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PAPER

Supramolecular binary hydrogels from calixarenes and amino acids and their entrapment–release of model dye molecules†

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A series of supramolecular binary hydrogels based on tetra-proline modified calix[4]arenes and basic amino acids (arginine, histidine and lysine) were constructed in acidic condition. The obtained results show that different amino acids lead to distinguishable backbones of hydrogels, which were identified by the combination of atomic force microscopy, transmission electron microscopy and scanning electron microscopy. Moreover, all the prepared low molecular weight hydrogels are thermoreversible and the gel-to-sol transition temperature (T_{gel}) depends on the concentration of calixarene, which endows these hydrogels with the capability of entrapment–release of model dye molecules.

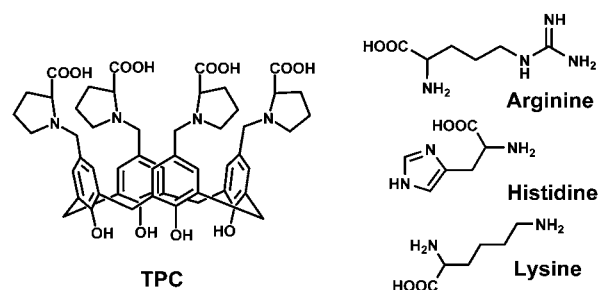
Introduction

Calixarenes are one of classical families of macrocyclic compounds in host–guest and supramolecular chemistry.¹ Benefiting from their facility of modification, calixarenes are described as ‘macrocycles with (almost) unlimited possibilities’,² and are widely used in molecular recognition, sensing, self-assembly, *etc.*³ Up to now, several kinds of nano-architectures have been fabricated from calixarene backbones, including capsules,⁴ rotaxanes/catenanes,⁵ 1D nanotubes/wires,⁶ 2D nanosheets/films,⁷ and also some amphiphilic assemblies of micelles/vesicles.^{8,9} At a higher aggregation level, low molecular weight gels based on calixarenes have also gained significant attention. Shinkai and coworkers reported the first example: that *p*-acylcalix[*n*]arenes ($n = 4, 6, 8$) act as excellent and unique gelators of various organic solvents.¹⁰ Xu and coworkers built a coordination-induced gel from 3-pyridine-azo-calix[4]arenes and metal ions, which can uptake neutral organic molecules from the aqueous phase.¹¹ Zheng and coworkers reported that chiral calix[4]arenes bearing long tertiary alkyl groups at the upper rim and *S*-1-phenylethylamine groups at the lower rim can form heat-set gels and egg-like vesicles enantioselectively with *D*-2,3-dibenzoyltartaric acid in cyclohexane, which is the first example of heat-set gels resulting from a difference in the interaction between two component chiral gelators.¹² They also found that chiral calix[4]arene bearing *L*-2,3-dibenzoyltartaric acid groups

at the lower rim can enantioselectively form gel only with one enantiomer of chiral amines.¹²

Among the family of gels, the growth of low molecular weight hydrogels (LMWH) has advanced many fields ranging from cosmetic products to pharmaceuticals to gene delivery, due to their characteristics of biocompatibility and biodegradation. In addition, the development of an ever-increasing spectrum of functional hydrogelators continues to broaden the versatility of hydrogel applications. LMWH now play a critical role in many tissue engineering scaffolds, biosensor and biological micro-electro-mechanical systems, and drug carriers.¹³ However, to the best of our knowledge, almost no studies have concerned calixarene-based hydrogels except one recent example that Mocerino and coworkers reported the first calixarene based hydrogelator, a proline-functionalised calix[4]arene,¹⁴ where its gelation was controlled by specific anions.¹⁵ This work is an interesting topic not only in the field of supramolecular chemistry but also in biomedical-engineering as water-soluble calixarenes are generally regarded as biologically friendly.^{1,16}

In this work, we constructed a series of supramolecular binary hydrogels based on tetra-proline modified calix[4]arene (TPC) and basic amino acids (arginine, histidine and lysine), as shown in Scheme 1. Binary gel systems offer a fine alternative candidate



Scheme 1 Structural illustration of TPC and basic amino acids (arginine, histidine and lysine).

