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# Temporally-Programmed Transient Supramolecular Gels

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**In living systems, self-assembly processes are driven by the consumption of chemical fuels. Synthetic adaptation of living systems can be achieved by coupling of competing pathways that drive the assembly and disassembly, respectively, under the influence of chemical fuels. Here, a pH-responsive transient gel system is created by simultaneous incorporation of two triggers, of which one is responsible for the initiation of the self-assembly by increasing the pH and the second trigger drives the disassembly by reducing the pH. This method allows us to prepare transient gels with a high degree of control over the self-assembly lifetime as well as the mechanical properties of the transient gels.**

Nature utilizes self-assembly to create molecular systems capable of carrying out biological processes with autonomous regulation.[1] Natural self-assembly systems operate out of equilibrium and so require a continuous energy input to sustain the assembly structures. As a result, the assemblies only exist whilst the energy source is available. Once the source of energy is removed, the assembled structures revert back to the original thermodynamically-stable precursor. This results in an aggregation-to-nonaggregation transition. The formation of these transient assemblies is thermodynamically unfavorable and can only be controlled by the kinetics of fuel consumption.[1b,c] An interesting and difficult challenge is to prepare synthetic analogues. Limited attempts have been reported to create transient assemblies; where successful, strategies like co-operative catalysis, chemically fueled assemblies, pH cycles, different enzyme programmed reactions and redox reactions have been used.[1b,2] It is now becoming clear that switchable assemblies with a tunable lifetime can be designed by balancing the activation and deactivation kinetics,[2c,e,f,j,3] where responsiveness of the precursors toward the environment is the key factor to determine the lifetime of the assemblies.

Supramolecular gels are receiving attention in artificial synthesis of out-of-equilibrium, or energy-dissipative, assemblies, because of their responsiveness toward signals like redox, pH, temperature, and specific ions.[2a–c,e,f,k,4] Whilst some success has been reported, there are drawbacks in many cases. In some cases, there is only a small operational pH range. For example, Wojciechowski et al. reported redox-responsive transient hydrogelation of *N,N′*-dibenzoyl-L-cystine at pH 2.70 while performing experiments at pH 3.12 only gave a viscous solution.[2c] Similarly, George and co-workers recently developed a transient supramolecular polymer gel involving cooperative catalysis at extremely high pH.[2e] In some cases, the reactions were performed in semi-aqueous solvents,[2e,k,5] which impose limitations on use in biological fields. In other cases, during the refueling experiments, the solvent compositions are changed.[5] Finally, there are potential temperature issues, where low temperatures are required for inducing gelation.[6]

We also note that in many cases gelation is triggered rapidly (within 30 s)[2c] and only the disassembly kinetics, if any, tend to be controlled.[2e,3a,5,7] Rapid gelation is often associated with mixing issues, and hence there are potential concerns over reproducibility and scale up if kinetics are not controlled.[8] We also note that rheological studies are rarely reported demonstrating the sol–gel–sol transitions.[2c,e,k,7,9] Simple vial inversion is the only demonstration of gelation and hence it is not possible to understand whether the reported gels have properties that are useful or reproducible. On top of this, the short lifetime of the transient assembled state means probing the microstructure of the gel is difficult.[2a,e,h] There is a real need for more detailed understanding in order to understand the assembly/disassembly process at the molecular level.

Here, we describe a pH-responsive transient supramolecular gel where we minimize the above-mentioned difficulties and drawbacks. This work builds from the work of others where enzymes have been used to control assembly and disassembly.[2i,j,3,5,7,9,10] The solubility is controlled by the degree of protonation of an amino group, as opposed to a specific reaction with a fuel.[2d] We use the cationic amphiphile **1** that forms a self-supported gel at basic pH in water.[8a] To construct the transient gel, we simultaneously use two triggers. The first is responsible for increasing the pH of the medium, causing gelation. The second trigger causes a subsequent decrease in pH (**Figure 1**). To minimize mixing issues during gelation, we utilize the autocatalytic reaction between urease and urea to generate $NH_3$ for a controlled and uniform pH increase that leads to homogeneous hydrogelation as we have shown previously.[8a,11] This method developed by Jee et al. allows a controlled increase in pH.[11] We also include methyl formate, which hydrolyses and causes the disassembly of the gel network by reducing of the pH of the medium. By simple

