition and molecular weight. *Carbohydrate Polymers*, 25, 31-38.

151 Draget, K.I., Skjåk Bræka, G., Christensen, B.E., Gaserod, O., and Smidsrød, O. (1996)
152 Swelling and partial solubilization of alginic acid gel beads in acidic buffer. *Carbohydrate*
153 *Polymers*, 29, 209-215.

154 Draget, K.I. (2000) Alginates. In: Handbook of hydrocolloids, pp. 379-396. Eds G.O.
155 Phillips, P.A. Williams. Woodhead Publishing Limited: Cambridge.

156 Draget, K.I., Stokke, B.T., Yuguchi, Y., Urakawa, H., and Kajiwara, K. (2003) Small-angle
157 x-ray scattering and rheological characterization of alginate gels. 3. Alginic acid gels.
158 *Biomacromolecules*, 4, 1661-1668.

159 Draget, K.I., Skjåk Bræka, G., and Stokke, B.T. (2006) Similarities and differences between
160 alginic acid gels and ionically crosslinked alginate gels. *Food Hydrocolloids*, 20, 170-175.

161 Draget, K.I., Moe, S.T., Skjåk Bræka, G., and Smidsrød, O. (2006) Alginates. In: Food
162 Polysaccharides and Their Applications, pp. 289-334. Eds A.M. Stephen, G.O. Phillips, P.A.
163 Williams. CRC Press, Boca Ranton, FL.

164 Keowmaneechai E, and McClements D.J. (2002) Influence of EDTA and citrate on
165 physicochemical properties of whey protein-stabilized oil-in-water emulsions containing
166 CaCl₂. *Journal of Agriculture and Food Chemistry*, 50 (24), 7145-53.

167 Kierstan, M. and Bucke, C. (1977) Immobilization of Microbial-Cells, Subcellular
168 Organelles, and Enzymes in Calcium Alginate Gels. *Biotechnology and Bioengineering* 19,
169 387-397.

170 Kubo, W., Miyazaki, S., Attwood, D., (2003) Oral sustained delivery of paracetamol

171 from in situ-gelling gellan and sodium alginate formulations. *International Journal of*
172 *Pharmaceutics*, 258, 55–64.

173 Hulst, A.C., Tramper, J., Vantriet, K., and Westerbeek, J.M.M. (1985) A New Technique for
174 the Production of Immobilized Biocatalyst in Large Quantities. *Biotechnology and*
175 *Bioengineering*, 27, 870-876.

176 Hunt, N., Smith, A.M., Gbureck U, Shelton RM & Grover L.M. (2010) Encapsulation of
177 fibroblasts causes accelerated alginate hydrogel degradation. *Acta Biomaterialia*, 6 (9), 3649-
178 3656

179 Jahromi S.H., Grover, L.M., Paxton J.Z. & Smith, A.M (2011) Degradation of
180 Polysaccharide hydrogels seeded with bone marrow stromal cells. *Journal of Mechanical*
181 *Behaviour of Biomedical Materials*, 4, 1157-1166

182 Matsumoto, S., Kobayashi, H., and Takashima, Y. (1986) Production of monodispersed
183 capsules. *Journal of Microencapsulation*, 3, 25-31

184 Miyazaki, S., Kubo, W., Attwood, D., (2000) Oral sustained delivery of theophylline
185 using in-situ gelation of sodium alginate. *Journal of Controlled Release*, 67, 275–280.

186 Sugiura, S., Oda, T., Izumida, Y., Aoyagi, Y., Satake, M., Ochiai, A., Ohkohchi, N., and
187 Nakajima, M. (2005) Size control of calcium alginate beads containing living cells using
188 micro-nozzle array. *Biomaterials*, 26, 3327-3331.

