

# Salt, pH and thermoresponsive supramolecular hydrogel of *N*-(4-*n*-tetradecyloxybenzoyl)-L-carnosine†

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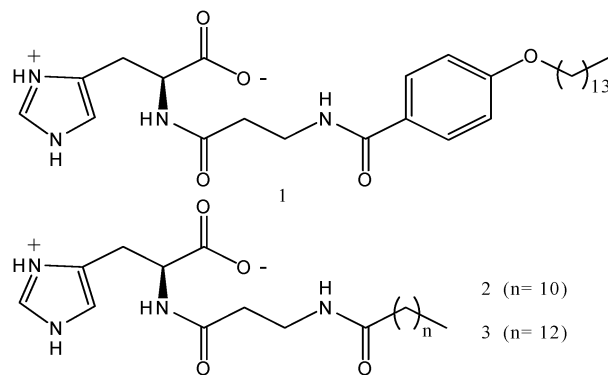
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**A carnosine based amphiphilic hydrogelator has been developed which efficiently gels water and exhibits salt, pH and thermo-responsive gelation properties.**

Stimuli-responsive hydrogels of low-molecular-weight-gelators (LMWGs) have attracted tremendous attention in the recent literature because of their potential applications in the pharmaceutical industry as soft materials for drug delivery and tissue engineering.<sup>1</sup> In the field of biological applications, desired responsiveness of the hydrogel to one or more external stimuli, such as pH, light and salts is essential. Also for these applications, biocompatibility of the gel-forming materials is very important. Various LMWGs capable of forming hydrogels have been reported in the literature.<sup>2,3</sup> Many of these LMWGs are derivatives of sugars, amino acids and nucleotides. Some of the hydrogels formed by these gelators have also exhibited temperature and pH-responsive character, and tolerance to additives, such as salt and surfactants, *etc.*<sup>4</sup> Peptide-based gelators have gained importance because of biocompatibility of the constituent materials (amino acids).<sup>5</sup> Recently, the gelation ability of *N*-acyl peptide amphiphiles also have been reported.<sup>6</sup>

L-Carnosine is a dipeptide normally found in mammalian skeletal and brain tissues and consists of two amino acids L-histidine and  $\beta$ -alanine. L-carnosine has been found to (i) scavenge peroxy radicals,<sup>7</sup> (ii) inhibit copper-catalyzed oxidative reactions,<sup>8</sup> and (iii) exhibit efficient singlet oxygen quenching.<sup>9</sup> On the other hand, *N*-(long-chain-acyl)carnosine derivatives are known to have excellent emulsifying activity and possess antioxidant activity toward lipid peroxidation.<sup>10</sup> This attracted our attention toward self-organization of *N*-(long-chain-acyl)carnosine in water. In this communication, we report the gelation behavior of *N*-acyl-L-carnosines **1–3** (Chart 1) at different pH in the absence as well as in the presence of inorganic and organic additives.

It was observed that when aqueous dispersions of *N*-(4-*n*-tetradecyloxybenzoyl)-L-carnosine, **1**, were allowed to stand at room temperature, after dissolution of the gelator in phosphate buffer (pH 7.0) by heating at  $\sim 368$  K, opaque hydrogels were formed. The gelation was confirmed by resistance to flow upon inversion of the screw-capped vials. The hydrogels thus formed are stable at room temperature



**Chart 1** Chemical structure of *N*-acyl-L-carnosine amphiphiles.

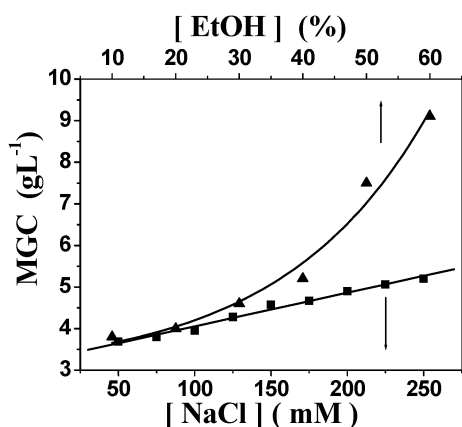
( $\sim 303$  K) for months when preserved under a constant condition (pH and temperature). The gelation was observed in all heating-cooling cycles attempted suggesting thermoreversibility of the hydrogel. Gelation was studied using buffers of various pH. Gelator **1** was found to gelate water in both alkaline ( $7.0 < \text{pH} < 11.0$ ) and acidic ( $0.3 < \text{pH} < 2$ ) pH ranges. However, under similar conditions, compounds **2** and **3** failed to gelate water. The gelation ability of the amphiphile at different pH as measured by minimum gelator concentration (MGC), which is defined as the minimum amount of gelator required to gelate a given volume of solvent, are listed in (Table 1). The gelation of water by the amphiphile at concentrations less than 1% (w/vol) (3000–11000 molecules of water per molecule of gelator) is of particular interest. Data in Table 1 show that MGC value is lowest at neutral pH and increases with both decrease and increase of pH. It is clear that the acid-base reactions involving the carboxylic acid group ( $\text{COO}^-$ ) and the imidazole ring of the histidine residue of the headgroup modulate the gelation ability of the amphiphile. The protonation of the carboxylate ( $\text{COO}^-$ ) group reduces hydration of the headgroup thereby increasing the MGC value. On the other hand, protonation of the nitrogen atom of the imidazole ring of the histidine residue at  $\text{pH} < 0.3$  transforms the gel to a sol or viscous dispersion as a result of increased repulsion among positively charged ionic headgroups of the amphiphile, which prevents growth of supramolecular aggregates. The inability of the amphiphiles **2** and **3** to gelate

