High-throughput characterisation of supramolecular gelation processes using a combination of optical density, fluorescence and UV-Vis absorption measurements†

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Herein, we showcase the use of high-throughput microplate reader methodologies for the characterisation of supramolecular gels. We demonstrate how UV-Vis absorption, optical density and fluorescence measurements can selectively define gel fibre assembly/disassembly processes, casting a new light on the construction of these materials.

Supramolecular gels are a class of soft material, formed through non-covalent interactions between the monomeric units of low molecular weight gelators.1 These monomeric units self-associate to produce a fibrous network, trapping any residual solvent.2 The scalability3 and regenerative properties4 has increased the popularity of these materials, making them of interest for a variety of applications which include chemosensors,5 biomedicines6 and drug delivery vehicles.7

To enable the characterisation and effective comparison of supramolecular organo/hydrogels, elucidation of a minimum gelation concentration through inversion testing is often obtained.8 In addition, a variety of other complementary techniques are often employed to confirm the presence, or further investigate the properties of the material. However, all these experimental techniques exhibit some limitations which include: the removal of solvent to produce a xerogel; expensive often specialised equipment; low potential for accurate data interpretation; large sample sizes; and/or long experimental time frames. Examples of these methods include the use of conventional UV-Vis absorption spectroscopy to identify organogel formation,9 dynamic light scattering to monitor fibre construction,10 rheology to observe bulk material properties,11 solution12/HRMAS13/solid14 state NMR and confocal15 or fluorescence16 microscopy to observe the gel fibres in the native material state. Furthermore, transmission and scanning electron microscopy,17 in addition to powder X-ray diffraction techniques,18,19 have been used to define the structure of gel fibres obtained from xerogels. Finally, it has been shown that neutron diffraction techniques may be developed for selectively deuterated gels,19 to observe molecular packing arrangements in aggregated and gelated materials.20

Herein, we introduce a toolkit containing a series of high-throughput characterisation methods utilising microplate reader instrumentation. This methodology toolkit will be demonstrated using a molecular example from our extensively well characterised supramolecular self-associating amphiphil e (SSA) systems.16,21 Use of this instrumentation for supramolecular gel characterisation affords: (i) low sample evaporation; (ii) in situ gel–sol/sol–gel measurement; (iii) low sample volume E200 mL; (iv) multiple experimental measurements per sample; (v) retention of sample integrity; and (vi) up to 384 experiments to be performed simultaneously. Additionally, we identify new properties of this model SSA gel, previously unobservable by traditional methodologies.16

Fig. 1 Chemical structure of 1 and structures formed through the self-association of 1 (dimers, spherical aggregates and hydrogels) under different environmental conditions. TBA = tetrabutylammonium. H₄ = hydrodynamic diameter.
SSA 1 (Fig. 1) is an intrinsically fluorescent amphiphilic salt that has previously been shown to form anionic, hydrogen bonded dimeric species in DMSO (≈1.4 nm in diameter),\textsuperscript{21b} spherical aggregates in H\textsubscript{2}O or H\textsubscript{2}O:EtOH 19:1 mixtures (≈55–350 nm in diameter),\textsuperscript{21b} and hydrogels in aqueous salt solutions.\textsuperscript{16} Within the toolkit produced here, we describe the use of UV-Vis absorption, optical density and fluorescence measurements for the selective study of supramolecular gel formation/solution. To demonstrate the applicability of each method we present comparative data for 1 (1.5 mg mL\textsuperscript{-1}) in DMSO, H\textsubscript{2}O and in two aqueous salt solutions (NaCl and Na\textsubscript{2}CO\textsubscript{3} at 0.505 M), where molecular self-association events result predominately in dimerization, spherical aggregates and gel or incomplete gel formation respectively.

Initially, we study the self-associative structures formed by 1 using UV-Vis absorbance microplate reader measurements over a range of temperatures from 25 °C to 45 °C. Fig. 2 shows those results obtained for a solution of 1 containing: (a) anionic dimers; (b) spherical aggregates; (c) a hydrogel; (d) an incomplete hydrogel. Here, as for all UV-Vis, optical density and fluorescence microplate reader studies detailed within this work, the type of self-associated structure (dimer, spherical aggregate or gel fibre) present within the sample studied was verified through comparison with published bulk material/aggregate or gel fibre) present within the sample studied was verified through comparison with published bulk material.

Using UV-Vis absorbance microplate reader measurements over a range of temperatures from 25 °C to 45 °C (Fig. 2), we observe evidence for the NaCl containing hydrogel, which was only found at 45 °C. Therefore, by observing and/ or comparing the OD\textsubscript{450} spectral scan data, it is possible to observe any self-associated structures of 1 existing at the interface as opposed to those spherical aggregates which exist in the bulk solution. This is indicated by the comparatively high OD\textsubscript{450} measurements recorded at the periphery of the microplate well over the central regions. Where gel fibres are known to form – in the presence of NaCl (Fig. 2c) or Na\textsubscript{2}CO\textsubscript{3} solution data.\textsuperscript{16,21} verified through comparison with published bulk material.

