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Remarkable solvent isotope dependence on gelation strength in low molecular weight hydro-gelators

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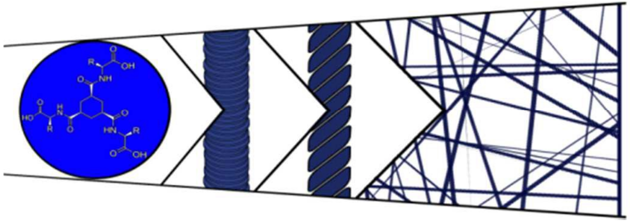
A delicate interplay of anisotropic hydrophobic/hydrophilic, π-π stacking, ionic and hydrogen bond formation interactions determine the strength of hydrogelators and are considered key factors in efforts to design potent small molecule hydrogelators. Here we show that solvent deuteration and electrolytic strength affect the strength of hydrogels formed from amino acid modified C3-symmetric cyclohexane trisamides profoundly. Gels formed by self-assembly through heating/cooling solutions or by pH switching show up to a 30 °C increase in their melting temperatures in D_2O compared to H_2O. The unusually large solvent isotope effect on gel formation and thermal properties indicates that, in contrast to expectations, hydrogen bonding is not the primary determinant of gel strength but instead that hydrophobic interactions between the gelator molecules and the terminal carboxylic acid units are of greater importance. A conclusion that is supported by a similarly large effect of electrolytes on gel strength.

Hydrogels are applied widely as biocompatible materials in medical, biological and the pharmaceutical applications and their use has increased dramatically in recent years.[1–7] Hydrogelators, either small molecule[8] or polymer based,[9,10] when present in minor amounts in water can form 3D networks to form a solid like material consisting of > 95% water by mass. Low molecular weight hydrogelators (LMWGs) are a subclass of hydrogelators, which aggregate anisotropically to form fibers and then bundles of fibres and finally the 3D network that holds water in place (Scheme 1).[11–15]

The utility of amino acids as a structural motif in LMWGs design is attractive not least because of their availability and synthetic versatility. Over the last decade, Feringa, van Esch and coworkers reported a cyclohexane based C_3 symmetric hydrogelator modified with three amino acid side groups (**A**),[16–20] and Meijer and coworkers reported analogous systems with a benzene core (**B**), Figure 1.[12,21–24]



Scheme 1. Hierarchical levels of interactions in the formation of gels from disc like low molecular weight gelators: stack, fibre and intertwined network.

More recently, Ulijn and coworkers showed that linear F-moc protected peptide chains (**C**),[25–28] and, together with Tuttle and coworkers even small tripeptides (**D**) can form hydrogels. The latter systems (**D**) demonstrated the potential of a combined molecular dynamics/quantum chemistry approach to rationally design gelators based on tripeptides.[29] All systems structure show that they are reliable in driving anisotropic fibre growth, Fig. 1, ascribed primarily to the triple set of anisotropic amide amide H-bonds interactions.[17,30] In all systems, variation of the amino acid side chains, *e.g.*, in **A**, methionine and norleucine derivatives form hydrogels, whereas those based on glycine do not. The end capping group used has a pronounced effect on gelation confirming that H-bond interactions are not the sole intermolecular forces involved.

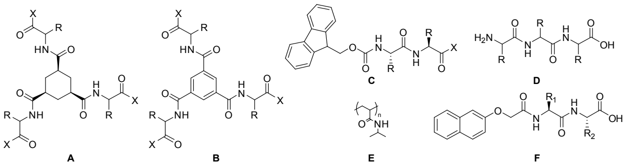


Figure 2. Compounds discussed in the text. R = amino acid groups, X = OH, OMe or ethylene glycol.

