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COMMUNICATION

Varying the hydrophobic spacer to influence multicomponent gelation

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Mixing low molecular weight gelators (LMWGs) shows promise as a means of preparing innovative materials with exciting properties. Here, we investigate the effect of increasing hydrophobic chain length on the properties of the resulting multicomponent systems which are capable of showing ambidextrous phase behaviour on pH perturbation.

Non-covalent interactions such as hydrogen bonding, aromatic stacking, electrostatic and hydrophobic interactions are the essence of most biological systems and processes. Incorporation of such interactions into synthetic materials can lead to various self-assembled systems; supramolecular hydrogels are one of these that have many applications. Typically, hydrogels are formed when small organic molecules self-assemble into long anisotropic fibres. These fibres then entangle to form a 3D-network that immobilizes the water. The intermolecular interactions essentially control the formation of such fibres, and their subsequent growth and distribution inside the matrix. A subtle change in the intermolecular interactions can significantly influence the gel properties; perturbation of non-covalent forces may even lead to destruction of gels and formation of sols.

In spite of reasonable progress in gel chemistry, it is still a difficult task to design molecules that form gels. Although computational techniques have been developed to predict gelation propensities of compounds, their use is still limited. As an alternative approach, it is common to adapt the properties of known gelators by following different approaches: first, by varying the route of self-assembly, specifically the self-assembly kinetics, keeping the final composition of the material identical; second, by exposing a pre-formed gel to an external stimulus like heat, pH, UV-light, or ions to thereby modify its properties; third, by varying the chemical structure of the gelator. Compounds with very similar chemical structures may have significantly different gel properties. Structure-property relationship studies are essential to not only understand new functional gelators, but also to adapt material properties through tuning of the noncovalent interactions.

A fourth approach, which has been getting increasing interest in recent years, is to synthesize multicomponent gels by mixing of two or more gelator building blocks. Multicomponent gels have several advantages over single component systems. The properties of multicomponent gels can often not be predicted from either of the individual components. For example, multicomponent gels can show a multistep gel-to-gel transition, pH responsive ambidextrous phase behaviour, improved behaviour in cell, and photoelectric properties that a single building block cannot display. Moreover, selective modulation of individual components can be used to modify the gel properties. In short, multicomponent gels are more complex materials than single component gels with enhanced morphogenesis and tuneable material properties.

Here, we investigate the effect of hydrophobic spacer on gel properties in a multicomponent system. Among various non-covalent forces, hydrophobicity plays a pivotal role in biology, particularly in protein folding and assembly. In synthetic hydrogels, hydrophobicity greatly influences the gel properties. Typically, gels are prepared by reducing the solubility of gelators in solution. If the molecules are more hydrophobic, wormlike micellar aggregates can be formed in the pre-gelled solution. We have pointed out that, during a sol-to-gel transition, the micellar aggregates can direct the gel properties. Furthermore, changing hydrophobicity is reported to influence gelation kinetics, morphology and thermal behaviour, mechanical properties, self-healing, and catalytic activity of single component systems. The hydrophobic microenvironments within the fibre network of hydrogels have also been found to enhance stability of encapsulated materials like enzymes and proteins.

The design principle of our system involves two different compounds with a pH-responsive carboxylic acid (compound 1)
and ammonium (compound 2) functionalities (Figure 1).\textsuperscript{15} As single building blocks, only the peptide 1 exhibits gelation at acidic pH. At high pH, both the components 1 and 2 produce sols. The components show opposite responses in their solubility and self-assembly attributes in water on pH-perturbation which allows the mixed system (1–2) to maintain the overall hydrophobic-hydrophilic balance enabling a gel state both at acidic and basic pH (ambidextrous phase behaviour).\textsuperscript{15} At low pH, while the gel formation occurs primarily due to self-sorting of 1, at high pH gelation was driven by the co-assembly of 1 and 2 (Scheme S1).\textsuperscript{15} We gradually increased the chain length of the ammonium component (compounds 3 and 4) and studied the effect of hydrophobic spacer on the properties of mixed gels at both acidic and basic pH (Figure 1). We observed that, while the properties of the multicomponent gels at low pH are hardly affected, the mechanical properties of the co-assembled gels formed at high pH increase linearly with the increase in hydrophobic structural feature of the ammonium salts. The underlying molecular packing as well as microstructures of the co-assembled gels are also influenced by the nature of the ammonium salts. We further employ the urea-urease reaction to achieve a gel-to-gel transition involving these multicomponent systems. The hydrophobicity of compounds 2–4 also influences the kinetics of pH-switchable systems.

