A supramolecular hydrogel that responds to biologically relevant stimuli†

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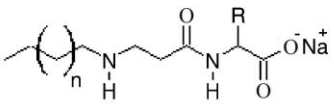
First demonstration of heat and pH-responsive hydrogel of SDS and a zwitterionic amphiphile, sodium *N*-(n-dodecyl-2-aminoethanoyl)-L-valinate with very low minimum gelation concentration.

Soft materials that can respond to biologically relevant stimuli have recently attracted huge interest because of their uses in a range of biomedical applications.[1] An example of biologically relevant stimuli responsive materials are hydrogels that are responsive to temperature or pH. Because of possible practical applications in tissue engineering,[1,2] vehicles for controlled drug release,[3–5] and water pollution control[6] the research into designing small organic molecules capable of gelling water has gained tremendous importance. The subject has been reviewed in some recent reviews.[2,7,8] Since specific noncovalent forces such as electrostatic, π–π stacking, dipole–dipole, and hydrogen-bonding interactions hold low-molecular-weight gelator molecules together, they offer various advantages over the traditional polymer gels. For example, it becomes easier for the body to degrade them. Also gels formed by low-molecular-weight hydrogelators (LMWH) are thermoreversible. The LMWH are capable of growing from homogeneous solution into fibrillar structures in water. The gelation is thought to arise from entanglement of the fibers or network structure formation and trapping solvent *via* surface tension. To date only a few hydrogels composed of such aggregates have been reported. The only LMWH, the molecular self-assembly of which results in water gelation, includes sugar-based gelators,[8] bis-urea carboxylate derivatives,[9] amino acid derivatives,[10] bis-oxalyl amides,[11] and tartarate-based gemini surfactants.[12] In general, gelation ability, efficiency, and properties change dramatically with small structural variations in the gelator molecule. In fact, control of hydrophobic forces is the key to designing organic hydrogelators.

In this work, we describe an amino acid derived zwitterionic amphiphilic molecule, sodium *N*-(n-dodecyl-2-aminoethanoyl)-L-valinate, **1**, (Chart 1) that exhibits complete pH- and thermo-reversible self-assembly formation into a hydrogel network in the presence of sodium dodecyl sulfate (SDS). The effects of three structural variations: (i) length of the hydrocarbon chain, (ii) hydrophobicity of the amino acid side chain, and (iii) chirality of the amino acid head group of the chiral amphiphile on the gelation process were investigated.



n	R	Name	n	R	Name
9	$CH(CH_3)_2$	**1**	9	H	**2**
9	CH_3	**3**	9	*$CH(CH_3)_2$	**4**
5	$CH(CH_3)_2$	**5**		*(DL – Valine)	

Chart 1 Chemical structure of amphiphiles 1, 2, 3, 4, and 5.

We have earlier shown that **1**, and the corresponding glycine (**2**) and L-alanine (**3**) analogs are efficient gelators of organic solvents.[13] These amphiphiles are sparingly soluble in water at neutral pH as they exist in the zwitterionic form. However, the amphiphiles, as expected, become soluble at pH $\leqslant$ 2 and at pH $\geqslant$ 11.5. In an effort to solubilize amphiphile **1** we used a 10 mM SDS solution instead of distilled water. But to our surprise the amphiphile **1** remains insoluble even in the presence of SDS at neutral pH. However, at pH = 2 (0.01 N HCl), the mixtures of **1** and SDS solutions with molar ratio [**1**] : [SDS] in the range 0.2–1.0 are opaque. The opaque solution slowly transforms into an almost clear gel (Fig. 1) at room temperature ($\sim$30 °C). The opacity of the gel decreases with the decrease in molar ratio. Depending upon the molar ratio and concentration of the gelator the gelation time varies in the range 3–48 h.