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Beyond structural modifications, changes in solvent properties influence gel properties through the addition of co-solutes, pH and isotope exchange. Indeed an ion specific influence (ΔT_m ca. 12 °C) on gel strength in the Fmoc protected dipeptides (**C**), Fig. 1, that follows the Hofmeister series was reported.[27,31,i] AFM studies indicated that the effect was due to changes in fibre morphology and not salting out. Solvent isotope effects (i.e. H_2O/D_2O) have received only limited attention to date despite the opportunities it presents to disrupt hydrogen bonding driven assemblies. The effects on gelation that have been report are however modest with a number of reports on poly(N-isopropylacrylamide) (**E**), that show an increase of 0.6 °C in gel melting point, and for **F** a 50% reduction in G'.[32–36]

Here we report that for a series of cyclohexane core based LMWGs (**A**, Fig. 2) both solvent deuteration and ionic strength have a profound influence on gel properties with as much as a 30 °C increase in melting temperature. Hence, although anisotropic hydrogen bonding interactions between amides in the **C** series of LMWGs has been focused upon to rationalize their gelation properties,[17] the unexpected and unprecedented major increase in gel strength upon substitution of H_2O with D_2O or the addition of electrolytes indicates that amide H-bonding is in fact of minor importance.

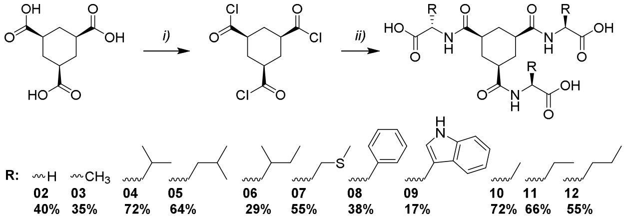

Figure 3: Reagents and conditions‡ *i)* $SOCl_2$, Δ, 20h, 96% *ii)* a) Amino acid methyl ester hydrochloride, DCM, trimethylamine, RT, 48h, 88%. b) Methanol, water, NaOH, RT, 20h, 62%. R: Amino acid side chain

Eleven LMWGs were prepared (Fig. 2) using both natural and unnatural amino acids and their hydrogelation behavior determined from both heat/cool and pH-jump formation thermotropic properties, rheological properties and TEM analysis (for synthesis and spectroscopic data see ESI).

The two approaches taken to form gels from **C** were to dissolve the LMWGs in water with heat or high pH (ca. 10) and then form gels by cooling or pH jumping[ii] (to below the isoelectric point, ca. pH 3), respectively (Table 1). Although both methods lead to the formation of gels, for several Gly and Ala based compounds solutions were obtained only and for Valine, Phe and Trp based compounds crystallization was observed instead of gelation. For Leu, gels formed only upon pH jumping; cooling from hot solutions led to crystal growth. By contrast Ile formed a precipitate with pH jumping but gels upon heating/cooling, which indicates that heating and cooling allows for anisotropic growth to take place whilst the sudden pH switching results in flash precipitation. These variations in behavior indicate that gel fibre growth may proceed differently with heat/cool cycles than by pH jumping. Gels with Abu form slower (min vs s) upon pH switching compared to the other gelators. The differences in the CGC of Abu and Nle, and of Ile and Leu indicate that CGCs decrease with an increase in alkyl chain length and increases with branching. Furthermore the lack of gelation by Gly and Ala indicate that hydrogen bonding is not the sole determinant of gelation properties.

Table 1. Appearance in water after heating/cooling or pH jumping. S: Solution, P: Precipitate, C: Crystal, G: Gel, value between bracket CGC in mg/mL

Compound (amino acid)	Heat/cool	pH
02 (Gly)	S	S
03 (Ala)	S	S
04 (Val)	C	C
05 (Leu)	C	G (5.0)
06 (Ile)	G (7.5)	P
07 (Met)	G (0.6)	G (0.6)
08 (Phe)	C	C
09 (Trp)	C	C
10 (Abu)	G (6.0)	G (7.5)
11 (Nva)	G (5.0)	P
12 (Nle)	G (0.6)	G (0.6)

The temperature dependence of the mechanical stability of the gels was determined by dropping ball measurements. In H_2O, gels formed by Ile (7.5 mg/mL), Nva (5.0 mg/mL) and Nle (2 mg/mL) do not melt below 130 °C.[iv] The Met and Abu gels show an increase in melting point with an increase gelator concentration, 50-110 °C (7.5 – 12 mg/mL) for Abu and 45-100 °C (2 – 7.5 mg/mL) for Met. As the other gelators melt only at high temperatures further studies on the effect of salts and D_2O focused on Abu and Met.