![Image](14x290 to 26x354)

![Image](42x271 to 287x487)

**Figure 1.** (a) pH-responsive changes in chemical structures of the compounds 1–4. The pictures of the vials represent gel/sol states formed by the corresponding species in solution. (b) Cartoon demonstrating the pH-driven changes in the self-assembled network for the multicomponent systems of 1 with 2–4.

Dipeptide 1 is a well-known gelator that forms a self-supporting gel in DMSO/H\textsubscript{2}O (20/80, v/v) with a pH of 4.3 (Figure S9).\textsuperscript{15, 26} At a pH above the apparent pK\textsubscript{a} of 1 (c.a. 6.4, Figure S10),\textsuperscript{26} due to deprotonation of the terminal carboxylic group, the solubility of the molecules increases, and no gel formation occurs (Figure 1, Figure S9). In contrast, compounds 2–4 are highly soluble in DMSO/H\textsubscript{2}O (20/80, v/v) resulting in free-flowing solutions with pH in the range 4.9–5.2 (Figure S9). A UV-vis study shows that solutions of 2–4 exhibit strong absorption at 265 nm along with a shoulder peak at 300 nm (Figure S11). They also exhibit very similar emission profiles. In all cases, a strong emission at 318 nm corresponding to the monomeric forms of 2–4 was recorded (Figure S12).\textsuperscript{27} pH titration data reveal that the apparent pK\textsubscript{a} of the amphiphiles gradually increases from 3.6 to 9.1 on moving from compound 2 to 4 (Figure S10). At high pH, deprotonation of the terminal ammonium groups generates the corresponding amines (pH 11.1–11.3 in presence of 0.01 M NaOH, Figure 1). There was a change in the UV-vis spectra of 2–4 at high pH (Figure S11). The intensity of the broad band which appeared at 258 nm for the amine form of 2 progressively decreased to 3 and 4. However, no substantial change in the absorption at 300 nm was noticed. At high pH, the monomer emission of 2–4 was red shifted by 4–5 nm along with the appearance of a new band in the region 425–525 nm (Figure S12). The emergence of the band at 425–525 nm suggests excimer formation in all cases due to overlapping of the fluorenyl groups at high pH.\textsuperscript{27} Interestingly, the emission intensity of both the monomer and excimer peaks increases from 2 to 4. The hydrophobicity of the amphiphiles affects the solubility of the components at high pH. In presence of NaOH, while compound 2 remained in solution, compounds 3 and 4 produced precipitation (Figure S9). Hence, none of the amphiphiles 2–4 have gelling capability on their own either at low pH or in presence of base.

We next prepared the multicomponent gels by mixing of compounds 1 with 2–4 in absence and presence of NaOH (0.01 M). Simple mixing of 1 and 2–4 individually results in a gel in all cases with a pH of 3.1–3.3 (Figure S13). These gels have very similar microstructure to that of the single component gel of 1 as observed by confocal fluorescence microscopy (Figure 2, Figure S14). In all cases, spherulitic domains of fibres were formed. The multicomponent gels, the density of the spherulitic structure increased. We previously suggested that the slight change in the microstructure of the multicomponent gels is due to the presence of organic salts (2–4)\textsuperscript{15} which influences the hydration of the peptide in water involving the Hofmeister effect\textsuperscript{28} endowing a change in the nucleation centre and subsequent growth of the fibres. The rheological moduli (the storage and loss moduli, G’ and G”) as well as the strain bearing capacity (critical strain or gel strength, the strain at which the gel breaks is 6–8%) of the multicomponent gels were also similar to that of the hydrogel of 1 alone (Figure S15, S16). These results show that for the multicomponent gels of 1 with 2–4 at low pH, the gel matrix was formed by self-sorting. 1 forms the gel network and compounds 2–4 behave as a non-gelling additive and hardly perturb the gel properties (Scheme S1).\textsuperscript{15}
At high pH, all the single-component systems 1-4 did not have gelling ability (Figure S9). However, mixing of 1 with 2-4 in presence of NaOH individually resulted in a gel in all cases (pH is 10.2-10.4 in presence of 0.01 M NaOH) (Figure S17). Hence, at high pH, gelation was driven by the co-assembly between the components (Scheme S1). Interestingly, the microstructure and the mechanical properties of the co-assembled gels are significantly influenced by the hydrophobicity of the ammonium salts. While the multicomponent gels of (1-2) and (1-3) exhibited long fibre formation, the mixed system (1-4) showed large spherulitic domains as the microstructure at high pH (Figure 2). A linear increase in stiffness (G’) of the gels was realized on moving from (1-2) to (1-4) (Figure 3a, Figure S16, S18). The hydrogel of (1-4) showed ~25 times higher stiffness compared to the (1-2) gel at high pH. Despite their high stiffness however, the gels break down at relatively low strain. The gel strength (critical strain) gradually reduced from ~13% to ~3% on moving from the system (1-2) to (1-4).

