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A hybrid gel has been developed by combining two supramolecular gelators. Each gelator endows the hybrid gel with its own characteristics. One gelator enables pH-mediated controlled release of the active pharmaceutical ingredient naproxen, while the other new gelator enhances mechanical stability. Self-assembly thus gives multi-functional gels with potential applications.

Supramolecular hydrogels, soft materials typically comprising ca. 99% water immobilised by a network of entangled fibres, are of increasing interest in biomedical applications. The fibres self-assemble into a 'solid-like' network from a low-molecular-weight gelator (LMWG). Self-assembled hydrogels have many attractive properties, particularly their responsive nature and generally high biocompatibility. There is also the potential to program desired functionality into the bulk gel, through simple molecular-scale modifications of the LMWG. This has led to increasing interest in their use in biomedical settings, including drug delivery. However, supramolecular gels often have low mechanical strength, making them difficult to manipulate.

One way of enhancing the mechanical strength of supramolecular hydrogels is to combine them with polymer gelators, which are generally more mechanically robust, albeit less responsive to external stimuli. Increasingly, hybrid gels comprising two or more supramolecular gelators are also being studied, with the hope that the properties of the overall gel can be modified by tuning the ratio of the components, although applications of this approach remain rare. Combining multiple gelators is potentially a simple way of accessing gels with different properties. When two LMWGs are combined, self-assembly can occur in different ways giving different types of self-organised material. The individual gelators may preferentially interact with other gelators of the same type, leading to 'self-sorted' fibres. Alternatively, each gelator may preferentially interact with the other gelator, and fibrils will assemble with alternating gelators. If the gelators have no strong preference for either interaction, then random mixing can occur. Finally, one gelator may disrupt the assembly of the other through non-productive interactions, causing loss of gelation. Self-sorting gelators are desirable because orthogonal networks can be exploited to control the properties of the gel (Fig. 1).

In recent years, we have investigated hydrogels based on the industrially-relevant 1,3:2,4-dibenzylidenesorbitol (DBS) framework. The parent molecule (DBS) and simple derivatives, have long been exploited for thickening properties, but were not previously capable of forming hydrogels as they are too insoluble in water. By modifying the aromatic 'wings' of DBS, we developed hydrogelators. One of these is DBS-CONHNH₂, with hydrophilic acyl hydrazide groups. It forms a hydrogel using a simple heat/cool cycle, and is stable across a range of pH values. Hydrogels formed from DBS-CONHNH₂ have been used for environmental remediation, drug delivery, and cell culture. Controlled release of active pharmaceutical ingredients (APIs) like naproxen (NPX) can be achieved as a result of interactions between the DBS-CONHNH₂ fibres and the API carboxylic acid. However, the gels are mechanically weak and difficult to manipulate. We have previously combined DBS-CONHNH₂ with polymer gelators to...
Interestingly, MBS-CO₂Me to physically manipulate than DBS-CONHNH₂† that formed by G. The effect of solubility on the presence of two additional OH groups increases hydrophilicity, making it sufficiently water-soluble. The effect of solubility on gelation is increasingly understood.⁰

We investigated the properties of this new hydrogelator. Gelation was achieved via a heat/cool cycle, giving transparent gels (Fig. S4, ESI†). The minimum gelation concentration (MGC) was 0.75% wt/vol, with lower loadings giving solutions. SEM and TEM indicated a fibrous network. TEM shows relatively homogeneous helical fibres, with a diameter of ca. 8 nm (Fig. 2, left). Drying gels can cause morphological change⁴¹ – in an attempt to limit this, we cryo-dried samples for SEM. The small nanofibres and relative lack of aggregation are consistent with the observation that gels formed by MBS-CO₂Me in water are transparent. This is similar to gels formed by DBS-CONHNH₂, which also have nanofibres ca. 10 nm in diameter.¹⁵

Rheology was carried out to explore the mechanical properties of the MBS-CO₂Me hydrogel. At a loading of 0.85% wt/vol, \( G' > G'' \) in the linear viscoelastic region (LVR), with \( G' \) being ca. 2800 Pa (Fig. S6a, ESI†). This is a significantly stiffer gel than that formed by DBS-CONHNH₂ (\( G' = 1200 \) Pa). The two gels had similar elasticity, with \( G'/G'' \) crossover points of ca. 12%. Interestingly, MBS-CO₂Me was much more robust and easier to physically manipulate than DBS-CONHNH₂, with gel discs being easily transferred for rheology, with no breakage.