Both Met and Abu gels prepared by pH jumping show an 45 °C increase in melting point compared with those prepared by a heating/cooling cycle. However, a gel prepared thermally from H_2O containing 0.1 M $NaCl_{(aq)}$ (a neutral salt on the Hofmeister series[27,31]) showed the same melting temperature as the pH jump prepared samples confirming that the increase was due to differences in electrolytic strength. Gels formed by heat/cool cycling with 0.1 M of kosmotropic (Na_2SO_4, $CaCl_2$) and chaotropic (NaI, NH_4Cl) salts as well as smaller and larger alkali salts of chloride (RbCl, KCl, LiCl), showed that the increase in melting temperature was approximately constant at 40 °C for Abu and 20 °C for Met, regardless of the salt used. The only exceptions were LiCl and RbCl, which yielded a gel melting point 8 °C lower than with all other salts. These data can be rationalized by a model in which the the cation occupies the space between the fibres and stabilizes the carboxylate groups. The concentration of salt used, however, was 100 times higher than the concentration of Met and 8 times higher than Abu. This prompted us to assess the effect where salt concentrations approached the gelator concentration. With 0.33 eq. and 1 eq. of salt with respect to the gelator, the melting point was the same as that of 0.1 M, however, with 5 eq. the melting temperature decreased again, in contrast to Abu which showed an increase as the amount of salt added increased.

A priori it is expected that hydrogen bonding makes a significant contribution to hydrogelation. Hence weakening the hydrogen bonds with H-D exchange would be expected to suppress the gels' melting points. Surprisingly in 100 % D_2O an increase in melting point by ~50 °C for Met and Abu was

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observed. FTIR and Raman spectra of lyophilized gelators confirmed that rapid exchange of the amide hydrogens occurred when dissolved in D_2O, manifested in a shift of the amide bands. The ratio of the intensity of N-H and N-D bands were used to quantify exchange, with the C-H stretch at 3000 cm^{-1} as an internal standard. A full exchange to N-D was observed (Figure S61-63) and can be reversed by rehydrating in H_2O. TEM analysis shows that fibers formed in D_2O are similar to fibers formed in H_2O.

Table 2. Melting points of Met and Abu LMWGs under various conditions

	Melting point (°C)	
Condition	**Met (2 mg/mL)**	**Abu (8 mg/mL)**
Heat gel	45	56
pH jump	65	99.5
0.1 M NaCl	64	105
0.33 eq. NaCl	65	25
1 eq. NaCl	63.5	27.5
5 eq. NaCl	50	38
0.1 M LiCl	50	88
0.1 M KCl	64	90.5
0.1 M RbCl	55	76
0.1 M NH_4Cl	61	97
0.1 M $CaCl_2$	64	95
0.1 M Na_2SO_4	61	90
0.1 M NaI	58	94
25 % D_2O	79.5	79
50 % D_2O	86.5	96
75 % D_2O	92	101
100 % D_2O	96	103

The storage (G') and loss (G") modulus for gels formed by Met and Abu in H_2O and D_2O were essentially the same as were the breaking points where G" becomes greater than G'. These data indicate that the structure formed in both solvents is in fact the same and that the change in hydrogen bonding does not affect the network. It should be noted that although the solvents differ in viscosity over the range of electrolyte concentrations used, the solvent viscosity does not change significantly[37] and the 20% increase of viscosity in D_2O vs H_2O does not contribute substantially to the rheology since the viscosity increase due to the gel is much greater than the increase due to D_2O.

TEM analysis of gels formed with Met and Abu by heat/cool cycling and pH jumping, Figure 4, indicates that for Met, pH jumping provides for a decrease in order compared to gels formed by heating and cooling. Notably, Abu gels formed by either method do not show differences in morphology, which reflects the slower formation of gel fibers compared to Met. The interlocking network of Met shows more crosslinks in the pH gel than in the heat gel which, considering the increase in melting point with the former method, suggests that gel stability is a result of the interlocking fibers. In the TEM images of gels formed in the presence of NaCl spherical objects are observed attached to the fibers in Met, which are most likely salt crystals.

