RHEODIALYSIS: A PLATFORM FOR PROBING RHEOLOGY IN EVOLVING CHEMICAL ENVIRONMENTS

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ABSTRACT

Many soft and complex materials exhibit rheological behaviour that evolve spatially in response to changes in their chemical environment, particularly at interfaces. Examples include biopolymer coatings, food products during digestion and personal care products. However, conventional rheological techniques typically operate under static conditions, limiting their ability to capture these coupled time and spatial effects. This presents a significant challenge for understanding processes such as gelation, transport-driven structuring, and interfacial material evolution. Here, we present rheodialysis as an experimental platform that enables in situ rheological measurements under dynamically controlled chemical conditions. The approach integrates a modified rheometer geometry with a membrane-separated flow system, allowing reagents to be introduced non-invasively via diffusion while continuously monitoring rheological properties. This configuration provides precise control over the local chemical environment without disrupting the sample structure. Using alginate hydrogels as a model systems undergoing chemically induced transformations, we demonstrate how rheodialysis can resolve both time-dependent rheological evolution. This work highlights the potential of rheodialysis as a versatile tool for studying soft matter in non-equilibrium conditions. The approach is broadly applicable to systems where external stimuli drive changes in structure and mechanics, offering new opportunities to investigate and optimise processes relevant to formulations, biological systems, and advanced materials.

Introduction

Hydrogels are three dimensional water-swollen networks that are corslsinked through either physical or chemical interactions. The physical properties of hydrogels have long been studied by rheologists and their industrial applications are myriad and growing, from simple applications such as structurants for food and personal applications, to advanced materials applications in tissue engineering, drug delivery, regenerative medicine and biosensing.[1] When manufacturing and using these materials, rheological changes commonly take place at boundaries and interfaces. For example, edible coatings such as meat casings are formed at air-water interfaces during co-extrusion, hydrogel bandages undergo changes in viscoelasticity when exposed to the surface of a wound, and anti-perspirant products undergo gelation-like behaviour when exposed to sweat from the human skin. Clearly, when gelation occurs in these conditions, the rheology of the interface and the rheology of the bulk material are interrelated. Both are sensitive to local changes in chemistry and these changes take place over different timescales that are defined by diffusion and chemical exchange at the surface of the material. This is a physically complex process and its industrial importance, there is a notable lack of

rheological techniques that can probe changes in viscoelasticity under changing chemical stimuli.

Recent rheological research has benefited from a number of novel custom-made platforms which have attempted to bridge this gap in instrumentation. In one study, chemical exchange was induced through a dialysis membrane soaked in an ionic solution that adhered to the lower plate of a parallel plate rheometer, enabling the gelation of the sample to be rheologically probed due to its direct external contact with membrane.[2] While effective at inducing and probing gelation, this approach did not allow for control of chemical release. In another study, this simple membrane was replaced by a hollow cell separated from the sample by a stainless steel mesh, with the chamber volume being continuously replenished using peristaltic pump.[3] This enabled the authors to carry out real time measurements of both gel formation and gel disassembly while the chemical environment was exchanged. More recently, a similar design was reported that involved injecting a crosslinking reagent into a chamber through a two holes on the lower plate using a manual syringe.[4] This enabled the process of gelation to be monitored from the onset of exposure to a crosslinking reagent. Further work by the same authors refined this design by adjusted number and arrangement of these holes to ensure more homogeneous chemical exposure to the sample. As expected, this also appeared to control the mass transfer of ions into the sample, with a larger number of holes leading to faster gelation.[5] In the same study it was reported that gelation process was sensitive to the oscillatory rheology protocol used, which was speculated to arise from induced mixing between the flow chamber and sample. While each of these published designs has it's own individual advantages, they also each have certain drawbacks. There is therefore a lack of a unified platform to enable in-situ chemical exchange for rheometers. In this work, we draw inspiration from these studies to present a new rheology accessory which we term Rheodialysis. Rheodialysis combines what we consider the best features of each of these previously reported approaches, while also incorporating a number of new design features.

Design and Implementation

The design of the Rheodialysis approach are defined by the following goals:

1. In-situ control of chemical environment though automated injection
2. Mechanical isolation of the sample from this chemical environment to minimise external pressure gradients
3. Control of injection rate and contact area to enable mass transfer into the sample to be adjusted.