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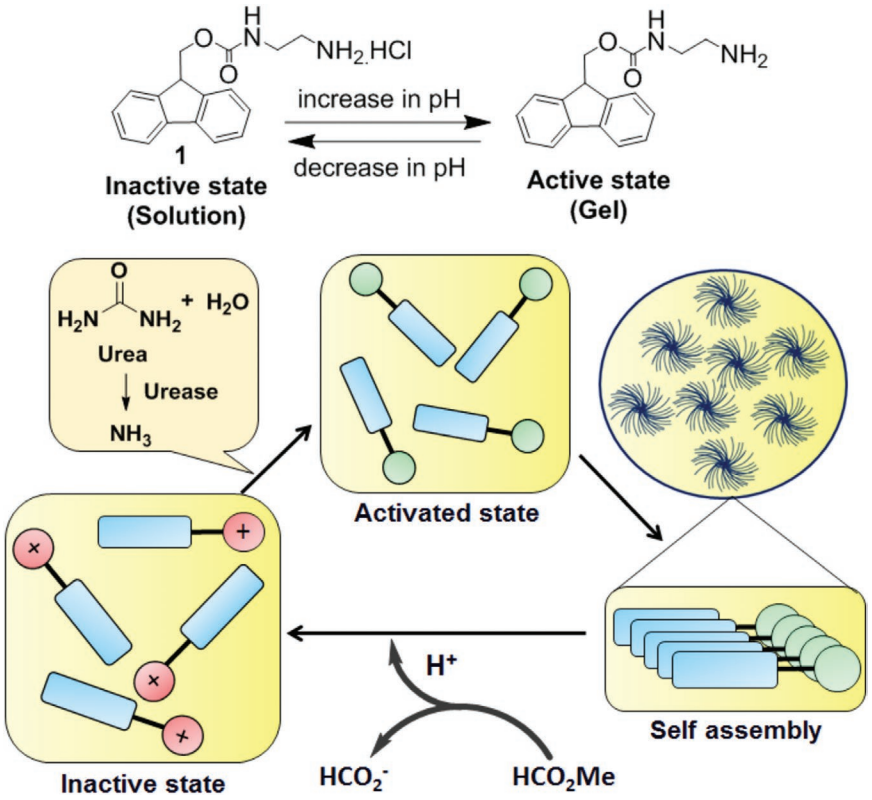


**Figure 1.** Cartoon of transient hydrogelation of **1** driven by simultaneous incorporation of a trigger and a counter trigger. The trigger is responsible to increase the pH while the counter trigger is accountable to revert the pH.