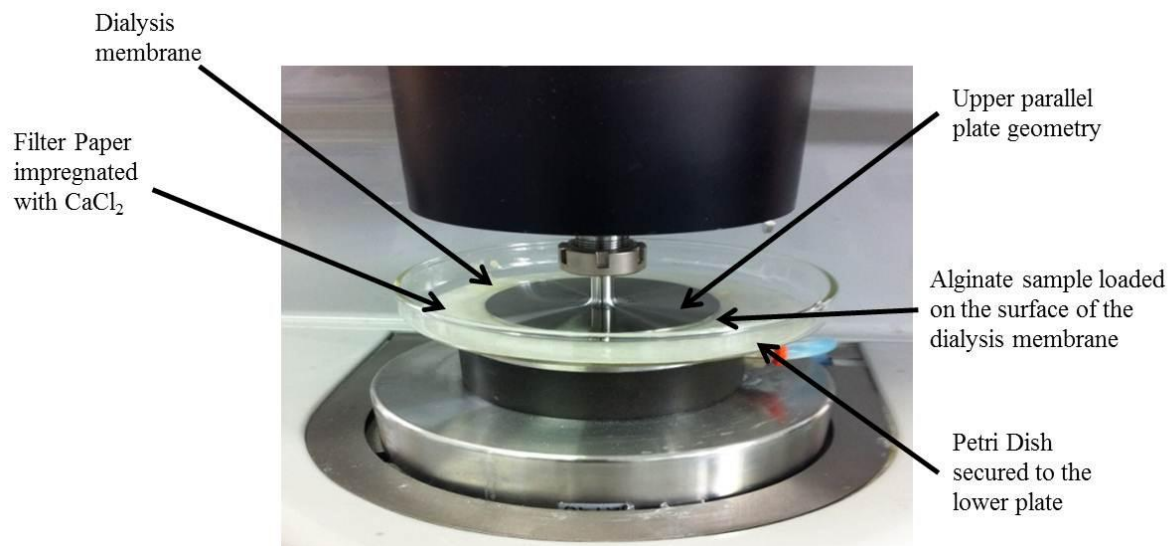
189 Smidsrød, O. & Draget K.I. (1996) Alginates: chemistry and physical properties.
190 *Carbohydrates in Europe*, 14, 6–13

191 Toft, K. (1982) Interactions between pectins and alginates. *Progress in Food and Nutrition*
192 *Science*, 6, 89-96.

193

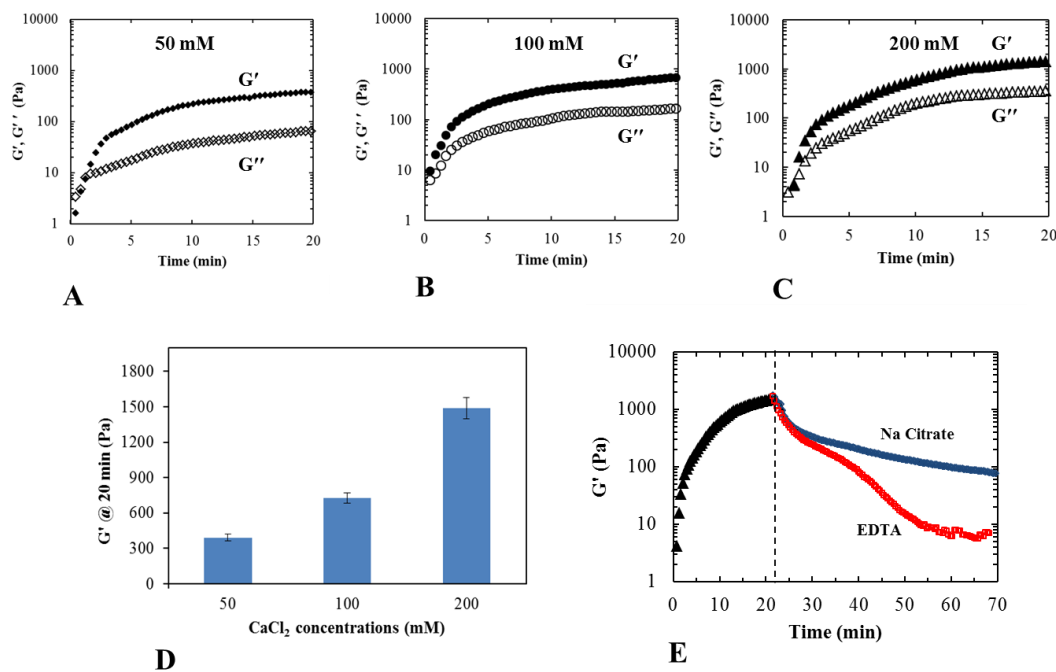
194

195



196

197 **Figure 1** *In situ* gelling experiment using a modified lower plate of a commercial rheometer



198

199 **Figure 2** Rheological measurements showing variation of G' (filled symbols), G'' (open
 200 symbols) vs time on exposure to A) 50 mM B) 100 mM and C) 200 mM; D) shows the values
 201 of G' after 20 min exposure to 50mM, 100mM and 200mM CaCl_2 ; E) shows the effect of the
 202 calcium chelators sodium citrate 500 mM and EDTA 500 mM on the variation G' for 4%
 203 alginate crosslinked with 200 mM CaCl_2 for 20min *in situ*. Dotted line indicates when the
 204 crosslinking source CaCl_2 was changed to either sodium citrate or EDTA.