As shown, these UV-Vis Abs\textsubscript{450} measurements could be used to verify the presence of gel fibres within a sample of 1 (Abs\textsubscript{450} > 0.05 AU), when compared to other self-associated species such as dimers or spherical aggregates (Abs\textsubscript{450} < 0.05 AU) at comparable concentrations within a microplate well. We next explored the use of optical density measurements at 450 nm (OD\textsubscript{450}) to further characterise this system. Spectral scanning experiments performed for those same samples of 1 as shown in Fig. 2, allow us to further observe any self-associated structures of 1 within a microplate well, over a 25 °C to 45 °C temperature range. Here, as shown in Fig. 3, the surface area of each well was divided into 177 sections, with an OD\textsubscript{450} measurement obtained for each. When only small dimeric self-associated species are known to exist (Fig. 3a) OD\textsubscript{450} measurements obtained for each section of the microplate well are shown to be uniformly low. However, moving into H\textsubscript{2}O only (Fig. 3b), we observe evidence of 1 primarily self-associating at the microplate well periphery. Amphiphilic compounds such as 1, that are shown to lower the surface tension of aqueous solutions,\textsuperscript{21} are known to preferentially self-associate at the interface until the critical micelle concentration (CMC) is reached.\textsuperscript{22} Therefore, by observing and/ or comparing the OD\textsubscript{450} spectral scan data, it is possible to observe any self-associated structures of 1 existing at the interface as opposed to those spherical aggregates which exist in the bulk solution. This is indicated by the comparatively high OD\textsubscript{450} measurements recorded at the periphery of the microplate well over the central regions. Where gel fibres are known to form – in the presence of NaCl (Fig. 2c) or Na\textsubscript{2}CO\textsubscript{3} solution data.\textsuperscript{16,21} verified through comparison with published bulk material.

Fig. 2 Average (n = 3) absorbance spectra of 1 (200 µL, 1.5 mg mL\textsuperscript{-1}) in; (a) DMSO; (b) H\textsubscript{2}O; (c) NaCl (0.505 M); (d) Na\textsubscript{2}CO\textsubscript{3} (0.505 M). Volume and concentration measurements result in saturation at 3.5 OD for wavelengths <400 nm. Error bars represent the full range of absorbance measurements obtained for n = 3 measurements.

Fig. 3 Spectral analysis well scans conducted at OD\textsubscript{450} with 1 (1.5 mg mL\textsuperscript{-1}) at 25 °C in; (a) DMSO; (b) H\textsubscript{2}O; and (c) NaCl (0.505 M). High OD\textsubscript{450} = red. Low OD\textsubscript{450} = purple. Each segment (outlined in black) represents a different portion of an individual microplate well as each measurement is recorded for the entire depth of the sample.
to form (Fig. 3e), uniformly higher OD\textsubscript{450} measurements are recorded across the entirety of the microplate well.

To further enable the analysis of these spectral scan data, two-dimensional OD\textsubscript{450} maps were also produced (Fig. 4). This representation of those data shown in Fig. 3 demonstrates lower OD\textsubscript{450} values occurring towards the centre of the well, with higher values towards the periphery, where 1 is present as a hydrogel or incomplete hydrogel (Fig. 4a and b). As for those Abs\textsubscript{450} measurements (Fig. 2c and d), we observed no notable change for these data displayed within the intensity map produced for the hydrogel of 1 containing NaCl as the temperature was raised from 25 °C to 45 °C. However, for the incomplete hydrogel formed in the presence of Na\textsubscript{2}CO\textsubscript{3}, we observe a decrease in OD\textsubscript{450} values across the majority of the 177 microplate well segments with increasing temperature. Through ratioing the OD\textsubscript{450} maximum and minimum values (the highest and lowest OD values recorded for a single well of a single sample at a single temperature) Table 1, it becomes possible to screen these data for the presence/amount of gel fibres, validated by those previously published data for the ‘bulk’ material summarised in Table 2, and obtain a gel–sol/gel fibre melting temperature (T\textsubscript{m}), as illustrated in Fig. 5. This proof-of-principle dataset indicates that at a ratio value <0.06, unless the material is uniformly gelled, proportionally very few if any gel fibres will be present. Here, 1 instead acts as an amphiphile, with only extensive self-associated structures observable at the microplate well interface. This data also suggests there is a different density of structures and/or different structures present at the surface–gel interface, as reported previously by Marlow and Zelzer.23

Due to the intrinsic fluorescent nature of 1, we were also able to investigate the self-assembly processes of this SSA using fluorescence spectroscopy. Interestingly, although this method was able to distinguish between the presence of dimers (Fig. 6a) and larger self-associated aggregates (Fig. 6b–d). This method could not verify the presence of spherical aggregates (Fig. 6b) over gel fibres (Fig. 6c and d), due to the lack of distinguishing spectral features observed for these samples. However, comparison of emission profiles we are able to follow the disassembly of those larger self-associated structures formed with increasing temperature (Fig. 6b and d). Here the disassembly process can be identified through increasing intensity of the emission spectrum maxima when excited at 435 nm. The lack of change in the emission spectra of that sample shown in Fig. 6c is due to the T\textsubscript{m} for this sample being > 45 °C. We hypothesise that both the decrease in experimental sensitivity towards aggregate identification and observation of the aggregate disassembly process can be explained as a result of incident light scattering by the sample.