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† Electronic supplementary information (ESI) available: ¹H-NMR, ¹³C-NMR spectra, crystal structure, CMC determination, zeta potential measurement, TEM and SEM images of TPC. AFM images of LMWHs, T_{gel} measurement, thermoreversible experiments, temperature dependent fluorescence spectra and process for model dye release test. CCDC reference numbers 793843. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0sm01041c

for tailoring the properties by changing one of the two components.¹⁷ Especially, the use of bio-safe natural products such as amino acids as one component is more advantageous over synthetic gelators. Natural amino acids are benign candidates to prompt gelation of LMWH for not only are they biocompatible¹⁸ but that they are also hydrotropes.¹⁹ In addition, despite hardly understanding complicated supramolecular interactions in TPC/amino acid systems, studying the influence of amino acid structures on the properties of the resulting hydrogels can also serve as a model to discover or design other new functional binary gel-phase materials.

Result and discussion

Formation of hydrogels

Twenty natural amino acids were all used to examine the gelation behavior of TPC; it was found that only three basic amino acids (arginine, histidine and lysine) can induce gelation of TPC successfully (Table 1), see Fig. 1 for a representative case of TPC/arginine LMWH. The hydrogelation conditions were then optimized, at ratios of arginine/TPC ranging from 1/1 to 10/1 (mol/mol), TPC concentrations of 1.0 mM to 100 mM, and the pH varying from 7.0 to 1.0. It was found that 5.0 mM of TPC with four times as much arginine yields a stable transparent hydrogel when the pH was decreased to 3.0 (Fig. 1). The same optimal conditions were also obtained in the cases of lysine and histidine. In fact, TPC can form LMWH upon the addition of a great excess of HCl (concentration up to 1.0 M), which somewhat resembles TPC gelation triggered by anions.¹⁵

The gelation mechanism of TPC with basic amino acids was hypothesized as shown in Scheme 2. Firstly, TPCs themselves form micellar aggregates when the concentration is over the CMC (critical micelle concentration). Secondly, basic amino acids non-covalently interact with the TPC micelles

Table 1 Gelation performances of TPC with various amino acids. The concentrations of TPC and amino acids are 5 mM and 20 mM, respectively.^a

	pH 7.1	pH 5.5	pH 3.0
phenylalanine	S	S	IG
tyrosine	—	—	IG
tryptophane	S	S	IG
arginine	S	IG	G
lysine	S	IG	G
histidine	S	S	G
proline	S	S	S
alanine	S	S	S
valine	S	S	S
leucine	S	S	S
isoleucine	S	S	S
glycine	S	S	S
serine	S	S	S
threonine	S	S	S
cysteine	S	S	S
methionine	S	S	S
asparagine	S	S	S
glutamine	S	S	S
aspartic acid	S	S	S
glutamic acid	S	S	S

^a G = gel; IG = incomplete gel; S = solution; "—" = poor solubility.

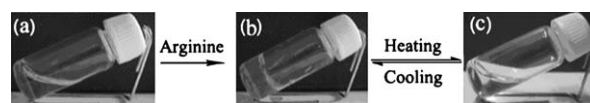
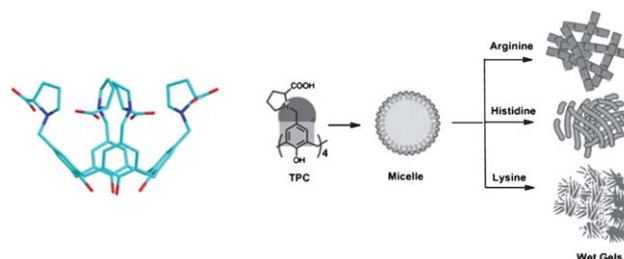


Fig. 1 Supramolecular LMWH obtained from TPC with arginine and its thermoreversible property. (a) TPC, (b) gelation upon addition of arginine, and (c) thermoreversible gel-sol transition.



Scheme 2 (left) Crystal structure of TPC. (right) Schematic illustration of gel generation from TPC gelator induced by basic amino acids.