The gelation was observed at a total concentration as low as 3.5 mM, which can be taken as the minimum gelator

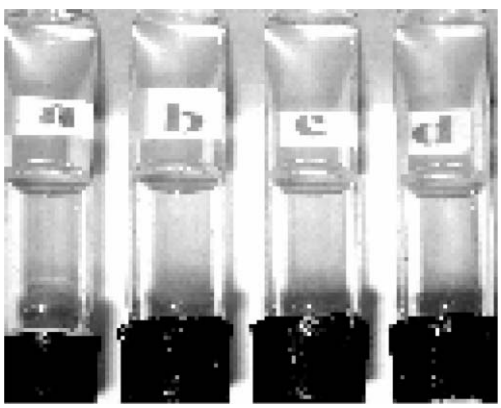


Fig. 1 Vials showing 4 mM hydrogel of **1** having [**1**] : [SDS] equal to (a) 0.25, (b) 0.5, (c) 0.67, and (d) 0.67 with 0.8 M NaCl; opacity of the hydrogels is shown by the visibility of the letters put at the back of the respective vial.

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concentration, MGC (0.05%, w/v). The MGC value increased to 0.08% when the [**1**] : [SDS] ratio decreased to 0.2. In fact, no gelation was observed below the molar ratio of 0.2. The gel thus formed is stable for many days. To our knowledge the only other LMWH that has a low MGC (0.05%) value is a bolaamphiphile 1,12-dodecanedicarboxylic-bis-(*p*-aminophenyl-β-D-aldopyranoside).[14]

The hydrogel of **1** produces a viscous liquid when shaken vigorously. However, the gel reformed again upon standing at room temperature. The hydrogel also shrinks while expelling water upon heating. Interestingly, the shrunken gel swells again by slow cooling to room temperature such that the macroscopic hydrogel reforms again. Thus the gelation is thermoreversible. This gel–sol transition was found to be totally reversible in subsequent heating cooling cycles. The gel-melting temperature, T_m (~315 K) is relatively low. The T_m value increases slightly when the molar ratio decreased to 0.25 (318 K) (Fig. 2).

The hydrogel was also found to shrink upon a slight increase of pH above 2.0. Raising the pH by the addition of a drop of NaOH solution destroyed the gel structure completely. But a clear gel reformed again when the pH was decreased to 2.0 by the addition of a drop of concentrated HCl. However, the gelation time increased to 72 h. This means that gel formation is also reversible with respect to a change of pH. The gel formed was found to be stable in the presence of high concentrations of salt (0.8 M NaCl). It is interesting to note that gelation did not occur when sodium dodecyl benzene sulfonate (SDBS) was employed in place of SDS.

In order to study the influence of different amino acid headgroups on hydrogel formation, we synthesized corresponding glycine (**2**) and L-alanine (**3**) derivatives. These amphiphiles are also insoluble in water but become soluble in the presence of SDS at a molar ratio <1.0. However, no gelation occurred except a small increase of viscosity of the solution with the increase in total amphiphile concentration. The test of gelation at pH ⩽ 2.0 also failed. Similarly, the D,L-valine derivative (**4**) could not gel SDS solution under similar conditions. The compound **5** with a shorter hydrocarbon chain also failed to produce gel in the presence of SDS.

Since the first stage of physical gelation involves self-assembling of gelator molecules, the molecular self-assembly feature was

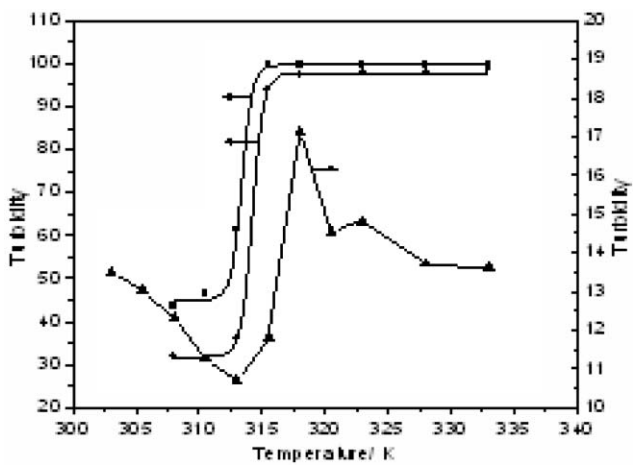

Fig. 2 Plot of turbidity of hydrogel (4 mM) *versus* temperature for [**1**] : [SDS] = 0.67 (■), 0.33 (●) and 0.25 (▲).