**Table 1** Minimum gelator concentration (MGC,  $\pm 0.1$  g L<sup>-1</sup>) at 25 °C of hydrogelator **1** in different pH solutions. Gel-to-sol transition temperatures ( $T_{\text{GS}}$ ,  $\pm 1$  K) of the hydrogels containing 0.02 M gelator are also listed

pH	1	2	7	8	9	10	11
MGC/g L <sup>-1</sup>	6.5	5.5	3.4	4.8	5.5	6.1	6.8
$T_{\text{GS}}/\text{K}$	334	344	338	337	335	333	330

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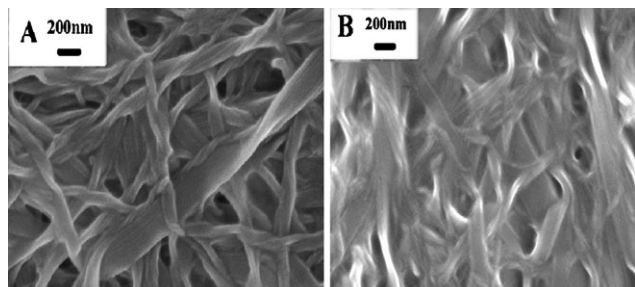


**Fig. 1** Variation of MGC value of **1** with [NaCl] (■) and [EtOH] (▲) in phosphate buffer (pH 7.4) at 298 K.

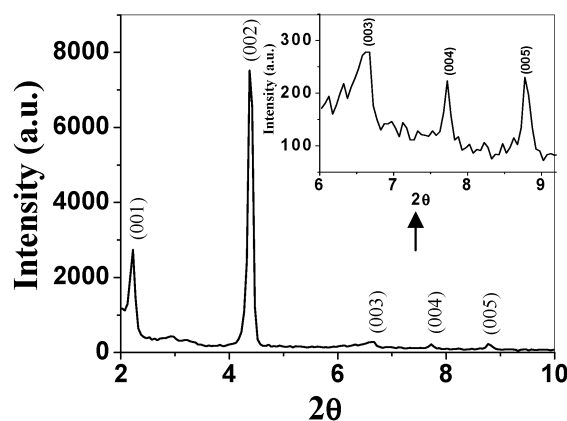
water suggest that removal of the phenyl ring from the hydrocarbon tail destroys its gelation ability. Thus it can be concluded that  $\pi$ - $\pi$  stacking interactions play an important role in the promotion of gelation.

Gelation of buffers by the gelator also occurred in the presence of additives, such as NaCl, and alcohol (EtOH). However, the MGC value of **1** increased with the increase of [NaCl] or [EtOH] as shown by the plots in Fig. 1. Hydrogelation also occurred in the presence of 60% EtOH. This might be due to the increased solubility of the gelator in the presence of NaCl or EtOH. In the presence of salt, the electrostatic interaction between the dipoles of the headgroup of the amphiphile is partially screened making the molecule partially charged which increases its aqueous solubility.

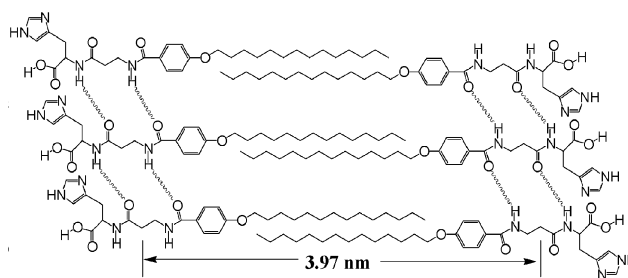
The microstructures (Fig. 2A and B) of the hydrogel at pH 2 and 8 were investigated by field-emission scanning electron microscopy (FE-SEM). As shown by the micrograph in Fig. 2A the hydrogel appears to be comprised of 3-D networks of intertwined ribbons that result in immobilization of water. A similar morphology of the hydrogel can also be observed at pH 8.0 (Fig. 2B). A close look at the micrograph also shows twisting of the ribbons, which are bilayer assemblies of the gelating molecule. The existence of bilayer structures is confirmed by X-ray diffraction patterns (Fig. 3) of the gel cast film at pH 2, which exhibits periodical reflection peaks. The XRD pattern of the hydrogel at pH 8 (not shown) was closely similar. The relative peak position  $q_1 : q_2 : q_3$  is 1 : 2 : 3 : 4, which is typical for a Bragg scattering pattern from a 1-D lamellar structure corresponding to 001, 002, 003 and 004