Variable temperature (VT) circular dichroism (CD) spectroscopy of MBS-CO₂Me had a maximum ellipticity at ca. 280 nm, showing the aromatic ring of MBS-CO₂Me experiences a chiral environment (Fig. S10, ESI†). This CD band was temperature-dependent, proving the chiral environment is generated by self-assembly. The most significant change in ellipticity was at 45–55°C, supportive of a gel–sol transition at this temperature.

Simple tube inversion methodology confirmed MBS-CO₂Me hydrogels indeed had \( T_{gel} \) values at ca. 55°C, significantly lower than DBS-CONHNH₂ (ca. 80°C), despite the higher concentration (Table S1, ESI†). We reasoned the greater hydrophilicity of MBS-CO₂Me makes it more temperature-sensitive, i.e., it dissolves more easily on heating. When the resulting hot solutions were left to stand overnight, gels re-formed. However, rather than being transparent, they were white, with some precipitate, indicative of greater aggregation and less controlled assembly. When \( T_{gel} \) values for these re-formed gels were determined, those at lower concentrations had lower \( T_{gel} \) values, while there was little impact on the \( T_{gel} \) values at higher concentrations. These gels at higher loadings also re-formed opaque gels for a second time. Rheology indicated a slight increase in \( G' \) values.

MBS-CO₂Me gels were then tested for their ability to encapsulate and release the API naproxen (NPX, Fig. 3). This was achieved by mixing the API and gelator as solids in advance of the heat/cool cycle. Hydrogels were still formed (Table S2 and Fig. S8, ESI†), but when API release was investigated, the MBS-CO₂Me hydrogel broke down rapidly (within 3 h) once the supernatant was placed on top of the gel (Fig. 3). This occurred in the presence of any supernatant, including water and a range of buffers. This was not observed with DBS-CONHNH₂, and was initially surprising given the MBS-CO₂Me gel is more robust. We reason that the higher solubility of MBS-CO₂Me makes the gel less stable to the presence of excess aqueous medium.

Fig. 2 Left: TEM image of an MBS-CO₂Me hydrogel; scale bar 100 nm. Right: SEM image of an MBS-CO₂Me hydrogel; scale bar 1 μm.

Fig. 3 Release of naproxen into pH 7 buffer from MBS-CO₂Me hydrogels showing rapid release driven by erosion of the gel.
NPX has a maximum absorbance at 329 nm, allowing API release to be quantified via UV-vis spectroscopy. When incubated at 37 °C with pH 7 buffer, around 85% of NPX was released within 3 h, roughly at the point at which the gel broke down completely – we thus propose an ‘erosion’ mode of API release for MBS-CO₂Me.

We combined MBS-CO₂Me and DBS-CONHNH₂, hoping their different properties would be synergistic. Our goal was a hybrid gel with mechanical properties enhanced by MBS-CO₂Me, but maintaining the controlled release and water stability of DBS-CONHNH₂. Initially, gelation was optimised. DBS-CONHNH₂ and MBS-CO₂Me hydrogels have very different MGCs (0.20% and 0.75% wt/vol respectively). Varying concentrations were explored, with the gelators mixed as solids. The total MGC for the hybrid gel was 0.3% wt/vol (DBS-CONHNH₂ at 0.2% wt/vol, and MBS-CO₂Me at 0.1% wt/vol). Hybrid hydrogels formed even with both gelators below their individual MGCs (DBS-CONHNH₂ at 0.16% wt/vol and MBS-CO₂Me at 0.2% wt/vol).