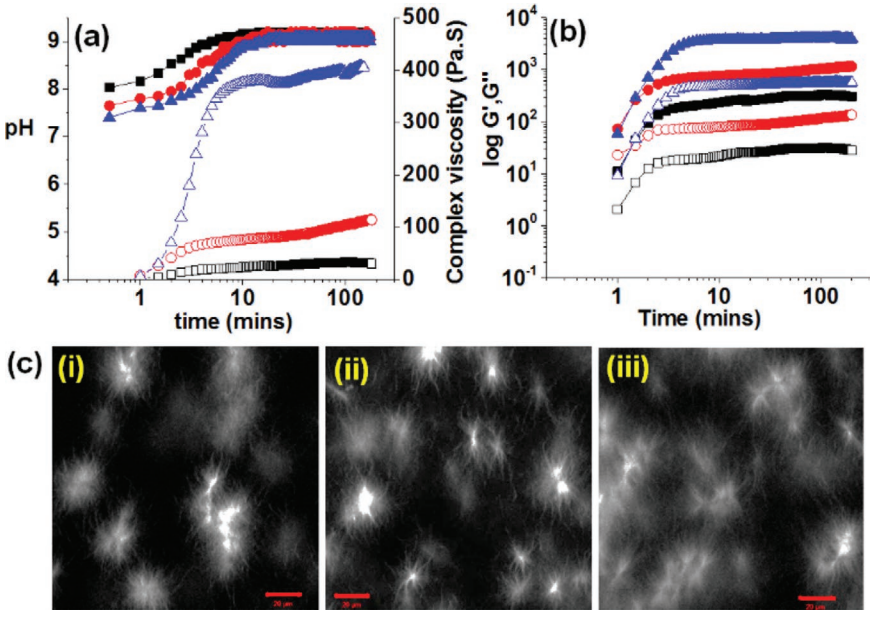


**Figure 2.** a) Variation of pH (closed symbol), complex viscosity (open symbol) (a) and G′ (close symbol), G″ (open symbol) b) with time for **1** in presence of urea–urease reaction. c) Confocal microscopy images of the hydrogels of **1** having gelator concentration 2, 3, and 4 mg $mL^{-1}$ for (i), (ii) and (iii) respectively (scale bar is 20 µm). For (a) and (b) the concentration of **1** is 2 (black), 3 (red) and 4 (blue) mg $mL^{-1}$. For (a)–(c) the initial reaction condition is [urease] = 0.2 mg $mL^{-1}$, [urea] = 0.02 M.

variation of the initial reaction conditions, we are able to prepare transient gels with a high degree of control over the lifetime as well as mechanical properties.

**1** is highly soluble in water. As the pH of the solution is increased, the corresponding less soluble amine becomes predominant and undergoes self-aggregation to form a self-supported gel network at a concentration of 2 mg $mL^{-1}$ or above.[8a] To prepare a homogeneous gel by eliminating mixing issues, we employ the autocatalytic reaction between urease and urea to generate $NH_3$ locally in order to promote a uniform pH increase.[2,j,8a,10a,11] When a mixture of urease (0.2 mg $mL^{-1}$) and **1** (2 mg $mL^{-1}$) was added to urea (0.2 M), the pH of the medium increased and reached the plateau at pH 9.1–9.2 within 10 min (**Figure 2**a and Figure S1, Supporting Information). An increase in gelator concentration resulted in a considerable decrease in the rate of pH change as observed from the pH-time profiles (Figure 2 and Figure S1, Supporting Information). Note, to control the final material properties, it is really important to control the onset of gelation as mentioned above. This allows reproducible materials to be prepared. To show this effectively, in all cases here we show the time axis on a log scale. Data where a linear scale is used is available in the Supporting Information. In all cases, a visual transformation occurred from a free-flowing solution to a highly viscous material that resisted inversion of the vial after 4–6 min. Time sweep rheology as well as viscosity measurements indicated the transition of a solution into a gel with time (Figure 2a,b and Figures S1 and S2, Supporting Information). For all gels, the storage modulus (G′) was significantly higher than the loss modulus (G″) as expected.

An increase in gelator concentration increases the stiffness (G′) as well as viscosity of the gels as expected. The microstructure of the respective gels was examined by using confocal microscopy imaging, showing the presence of spherulitic domains of fibers (Figure 2c). Increasing the gelator concentration resulted in gels that contain more spherulitic domains with a higher interlinkage of fibers. This difference in the network structure correlates with the higher stiffness as well as viscosity of the gels.

