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Molecular Hydrogels from Bolaform Amino Acid Derivatives: A Structure–Properties Study Based on the Thermodynamics of Gel Solubilization

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Abstract: Insight is provided into the aggregation thermodynamics associated to hydrogel formation by molecular gelators derived from L-valine and L-isoleucine. Solubility data from NMR measurements are used to extract thermodynamic parameters for the aggregation in water. It is concluded that at room temperature and up to 55 °C, these systems form self-assembled fibrillar networks in water with quite low or zero enthalpic component, whereas the entropy of the aggregation is favorable. These results are explained by considering that the hydrophobic effect is dominant in the self-assembly. However, studies by NMR and IR spectroscopy reveal that intermolecular hydrogen bonding is also a key issue in the aggregation process of these molecules in water. The low enthalpy values measured for the self-assembly process are ascribed to the result of a compensation of the favorable intermolecular hydrogen-bond formation and the unfavorable enthalpy component of the hydrophobic effect. Additionally, it is shown that by using the hydrophobic character as a design parameter, enthalpy-controlled hydrogel formation, as opposed to entropy-controlled hydrogel formation, can be achieved in water if the gelator is polar enough. It is noteworthy that these two types of hydrogels, enthalpy-versus entropy-driven hydrogels, present quite different response to temperature changes in properties such as the minimum gelator concentration (mgc) or the rheological moduli. Finally, the presence of a polymorphic transition in a hydrogel upon heating above 70 °C is reported and ascribed to the weakening of the hydrophobic effect upon heating. The new soft polymorphic materials present dramatically different solubility and rheological properties. Altogether these results are aimed to contribute to the rational design of molecular hydrogelators, which could be used for the tailored preparation of this type of soft materials. The reported results could also provide ground for the rationale of different self-assembly processes in aqueous media.

Keywords: hydrophobic effect · molecular gels · polymorphism · solubility · thermodynamics

Introduction

The detailed rationalization of the structural, kinetic, and thermodynamic parameters relevant for self-assembly processes that yield soft materials represents a very appealing goal.[1] In this context, aqueous environments are especially interesting due to the biological relevance of water. For example, a recent review highlights the interesting possibilities that emerge from the marriage of synthetic supramolecular chemistry and biological systems[2] and in other recent papers emphasis is put on the diversity of supramolecular complex assemblies that can be formed in aqueous media.[3] It has been pointed out that water-based non-covalent materials have the potential to replace conventional polymers and to promote novel applications that require the combination of robustness and adaptability.[4] There is a general agreement that the use of water as solvent in supramolecular chemistry research makes a significant difference when compared to any other solvent. For example, hydrophobic interactions are fundamental for the study of binding mechanisms in supramolecular chemistry.[5] As highlighted by Engerts and Blokzijl,[6e] water presents unique properties such as a small molecular volume, the capacity of forming hydrogen-bonded networks, and a very low isothermic compressibility. These remarkable properties are responsible both of water being the solvent of life processes[7] and of some awkward properties that emerge in the presence of this solvent such as an unusual reactivity[8] or the so called hydrophobic effect.[6] Leaving aside the controversy on the proper definition of the hydrophobic effect, it is well known that it plays a key role in the organization of living matter and, in general, of soft materials such as, for example, micelles or vesicles,[6a] as well as in protein science[6g] or for the properties of foldamers.[9]

Molecular gels represent an intriguing case of self assembly of low molecular weight species into nano(micro)fibrillar networks that percolate the solvent and transform it into a viscoelastic material, namely into a molecular gel.[10] Key

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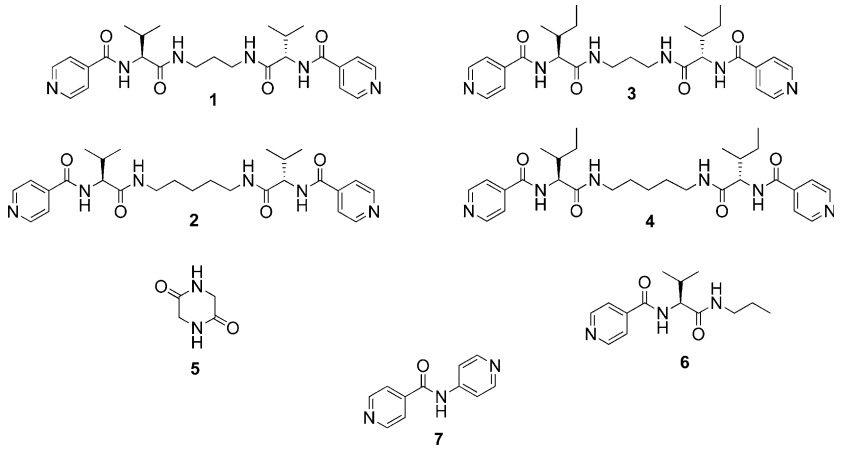
features of this type of soft matter when compared to most cases of gels formed by polymers are the remarkable lyo- and thermoreversibility together with the precise structural arrangement associated to the self-assembly process that generates molecular gels. These materials present applications in a wide range of areas such as regenerative medicine, controlled drug release, optoelectronic materials, or catalysts among others.[11] As an example of the above-mentioned special behavior of water, commonly molecular gels are divided into organogels (gels in organic solvents) and hydrogels. The preparation of molecular hydrogels has been reviewed[12] and, among the structural diversity found in hydrogelators, common structural motifs include the presence of amino acid or peptide moieties, hydrophobic aliphatic tails, and aromatic moieties. For example, a very detailed and illustrative report on the hydrogelation capabilities of some amino acid derivatives was reported by Menger and Caran.[13] The ab initio design of molecules capable to generate molecular gels or the prediction of the gel properties based on the structure of the gelator are major challenges in this field.[14] In this sense, the relationship among solubility, minimum gelator concentration (mgc), and gel–sol transition temperature (T_{gel}) was highlighted for a series of molecular gels formed in toluene.[15] Very recent work also points to the use of solubility parameters as predictors for the formation of molecular gels.[16] It has been stressed that in order to achieve rational design of molecular gels a combination of thermodynamic and kinetic studies is required.[17] Although thermodynamic parameters associated to molecular gel formation in organic solvents are described in many cases,[15,18] in the case of molecular hydrogels there is a lack of data on this respect. For example, no thermodynamic data are reported in the above-mentioned reviews on molecular hydrogels and, up to our knowledge, only in some cases thermodynamic parameters have been extracted for the aggregation of molecular gelators in mixtures of water with organic solvents.[19] Overall, the role played by hydrophobic effects in molecular gels and its regulation by means of structural changes has not been studied in detail. Recently, Adams et al. reported a clear correlation between the hydrophobic character and the gelation efficiency for a series of 9-fluorenylmethoxycarbonyl (Fmoc)-protected dipeptides.[20] In particular, the role played by intermolecular hydrogen bonding versus hydrophobic effects in the formation of hydrogels by dipeptides has been pointed out recently, as a source of interest in this field and the presence of both hydrogen-bonding interactions and hydrophobic effects in the formation of molecular hydrogels has been pointed out in some cases.[21]



