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Controlling supramolecular gels

Kinetic trapping in supramolecular gels leads to varied morphologies and macroscopic properties. Emily R. Draper and Dave J. Adams discuss subtle experimental effects that can lead to reproducibility issues in these systems.

Supramolecular gels are of huge interest in many areas, from cell culturing and drug delivery to dissipative systems and optoelectronics[1]. Supramolecular systems are held together by many weak intermolecular and intramolecular forces. Individually, each is insufficient to lead to persistent structures, but together, they are sufficient to lead to aggregation. It is not uncommon to assume that these systems are at the (or at least a) thermodynamic minimum, but this is not always the case. Kinetically trapped states are common. The result of this is that there are often issues with reproducibility for many supramolecular gels.

Supramolecular gels are formed via multiple levels of hierarchical assembly. Initially, small molecules aggregate into one-dimensional structures such as nanofibres or nanotubes[2,3]. The further aggregation and interaction of these structures leads to a three-dimensional network that immobilizes the solvent, leading to gel formation. Even if one assumes that the first level of assembly (where the molecules aggregate to form fibres or tubes) is well defined, the next levels of hierarchy (where the fibres somehow form a network via varied supramolecular interactions) is, in our experience, often hard to reproduce.

As a single example, even for a gel measured with a specific volume or in a particular container, it is not necessarily true that this nominally identical gel will have the same rheological properties when prepared at a larger volume. By changing the gel volume, the ratio of interfaces to the bulk gel changes, which can lead to a change in the material properties. Similarly, there is evidence that gels formed in either glass or plastic containers can have different rheological properties[4]. This is probably due to different air–gel interfaces and gel–container interfaces directing or templating the assembly. Even for the same gelator molecule, different gelation methods often lead to gels with different properties[5].

As such, although there are many different molecules that form gels, it is not just the primary chemical structure that is important, but also the control over the gelation process. There are many possible permutations in processing, many of which are often not discussed, such as laboratory ambient temperatures, stirring speeds and the rate of addition of antisolvents, to name a few. Although these extra variables add a lot of complexity, the advantage is that once they are understood, there is huge potential and tuneability available using such gels (Fig. 1).

It is also not uncommon to see data in the literature that are presented as single-data-point measurements, such as some rheology data for a single gel sample. If one then repeats the gelation process and obtains a gel with different rheological values, it is never clear whether this is due to the gel-formation process, the inherent (lack of) reproducibility in the system, the method of loading the gel on to a rheometer (which is often not specified) or a differing rheometer or measuring system. Since there are many reports linking a rheological value such as the storage modulus (G') to properties such as stem-cell differentiation[6], it is obviously important to know which gels have specific values and to be sure that this is indeed the gel-specific value as opposed to a value that was measured just once for that gel.

There are further complications once the gels have been prepared. There are many gels reported in the literature. Differences between the gels will often be explained with techniques such as transmission or scanning electron microscopy to understand the network. A real problem here is that drying often leads to changes in morphology and network[7] (unsurprisingly, given that the molecules are weakly held together, and the network needs to withstand pressures when the solvent is removed; on top of this there is clearly a large increase in concentration during the drying process). From this perspective, it is important to consider why these systems may be difficult to reproduce. This has two aspects: (1) the difficulty of reproducing a gel in a single laboratory; and (2) the difficulty of reproducing a gel by others.

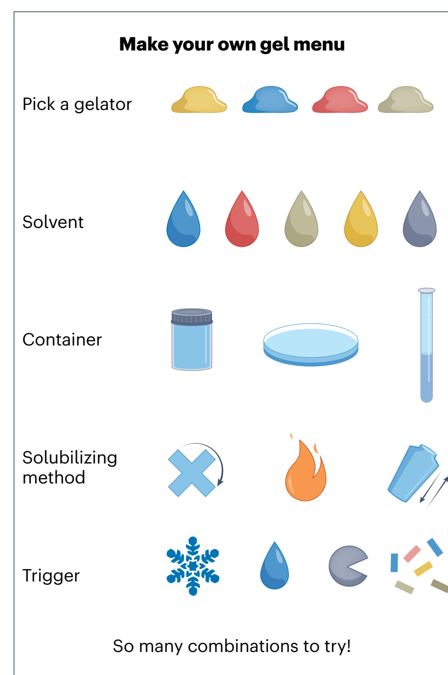

Fig. 1 | Processing controls the properties of many supramolecular gels. Many permutations in experimental protocols lead to high tunability but also issues with reproducibility.

