In situ rheological measurements of the external gelation of alginate

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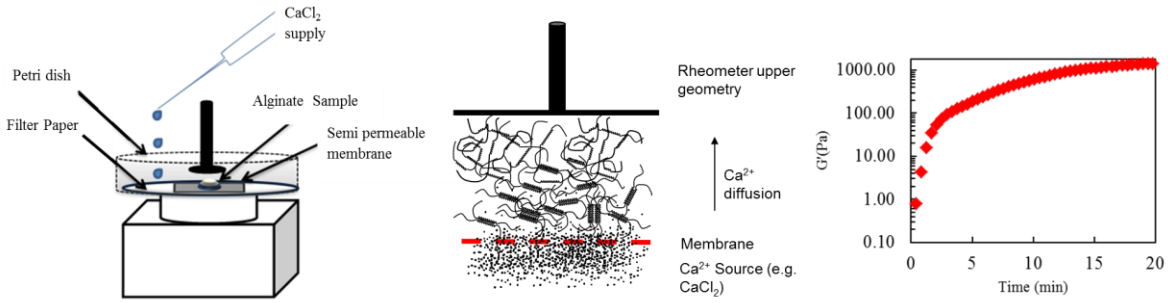
Food Hydrocolloids

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. Introduction

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

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

2. Materials and Methods

2.1 Materials

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

2.2 Methods

2.2.1 Preparation of alginate solutions

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

2.2.2 Preparation calcium chloride solution

Three different concentrations of $CaCl_2$ (50,100 and 200 mM) were prepared by dissolving the correct weight of calcium chloride dihydrate powder in 100 ml deionized water.

2.2.3 Preparation of EDTA solution

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

2.2.4 Preparation of sodium citrate solution

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

2.2.5 In situ gelation

The experimental setup used a Malvern Gemini Nano HR rheometer with a modified lower plate as shown in **Figure 1**. Briefly, a petri dish containing a filter paper soaked with $CaCl_2$ solution was securely attached to the lower plate of the rheometer. The theoretical amount of total calcium added was estimated by weighing the filter paper before and after soaking. This was calculated as 2.5, 5 and 10 mg of calcium for 50, 100 and 200 mM $CaCl_2$ solutions respectively. A dialysis membrane (MWCO 14000 Da) which had previously been hydrated

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

2.2.6 In situ gel degradation

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

3. Results and Discussion

3.1 In situ gelation

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

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

3.1 In situ gel degradation

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

4. Conclusion Limitations and Future Perspectives

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

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

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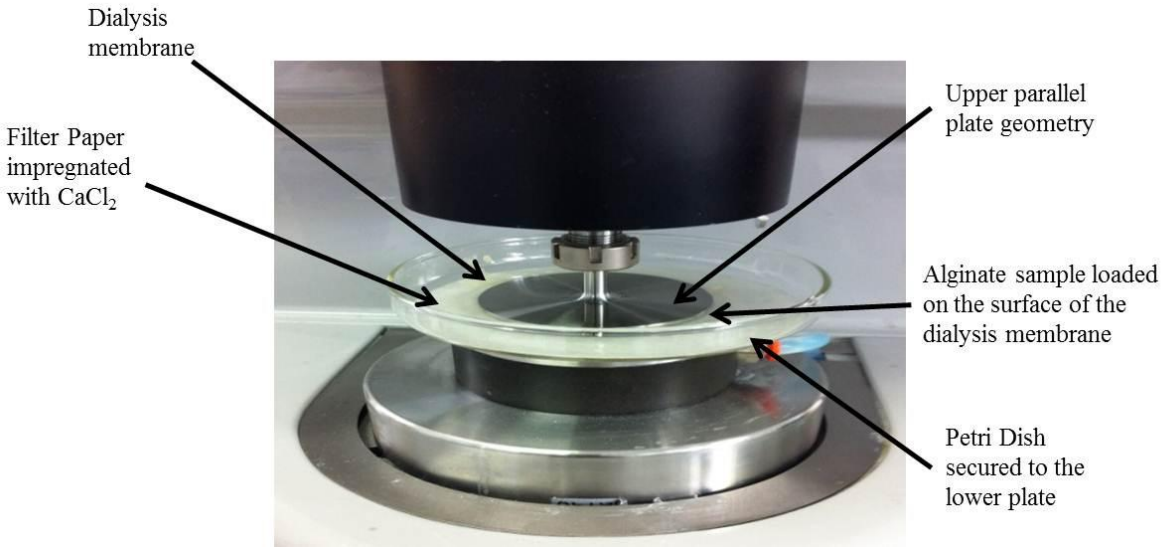

Figure 1 *In situ* gelling experiment using a modified lower plate of a commercial rheometer

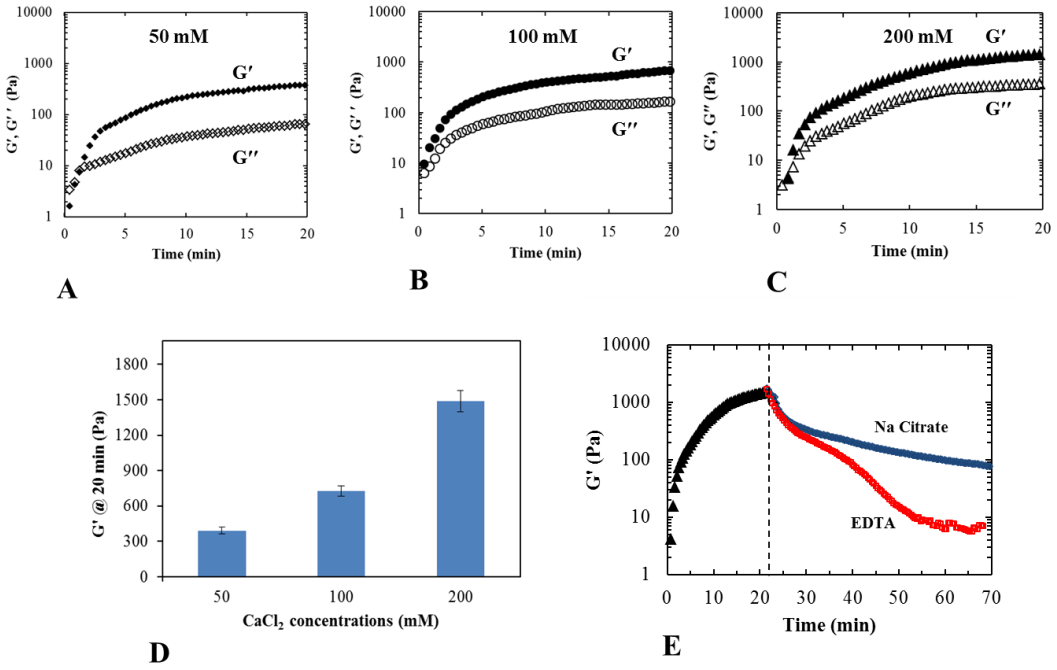

Figure 2 Rheological measurements showing variation of G′ (filled symbols), G″ (open symbols) *vs* time on exposure to A) 50 mM B) 100 mM and C) 200 mM; D) shows the values of G′ after 20 min exposure to 50mM, 100mM and 200mM $CaCl_2$; E) shows the effect of the calcium chelators sodium citrate 500 mM and EDTA 500 mM on the variation G′ for 4% alginate crosslinked with 200 mM $CaCl_2$ for 20mim in situ. Dotted line indicates when the crosslinking source CaCl2 was changed to either sodium citrate or EDTA.