Scheme 1. Chemical structure of the compounds discussed in this paper.

Here, we report on a detailed study of the formation of molecular hydrogels by a derivative of L-valine **1** and the closely related compounds **2**–**4** (Scheme 1). Additionally, hydrogels formed by compound **7** are analyzed and compared to those formed by compounds **2**–**4**. The results shown here highlight the role that entropy and enthalpy may play in any self-assembly process in water and could provide a ground for the rational design of hydrogels and, in general, of soft matter building blocks. In the present work, special emphasis is placed on the role that hydrophobic interactions play in the aggregation process. This study takes advantage on the fact that compound **1** is known to be an "ambidextrous" gelator capable of forming gels in organic solvents and water.[22] Therefore, the thermodynamic driving forces for the aggregation of this molecule in both solvents are compared.

Results and Discussion

Aiming to evaluate thermodynamic parameters for the process of hydrogel formation by compound **1**, the solubility of the networked gelator in acetonitrile and water were compared by means of NMR measurements.[23] As shown in Figure 1, an exponential relationship of the solubility with the temperature was measured for the gel formed in acetonitrile, a pattern which has been commonly observed for different gelators. However, the solubility of the gelator in water shows a much lower dependence on the temperature values in the range $T = 20$–$55\,^{\circ}C$. What is particularly remarkable is that the measured solubility decreases slightly from 9.3 to 8.7 mM upon going from 25 to 55 °C. At this point, the solubility of the gelator increases with the temperature in a related way to that observed in acetonitrile. A similar solubility profile has been previously reported for the aggregation of a modified amyloid beta heptapeptide, and was ascribed to the enthalpy and entropy dependence on temperature for the peptide solubilization in water.[6i]

Thermodynamic parameters involved in the aggregation can be obtained from the data shown in Figure 1. Firstly, a

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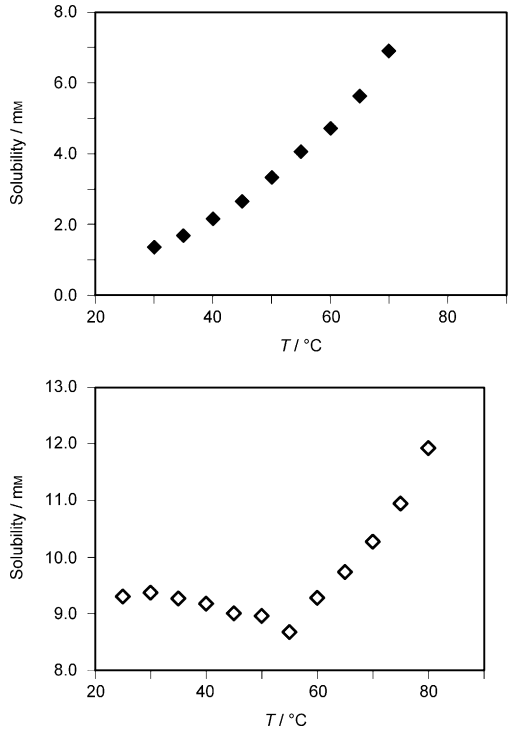

Figure 1. Temperature dependence of the solubility of compound **1** in the gels formed in acetonitrile (top) and water (bottom). The data were obtained by NMR analysis.[23]

van't Hoff treatment of the data for the gel formed in acetonitrile with the thermodynamic parameters for the solubilization of the fibrillar network, results in positive values for the enthalpy change (unfavorable) and entropy change (favorable) associated to this process (Table 1). Regarding the hydrogel, a similar analysis of the solubility data is not feasible as a result of the non-exponential relationship between solubility and temperature for this gelator in water. However, the solubility versus temperature profile in water allows for the easy extraction of some thermodynamic parameters of this system. The relative invariance of the solubility in water from 25 to 55 °C must be associated to an almost negligible enthalpic component in the solubilization process[6c] (see the Supporting Information for equations). As a matter of fact, the solubility of the gel network in water at 55 °C represents a minimum and, therefore, at this temperature the enthalpy change associated to the solubilization process must be zero. Therefore, the entropy change of the solubilization can be calculated at 55 °C, taking advantage of the lack of an enthalpic component at this temperature, being the entropy associated to this process equal to the natural logarithm of the solubility divided by the gas constant (see the Supporting Information for equations). Noticeably, a negative, unfavorable, solubilization entropy value (see Table 1) is obtained for the gel network formed in water. This negative entropy value obtained can be considered in a first analysis a counterintuitive result considering that the solubilization process transforms a condensed phase into a solution, which would present more degrees of freedom associated, for example, to molecular translation and rotation. However, this result is closely related to those obtained in the study of the solubility of alkanes in water and can be rationalized considering the so-called hydrophobic effect. In brief, the solubilization of hydrophobic components in water can result, as noted for example by Engberts and Blokzijl,[6e] in negative entropy values associated to "the small molecular volume of the water molecules" and to the "reduction of rotational freedom of water molecules in the first hydration layers". The decrease of solubility with temperature described is to some point related with the so called "inverse temperature transitions" reported in aqueous media that result, for example, in crystallization of polypeptides or in enhanced protein stability on raising the temperature.[24]

Table 1. Solubilization enthalpy and entropy for the fibrillar gel network of compound **1** at 55 °C.[a,b]

Solvent	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
water	0 (1)	−11 (1)
acetonitrile	35 (2)	19 (1)

[a] [**1**] = 19 mM. [b] Estimated error in parentheses.