(electrostatic, hydrogen-bonding, host-guest and van de Waals interactions can occur between TPC and amino acids), forming joint micelles, leading to the formation of a hydrogel matrix. As reported by Mocerino and coworkers, TPC is a kind of amphiphile that can form micellar aggregates with a CMC of 5.6 mM in neutral conditions.¹⁵ From another viewpoint, TPCs can be regarded as kinds of hydrophobic groups modified by amino acids or peptides that have a tendency to amphiphilically aggregate in various pH conditions.²⁰ X-Ray crystallography measurements shows that TPC maintains the preferable pinched-cone conformation (left in Scheme 2). Such a truncated cone structure is favourable for the formation of micellar aggregates. The CMC of TPC at pH 3.0 was measured by circular dichroism (CD) spectroscopy, giving value of 1.2 mM (Fig. S4[†]). The TPC concentration employed in gelation is over 4 times higher than its CMC, which ensures the formation of micellar aggregates before gelation. Dynamic laser scattering (DLS) and zeta potential measurements were further performed to identify the TPC micellar size and surface charged distribution, giving results of an average diameter of 124 nm (Fig. 2a) and an average zeta potential of -2.32 mV (Fig. S5[†]). DLS analysis further shows that the majority of the particles exhibit a mean diameter of 190 nm, whereas smaller particles with a mean diameter of 42 nm also exist. Nanoscaled spheres were also observed from atomic force microscopy (AFM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images. As shown in Fig. 2, S6 and S7,[†] the diameters were measured as in the range of 40–200 nm, which is in agreement with the DLS data. It can be seen that it forms nanoscaled particles with negative charges in free TPC solution (5.0 mM, pH 3.0). At pH 3.0, these three basic amino acids obviously present positive charges according to their pK_a values.¹⁸ As a consequence, arginine, lysine and histidine can interact with TPC micelles well where Coulombic forces play a large role along with other possible interactions, while the other amino acids can not. Based on this assumed mechanism, TPC concentration, component ratio and pH are three key factors controlling the formation of supramolecular binary hydrogels.

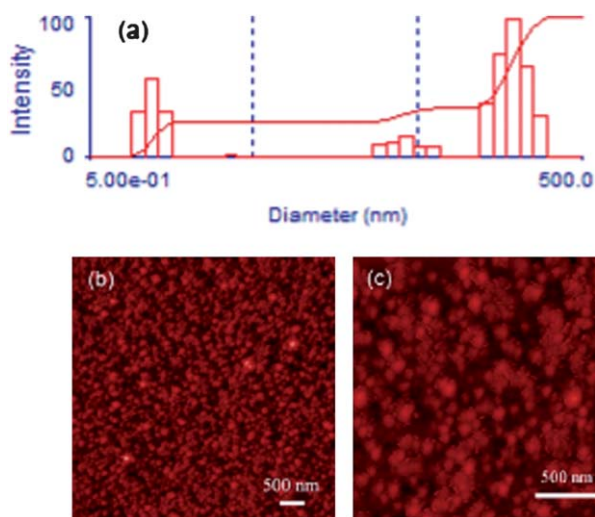


Fig. 2 (a) DLS profile of the nanoscale micelles of TPC. (b) AFM morphological image of the micelles of TPC deposited on mica, showing that these nanoscale spheres stack together. (c) Higher magnification of (b). TPC dissolved in water at 5.0 mM at pH 3.0. The scales are marked in each panel.

Morphological studies of hydrogels

AFM has been performed to study the morphological features of wet gels.¹⁵ In a previous report, TPC is an electrolyte activated hydrogelator and the density of the resulting hydrogels depends on the particular salts used.¹⁵ As we know, basic amino acids can exist in an anionic or cationic form, and have more complicated structures than inorganic electrolytes. Hence, we hypothesized that all three LMWHs may display different structures of networks although they have similar components and were prepared under exactly the same conditions. As shown in Fig. 3a–c, the TPC/amino acid LMWHs possess frameworks of nanofibers, and the fiber shapes differ when the basic amino acid was changed. In Fig. 3a and S8†, the fibers of the TPC/arginine LMWH are composed of many long and branched fibers of 1.0–10.0 μm long. For the TPC/histidine LMWH, the fibers become shorter and twisted with lengths of 1.0–3.0 μm (Fig. 3b and S9†). Fig. 3c shows a denser network with stacks of rodlike nanofibers in the TPC/lysine LMWH. Therefore, distinguishable microstructures are observed in LMWHs, indicating that basic amino acids with side chain variations can indeed induce significant morphological changes in binary hydrogels. Similar phenomena were also observed in SEM and TEM studies. Specifically, the SEM and TEM images reveal three dramatically different types of xerogels: fibrous, porous and lamellar. The TPC/arginine xerogel shows a fibrous framework (Fig. 3d and 3g). Fig. 3e and 3h show a porous material with stacks of sheets for the xerogel based on TPC/histidine. A lamellar structure with less thickness exists for the TPC/lysine xerogel (Fig. 3f and 3i). Here, it is important to note that the structures obtained from AFM experiments are different from the structures obtained from SEM and TEM. This is due to the fact that the method of sample preparation is different for different microscopic experiments. In comparison with AFM experiments, SEM and TEM studies require complete drying of the LMWHs, and the preparation of xerogels may result in artifacts for gel systems that inherently exist in their solvated states.¹³