Hydrogels with a range of LMWG concentrations were prepared, and their thermal properties investigated. Given that MBS-CO₂Me hydrogels have lower T_gel values (ca. 55 °C) than DBS-CONHNH₂ hydrogels (ca. 80 °C), it was predicted that increasing the proportion of MBS-CO₂Me in the hybrid gel may lower T_gel, providing access to a range of T_gel values. This was indeed the case, with T_gel values from 27 °C to >100 °C being obtained (Tables S3 and S4, ESI†). In general, a greater proportion of MBS-CO₂Me gave a lower T_gel and a greater proportion of DBS-CONHNH₂ gave a higher T_gel. Total gelator loading was also important – more gelator gave higher T_gel values.

SEM and TEM indicated nanofibres of ca. 10 nm diameter (Fig. S4 and S5, ESI†). It was not possible to differentiate nanofibres for the individual gelators, as they are similar in morphology, but it was clear self-assembly in the mixed system was not disrupted, nor did different types of assembly arise as a result of mixing.

VT ¹H NMR studies investigated gelator immobilisation in the hybrid gel, and understand how the LMWGs respond to an increase in temperature (Fig. 4). Hybrid gels (0.80% wt/vol MBS-CO₂Me and 0.22% wt/vol DBS-CONHNH₂) were prepared in an NMR tube using D₂O : H₂O (50:50, D₂O alone leads to reduced solubility of MBS-CO₂Me), with DMSO (2 µl) as an internal standard. This allows determination of the gelator assembled in the ‘solid-like’ network, because mobile gelator in the ‘liquid-like’ phase is visible in the ¹H NMR, and can be quantified by relative integration. At room temperature, neither gelator was observed in the spectrum, indicating both are assembled into ‘solid-like’ networks. As temperature increased, the signals for MBS-CO₂Me became visible first, from around 40 °C with around 90% being free at 65 °C. This reflects the higher solubility of MBS-CO₂Me and its lower T_gel value (see above). The signal corresponding to mobile DBS-CONHNH₂ first appears at around 55 °C, and 90% of the gelator is free at 85 °C, in-line with its higher T_gel value. A small amount of the DBS-CONHNH₂ disassembles at ca. 55 °C before MBS-CO₂Me disassembly is complete. It is therefore possible that self-sorting in this system is not perfect, but nonetheless, the networks do still largely disassemble in a sequential way and thus with a good degree of self-sorting.

It was hoped the stiffer MBS-CO₂Me would reinforce the more interactive, but mechanically weak, DBS-CONHNH₂, giving a gel that was easier to manipulate. Hybrid hydrogels with varying loadings of MBS-CO₂Me (‘high’: 0.80% wt/vol, ‘low’: 0.10% wt/vol) and DBS-CONHNH₂ (‘high’: 0.28% wt/vol, ‘low’: 0.24% wt/vol) were tested by rheology (Table 1 and Fig. S6, S7, ESI†). Pleasingly, with higher proportions of MBS-CO₂Me, the hybrid gel was stiffer than either DBS-CONHNH₂ or MBS-CO₂Me alone (G’ = 9600 Pa). At higher MBS-CO₂Me concentrations, increased loading of DBS-CONHNH₂ leads to a slightly weaker gel, perhaps as a result of the more extensive weaker DBS-CONHNH₂ network being more dominant. However, at the lowest concentrations of MBS-CO₂Me, where the concentration of MBS-CO₂Me here is below the MGC, the gels are slightly weaker than DBS-CONHNH₂ alone, suggesting a small amount of MBS-CO₂Me network can disrupt the DBS-CONHNH₂ network. Therefore, there is scope to tune the rheological properties of the hybrid hydrogel, to give properties suitable for the desired application.