Finally, we detail the additional self-associative system information generated for 1 using this combination of plate reader methodologies, unobtainable through our previous characterisation of these systems.16 As shown in Table 2 when supplied at a concentration of 5 mg mL\textsuperscript{-1}, 1 gelated all salt

### Table 1

| Soln      | Temperature | Temperature | Temperature |
|-----------|-------------|-------------|-------------|
| DMSO      | 25          | 45          | 25          | 45          |
| H\textsubscript{2}O  | 0.01        | 0.02        | 0.02        | 0.03        |
| Na\textsubscript{2}CO\textsubscript{3} | 0.35        | 0.05        | 0.35        | 0.05        |
| NaF       | 0.55        | 0.48        | 0.55        | 0.48        |
| Na\textsubscript{2}SO\textsubscript{4} | 0.62        | 0.56        | 0.62        | 0.56        |
| Na\textsubscript{2}HPO\textsubscript{4} | 0.57        | 0.48        | 0.57        | 0.48        |
| NaH\textsubscript{2}PO\textsubscript{4} | 0.70        | 0.65        | 0.70        | 0.65        |
| NaOBz     | 0.64        | 0.58        | 0.64        | 0.58        |

### Table 2

| Soln            | T\textsubscript{m} (°C) | T\textsubscript{fa} (°C) | Mat. prop. | Soln            | T\textsubscript{m} (°C) | T\textsubscript{fa} (°C) | Mat. prop. |
|-----------------|------------------------|------------------------|------------|-----------------|------------------------|------------------------|------------|
| Na\textsubscript{2}HPO\textsubscript{4} | i                      | < 25                   | i          | NaOAc          | 46                     | > 45                   | i          |
| Na\textsubscript{2}CO\textsubscript{3} | 44                     | 40–45                  | g          | NaF            | 47                     | > 45                   | i          |
| Na\textsubscript{2}SO\textsubscript{4} | 43                     | 40–45                  | i          | NaCl           | 51                     | > 45                   | g          |
| Na\textsubscript{2}HPO\textsubscript{4} | 45                     | > 45                   | i          | NaNO\textsubscript{3} | 52                     | > 45                   | g          |
| NaH\textsubscript{2}PO\textsubscript{4} | 54                     | > 45                   | i          | NaOBz          | 54                     | > 45                   | g          |

Experimental T\textsubscript{m} and T\textsubscript{fa} details are provided within the ESI for convenience.
solutions listed, apart from Na₂HPO₄. This enabled the elucidation of comparative gel–sol Ƭₘ values. However, those same samples presented to the microplate reader for analysis proved too concentrated for accurate measurements to be obtained. For this reason, in the case of these microplate reader studies, the concentration of 1 was reduced to 1.5 mg mL⁻¹ for all samples. This resulted in a mixture of gels, incomplete gels and solutions. However, as confirmed by the results of both comparative UV-Vis absorption and optical density measurements, the fibre assembly temperature range (Ƭₐ) calculated using these methods at 1.5 mg mL⁻¹ was found to correlate with the results of gel–sol Ƭₘ studies conducted at 5 mg mL⁻¹. This leads us to hypothesise that the Ƭₘ is not only representative of the gel–sol transition temperature but also gelator monomer dissociation, resulting in fibre solvation and is concentration-independent between 1.5 and 5.0 mg mL⁻¹. Additionally, the OD₄₅₀ intensity maps produced for hydrogels/incomplete hydrogels of 1 show a greater proportion of gel fibres to exist at the sample periphery in comparison to the well interior, giving us new information about material formation and construction.

We have introduced a toolkit of high-throughput gel characterisation methods using microplate reader technology. It is envisioned that this toolkit maybe used for standard supramolecular gel characterisation, removing those limitations overviewed herein. Using UV-Vis absorption measurements we have shown that for solutions/gels/incomplete hydrogels of 1, increased Abs₄₅₀ values selectively indicate gel fibre formation. OD₄₅₀ measurements may also be used to selectively observe the formation of gel fibres. Interestingly, spectral well-scans confirmed SSA self-assembly processes at the interface in the presence and absence of gel fibre formation in aqueous solutions. The use of the well scan measurements could be used to illustrate uniformity of gelation or be used to characterise gel patterning or gradients. This would be a huge advantage as it is non-destructive and is carried out in-situ. We also established that although fluorescence spectroscopy is an incredibly sensitive technique for molecular/complex characterisation, here this technique is unable to reliably distinguish between spherical aggregate and gel fibre formation. Finally, we propose that the Ƭₘ of a supramolecular gel may be due to fibre disassembly and therefore concentration-independent to the point of solution saturation, although verification of this hypothesis remains the subject of ongoing investigation.

**Conflicts of interest**

There are no conflicts to declare.

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