Thermal behavior of hydrogels

Thermal stability and thermoreversibility of gels are of interest with respect to their various applications, such as drug delivery.^{13,21} We have studied the thermal stability of gels based on TPC/basic amino acids in an aqueous medium at acidic pH 3.0 across a range of concentrations (Fig. S11†). The gel-sol transition temperature, T_{gel} , increases along with increasing the concentration of TPC. As can be seen, when the concentration of TPC is below 5.0 mM, all the mixtures remain as solutions, in contrast, at high concentrations, TPC can easily form gels with basic amino acids; for instance, even at 50 $^{\circ}\text{C}$, TPC still exhibits a powerful gel capability at 20.0 mM. Furthermore, a high concentration of basic amino acids can also be employed to encourage the system to form stable gels. Therefore, basic amino acids play a crucial role in impacting and stabilizing the hydrogels. Accordingly, these results clearly demonstrated that the concentrations of TPC and basic amino acids have profound effects on the thermal stability of the LMWHs discussed here.

To further investigate the thermoreversibility of the LMWHs, we performed fluorescence spectroscopic studies using 8-anilino-1-naphthalenesulfonic acid sodium salt (ANS) as a probe, which is sensitive to a hydrophobic environment.²² LMWHs containing 10.0 mM TPC were taken as representative examples. The emission intensity of LMWH-loaded ANS was measured in the temperature range of 25–70 $^{\circ}\text{C}$. The fluorescence decreases gradually with elevating temperature (Fig. 4 and S12†), which indicates that ANS is exposed to a more hydrophilic environment as the gel phase turns into the sol phase. While in the cooling cycle, the emission intensity increases when temperature decreased from 70 to 25 $^{\circ}\text{C}$, and the curves almost coincide with those in the heating cycle in shape (Fig. 4a, 4b and 4c). Moreover, obvious inflection points are observed in the plots of emission intensity *versus* temperature (Fig. 4a, 4b and 4c). The inflection points, T_{gel} , appear at 48, 45 and 46 $^{\circ}\text{C}$ for LMWHs of TPC/arginine, TPC/histidine and TPC/lysine respectively, for samples being heated. Similar values of 50, 45, and 45 $^{\circ}\text{C}$ were also observed with decreasing temperature. Furthermore, we investigated the changes in fluorescence intensity of ANS as a function of cyclical variation of temperature between 25 and 70 $^{\circ}\text{C}$. The results are shown in Fig. 4d and S13†, in all LMWHs, these temperature dependent changes in intensity are reversible and can be observed for several cycles. These results indicate a thermoreversible gelation process for TPC/amino acids in water.

Release of model compound from hydrogels

Concentration-dependent T_{gel} measurements for LMWHs show that T_{gel} increases as the concentration of TPC increases, and the resulting hydrogels are stable above the room temperature (*ca.* 25 $^{\circ}\text{C}$) when the concentration of TPC is equal to or below 10.0 mM. Hence, we selected doxorubicin hydrochloride, a kind of water-soluble fluorescent dye and anticancer drug,²³ to elucidate the potential capability of model compound release, and the concentration of TPC was fixed at 10.0 mM. TPC/basic amino acids were allowed to form uniform hydrogels in a 1.0 mM solution of doxorubicin. The further evidence of doxorubicin being loaded in LMWHs was supplied by optical and fluorescence microscopic images (Fig. 5) as doxorubicin has an intrinsic