To drive the system toward disassembly, we need to drop the pH of the medium

from basic to acidic. To do this, we utilized the base-catalyzed saponification reaction as a counter trigger/deactivator, releasing protons during the hydrolysis reaction. We used methyl formate as the deactivator, which produces formic acid on hydrolysis, lowering the pH. In the absence of gelator, the enzymatic reaction initially exhibits a slow decrease in pH, followed by a rapid decrease in the overall pH of the medium (Figure S3, Supporting Information). However, the hydrolysis of methyl formate depends upon the concentration of the methyl formate used. An increase methyl formate concentration not only showed significant reduction of the maximum and final pH of the medium but also exhibited a much faster rate of decreasing in pH.

To construct a dissipative self-assembly system involving **1** we simultaneously incorporated both the trigger (enzymatic reaction) and the counter trigger (methyl formate). Balancing the rate of activation and deactivation determines the extent of self-aggregation and allows programmable autonomous self-regulation of the corresponding hydrogel assembly in time. Initially, we used 25 µL of methyl formate and recorded the change in pH with time in presence of **1** with the enzymatic reaction (**Figure 3**, top left) (Figure S4, Supporting Information). The pH-time profile clearly indicates existence of three different zones. Initially, the pH of the medium increases as the production of ammonia is faster than the hydrolysis of methyl formate. The pH then becomes almost constant at pH 8.5–8.6 for a considerable time. The pH then decreases as the production of formic acid becomes accelerated.

The physical behavior correlates with the changes in pH. Gelation occurs at the beginning (after 4–5 min). Note, the time for gelation is based on the observation that the vial can be inverted. This does necessarily not correlate with a specific value of G′ (vial inversion is determined by yield stress,[12] not G′). Since the system is constantly evolving and it is likely that repeated checking of inversion will lead to an effect on the gelation, we provide gelation times as approximate values. Even at the beginning, G′ is greater than G″ as this is a structured liquid. However, comparison of tan delta (G″/G′) shows that this is not a true gel. It is worth noting that this is not an uncommon observation, with a number of other studies showing similar behavior where G′ is greater than G″ or that there are structures present prior to addition of the trigger.[2c,i,k,13] With time, the gels started to disintegrate (after 25 min) and a clear solution is formed after 15 h (Figure 3, top right). Time sweep rheology also showed an initial increase in G′ and G″, which became almost constant for ≈20 min before starting to decrease again (Figure 3, top left) (Figure S4, Supporting Information). Similarly, the viscosity data exhibits a bell-shaped curve, showing a maximum viscosity at pH 8.5–8.6 followed by a gradual decrease with the decreasing pH of the medium (Figure 3, top left) (Figure S4, Supporting Information). Confocal microscopy with time shows aggregation begins immediately (within 2 min) after starting of the enzymatic reaction, with the appearance of spherulitic domains of fibers observed at the early stage