Noticeably, the different thermodynamic parameters associated to gels formed in water and acetonitrile by compound **1** result in some unusual properties of the hydrogels when compared to common molecular organogels. For example, an interesting consequence of the flatness of the solubility versus temperature graph in the temperatures range of approximately 25–70 °C is that the minimum gelator concentration value, 17 mM, is virtually independent of the temperature in this interval. A curious consequence of this property is evidenced in the graphs of temperature for the gel-to-solution transition (T_{gel}) versus concentration. Typically this type of graph shows a marked slope in the initial range of concentration and then a flat profile at higher concentrations, which can be related to the exponential relationship of concentration and temperature (Figure 2, top). Notably, the T_{gel} versus concentration graph obtained for the hydrogel derived from **1** is atypical because T_{gel} = 85 °C represents an absolute minimum in the T_{gel} values. No gel can be prepared with thermal stability below this point (in the temperature range studied, which starts at 25 °C) because the concentration at which a gel melts at 85 °C can also be considered the mgc value at 25 °C.

Of special interest is the amazing variation of the rheological properties of the hydrogel with the temperature. As can be seen in Figure 3, the storage modulus, G', measured for the hydrogel formed by **1** shows an intriguing big increase (notice the logarithmic scale) in the temperature range 25–55 °C. Namely, the gel network seems to be "stronger" as the temperature is raised. At higher temperatures the G' module decreases clearly, a behavior which is expected for this type of supramolecular systems. A related case has been reported for an aqueous triblock copolymeric hydrogel[25a] and a similar trend has been reported, although the ob-

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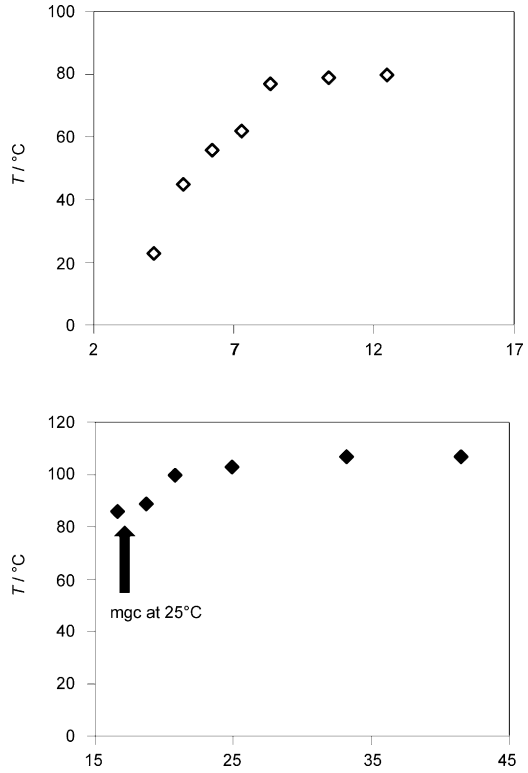

Figure 2. Temperature measured for the gel-to-solution transition for the gels formed by compound **1** in acetonitrile (top) and water (bottom). The data were obtained from oscillatory shear rheology studies (see the Supporting Information).

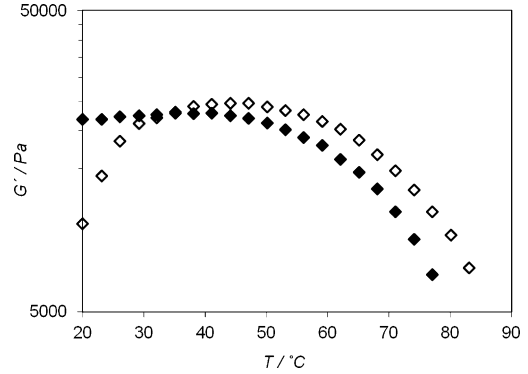

Figure 3. Variation of the rheological storage modulus (G', logarithmic scale) with the temperature for the gels formed by compound **1** in water (◇, 19 mM) and acetonitrile (◆, 6 mM). Oscillation frequency = 1 Hz and oscillatory stress = 130 Pa. For clarity the variation of loss modulus (G'') is omitted. See the Supporting Information for a full set of data.

served variation is much smaller, for a peptide amphiphile capable of forming hydrogels in the presence of calcium chloride.[25b] Noticeably, the rheological profile of Figure 3 shows similarities with the solubility profile shown in Figure 1, being the maximum G' value coincident with the minimum observed solubility. On the other hand, the rheological analysis for the gel formed in acetonitrile shows the expected trend, becoming the gel progressively weaker upon increasing the temperature, and revealing a nice correspondence with the solubility increase shown in Figure 1.

