This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Gel to gel transitions by dynamic self-assembly
Santanu Panja and Dave J. Adams*

Received 00th January 20xx, Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

Dynamic systems are of great interest from the perspective of mimicking biology through to preparing useful and exciting materials. Transient supramolecular gels are potentially useful, but there are limited applications that require a gel that only exists for a short length of time. Here, we show how a dynamic system can be designed to prepare materials with properties that cannot be directly accessed using the same gelator.

Gels are important and useful materials, both academically and industrially. These materials contain significant amounts of liquid, but behave as solids. The solid-like behaviour arises from a network, which immobilises the solvent. The networks can be essentially permanent (as in cross-linked polymer gels). In comparison, supramolecular gels are formed by networks that are a result of non-covalent interactions; as a result, these networks can be readily disassembled, resulting in a return to the solution state.

Typically, gels are formed and then used, generally with the aim that their mechanical properties do not change over their useful lifetime. However, dynamic systems are becoming increasingly of interest. In these dynamic gel systems, typically a supramolecular gel is formed on application of an energy input or fuel, which drives the formation of a molecule capable of forming a gel. When the energy source is turned off or the fuel runs out, the system returns to its original, non-gelling state. Hence, transient gels can be formed, the duration of which is controlled by the concentration of the fuel or energy inputted. Such gels are interesting, and there have been a small number of suggested applications. As a specific example describing a change in state from a solution to a gel phase, followed by a return to the solution phase when the fuel runs out or energy input ceases, Tena-Solsona et al. have shown that a transient gel can be formed using the addition of a fuel to a Fmoc-amino acid, which can be used to form self-erasing inks. However, it has been pointed out that most (if not all) of the dynamic assemblies reported so far have no practical application. For the field to progress, this needs to change.

Another interesting, but as yet unrealised, possibility is a reconfiguring of state to give a different material at the end point, for example a change from a solution to a gel, followed by formation of a different type of gel network (Figure 1). This could be an extremely powerful approach to adapt material properties, annealing the properties, controlling diffusion or synergistic catalytic effects by changing the network for example. It may also be possible to access materials which cannot be formed directly. This could be potentially be achieved in a spatially controlled manner.

![Figure 1](image1.png)

**Figure 1.** Inducing gel to gel transitions by dissipative assembly. The gelator used here can be used to form a transient gel, with disassembly driven by an increase in pH. At high pH, a second type of gel network is formed.

When a DMSO solution of 1 (Figure 1) is diluted with H₂O (final ratio 20/80 DMSO/water (v/v)), phase separation occurs and a self-supporting gel is formed at a concentration of 1 of 2 mg/mL (Figure S1). The gel consists of densely packed spherulitic domains of fibres as observed from confocal microscopy imaging (Figure S2). Rheological studies showed that the storage modulus (G’) is significantly higher than the corresponding loss modulus (G’’) and both are independent of frequency (Figure S3 and S4). The gel has strain bearing capacity of ~5 % before it started to collapse. The pH of the gel was...
measured to be ~4.1. A stable gel was also formed when urease is present, or when urease and a calcium salt is present (Figure S1), with the gels having very similar microstructure (Figure S2). The rheological moduli are slightly lower in the presence of a calcium salt and/or the enzyme (Figure S3, S4).

At this ratio of DMSO and H2O, 1 exhibits a pKd of 5.7 (Figure S5); therefore, if we increase the pH of the gel above a pH 5.7, the gel would be expected to return into a solution state. This is indeed the case if we adjust the pH with sodium hydroxide (Figure S6). Gel formation does not occur if the initial pH is above 5.7 (Figure S7).

As a first step towards constructing an out-of-equilibrium system involving the DMSO/water gel of 1, we used a slow increase in pH as a counter trigger to drive the self-assembly towards non-assembled states. The autocatalytic reaction between urease and urea produces NH3,23, 24, 25 that can trigger homogeneous hydrogelation of cationic amphiphiles at high pH.26 We therefore examined the rate of pH change involving urease-urea reaction in presence of gelator 1. When an aqueous solution of the enzyme was added to the vial containing a mixture of urea (in water) and gelator (in DMSO), initially the pH dropped to ~pH 4.1 and a gel was formed as above (Figure 2).