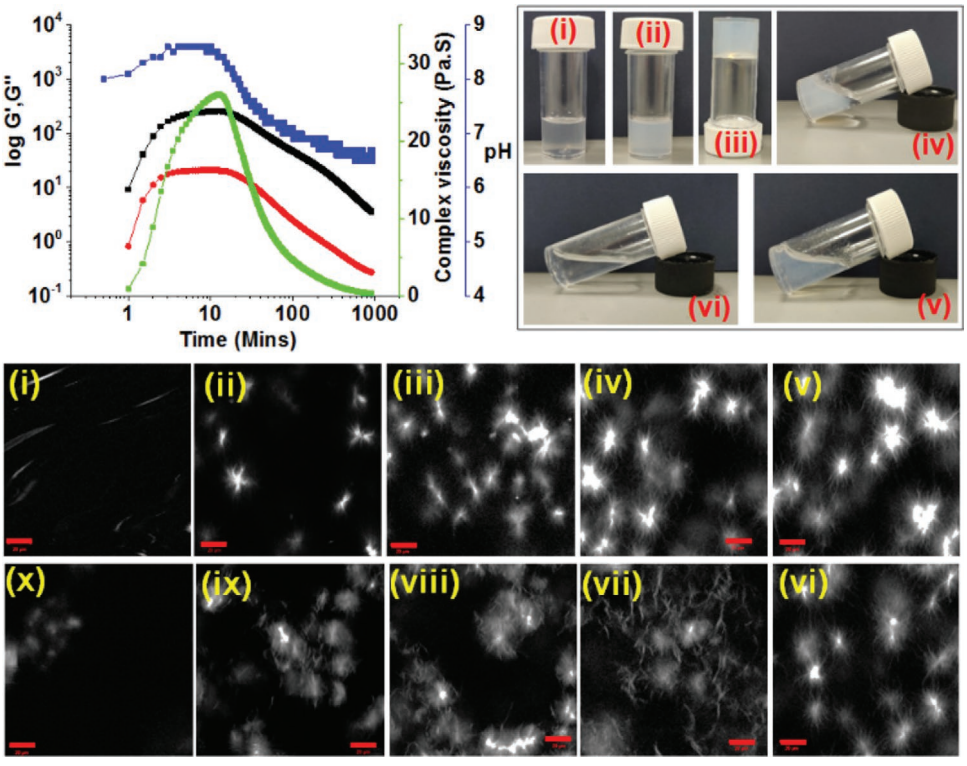


**Figure 3.** (Top left) Variation of pH (blue), complex viscosity (green), G′ (black) and G″ (red) with time for **1** from the urea–urease reaction in presence methyl formate. (Top Right) Photographs showing the phase change of **1** with time when a mixture of **1** and urease (i) was added to the mixture of urea and methyl formate; after (ii) 2 min, (iii) 5 min, (iv) 25 min, (v) 3 h, (vi)15 h. (Bottom) Time-dependent confocal microscopy images of **1** in presence of urea–urease reaction involving methyl formate (scale bar is 20 µm): (i) 0 min, (ii) 2 min, (iii) 5 min, (iv) 10 min, (v) 20 min, (vi) 30 min, (vii) 1 h, (viii) 3 h, (ix) 5 h, (x) 15 h. In all cases, initial reaction condition is [**1**] = 2 mg mL$^{-1}$, [urease] = 0.2 mg mL$^{-1}$, [urea] = 0.02 M, volume of methyl formate = 25 µL.

(Figure 3, bottom). With further time, the density of the spherulitic structures increases and interlinked fibers can be observed around the same time as when the pH and the rheological moduli reach their maximum. As time proceeds, the fibers start to break down as the pH of the medium decreases and discrete spherical aggregates are formed. With further time, the concentration of these spherical aggregates decreases and after 15 h, they almost disappeared correlating with the conversion to a solution.

In order to monitor the transient behavior further, UV–vis spectra were recorded. As the pH of the medium increases, the absorbance at 265 nm started to decrease with the appearance of a new band at 303 nm (Figure S5, Supporting Information), attributed to aggregation of the gelator.[8a] As the pH of the medium began to decrease, the opposite trend was noticed indicating the transient existence of the aggregates. A plot of absorbance at 303 nm against time showed a similar profile to the rheology (**Figure 4**a). Time variable $^1$H NMR experiments also back up the sequential assembly and disassembly of **1** (Figure 4a and Figures S6 and S7, Supporting Information), where aggregation leads to disappearance of the signals from **1**. These reappear as the pH decreases again at later times. Calculation of signal intensity shows that ≈98% of molecules of **1** in solution disappear within 5 min. After 30 min, the signals of **1** gradually increase again and around 90% of the signal intensity is recovered after around 15 h. Importantly, these NMR data also shown that no deprotection of the Fmoc group occurs at the pH used here.