Summing up, the aggregation of compound **1** into fibrillar networks in acetonitrile is controlled by the enthalpy and in water (at temperatures below 55 °C) by the entropy. To ascertain how this observation correlates with the structure of the assemblies formed in both solvents structural studies by using NMR and IR spectroscopy were performed. The aggregation of compound **1** in organic solvents most likely proceeds through a structural arrangement related to that found for analogues, which present terminal benzoyl of benzyloxycarbonyl (Boc) units. The proposed structural arrangement shown in previous work involves multiple hydrogen bonds among the molecules of gelator yielding a β-sheet-like structure (see Figure 4).[26] A similar aggregation

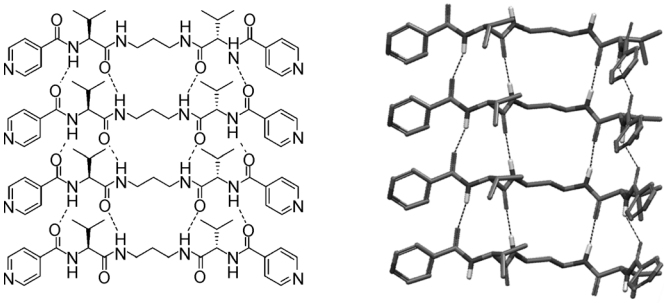

Figure 4. Schematic aggregation model of for compound **1** and molecular mechanics energy minimized model (non-polar hydrogen atoms are removed for clarity; MACROMODEL 9.0, AMBER*, GB/SA simulation of chloroform as solvent).

pattern is reported for the crystalline structure of closely related Boc-protected analogues.[27] Although the possibility of pyridine units participating in intermolecular hydrogen bonding is feasible, in the case of compound **1** previous studies regarding the interaction of the gel network with metal cations and guest species with hydrogen-bonding donor capabilities seem to discard this possibility in our system.[22,28]

A ^{1}H NMR study of compound **6** in CD_3CN, a fully soluble analogue of compound **1**, confirms that hydrogen bonding is a driving force for the aggregation of this type of molecules in acetonitrile, as shown in the shift of the NH signals upon increasing concentration (Figure 5). The aggregation model proposed in acetonitrile (Figure 4) would be in ac-

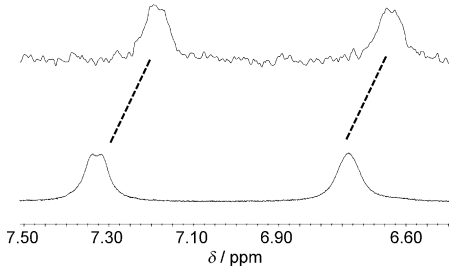

Figure 5. Fragment of the ^{1}H NMR spectrum of compound **6** in CD_3CN, showing the resonance of the amide NHs. [**6**] = 1mM (top), 76 mM (bottom).

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cordance with the important enthalpic component (35 kJ mol^{-1}) of the solubilization process which would be related mainly to the breakage of several intermolecular H-bonds.

On the other hand, the involvement of intermolecular hydrogen bonding in the aggregation of compound **1** in water was nicely confirmed by IR spectroscopy studies.[29] As shown in Figure 6, upon cooling down a hot solution of compound **1** in water, new bands in the C=O region can be observed, which should be ascribed to the formation of intermolecular hydrogen bonds as the aggregation proceeds. Similar conclusions were obtained in the study of compounds **2** and **3** when their IR spectra were recorded in mixtures of water and DMSO (these compounds presented very fast aggregation kinetics that precluded the variable temperature analysis performed for compound **1**). Upon increasing the percentage of water in the water/DMSO mixtures the C=O stretching shifted to lower wavenumbers, in agreement with the participation of hydrogen bonding in the aggregation process (see the Supporting Information).[30] Additionally, the IR spectrum reveals changes in the CH stretching region, As can be seen in Figure 7 there is a shift of these bands to lower wavenumbers as the concentration increases, which has been ascribed to the packing of aliphatic chains.[31]

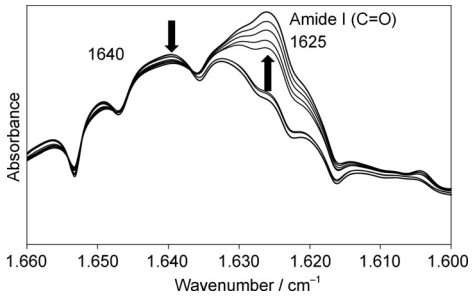

Figure 6. Variation of the FTIR spectrum of a hot solution of compound **1** (14.5 mM) in water upon cooling down to room temperature. Arrows indicate how the signals change with time.

The fact that the formation of hydrogel **1** is entropy driven and, at the same time, hydrogen bonding is a driving force for the aggregation, seem to be to a certain point contradictory results. As noted above, the solubilization of the hydrogel formed by compound **1** in water presents a low enthalpic component, especially in the temperature range 25–50 °C, being zero at 55 °C. These data at first glance suggest that the energy associated to the breakage of hydrogen bonds of the gelator in the solubilization process is almost negligible. If we assume this statement to be correct, the introduction of hydrogen-bonding groups in the design of hydrogelators would seem pointless. Fortunately for this discussion, the relevance of hydrogen bonding in aqueous environment has been studied in detail due to the key role that this non-covalent interaction plays in protein folding and stability. As pointed out by Murphy and Habermann,[32] although some authors have proposed that the loss of amide–amide hydrogen bonds is compensated energetically by the formation of amide–water hydrogen bonds (resulting in a zero enthalpy gain for the process or disassembly), now there is a general agreement that the enthalpy of amide–amide hydrogen-bonding formation in water is favorable. For example, the results shown by this authors in the solubilization of crystalline cyclic dipeptides clearly reveal positive (unfavorable) enthalpies associated to the dissolution (breakage of intermolecular hydrogen bonds) of the cyclic peptides in water. The values reported range from 13 to 26 kJ mol^{-1} and, for example, the cyclic dipeptide **5** (Scheme 1) is reported to present a solubilization enthalpy of 26 kJ mol^{-1}. Obviously, these data are in contradiction with the zero enthalpy change observed for gelator **1** in water. However, the consideration of the hydrophobic effects can rationalize these results. The zero enthalpic component of the solubilization process of compound **1** in water at 55 °C should be seen as the result of the sum of the unfavorable enthalpy associated to intermolecular bond dissociation (mainly hydrogen bonds) and the enthalpy associated to the hydrophobic effect at that temperature, which must be favorable. Indeed, it is well reported that the enthalpic component of the hydrophobic effect is quite variable with the temperature, being favorable for the dissolution of apolar substrates into water at low temperatures (for example 25 °C) and becoming unfavorable at higher temperatures.[6b,f] As for the cyclic dipeptide **5**, the unfavorable dissolution enthalpy reported, can be rationalized considering that the hydrophobic effects associated to the dissolution of this rather polar molecule are negligible. For example, a quantitative estimation of polarity reveals a tremendous difference in the polar character of compounds **1** and **5** being the values of the calculated partition coefficients between water and octanol (*c*log *P*) 1.9 and −1.7, respectively (in this scale the value of *c*log *P* is 2.0 for benzene and −0.7 for methanol).