With time, the pH of the medium increased slowly and reached above pH 5.7 (pKd of 1) within ~30 mins (Figure 2a). As expected, at a pH above the pKd, the gel was not stable, but rather a highly viscous material was formed over time which did not allow the inversion of vial. The viscous material progressively converted to a clear solution with time (Figure 2b). Interestingly, the final viscosity of the solution is higher than if a solution is prepared directly at high pH (Figure S8). Hence, as expected, this method allows the preparation of gels with a transient lifetime. The lifetime of the gel could be easily controlled by the concentration of the enzyme and urea (Figure S9 and S10).

The resulting non-equilibrium assemblies can be further understood using time sweep rheology (Figure 2c), viscosity (Figure 2d) and confocal microscopy (Figure 2e). From the rheology, initially the storage modulus (G′) was considerably higher than the loss modulus (G″), showing that a gel is formed quickly on addition of water to the DMSO solution of 1. As the pH of the system increases, G′ begins to decrease indicating destruction of the intermolecular associations. The rate of pH change can be controlled by varying the concentration of urease or urea (Figure 2a). When the rate of pH change was slow, the decrease in G′ was significantly delayed as compared to a system where the rate of pH change was higher (Figure S9). In both cases, initially tan δ (G″/G′) decreases and becomes almost constant for a limited time frame before it started to increase again. The viscosity data also depends on pH and mirrors the rheology. Time dependent confocal microscopy imaging was used to show the presence of spherulitic domains of fibres initially (as expected from this solvent-triggered approach22). With time, these domains become less distinct and disappear, leading to the gel converting to a solution (Figure 2e and S9).

Homogeneous hydrogelation of solutions of 1 directly at high pH can be achieved in presence of Ca2+ ions.22 The divalent ion cross-links the worm-like micelles that are formed at high pH. With this in mind, we incorporated a calcium salt in our systems, initially at a concentration of 0.5 molar equivalents with respect to 1. As mentioned above, there is little effect on the initially-formed gels. In the presence of urease and urea, the pH again increased with time (Figure 3a and S12). The rate of pH change was again dependent on the concentration of the urease and urea; the rate was also dependent on the concentration of Ca2+ (Figure S12). At higher concentrations of Ca2+, a slight decrease in pH is observed at longer times. It is not currently clear why this occurs. Under all conditions, instead to the formation of solutions as in the absence of Ca2+, at high pH we obtained gels that were transparent in comparison to the DMSO-H2O gel (Figure 3b and S13). Hence, the dissipative assembly here leads to an unusual gel-to-sol-to-gel transition.

The pH-dependent destruction and salt-induced reconstruction of the assemblies was studied by time sweep rheology (Figure 3c and S14). At low Ca2+ ion concentration, self-assembly begins (~pH 4) immediately after the addition of H2O (containing urease) as observed from the gradual increase of G′ with time. As the pH of the medium progressively increases, G′...
started to decrease and reached minimum at pH 5.7 (the pK_a of 1). This indicates that the primary aggregated structures are disassembling near pH 5.7. However, further increase in G with an increase in pH confirms the reconstruction of a new type of aggregation involving the Ca^{2+} ions which crosslink the carboxylate groups of 1. Analysis of tanδ also confirms disassembly and rebuilding of aggregation below and above the pK_a of the gelator respectively. A similar trend in the variation of G' and tanδ was also found in presence of 2 equivalents of Ca^{2+} ions (Figure S14). At a fixed concentration of Ca^{2+}, G' increases as the rate of pH change is decreased.

Interestingly, at a fixed Ca^{2+} ion concentration, when the rate of pH change is high, a sudden decrease in G' was noticed just after when the pH had reached a plateau at pH 9 (Figure S14). This was not observed when the pH change was slow. We hypothesise that when the pH change is fast, the Ca^{2+} binds structures reorganize again at high pH. In this case, analysis of tanδ confirms the existence of solid-like nature of the gels throughout the high pH regime.