Importantly, it is possible to control the properties and lifetime of the gel. Increasing the concentration of **1** results in only slight changes in the rate of gelation and reformation of the solution phase. There were also no changes in maximum pH (pH 8.4–8.6). However, as expected, there was an increase in the maximum viscosity and rheological moduli in the high pH gel state (Figure 4b and Figures S8 and S9, Supporting Information). An increase in gelator concentration from 2 mg mL$^{-1}$ to 4 mg mL$^{-1}$ resulted in ≈10 times enhancement of G′ and viscosity of the assembled states at transient high pH-regime. There was also a considerable improvement of the lifetime of the gel from ≈20 min (2 mg mL$^{-1}$) to ≈12 h (4 mg mL$^{-1}$) (Figure 4c). Again, these data correlate with the rate of decay in absorption at 303 nm (Figure S10, Supporting Information). Compared to the gels formed in the absence of methyl formate, the transient gels exhibit a lower gel stiffness as well as in viscosity, even at their maximum value.

The lifetime of the transient gel can also be controlled by adjusting the concentration of methyl formate, whilst keeping all other parameters fixed. Increasing the concentration of methyl formate results in an increase in the rate of pH change. The maximum pH of the reaction medium also reduced from pH 8.5–8.6 (at 25 µL of methyl formate) to <pH 8.0 (using 100 µL) (Figure S11, Supporting Information). There was also a significant increase in the rate of decrease of pH. The change in the kinetics of change of pH over the course of the experiment influences the stability of the aggregated structures. An increase volume of methyl formate from 25 to 100 µL resulted in a significant delay of the appearance of the gel (from around 5 to around 10 min), supported from the rheology and viscosity data (Figures S11 and S12, Supporting Information). The absolute stiffness (G′) as well as the complex viscosity values of the transient gel state notably decreased (>80%) which ultimately reduced the gel lifetime from ≈20 min (at 25 µL of MF) to ≈2 min (at 100 µL MF). Similar trends in the pH, rheology as well as in viscosity data were recorded when we performed the reactions at gelator concentration of 4 mg mL$^{-1}$ (Figures S13 and S14, Supporting Information). In this case, increase in methyl formate volume from 25 to 100 µL not only resulted