Given DBS-CONHNH₂ can achieve pH-mediated naproxen release,¹⁸ we wanted to determine if this ability was retained by the hybrid gel, and thus investigated encapsulation and release of NPX. Stable gels were still formed in the presence of NPX, and the addition did not have a significant impact on the rheological properties of the hydrogels – there was perhaps a slight stiffening reflected by a small increase in G’ (Fig. S8 and S9, ESI†). We studied the interaction of the hybrid gels with NPX by ¹H NMR. A known mass of each gelator and NPX, were dissolved in D₂O : H₂O

![Fig. 4](image-url) The breakdown of the hybrid hydrogel on increasing temperature, with free gelator monitored by ¹H NMR spectroscopy. The loading of MBS-CO₂Me is 0.80% wt/vol, and of DBS-CONHNH₂ is 0.22% wt/vol.

Table 1  G’ and G” values, from the LVER, for MBS-CO₂Me (high – 0.80% wt/vol, low – 0.10% wt/vol) and DBS-CONHNH₂ (‘high’: 0.28% wt/vol, ‘low’: 0.24% wt/vol) gels

| Gelator    | G’/Pa | G”/Pa |
|------------|-------|-------|
| DBS-CONHNH₂ | 1200  | 60    |
| MBS-CO₂Me  | 2800  | 550   |
| High MBS, low DBS | 9600 | 700   |
| High DBS, high MBS | 6300 | 900   |
| Low MBS, low DBS | 300  | 25    |
| Low MBS, high DBS | 500  | 30    |
mechanical strength as a result of facilitating controlled drug release, while having improved release observed when using DBS-CONNH$_2$ (50:50), and the hydrogel formed in an NMR tube. An internal standard (DMSO) was added to allow quantification of any ‘mobile’ NPX. These studies indicated that, like DBS-CONNH$_2$ gels, over 90% of the NPX was not visible in the NMR spectrum, and is therefore likely bound to the gel network as a result of non-covalent interactions between the acid on NPX and the hydrazide. The interactive properties of DBS-CONNH$_2$ thus appear to translate into the mechanically-robust hybrid gel.

The release of NPX from the hybrid gels, into buffers with different pH values, was then investigated by monitoring the UV-vis absorbance at 329 nm. Pleasingly, the supramolecular hybrid gel showed pH-dependent release (Fig. 5). The NPX release profile at pH 7 is similar to that observed previously for DBS-CONNH$_2$ with rapid initial release, and total release of 85–95%. In contrast, release at pH 5.5 is slower and much less favoured, reaching only ca. 35% in the first 3 h, and a maximum of just 55%. This is a result of the interactions between DBS-CONNH$_2$ fibres and NPX, which occur when NPX is protonated. The $p_K_a$ of NPX is 4.15,22 and at pH 5.5 a greater proportion of NPX will be protonated than at higher pH values, and thus able to interact with gel nanofibres, limiting release. This controlled release is very different to the erosion release of 85–95%. In contrast, release at pH 5.5 is slower and total release of just 55%. This is a result of the interactions between DBS-CONNH$_2$ fibres and NPX, which occur when NPX is protonated. The $p_K_a$ of NPX is 4.15,22 and at pH 5.5 a greater proportion of NPX will be protonated than at higher pH values, and thus able to interact with gel nanofibres, limiting release.

In summary, a two-component supramolecular hydrogel, formed from two gelators (DBS-CONNH$_2$ and novel MBS-CO$_2$Me), has been used to encapsulate and release NPX. NMR studies indicate that the gel has sequentially assembled networks. Each gelator has its own unique characteristics programmed into the performance of the gel. Specifically, the hybrid gel retains the interactive nature of DBS-CONNH$_2$, facilitating controlled drug release, while having improved mechanical strength as a result of MBS-CO$_2$Me. NPX release is pH-mediated, lower pH leading to less release, with potential for controlled release in the small intestine. Further work will focus on tuning multi-component hydrogels to give different release profiles, enabling other drug delivery applications, as well as investigating such gels in wider biomedical applications like tissue engineering or wound healing.

Conflicts of interest
There are no conflicts to declare.

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