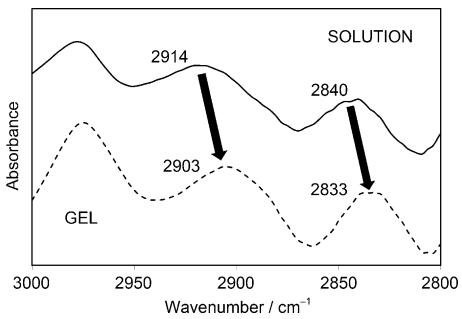

Figure 7. FTIR spectra of compound **1** in D_2O; solution (top, 8 mM) and gel (bottom, 14.5 mM).

Further insight in the structural properties of the gel formed by compound **1** in water was obtained from NMR studies. The comparison of the ^{1}H NMR spectra of compound **1** in solution (2 mM) and after formation of a gel (19 mM) in H_2O is puzzling. As can be seen in Figure 8, the spectrum of the solution does not show any NH resonance but the spectrum corresponding to the gel system reveals an

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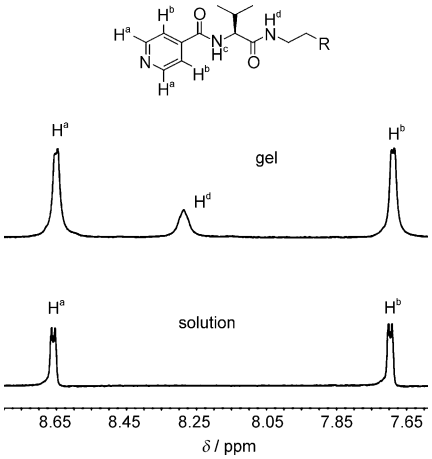

Figure 8. Fragment of the ^{1}H NMR spectra of compound **1** in solution (bottom, 2 mM) and gel (top, 19 mM) in H_2O. Solvent signal was presaturated.

NH signal corresponding to the central amide unit. This fact should be ascribed to the exchange of the amide NHs with the solvent. If this process is fast enough, the NH signals vanish as a result of the solvent presaturation technique used to record the spectrum. The results obtained show that this exchange is slowed down in the gel system, most likely due to the involvement of the amide in intermolecular hydrogen bonding. Therefore, in an indirect way, this result confirms that hydrogen bonding plays a role in the self-association of this molecule in water. Particularly, the central NH unit would be involved in the aggregation mechanism, whereas the terminal one, which is not visible in any spectra, would be weakly involved, if at all, in intermolecular hydrogen bonding.

An interesting difference in the intermolecular interactions found for compound **1** in acetonitrile and water is the probable presence of aromatic π–π stacking in water. The ^{1}H NMR spectrum of the model compound **6**, which is soluble in water in the studied concentration range, reveals a shielding of the aromatic pyridine units upon increasing the concentration, which is indicative of π–π stacking interactions (Figure 9). This behavior was not detected in acetonitrile, suggesting that the hydrophobic effect may contribute

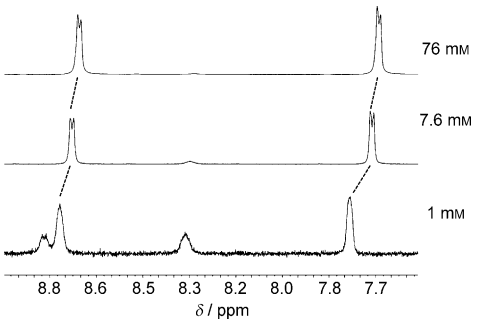

Figure 9. Fragment of the ^{1}H NMR spectra of compound **6** showing the shielding experienced by the protons of the pyridine unit upon increasing the concentration (see Figure 8 for labeling).

to this aromatic stacking. Additionally, it is worth to mention the intriguing disappearance of the NH resonances in the top spectrum of Figure 9. The fact that the NHs resonances of compound **6** can be observed at a concentration of 1 mM but not at higher concentrations (76 mM) indicates an acceleration of the exchange process between the hydrogen atoms from the amide NHs and water associated to a concentration increase. A rationale for this concentration effect is that the pyridine groups catalyze the NH–H_2O proton exchange and that a bimolecular event (collision of two molecules of **6**) is required for the catalysis to be effective.

All the results shown above can be used advantageously for the rational design of low molecular weight hydrogelators. Compound **1** is a hydrogelator with moderate efficiency in terms of minimum gelator concentration at 25 °C (mgc = 14 mM). Considering that the entropy change associated with the hydrophobic effect is the driving force for the aggregation at this temperature, it seems reasonable to introduce groups with enhanced hydrophobicity in the molecule to boost the gelation properties as a result of an improved favorable entropic term. With this purpose compounds **2**–**4** were prepared and studied (see Scheme 1). In compound **2** the hydrophobic character is increased by employing a central pentamethylenic spacer as compared to compound **1**, which presents a propylenic one. Compound **3** is an isomer of compound **2**, which derives from L-isoleucine instead of valine and presents a propylenic aliphatic spacer. Finally, compound **4** presents both a pentamethylenic spacer and derives from the amino acid L-isoleucine. The study of these compounds permits to evaluate how the introduction of hydrophobic moieties in different parts of the molecule (amino acid side chain vs. aliphatic spacer) affects the hydrogelation properties. It was found that compounds **1**–**4** form hydrogels that were made of fibrillar networks (see the Supporting Information for electron microscopy images). The results obtained in the study of the hydrogelation capabilities of the compounds mentioned above are summarized in Table 2. Remarkably, the rational design of improved hydrogelators by means of increasing the hydrophobic character of the molecule was very satisfactory and agrees with a recent report from Adams et al. on the correlation of the hydrophobic character and the gelation efficiency.[20] On