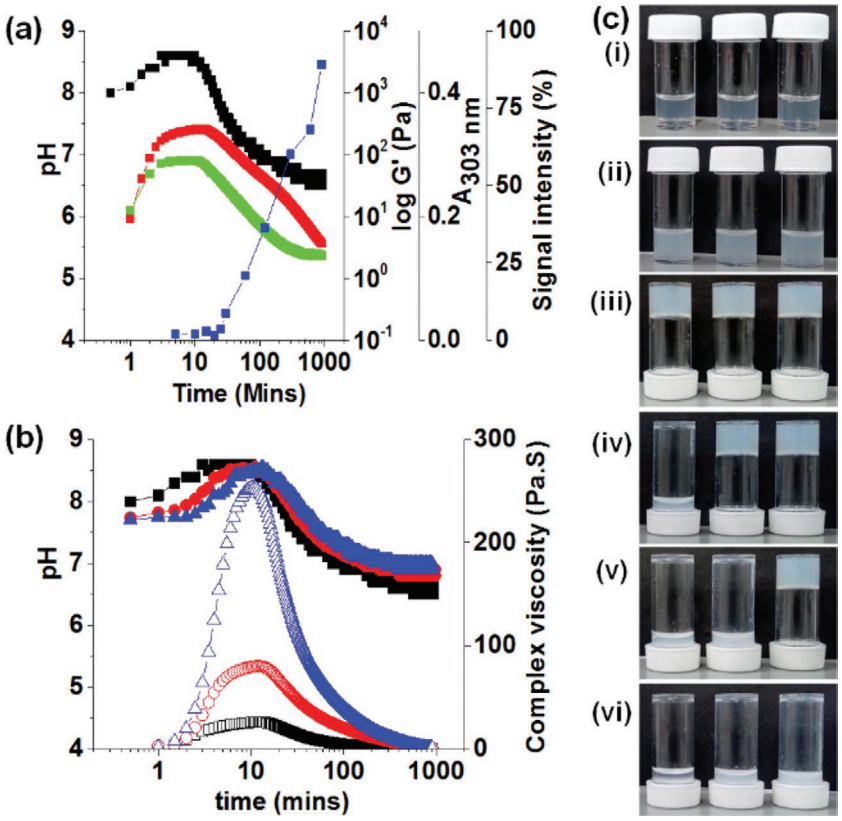


**Figure 4.** a) Variation of pH (black), G′ (red), absorbance at 303 nm (green) and $^1$H NMR signal intensity (blue) with time for **1** from the enzymatic reaction in presence methyl formate. Initial reaction condition is: [**1**] = 2 mg mL$^{-1}$, [urease] = 0.2 mg mL$^{-1}$, [urea] = 0.02 M, volume of methyl formate = 25 µL; b) Variation of pH (close symbol) and complex viscosity (open symbol) for **1** in presence of urea–urease reaction involving initial reaction conditions: [urease] = 0.2 mg mL$^{-1}$, [urea] = 0.02 M, volume of methyl formate = 25 µL, [**1**] = 2 mg mL$^{-1}$ (black), 3 mg mL$^{-1}$ (red) and 4 mg mL$^{-1}$ (blue); c) Photographs showing the phase change of **1** with time when a mixture of **1** and urease (i) was added to the mixture of urea and methyl formate: after (ii) 2 min, (iii) 5 min, (iv) 25 min, (v) 2 h, (vi) 12 h. In each photograph, from left to right, concentration of **1** is 2, 3, and 4 mg mL$^{-1}$, respectively. Initial reaction condition is [urease] = 0.2 mg mL$^{-1}$, [urea] = 0.02 M, volume of methyl formate = 25 µL.