The design of our device is shown in **Fig. 1(a), (b)**. We use a 20 mm parallel plate configuration with the flow cell taking the place of the lower plate, which is mounted on a Netzsch Kinexus Pro stress-controlled rheometer. The Rheodialysis device comprises a conical flow chamber with a flow inlet at it's base and a flow outlet at it's side. Two rigid plates with 0.16 mm holes are screwed in place on top of the chamber, with a 0.22 μm cellulose membrane between them. This combined assembly essentially replaces the lower plate of the rheometer. Therefore our three goals are addressed through (1) the use of the conical chamber ensures in-situ control, (2) the separation of the chamber using a semi-permeable membrane, and (3) the use of a syringe pump with controllable rate to enable control of the injection rate.

Our method is important that the device does not induce undesirable pressure to the sample between the during chemical exchange. To investigate this, we carried out a simple computational fluid dynamic simulation. **Fig. 1 (c)** shows the modelling of the fluid velocity profiles at an injection speed of 0.1 ml/min with the colour . The result shows that the design enables the inlet to expose the sample to a new chemical environment from below and minimises

the pressure gradient on the sample since the flow is directed parallel to the plate and flow rate (colour bar in **Fig. 1 (c)**) and is lowest at the top of the chamber, directly below the membrane.

Results

We test our device using sodium alginate, a well-studied model gel-forming biopolymer. Alginate is a marine-derived polysaccharide that is widely used in the food,[6] packaging,[7] and biomedical industries.[8] Its crosslinking mechanism arises from its molecular heterogeneity, as it has a block copolymer structure comprising mannuronic acid groups, known as M-blocks, and guluronic acid groups, known as G-blocks as depicted in **Fig. 1 (d)**. When exposed to divalent cations such as calcium, these ions bind to backbone of the negatively charged G-blocks, leading to ionic crosslinking between chains and the formation of a water-swollen hydrogel network.

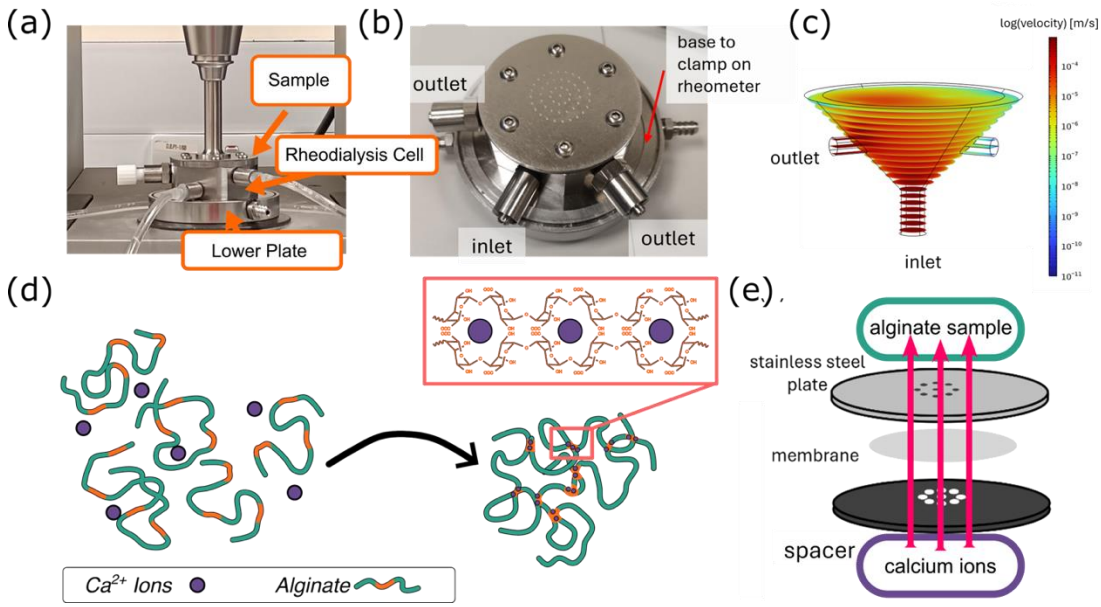

FIGURE 1: The rheodialysis approach comprises a custom-built flow cell that replaces the lower plate of a paralell plate rheometer (a). The flow cell's is separated from the sample through a semipermeable membrane. It's shape is conical with an inlet at the base, and two outlets a larger positions (b). A syringe pump exchanges the sample environment during rheological testing and the geometry ensures that maximum flow rates (c) are reached far from the membrane surface, ensuring minimal pressure gradients on the samples. As a proof-of-concept, we monitor the gelation of alginate, when exposed to calcium ions. The alginate biopolymer backbone consists of two groups: mannuronic acid and guluronic acid. Binding of calcium to the guluronic acid (inset) leads to the formation of a hydrogel network (d) We probe this dynamically by delivering calcium ions to an alginate soluton, through the flow cell.