To get more insight into the development of the microstructure of the respective gels, confocal microscopy imaging was conducted at different time intervals (Figure 3c and S15-S17). As soon as water is added to the DMSO solution of 1, spherulitic domains of fibres were observed. With time, these structures disappear, and new types of fibres appeared. This structural reorganization with time was more prominent when the rate of pH change was slow. Interestingly, an increase in Ca^{2+} ion concentration resulted in networks containing fewer spherulitic domains and instead a higher density of long fibres. Moreover, at a fixed Ca^{2+} ion concentration, gels obtained from slow rates of pH change were found to contain fibres which are relatively long and more interlinked than gels formed at a higher rate. We also followed the process by UV-Vis and fluorescence spectroscopy (Figure S18-S21). Whilst changes can be found, it is difficult to correlate the changes exactly as there are also turbidity changes throughout the process (Figure S22).

The final gels exhibit different mechanical properties depending on the kinetics of hydrogel formation and showed significant differences in G' and G'' values, as well as in gel strengths (Figure 4a and 4b, Table S1). For all the gels, G' was considerably higher than G''. At a fixed enzyme and urea concentration, an increase in the concentration of Ca^{2+} ions resulted in substantial increase in stiffness (G') of the gels (>2 times). In the strain sweeps for all gels, both G' and G'' were essentially constant at low strain but deviated from linearity after a certain strain (>20%) strain indicating breaking of the gels (critical strain). At a particular Ca^{2+} ion concentration, the gels formed at higher rates break at higher strain and exhibited a high elastic nature (high strength) of the gels. In contrast, a decrease in gelation rate resulted in significant increase (>3 times) in G' and G'' of the gels. However, irrespective of formation conditions, all these gels were essentially frequency independent (Figure S23).

Interestingly at a fixed Ca^{2+} ion concentration, when the rate of pH change is high, a sudden decrease in G' was noticed just after when the pH had reached a plateau at pH 9 (Figure S14). This was not observed when the pH change was slow. We hypothesise that when the pH change is fast, the Ca^{2+} binds structures reorganize again at high pH. In this case, analysis of tanδ confirms the existence of solid-like nature of the gels throughout the high pH regime.
Ca\textsuperscript{2+} ions. Then in both cases we added Ca\textsuperscript{2+} ions on the top of the solutions and left the samples undisturbed for \textasciitilde16 hours to obtain gels. These gels showed formation of long fibers in the aggregated states (Figure S24). A significant difference in visual appearances was noticed as turbid inhomogeneities are prominent in these gels compared to the transparent homogeneous gels obtained through controlled pH change (Figure 4c). In comparison to the gels formed by the dissipative process, the gels formed directly at high pH exhibits considerably higher \(G'\), but break at a lower strain (Figure 4d and S20, Table S2). Evaluation of the rheological data emphasizes that gels obtained from the dissipative pH change could withstand a strain (20-40\%) with higher crossover points (yield points are \textasciitilde450\% strain where \(G'\)=\(G''\)), than the gels formed directly at high pH (critical strains are \textasciitilde10\%, yield points are \textasciitilde100\% strain). This emphasizes that this unusual gel-to-sol-to-gel approach allows access to materials that cannot be directly formed under the final conditions. Interestingly, adding a Ca\textsuperscript{2+} salt to a solution prepared by the dissipative process after the pH has increased results in a lower \(G'\), again showing how important the process is to the final gel properties (Figure 4d and S25, Table S2).

Finally, our approach can be exploited to prepare an interesting case where gels are initially formed. After a pre-determined time, the return to the sol state allows flow, before the re-gelation immobilized the material once again. This is shown schematically in Figure 5.

In conclusion, we have shown how a pH responsive out-of-equilibrium system can be programmed to drive an unusual gel-to-sol-to-gel transition. Unlike all other current examples of gel-based dissipative assembly, we have a system where we do not simply form a single system and then revert to the original state. Kinetic control over gelation is achieved by simple modulation of the reaction conditions that allows us to prepare homogeneous gels with improved mechanical properties. As mentioned above, there is a real need in this field for real applications to be developed. Unlike conventional dissipative assembly, where the transient formation of a gel means their use is limited to the highly specific applications where short-lived networks are needed, our gel-to-sol-to-gel transition allows us to prepare gels with properties that cannot be directly accessed for example. This makes our approach analogous to annealing.

Acknowledgments. S.P. thanks the Royal Society and SERB for an India for a Newton International Fellowship. D.A. thanks the EPSRC for a Fellowship (EP/L021978/1).

Conflicts of interest There are no conflicts to declare.

Notes and references