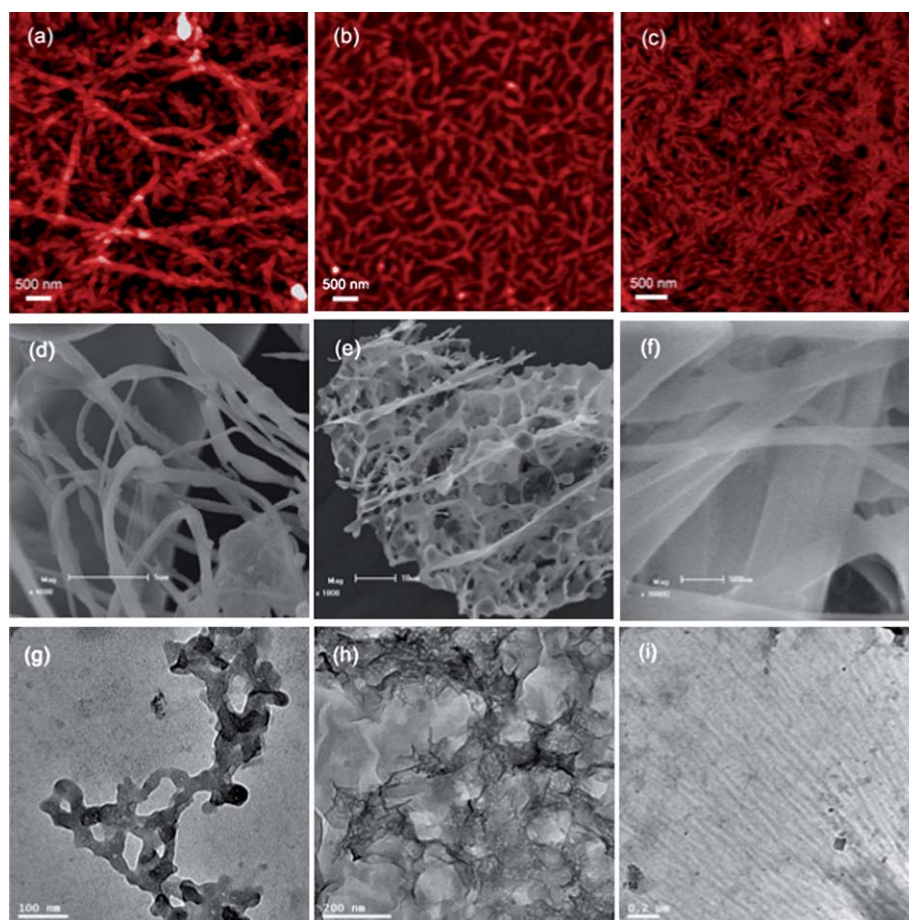


Fig. 3 AFM images of wet gels: (a) TPC/arginine LMWH, (b) TPC/histidine LMWH and (c) TPC/lysine LMWH. SEM images of xerogels: (d) TPC/arginine LMWH, (e) TPC/histidine LMWH and (f) TPC/lysine LMWH. TEM images of xerogels: (g) TPC/arginine LMWH, (h) TPC/histidine LMWH and (i) TPC/lysine LMWH.

fluorescent emission. In comparison with the optical microscopy images, the images of fluorescence microscopy show that the dispersion of doxorubicin is homogeneous throughout the LMWHs, indicating that the interaction with the hydrophobic fibers is not expected to be particularly favourable, and hence release should be determined by diffusion through the matrices.²⁴

The loaded doxorubicin molecules were successfully released upon the gels immersion into water, and we monitored the dye release from the gel phase into water by recording the UV–vis spectroscopic absorption at 490 nm (assigned to doxorubicin) with respect to time. It was found that 12.0% (TPC/arginine LMWH), 8.7% (TPC/histidine LMWH) and 8.2% (TPC/lysine LMWH) of doxorubicin were diffused from the respective gel matrix after 5 h (Fig. 6). The diffusion coefficients calculated from the release curve were $1.7 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ for TPC/arginine LMWH, $1.3 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ for TPC/histidine LMWH, and $1.3 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ for TPC/lysine LMWH.²⁵

Conclusions

We have successfully constructed a series of supramolecular binary hydrogels from TPC gelator triggered by basic amino acids. The present case highlights the fascinating feature that controlling the microstructures of hydrogels by tuning the basic amino acids. Furthermore, the obtained hydrogels are

thermoreversible, and T_{gel} depends on the concentration of TPC, which endows them to potential application as small molecule carriers, such as dyes and drugs. Endeavors to construct novel supramolecular hydrogels based on other water-soluble calixarenes and suitable guests are ongoing, in which of particular interest is the exploration of species with biocompatibility and gelation at physiological conditions.