Table 2. Comparison of the minimum gelator concentration, solubility, and entropy of the solubilization values for the hydrogels formed by compounds **1**–**4**.[a]

Compound	mgc [mM]	Solubility (25 °C) [mM]	$\Delta S_{gel-sol}$ (55 °C)[b] [J mol^{-1} K^{-1}]
1	14 (17)[c]	9	−35
2	8	3	−46
3	5	1	−61
4	≤2	≤0.1[d]	< −77

[a] Estimated errors: mgc = 0.5 mM, mgc = minimum gelator concentration; solubility = 5 %; $\Delta S_{gel-sol}$ = 1 J mol^{-1} K^{-1}. [b] Calculated considering $\Delta H = 0$ at this temperature. [c] 14 mM: gel formed after 72 h; 17 mM: gel formed after 24 h. [d] Estimated from the detection limit by ^{1}H NMR spectroscopy under the conditions of the experiment.

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going from compound **1** to the most hydrophobic compound in the series, that is compound **4**, a very significant improvement in the gelation capabilities was observed. The minimum gelator concentration required to form a gel was reduced from 17 to less than 2 mM. The gelation efficiency in water was found to be directly related to the solubility, as shown in previous work in organic solvents.[15]

The results in Table 2 reveal that the solubility in water at 25 °C experienced a dramatic reduction upon going from gelator **1** (9 mM) to gelator **4** (0.1 mM), which must be associated to the entropic term of the aggregation free energy that is much higher in absolute value for the more hydrophobic compound **4** ($\Delta S < -77\,J\,K^{-1}mol^{-1}$) than in the case of the system formed by compound **1** ($\Delta S = -35\,J\,K^{-1}mol^{-1}$). These results agree with the different water–octanol partition coefficients (*c*log *P*) estimated for compounds **1** and **4**, which are 1.9 and 2.9, respectively. The data from Table 2 corresponding to the isomeric compounds **2** and **3**, show that the introduction of hydrocarbonated fragments in the amino acid side chain (isoleucine instead of valine residue, compound **2**) results in quite stronger hydrophobic effects than those obtained by increasing the aliphatic central spacer (pentamethylenic instead of propylenic central aliphatic spacer, compound **3**). The relevant difference between the amino acids valine and isoleucine in terms of hydrophobic effects has been pointed out previously in the context of protein science.[6g]

It is worthy to comment on the solubility versus temperature profile obtained for compounds **1**–**3** (compound **4** was too insoluble for the determination of its solubility by NMR spectroscopy). It can be seen in Figure 10 that compounds **1** and **2** show related solubility profiles, both presenting a minimum value at 55 °C. In the case of compound **2**, the solubility increase above this temperature is significantly smaller in absolute terms when compared to that experienced for compound **1**, but comparable in relative terms. Having in mind the exponential relationship between the solubility and the entropy changes, this result agrees with a similar absolute change in the entropic term of the aggregation on going from 55 to 70 °C in both systems.

Compound **3** deserves a special mention due to its weird solubility versus temperature profile. As shown in Figure 10, the solubility of the hydrogelator is almost constant from 25 to 60 °C (this implies a negligible association enthalpy in this temperature range) but at higher temperatures a dramatic decrease of the solubility is observed, making the compound undetectable by NMR spectroscopy above 70 °C. This behavior is just the opposite of that shown for compound **1**, and instead of the expected solubility increase with the temperature the formation of a very insoluble gel network is observed. Considering the precedents in supramolecular gelation, this result should most likely be ascribed to a structural rearrangement of the aggregates (formation of a different polymorph) as reported in some previous cases, including closely related analogues of compound **3**, which were studied in organic solvents.[33] In the present case, the significant changes in the hydrophobic effect expected to take place above 55 °C could be the driving force for this structural rearrangement. A nice experimental proof of the polymorphic change experienced by the hydrogel formed by compound **3** was obtained from CD spectroscopy (Figure 11). The CD spectra obtained for hydrogels of compound **3** at 20 and

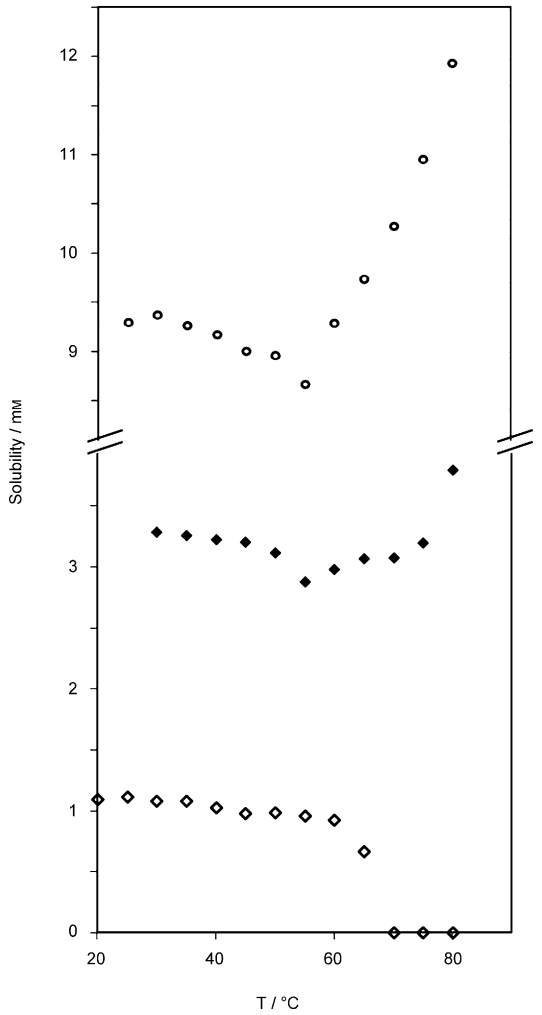

Figure 10. Solubility variation with the temperature for the hydrogels formed by compounds **1** (○, 19 mM), **2** (◆, 14 mM), and **3** (◇, 6 mM).