**Fig. 2** FESEM pictures of air-dried hydrogels of **1** in 20 mM phosphate buffer of pH 2.0 (A) and pH 8.0 (B).



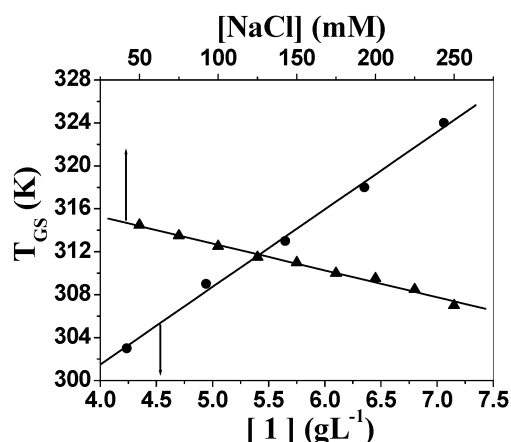
**Fig. 3** XRD Pattern of the hydrogel of **1** in 20 mM phosphate buffer, pH 2.0.



**Fig. 4** Molecular arrangement of **1** in the lamellar structure.

planes. This suggests that gelator **1** assembles into an ordered lamellar structure as shown in Fig. 4. This bilayer thickness of the lamella, which is equal to the interlayer distance ( $d$ ) of the 001 plane, is 3.97 nm. It is important to note that the bilayer thickness is smaller than twice the extended molecular length of the hydrophobic tail of **1** (2.52 nm)<sup>11</sup> molecule, but larger than the length of a single hydrophobic tail. This implies that the aggregate has interdigitated hydrocarbon tails, which facilitates hydrogen-bonding interactions at the headgroup of the amphiphile.

The thermal stability of the hydrogel was investigated by measuring gel-to-sol transition temperature ( $T_{GS}$ ), which depends on the concentration, as shown by the representative plot in Fig. 5. The higher thermal stability at higher concentration can be attributed to tight packing of the gelator molecules due to enhanced hydrogen-bonding and hydrophobic interactions as a result of incorporation of a larger number of gelator molecules in the aggregate. In other words, the increase of  $T_{GS}$  is linked to growth of the one-dimensional aggregates, which makes them more flexible, causing more entanglement of the fibres, and thereby increasing the  $T_{GS}$  value. A known amount of the gelator (10.8 g L<sup>-1</sup>) was employed for determination of  $T_{GS}$  in different pH solutions and the data are listed in Table 1. As observed, the hydrogel at pH 2 has the highest melting temperature suggesting its highest stability in acidic pH. The gel melting temperature decreases slightly with increase of pH above 7.0. This must be associated with the acid-base reaction involving histidine residues as discussed above. At lower pH, the COO<sup>-</sup> groups become protonated, and thus intermolecular hydrogen-bonding interactions involving COOH groups is facilitated, thereby increasing the melting temperature; at



**Fig. 5** Variation of gel-to-sol transition temperature ( $T_{GS}$ ) of **1** with concentration, and with [NaCl] in 20 mM phosphate buffer, pH 7.4.

neutral or alkaline pH the COOH group is dissociated, the intermolecular interactions become weaker, and hence the  $T_{GS}$  value decreases. The decrease of  $T_{GS}$  value with [NaCl] (Fig. 5) suggests that the hydrogels become thermally less stable upon addition of salt. This, as discussed above, can be attributed to increased solubility of the zwitterionic amphiphiles in the presence of salt.

In summary, we have developed a peptide based LMWG that efficiently gels water at a concentration less than 1% (w/v). Also the hydrogelation ability in a wide pH range shows its versatility. The hydrogels entrap a relatively large amount of water in a thermoreversible manner. The  $\pi$ - $\pi$  stacking interaction of the phenyl rings in the hydrocarbon chain of the amphiphiles was found to be essential for the gelation. The gelation occurred as a result of entanglement of twisted ribbons that are formed by the self-assembly of the amphiphilic molecules. These ribbons are comprised of interdigitated bilayer aggregates. Although the hydrogels are quite stable in the presence of salt and alcohol the gelation properties can be modulated by changing pH, salt and alcohol concentration. The hydrogels in different pH media have melting temperatures higher than the physiological temperature. Thus the hydrogel may have potential application in drug delivery.

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- 11 The length of the hydrophobic chain (including the benzene ring) was calculated using the MM2 program of CS Chem3D Std software.