As a proof-of-concept test, we probed an aqueous sodium alginate sample using the rheodialysis approach, exposing it calcum ions in-situ to induce gelation (**Fig. 1 (e)**). An alginate solution of concentration 5.4 % (w/v) was prepared with Sodium Alginate Protanal 8133 (Dupont, M/G ratio of 65/35, molecular weight 90-180 kDa) and Milli-Q water. The mixture was heated to 80°C and stirred for 30 minutes at 150 rpm to enable complete dissolution and was then cooled to room temperature. The sample was loaded on to the rheodialysis cell and a solution of 0.1 M Calcium Chloride ($CaCl_2$, Thermo Scientific) was injected into the chamber using a syringe pump constructed specially for this work. We explored three separate

injection rates: 0.05 ml/min, 0.1 ml/min, and 0.2 ml/min. The inlet piping was initially purged with the calcium solution carefully, ensuring that the chamber did not fully fill as this would induce gelation. Once complete, the rheometer inertia and torque was calibrated, using a 20 mm parallel plate and the alginate solution was loaded between the plates at a gap separation of 0.5 mm. A 5 rads.s^{-1} pre-shear protocol was then applied to ensure the sample was evenly spread between the plates and injection was initiated.

To monitor the gelation process we carried out a repeated oscillatory measurements across four frequencies 0.2 Hz, 0.4 Hz, 0.8 Hz and 1.6 Hz. This protocol allows observation of the phase angle (δ), elastic modulus (G') and viscous modulus (G") values during the gelation process. It is common to identify the gel point by the point at which G' exceeds G'', however this a crude measure since it invariably depends on the measurement frequency. The true gel point is generally defined through the Winter-Chambon criterion,[9] which posits that G' and G'' both scale with the same power-law relationship in frequency ω. This allows the gel formation time t_g to be accurately quantified through the frequency independence of δ, since, by definition, tanδ = G''/G'.

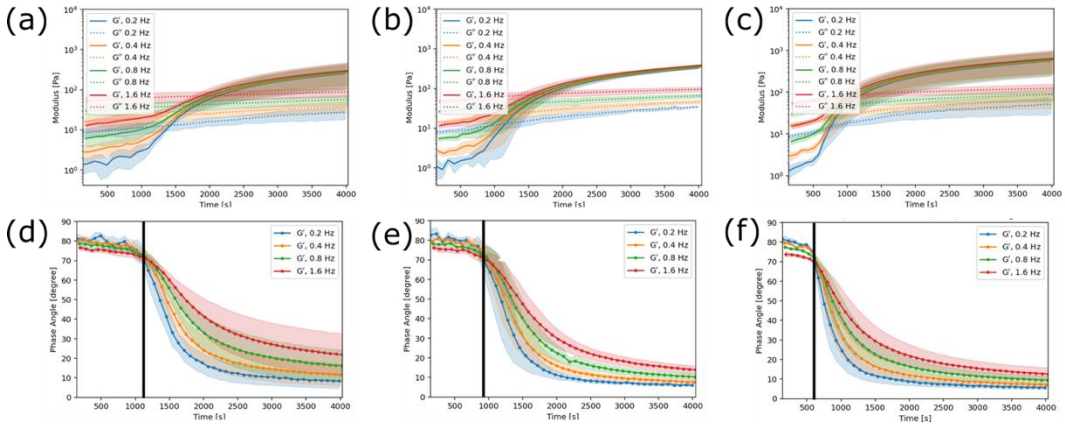

FIGURE 2: Oscillatory shear moduli of alginate samples, resolved over measurement time during in-situ exposure to calcium ions at three different injection speeds: 0.05 ml/min, (a) 0.1 ml/min, (b) and 0.2 ml/min (c). Crossover between storage modulus (solid lines) and loss modulus (dashed lines) is frequency dependent and indicates the approximate onset of gelation. Precise gel points are determined through use of the Winter-Chambon criterion, as the point at which the phase angle is frequency dependent, shown as vertical solid lines (d)-(f). Increasing the injection speed, results in gelation at earlier timescales.