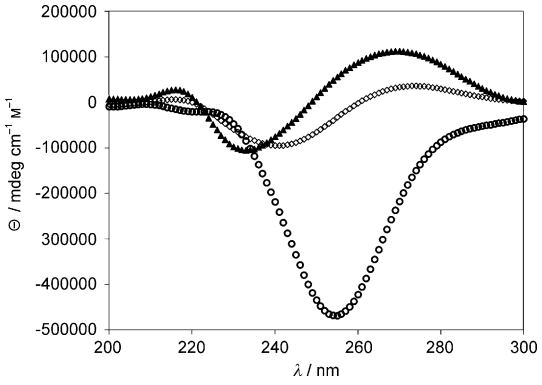

Figure 11. CD spectra for the hydrogel formed by compound **3** (4 mM) at different temperatures (◇ = 20, ▲ = 60, ○ = 70 °C).

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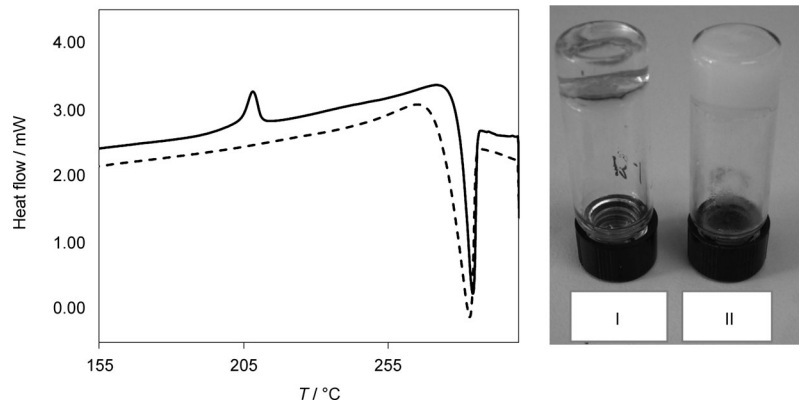

Figure 12. DSC thermograms of the xerogels prepared from hydrogels formed by compound **3**. The gels were obtained by cooling a hot solution either to 20 (polymorph I, solid line) or 70 °C (polymorph II, dashed line). Left: Pictures of the polymorph I and II.

70 °C showed remarkable differences, for example, the molar ellipticity at 260 nm being of opposite sign at these temperature values. This result indicates a significant reorganization of the aggregates that form the fibrillar network. The positive Cotton effect observed for the samples corresponding to the hydrogel at 20 and 60 °C agrees with previous work on aggregation of pyridine derivatives.[34] Further evidence of this polymorphic transition was provided by differential scanning calorimetry (DSC) analysis of xerogels prepared from a hydrogel of compound **3**. In one case, a hydrogel was obtained by cooling down the hot solution of **3** in a thermostated bath at 25 °C (polymorph I) and in the other case the system was thermostated at 70 °C (polymorph II). Notice in Figure 12 the different aspect that both gels present, being one transparent and the other one opaque. The DSC graphs obtained revealed that the xerogel obtained at 20 °C presented an exothermic peak at 220 °C, which should be ascribed to a polymorphic transition (Figure 12).

Additionally, scanning electron microscopy (SEM) images of the xerogels were remarkably different. In the case of polymorph I quite thin fibers were observed, whereas polymorph II presented the formation of tapes of approximately 1 μm width (see the Supporting Information).

An interesting consequence of the polymorphic change experience by the hydrogel formed by compound **3** is its unusual variable temperature rheological profile. As shown in Figure 13, the gel formed by cooling a hot solution to 20 °C (polymorph I) is strengthened upon heating from 25 to 70 °C and at this point an abrupt tendency change is recorded, which yields a gel with lower storage module and poor temperature dependence ascribed to the progressive formation of polymorph II. In fact, when the gel was formed by cooling down a hot solution to 70 °C, this hydrogel (polymorph II) presented a different rheological profile (Figure 13).

Up to this point, the improvement of gelation capabilities by using a rational design based on the consideration of hydrophobic effects has been shown. Following the line of reasoning presented above, it seems feasible to move from entropy- to enthalpy-directed hydrogelation. For this purpose the hydrophobic effect should be minimized, namely, hydrophilic gelators should be considered. With this idea in mind, compound **7**, which was reported in the literature as hydrogelator was chosen for the study.[35] This compound is quite more hydrophilic than, for example, compound **1** ($c\log P$ = 0.8 and 1.9, respectively). Indeed, the solubility profile obtained for compound **7** in water is significantly different from that of compound **1** (Figure 14). The solubility of compound **7** shows a very strong, exponential dependence with the temperature, revealing an enthalpy-driven aggregation. As a matter of fact, the solubility data can be fitted to a van't Hoff analysis, yielding an unfavorable solubilization enthalpy of 49 kJ mol^{-1} and a favorable solubilization entropy of 116 J K^{-1} mol^{-1}. The strong enthalpy component when compared to that obtained for compound **1** in acetonitrile

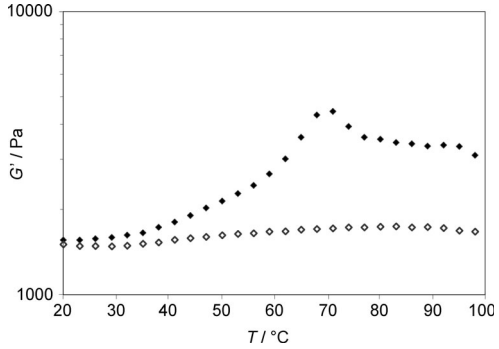

Figure 13. Variation of the rheological storage modulus (G') with the temperature for the gels formed by compound **3** (6 mM) in water, by cooling down a hot solution to 20 (polymorph I, ◆) and to 70 °C (polymorph II, ◇). Oscillation frequency = 1 Hz and oscillatory stress = 1 Pa.