The differences in the properties of the multicomponent gels at different pH can be ascribed to the change in underlying molecular packing due to the existence of different non-covalent interactions at acidic and basic pH. To confirm this, FTIR, UV-vis and fluorescence studies were conducted on the gels. In the FTIR spectra of 2-4, the carbamate carbonyl stretching appeared at 1687-1689 cm⁻¹ (Figure S19). The black, red, and blue data represent hydrogels of (1-2), (1-3), and (1-4), respectively. In all cases, initial concentrations of 1-4 are 2 mg/mL, concentration of NaOH is 0.01 M, solvent is DMISO/H₂O (20/80, v/v).

The ambidextrous phase behaviour of the multicomponent systems can be utilized to construct pH-driven self-regulating switchable materials. To execute this, we coupled our systems with the urea-urease reaction. In water, urease hydrolyses urea into ammonia. This reaction is widely used to construct various pH-responsive switchable systems with a precise control over the rate of pH change. Combination of 1 with the Fmoc-amphiphiles in presence of urease and urea initially resulted in a gel at pH 3.3-3.4 with G’ being considerably greater than G” in all cases (Figure 4, Figure S25). The pH-time profiles showed a sigmoidal curve where at the beginning the pH change was slow. Over time, the concentration of ammonia increases, and the pH reached a plateau of 9.3 for all systems. The variation of G’, G” and complex viscosity with time followed a similar trend as that of pH. However, depending on the Fmoc-component, different pH-feedback was recorded during the pH change. The rate of pH change and so the change of rheological moduli and
complex viscosity gradually increases from the (1+2) system to the (1+4) system. Throughout this pH change no sol formation occurs. Indeed, in all variations, the rheological moduli of the final gels were substantially higher (>3-6 times) than the initially formed materials (Figure 4, Figure S26-S28). Again, the stiffness of the high-pH gels follows the order of hydrophobicity of the Fmoc-amphiphiles (Figure S29). Time-variable fluorescence data showed gradual appearance of excimer peaks in the region 425-525 nm along with a 7-16 nm red shift in emission of the initial gels during the pH change (Figure S30). Again, the relative intensity of the excimer peaks increases in the order (1+2) to (1+4) at high pH (Figure S31). Furthermore, 3-4 nm red shifts in the absorption spectra of the gels intimate that there is a change in the molecular packing as the pH increases (Figure S32). Compared to the NaOH-triggered gels, the self-regulating approach leads to gels that are more reproducible as observed from small error bars in the rheological data (Figure S16, S29). The microstructures of the high pH gels obtained by the enzymatic reaction are also different to those of the NaOH-triggered gels. Here, all the high pH gels exhibit densely packed large spherulitic domains of fibre as the underlying network (Figure S33).

In conclusion, we demonstrated a simple method to adapt material properties of pH responsive multicomponent gels comprising of gelators with two oppositely ionizable pendant groups. We found that, when a component acts as a non-gelling additive, increasing the size of a hydrophobic structural feature on that component hardly perturbs the self-sorting of the second gelator building block at low pH. However, increasing hydrophobic chain length significantly influences the underlying molecular packing as well as microstructures and mechanical properties in co-assemblies at basic pH. Furthermore, a pH driven self-regulating switchable system is constructed by incorporating the urea-urea reaction into the multicomponent gel which allows us to achieve precise control over the final mechanical properties of the materials. These insights should be useful in devising new multicomponent hydrogels particularly with ambidextrous phase behaviour.

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Notes and references

Conflicts of interest
There are no conflicts to declare.