The resulting time-resolved multi-frequency data is shown in **Fig 2 (a), (b)** and **(c)** for each of the three injection rates, respectively. As expected, gelation can clearly be seen to have occurred through the steady increase of G' and G'' over all frequencies. The final values of both metrics show dominance of G', indicating the presence of a soft viscoelastic solid. We interpolate the time-dependent values of δ and define the gel point as as the time of the minimum variance between values of δ collected at the four frequencies. The values of t_g with respect to time at different frequencies are shown in **Fig 2. (a), (b)** and **(c)** for each of the three injection rates, respectively in which the error bars denote the standard deviation from three independent experiments. The measured values of t_g are denoted as vertical bold lines in these plots and are also summarised in **Table 1**.

As shown in **Table 1**, the time taken to gelation is inversely proportional to the injection rate of calcium ions. We postulate that this time dependence arises from two key metrics: **(1)** t_m the rate of mass transfer of calcium ions across the membrane, which is defined by the concentration of calcium ions in the solution and the injection rate and **(2)** the binding rate of

calcium which arises from its association constant K_a and is defined by the alginate molecular structure and charge. Alginate biopolymers differ between sources but are generally known to have a particularly high value of K_a (of order 4×10^3 M^{-1} [10]) which results in fast association of alginate polymers to calcium as they are transported across the membrane during the experiment. The high value of K_a also means that calcium-alginate binding is long lived, which would necessitate the gradual depletion of calcium within the flow cell if there was no flow present. The presence of an injection rate directly determines t_m and acts to replenish these calcium ions in the reservoir. We therefore speculate that the observed sensitivity of the gelation time t_g on injection rate in **Table 1.** This sensitivity suggests that the mass transfer rate and the binding rate in our experiment must be of similar orders of magnitude.

TABLE 1: Calculated values of the gel point using the Winter-Chambon criteron, t_g at three different injection rates. Error bars are standard deviation results from three independent experiments.

Injection Rate (ml/min)	T_g (s)
0.05	1166 ± 80
0.10	972 ± 155
0.20	613 ± 9

Having established that the gelation kinetics can be tuned by injecton rate, we now consider how these kinetics influence hydrogel structure. **Fig. 3 (a)-(c)** show the frequency spectra, at a strain amplitude $\gamma = 0.004$, which are collected once G' and G'' reach a steady state value. To understand the nonlinear rheology of these hydrogels, we also carry out oscillatory amplitude sweeps by logarthimically increasing γ from 0.001 to 1 at a frequency of $\omega = 5$ s^{-1}, shown in **Fig. 3 (d)-(f)**.