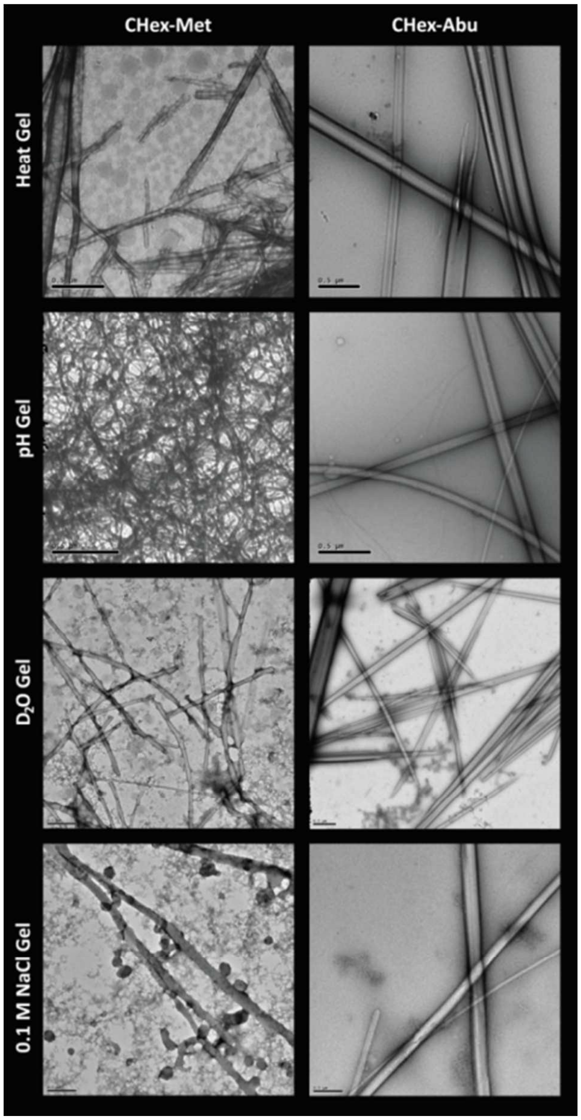

Figure 4. Representative TEM images of Met and Abu made by heating/cooling cycle in H_2O, pH jumping in H_2O, heating/cooling cycle in D_2O and heating/cooling in 0.1 M $NaCl_{(aq)}$ in H_2O. Scale bar 0.5 µm.

In conclusion fibres formed by the C_3-symmetric **A** series hydrogelators show a decrease in CGC with longer linear alkyl chains, however, branched alkyl chains disrupt aggregation. The effect of ionic strength on gelation by **A**-type LMWGs is equally pronounced, however, the magnitude of the effect and the lack of specificity contrast sharply with observations made by Ulijn *et al.* for gels of **C**-type LMWGs.[27] The lack of a dependence of the effect on the electrolyte used, with the notable exceptions of LiCl and RbCl, indicates that a reduction in carboxylate-carboxylate repulsion may play a role. Taken together with the remarkable and unprecedented increase in gel strength upon solvent deuteration, the data indicate that amide H-bonding is not the dominant interaction in driving anisotropic fibre growth but instead side chain hydrophobic interactions dominate. These observations and the differences

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in effects observed for **A** and **C**-type hydrogelators hold considerable implications in regard to efforts to develop general rules for the rational design of low molecular weight hydrogelators.

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Notes and references

i The salts examined range from chaotropic (water structure breaking) to kosmotropic (water structure making) salts.

ii Gelation of the **C** series LMWGs based gelators is pH dependent by virtue of the terminal acid groups, which disrupt aggregation when deprotonated through charge repulsion.

iii Gel preparation on the plates of the rheometer preclude use of heating and cooling to form gels for these experiments.

iv The thermal stability of CH-Ile, CH-Nva and CH-Nle is greater than previously highest reported by Friggeri *et al*.[18]

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