For the difficulty of reproducing a gel in a single laboratory, our experience is that many of these gel systems are far more process-dependent than is usually thought. There is often an assumption that the materials are initially molecularly dissolved and then aggregate as the system properties are changed to induce gelation. Typical triggers would be a change in temperature, a change in solvent composition or a change in a property such as pH or ionic strength. In every case, the gelator must be largely insoluble in the solvent at the end, with the process of gelation leading to the network formation as opposed to crystallization or precipitation.

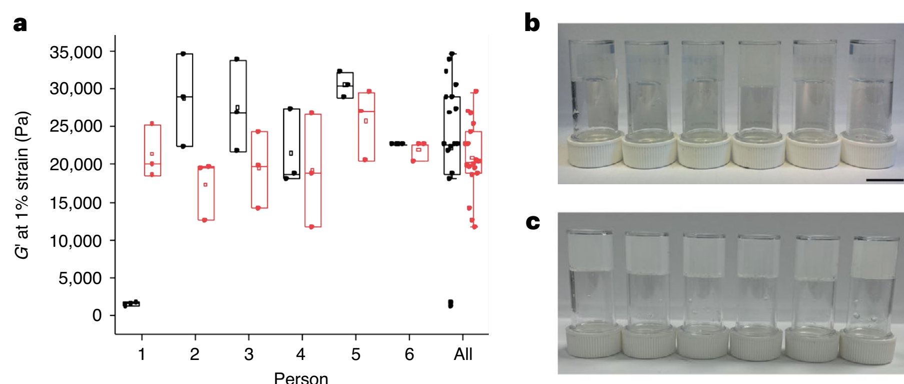

Fig. 2 | Results from the 'Great British Bake-off blind bake'. **a**, The storage modulus *G′* for the initial set (black data) and second set (red data) of gels formed from a Fmoc dipeptide. The data for six different people (1 to 6) are shown, with error bars representing the standard deviation from six gels per person. The average values are from all the data of the six people. The black data are for the original, less detailed protocol and the red data when using the more defined protocol. **b**, Photographs of six gels formed by person 5 from the first set of data. Scale bar, 2 cm. **c**, Photographs of six gels formed by person 5 from the second set of data.

For example, a typical gelation protocol would be to suspend a gelator in a solvent in which it is not soluble, followed by heating to dissolve the gelator, followed by cooling to give a gel. It is often unclear what temperature to heat to (often, a heat gun is used until dissolution occurs by eye), and then the cooling is often simply done ambiently. The cooling rate will depend on the temperature to which the solution was originally heated and the laboratory ambient temperature, as well as the volume of liquid used. Hence the cooling rate, and so the kinetics of assembly, would be expected to determine what self-assembled structures form and the final network. Indeed, there are a small number of examples that explicitly show this[8,9].

Likewise, when an antisolvent is added to induce gelation, the rate of addition and how mixing is carried out (probably also affected by the absolute volume used) will lead to different networks and hence different properties. These are all simple parameters that are often ignored. Similarly, in many water-based systems, complete dissolution of the gelator does not occur and micellar phases are formed instead. These can lead to processing issues. Indeed, we have recently shown that how long such solutions are stirred for, the size of the stirrer bar and the sample volume as well as the rest time after stirring all affect the aggregates present before gelation is triggered[10], and so can lead to differences in the network and hence the properties of the gel. In all cases, the network is formed as the gelator becomes insoluble.