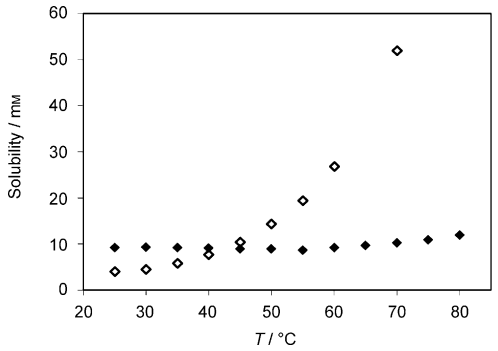

Figure 14. Solubility variation with temperature for the hydrogels formed by compounds **1** (◆, 19 mM) and **7** (◇, 106 mM).

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(38 kJ mol^{-1}) is difficult to rationalize if an aggregation based mainly on intermolecular hydrogen bonding is proposed for **7**. This compound presents only one amide group available for this purpose as compared to the four amide groups present in compound **1**. Most likely, aromatic stacking is a major driving force for the aggregation of compound **7**, which presents a planar geometry and two conjugated aromatic rings, as suggested in the original study of hydrogel formation by this molecule.[35]

Obviously, switching from an entropy-driven hydrogel to and enthalpy-driven one also has consequences on practical properties of these soft materials such as the rheological profiles. As shown in Figure 15, the enthalpy-driven hydrogel formed by compound **7**, presents a dependence of the storage modulus on the temperature, which could be considered as usual for molecular gels, and which correlates well with the solubility pattern depicted above. On the other hand, the poor temperature dependence of the solubility of compound **5** results in good mechanical properties (high storage modulus) in the range of temperatures shown in Figure 15.

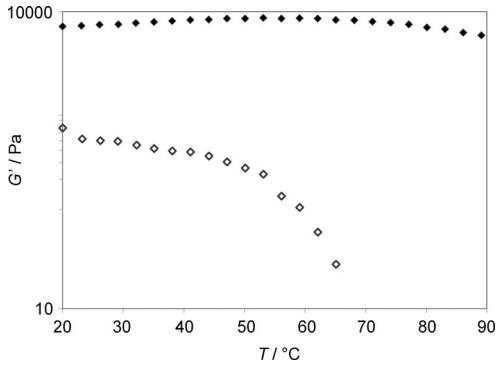

Figure 15. Variation of the rheological storage modulus (G') with the temperature for the hydrogels formed by compounds **2** (◆, 9.8 mM) and **7** (◇, 75 mM). Oscillation frequency = 1 Hz and oscillatory stress = 1 Pa.

Conclusion

Remarkable differences in the self-assembly process that takes place in acetonitrile and water have been found for the ambidextrous gelator **1**. Thermodynamically, the aggregation process is found to be enthalpy driven in the organic solvent but entropy driven in water. Therefore, the hydrophobic effect is shown to play a key role in the formation of the hydrogel by compound **1**. Improvement of the gelation efficiency is easily achieved by increasing the hydrophobic character of the gelators, by using, for example, isoleucine derivatives instead of valine ones. Noticeably, regarding the intermolecular driving forces for aggregation, it is found that aromatic stacking plays a role in the aggregation of model compound **6** in water, but this interaction is not detected in acetonitrile. Among the results reported, it should be highlighted that it is possible to have either enthalpy-driven or entropy-driven molecular gel formation in water. This fact is demonstrated by the study of hydrogelator **7**, whose aggregation is controlled by enthalpy. The low hydrophobic character of this compound explains this property. It is outstanding that moving from an enthalpy-driven to an entropy-driven aggregation process results in some noticeable changes in the properties of the hydrogels, which can potentially be used for different applications. Firstly, the entropy-driven hydrogels studied here, show poor temperature dependence of their properties below 55 °C, resulting in low solubility changes and therefore, almost invariable minimum gelation concentration values. As a result of this, in difference with common molecular gels, it is not possible to modulate the thermal stability with concentration changes in the range of about 25–80 °C in the entropic-driven hydrogels, being the gels either stable up to 85 °C or not formed. In addition to this, rheological studies reveal a quite unusual profile of the temperature-dependant viscoelastic properties, resulting in a strengthening of these materials upon heating up to approximately 55 °C. Another point of interest is the fact that hydrogen bonding is shown to play a key role in the aggregation of hydrogelator **1** in water, as demonstrated by IR spectroscopy. This fact agrees with previous work on the relevance that intermolecular hydrogen bonding may have in aqueous environments. All these results seem to contribute to provide a ground for the rational design of molecular gelators, a task that has been considered by different authors as a major challenge. The results reported should contribute to achieve a tailored design of molecular gels in order to prepare materials with the required properties. For example, enthalpic gels could be useful in controlled release based on temperature changes, whereas entropic gels would yield robust materials relatively insensitive to temperature changes. Noticeably, the identification of entropic or enthalpic gels is experimentally simple. For example, solubility versus temperature studies or, variation of T_{gel} with temperature can be used for this purpose.

Aside of these considerations, the study of the hydrogel formed by compound **3** underlines the importance that polymorphic transitions may have in soft materials. The reported structural change above 70 °C results in a dramatic change in the solubility and rheological properties.

Experimental Section

For experimental details, see the Supporting Information.

Acknowledgements

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