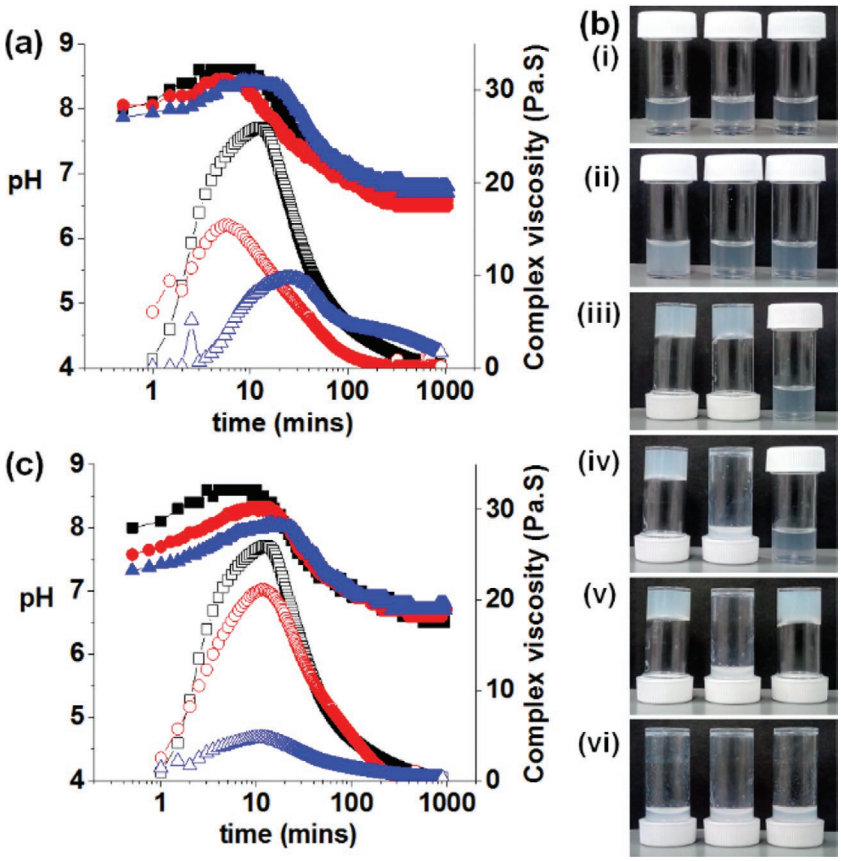


**Figure 5.** a) Variation of pH (close symbol) and complex viscosity (open symbol) for **1** (2 mg mL$^{-1}$) in presence of urea–urease reaction under different conditions: (black) [urease] = 0.2 mg mL$^{-1}$ and [urea] = 0.02 M, (red) [urease] = 0.2 mg mL$^{-1}$ and [urea] = 0.01 M, (blue) [urease] = 0.1 mg mL$^{-1}$ and [urea] = 0.02 M. In all cases, volume of methyl formate used was 25 µL; b) Photographs showing the phase change of **1** with time when a mixture of **1** and urease (i) was added to the mixture of urea and methyl formate: after (ii) 2 min, (iii) 5 min, (iv) 10 min, (v) 15 min, (vi) 25 min. In each photograph, from left to right: [urease] = 0.2 mg mL$^{-1}$ and [urea] = 0.02 M, [urease] = 0.2 mg mL$^{-1}$ and [urea] = 0.01 M, [urease] = 0.1 mg mL$^{-1}$ and [urea] = 0.02 M. In all cases, concentration of **1** is 2 mg mL$^{-1}$, volume of methyl formate = 25 µL; c) Variation of pH (close symbol) and complex viscosity (open symbol) for **1** (2 mg mL$^{-1}$) in presence of urea–urease reaction involving initial reaction conditions: [urease] = 0.2 mg mL$^{-1}$, [urea] = 0.02 M, volume of methyl formate = 25 µL, [**1**] = 2 mg mL$^{-1}$ for the first cycle (black), second cycle (red), and third cycle (blue).

in substantial decrease in G′ and viscosity of the transient gel states, but also reduced the gel life time from ≈12 h to ≈3 h. Again, all of these data correlate with the changes in the UV–vis spectra. (Figure S15, Supporting Information).

A decrease in enzyme concentration (keeping all other parameters constant) also significantly reduces the rate of pH increase, which in turn resulted in substantial delay in the appearance of the gels (after ≈15 min) (**Figure 5**a,b and Figure S16, Supporting Information). The stability of the transient gel reduced to ≈10 min. Reduction in urea concentration reduces the gel lifetime to ≈5 min. Either a decrease in urease or urea concentration produced transient gels with inferior mechanical properties (in terms of G′ and viscosity) (Figure S17, Supporting Information). All these observations indicate that the production of $NH_3$ relies on both the concentration of urease and urea. If either parameter is reduced, there is a reduction in the pH at the assembled state, and hence reduction in gel lifetime as well as gel strength.

An interesting question is whether the system can be reused repeatedly by refuelling after the gel-to-sol transition. After the first cycle, further addition of urea and methyl formate (carried out after 15 h) resulted in the reformation of the gel, which showed similar dissipative nature but exhibited a slight decrease in gel stiffness (G′), viscosity, and lifetime (gel lifetime ≈15 min) (Figure 5and Figures S18–S20, Supporting Information). We were able to cycle the system three times in total. However, in the third cycle, the appearance of the gel was significantly delayed (to around ≈10 min), and the gel collapsed very quickly after formation (gel lifetime ≈1 min). We presume that the build-up of methyl formate/acid within the reaction medium is responsible for the number of assembly–disassembly cycles being limited to three.

In conclusion, we have successfully created a pH-responsive transient gel system with a high degree of control over the lifetime as well as the mechanical properties of the transient gels by simultaneous incorporation of two triggers. Temporal control over the assembly and disassembly is achieved in a number of ways by simply varying the initial reaction conditions. Applying different conditions, it is possible to control the stability of the transient gel over a wide time range from ≈1 min to ≈12 h. We are also able to prepare gels with different and controllable mechanical properties.

## Experimental Section

See Supporting Information for full details.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

dissipative self-assembly, kinetic control, pH responsiveness, self-regulation, supramolecular gels

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