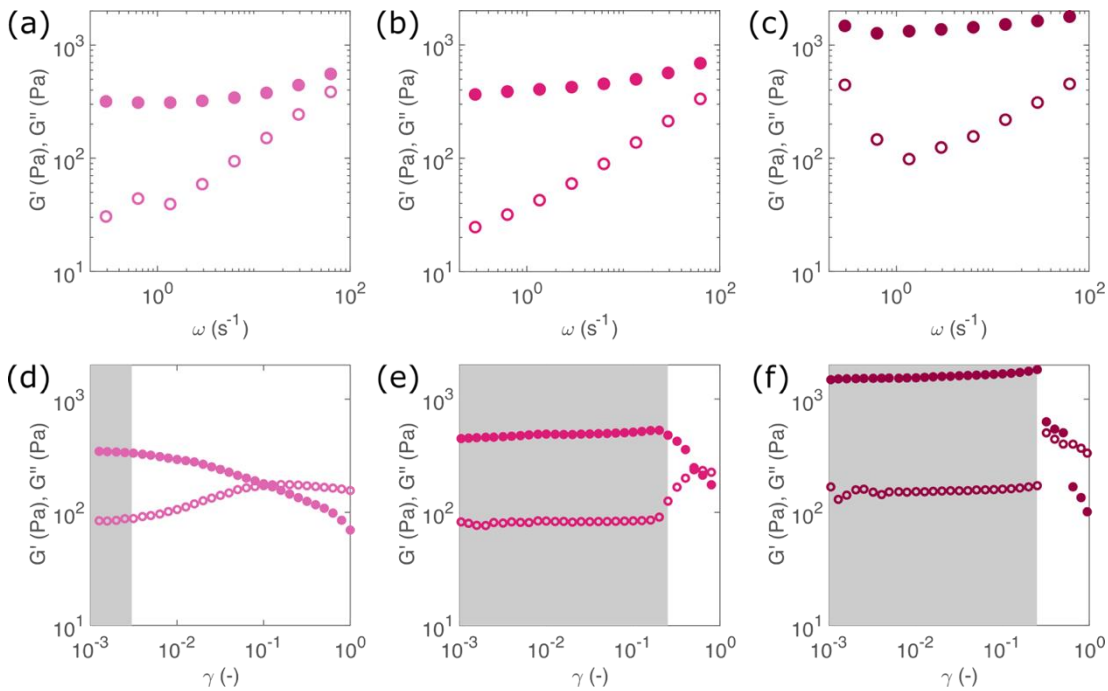

FIGURE 3: Final oscillatory frequency spectra (top) and amplitude sweep data (bottom) for alginate hydrogels formed using the rheodialysis approach. Data is shown for the three different injection speeds: 0.05 ml/min, ((a),(d)) 0.1 ml/min, ((b),(e)) and 0.2 ml/min ((c),(f)). Shaded regions in the lower panels indicate the linear viscoelastic regimes, where the apparent G' and G'' are strictly defined. Faster injection speeds are shown to result in higher values of G' and G'', with more extended linear viscoelastic regimes.

All three hydrogels show frequency spectra that are approximately level across the frequency range measured, which is a definitive characteristic of crosslinked hydrogels. Furthermore, the fastest injection rates appear to lead to hydrogels with significantly higher elastic moduli. The value of G'~ 300 Pa at 0.05 ml/min, while at 0.2 ml/min G'~ 1300 Pa. Similarly the amplitude sweep data show that, as the injection speed is increased, the hydrogels exhibit a more extended linear viscoelastic regime. Within the nonlinear viscoelastic regime, the yielding process of the hydrogels (intepreted as a decrease in the apparent values of G' and G'') has a distinctly different character. At the lowest injection speeds, (**Fig. 3 (d))** there is a gradual decrease in G' and gradual overshoot in G''. At the highest injection speeds, the opposite is observed: G' decreases sharply and G'' similarly overshoots abruptly at the onset of nonlinearity. While deeper analysis of this data is beyond the scope of this work, the observed trent in **Fig. 3 (d)-(f)** is characteristic of a transition between ductile yielding and brittle yielding, suggesting that the different gelation kinetics between the three samples result in hydrogels with distinctly different structures.

Conclusions

In summary, we have developed a simple experimental approach that enables the in-situ control of chemical environment during rheological testing for any viscoelastic material. Although our device stands on the shoulders of other devices reported previously, we have also introduced a number of novel innovations that we believe will broaden the applicability of this approach to other applications. Firstly, the use of a conical flow cell moves the point of injection into the flow cell from the sample surface and thereby minimises the possibility of any pressure graidents. Secondly, by separating the flow chamber from the sample through a semi-permeable membrane, we mechanically isolate the process of chemical exchange from the sample which also minimises the potential of pressure from injection from disrupting the sample. Finally, our Rheodialysis approach allows reaction kinetics to be controlled by adjusting the flow rate. Using alginate that is crosslinked in-situ by calcium chloride as model system, we have shown that the gelation time t_g, defined precisely through the Winter-Chambon criterion can be controlled through adjusting the injection rate. We rationalise this by considering the interplay between the injection rate and binding rate of calcium and postulate that the sensitivity of gelation kinetics to the former suggests that the two rates must be of similar order of magnitude. Intrigingly, the resulting hydrogels appear to have distinctly different rheological properties, both in the linear regime and in the nonlinear regime. This has clear implications for any industrial or biological application in which hydrogel crosslinking occurs at an interfaces, which includes wound healing, coatings, blood clotting and food products.

There are number of considerations regarding the Rheodialysis approach that remain inexplored in this work. More detailed complex fluid dynamics analysis of the binding of alginate and calcium with the hydrogel will allow us to better understand the structural changes that take place during gelation. Similarly, it is likely that the results presented in this work will be sensitive to the gap separation of the plates since our flow cell is incorporated into the lower plate. Finally, more detailed analysis of the nonlinear rheological response will enable us to better appreciate the structural changes

Our view is that there a wide diversity of potential applications for the Rheodialysis approach. Our approach could, for example be used to inform end-use industrial processes that involve rheological changes under shear, for example the topical application of creams and lotions, digestion of food products and rapidly dilution of detergents and cosmetics. Each of these applications have a co-dependence between shear and chemical exchange, which remain relatively unexplored and could be addressed using the approaches and principles set out here.

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