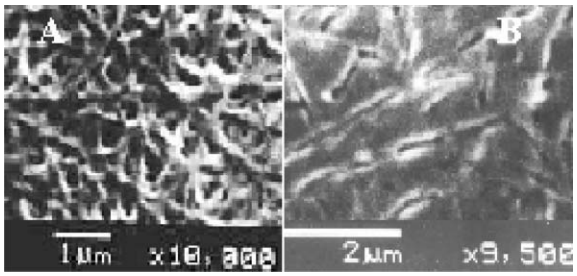

Fig. 3 SEM pictures of fully formed (A) and preformed (B).

observed by electron microscopy. Scanning electron microscopic (SEM) images of the gel display fibrous aggregation features (Fig. 3).

The hydrogel appears to be composed of intertwined fibrous networks that result in immobilization of water. The micrograph, B, of the aqueous gel of **1** (2.0 mM) that was taken prior to gel formation reveals thin twisted ribbons. We believe that the rope-like structures in swollen gel are composed of twisted ribbons, which in fact, are bilayer assemblies of the gelator molecules. Indeed, the X-ray diffraction patterns (Fig. 4) of the gel cast film exhibit periodical reflection peaks, which indicate that **1** assembles into an ordered lamellar structure. The long spacing (d) corresponding to the high and sharp reflection peak in the XRD pattern is 3.72 nm, which is smaller than twice that of the extended molecular length of **1** (2.45 nm as obtained from MM2 calculation), but larger than the length of one molecule. This implies that the aggregate has an interdigitated bilayer structure with a thickness of 3.72 nm. This is consistent with the strong hydrophobic interaction of the hydrocarbon tails.

The strong hydrophobic interaction facilitates intermolecular hydrogen bonding interactions at the headgroup of the complex

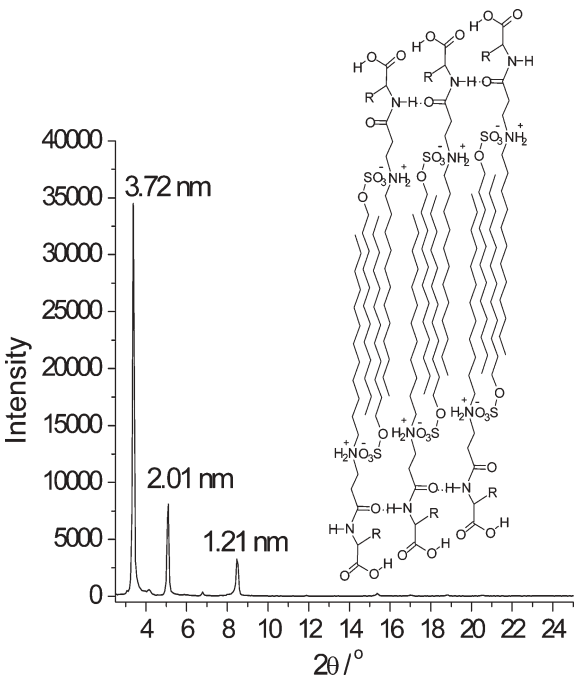

Fig. 4 X-ray diffraction pattern of hydrogel film, inset: schematic presentation showing arrangement of molecules in the bilayer self-assembly.

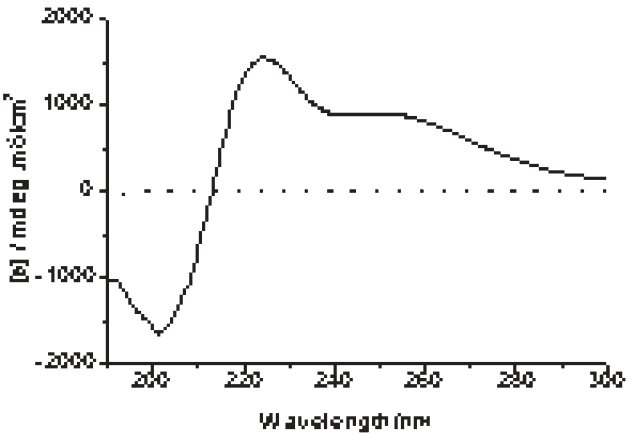

Fig. 5 CD spectrum of the hydrogel (4 mM, [**1**] : [SDS] equal to 0.67).

