***In situ* real-time rheological characterization of calcium alginate hydrogels**

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**Highlights**

* Design of a custom-made geometry for the rheological study.
* *In situ* gelation kinetics of sodium alginate and calcium chloride.
* Understanding the initial phase of the reaction kinetics.

**1. Introduction**

Polysaccharides are among the fundamental components of biosystems, and they are responsible for a variety of functions. Alginate is an important polysaccharide and forms hydrogels. [1,2] As a linear negatively charged high-molecular-weight copolymer of two monosaccharides, it reacts with multivalent cations (e.g. Ca2+, Ba2+) in aqueous solutions and forms hydrogels through ionic crosslinking, also referred to as ´´egg-box`` structural formation. [3] The motivation was to develop an understanding of the fast gelation kinetics of this model system using a custom-made rheometric setup. In the future, this investigation can be extended to similar reactions and controlled drug delivery studies.

**2. Methods**

We studied the kinetics of the *in situ* gelation of alginate in the presence of Ca2+ ions during the initial gelation phase using a custom-made cone and plate geometry (Figure 1a). The lower plate has been altered, providing a cavity where the Ca2+ solution can exist. Only upwards flow to the surface can be achieved where the alginate solution is placed. The alginate solution covered the lower plate and the upper cone was adjusted at the corresponding gap. Using a volumetric syringe to supply the appropriate volume of the Ca2+ solution, instant gelation can be achieved and the rheological response can be recorded. In this study, CaCl2 solutions were utilized with concentrations up to 200 mM and volumes between 0.1 to 0.25 mL. We performed small amplitude oscillatory shear time sweeps at alginate concentrations up to 4 wt.%, by applying 3% strain amplitude at 1 rad/s angular frequency. In all measurements, injection occurred after the initiation of the oscillatory time sweep.

**3. Results and discussion**

We found that with a sampling rate of 3 points per second, the rheometer can successfully record the fast gelation reaction during the initial ten seconds (Figure 1b, indicated area). For increased volumes of the injected Ca2+ solution, the elastic modulus G΄ increases continuously in time while the loss modulus G΄΄ forms a plateau (Figure 1b). Moreover, the magnitudes of the dynamic moduli were significantly higher. When the injection volume was constant, the same effect was noticed for the Ca2+ concentration. For instance, by supplying twice the CaCl2 concentration, the storage modulus of the hydrogel was doubled. At the end of the experiments, a macroscopic evaluation was performed and typical flowable soft gels were observed in all cases.

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| **(a)** | **(b)** |

**Figure 1.** (a) Custom-made lower plate placed on rheometer. A volumetric syringe is used for supplying a tracing liquid through the internal cavity and the two micro-holes (approx. 250 μm) to the surface of the lower plate. (b) Storage modulus (G’, filled square symbols) and loss modulus (G΄΄, nonfilled triangle symbols) vs. time. The *in situ* conditions correspond to 1 wt.% sodium alginate and 50 mM calcium chloride for injected volumes of 0.10 mL (black), 0.15 mL (red), 0.20 mL (blue), and 0.25 mL (magenta).

**4. Conclusions**

In this work, we present a method for recording *in situ* real-time gelation kinetics, like fast changes of storage and loss moduli. By studying the Ca2+/alginate system at various concentrations and volumes, we found that two distinct regions exist. The initial rapid region seems to be independent of the applied Ca2+ volume while the plateau region indicates a quasi-stable structure. The knowledge gained using this probing technique in this research can be applied in many engineering fields, such as food technology or tissue engineering.

**References**

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