All of these aspects can lead to issues with reproducibility within a laboratory, but also across different laboratories, depending on how well experimental protocols are described. This is one area where we believe everyone in the community could all improve dramatically. It is often impossible to know exactly what was done from the written experimental details. As part of writing this article, we thought it would be interesting to see the difficulty of reproducing a gel by others in action.

We picked the experimental details from an article from one of our groups, in which we were explicitly showing how to improve reproducibility in Fmoc-dipeptide-based gel systems[11]. At the time of writing, the state of the art was less well developed and in hindsight, it is obvious that insufficient details were provided for clear use of the protocol. We asked six people to follow this protocol under standard 'Great British Bake-Off blind bake' rules: with no conferring and no discussion of approach allowed. Two of the people regularly make gels, but not using these Fmoc dipeptides, two of the people were visiting students from elsewhere who had made gels but are far less experienced with these protocols and the final two had never made gels before. The results are shown in Fig. 2.

Even though these protocols can lead to extremely reproducible materials, the results from this group show variability (between individual people and between gels formed by the same person), both in terms of gel homogeneity by eye (Fig. 2b) and by the rheology results (Fig. 2a). All the rheology was performed by one individual to minimize measurement reproducibility issues. This variability was due to a range of issues in terms of following the protocol. One person, for example, used a stock solution for one component despite this not being in the methodology. Mixing was done in different ways even though the mixing method was specified. Finally, experimental issues arose – for example, some people used the sonication bath, which led to the temperature of the gelator solution rising significantly.

After this, one individual prepared a clear set of instructions with images and the same individuals made more gels. This time, the rheology data were far more consistent (Fig. 2a), and the gels were visually more similar (Fig. 2c).

There is still room for improvement, but this perfectly demonstrates two things. First, without a good methodology, things are hard to repeat. This is self-evident, but we all need to improve here. Second, things become reproducible by conversation and discussion; in our laboratories, we have excellent reproducibility, but this undoubtedly comes from method knowledge being shared and may not always translate into how procedures are described in papers.

In conclusion, supramolecular gels are useful, but extremely difficult to control. Processing when forming these gels is an often-overlooked issue and it is often extremely difficult to reproduce published work. It is often possible to prepare gels with different properties on multiple length scales by varying what can seem to be minor changes, such as the laboratory ambient temperature or the rate of cooling. Many of these parameters are usually not reported. Despite this, we emphasize that once these variables are controlled, the gels can be highly reproducible, although this concept can take some time to understand. The variation in properties that can be accessed by varying the processing allows different gel properties to be targeted, opening up many opportunities to expand the range of properties that can be accessed using a single molecule.

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References

1. Amabilino, D. B., Smith, D. K. & Steed, J. W. *Chem. Soc. Rev.* **46**, 2404–2420 (2017).
2. Terech, P. & Weiss, R. G. *Chem. Rev.* **97**, 3133–3160 (1997).
3. Draper, E. R. & Adams, D. J. *Chem* **3**, 390–410 (2017).
4. Angelerou, M. G. F. et al. *Chem. Commun.* **52**, 4298–4300 (2016).
5. Greenfield, M. A., Hoffman, J. R., Olvera de la Cruz, M. & Stupp, S. I. *Langmuir* **26**, 3641–3647 (2010).
6. Smith, L. R., Cho, S. & Discher, D. E. *Physiology* **33**, 16–25 (2018).
7. Mears, L. L. E. et al. *Biomacromolecules* **18**, 3531–3540 (2017).
8. Cui, J. et al. *Langmuir* **26**, 3615–3622 (2010).
9. Li, J.-L., Liu, X.-Y., Wang, R.-Y. & Xiong, J.-Y. *J. Phys. Chem. B* **109**, 24231–24235 (2005).
10. Thomson, L. et al. *ACS Nano* **16**, 20497–20509 (2022).
11. Adams, D. J. et al. *Soft Matter* **5**, 1856–1862 (2009).

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Competing interests

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