molecules. Indeed the FT-IR spectrum (Fig. S1 in the ESI†) of the xerogel exhibits a broad amide N–H band around 3247 cm^{-1} and C=O (amide I) band at 1655 cm^{-1}, clearly indicating the formation of amide hydrogen bond chains within the self-assembly (inset of Fig. 4). Gelator compounds bearing α-amino acids have been claimed to form helical fibers and ribbons in organic solvents and water.[7] It has also been demonstrated that only enantiomerically pure samples can form gels.[7] The presence of a stereogenic center at the headgroup causes twisting of the ribbons forming helical structures. In order to examine formation of chiral aggregates, we measured the CD spectrum (Fig. 5) of the pure compound, **1** at pH = 2.0 and the hydrogel. The spectrum of the gel clearly shows positive bands in the range 220–250 which is characteristic of right-handed helical structures. This band system is absent in the spectrum (not shown) of the pure amphiphile, suggesting that the peaks are due to helical aggregates and not due to molecular chirality. The physical entanglement of such helical fibers transforms water into gel. Since **2** has no stereogenic center and **4** is a racemate, they failed to gel water. On the other hand, the amino acid side chain of **3**, being less hydrophobic compared to that of **1**, means gelation does not occur. This implies that the geometry of the headgroup is responsible for the packing, which is translated into the morphology of the aggregate.

It seems packing of the hydrocarbon chain in the self-assembly is also important. The hydrophobic tail of SDBS having an aromatic ring is unsymmetrical with respect to that of **1**, which affects chain packing in the aggregate and forms only mixed micelles in the presence of the zwitterionic amphiphiles. This is further demonstrated by the failure of gelation by **5** the hydrocarbon chain of which is shorter than that of **1**.

The hydrogel of **1** can release various hydrophobic and hydrophilic drugs trapped in the gel matrix in a controlled manner when heated. For example, Fig. 6A shows release of coumarin-1(C-1), a hydrophobic fluorescent probe the fluorescent intensity of which increases upon gel melting. The leakage of the drug molecules exactly corresponds to the phase transition temperature (see Fig. S2 in the ESI†) suggesting concurrent release of the molecules upon gel shrinkage. Such a catch-and-release behavior can be associated with the amphiphilic characteristic of the hydrogel. Similar experiments were also carried out to study the

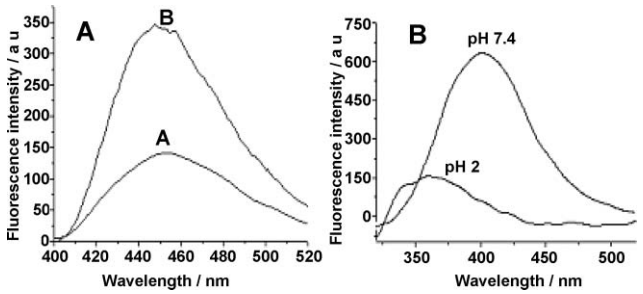

Fig. 6 (A) Fluorescence emission spectra of C-1 in gel at 25 °C, A, and in broken gel at 60 °C, B. (B) Fluorescence emission spectra of warfarin in gel (pH 2) and in broken gel (pH 7.4) measured at 30 °C.

pH-responsive release of drugs using warfarin. This is demonstrated by the shift of the emission maximum as well as by the rise of intensity (Fig. 6B) of the fluorescence spectrum of the drug.

In summary, to our knowledge, this is the first demonstration of gelation of an SDS solution by a zwitterionic amphiphile. Also, this is the second example of a hydrogel with very low MGC value. The gelation occurred as a result of entanglement of helical ropes formed from twisted ribbon-like structures generated by the ion-pair complex. The aggregate has an interdigitated bilayer structure. The studies have clearly shown that hydrophobicity as well as chirality of the amino acid headgroup is important for gelation. The packing of the hydrocarbon chains in the aggregate is another factor for gelation to occur. The gelling of aqueous SDS solution is thermo-reversible and also sensitive to change of pH. Thus the hydrogel may have potential applications in drug delivery and in formulations of healthcare products.

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