Experimental

Materials

Amino acids and doxorubicin were purchased from Aladin Chemicals. ANS was obtained from TCI. All the chemicals were used without further purification. Deionized water was used in this work to minimize the interference of metal ions. TPC was synthesized and purified according to the reported procedures and was characterized by ¹H- and ¹³C-NMR spectroscopy^{14,15} and X-ray crystal structure analysis. NMR studies were carried out on a Varian 400 MHz spectrometer at 300 K.

Synthesis of TPC

Calix[4]arene (1.0 g, 2.4 mmol), tetrahydrofuran (20 mL), proline (1.5 g, 13.0 mmol) dissolved in water (4.0 mL), glacial acetic acid

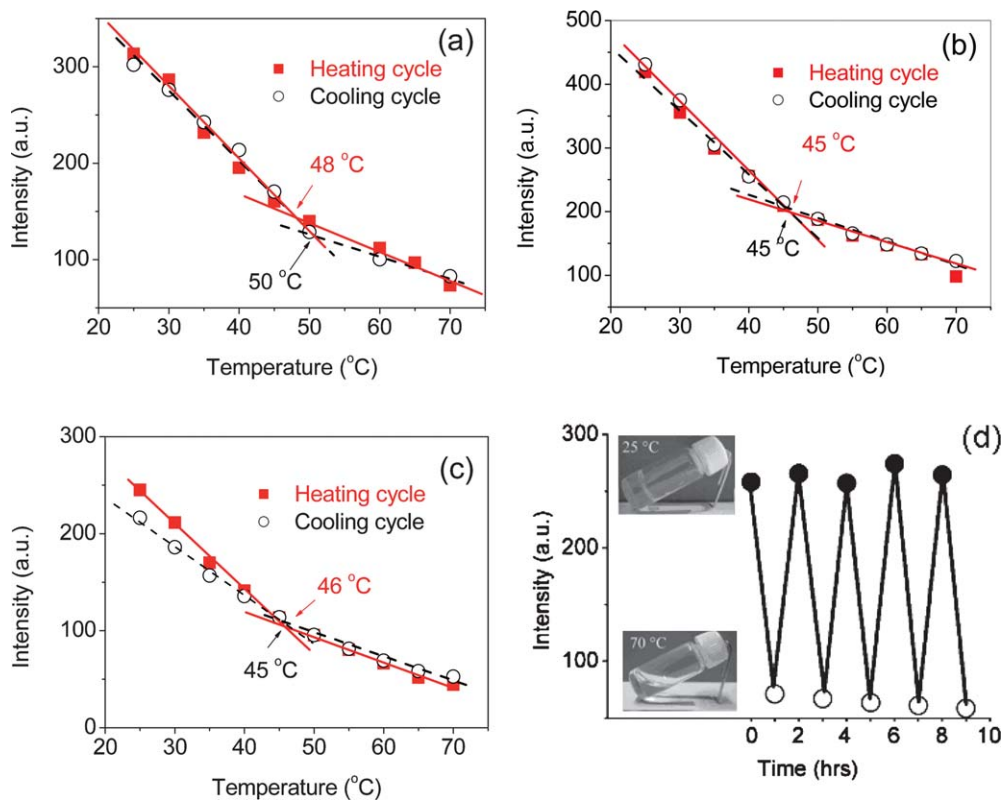


Fig. 4 Changes in fluorescence intensity of ANS owing to sequential variations in temperature in (a) TPC/arginine, (b) TPC/histidine and (c) TPC/Lysine LMWHs. (d) Changes in fluorescence intensity of ANS as a function of cyclical variation of temperature between 25 and 70 °C in TPC/arginine LMWH.

(3.0 mL) and formaldehyde (1.1 mL, 37%w/v) were combined and left stirring for 72 h. The reaction mixture was filtered and the precipitate was washed with acetone, and then recrystallised from water–ethanol–acetone to yield a white solid (1.4 g; yield, 64%). ¹H-NMR (D₂O): δ, 1.40–1.75 (broad m, 12H, CHCH₂), 1.75–2.20 (broad m, 4H, CHCH₂), 2.90–3.05 (broad m, 4H, CH₂N), 3.20–3.40 (broad m, 4H, CH₂N), 3.50–3.75 (broad t, 4H, NCH), 3.75–4.10 (broad m, 8H, ArCH₂N and 8H, ArCH₂Ar), 7.05 (s, 8H, ArH). ¹³C-NMR (D₂O): δ, 22.2, 28.0 (2 × CH₂), 30.0 (ArCH₂Ar), 54.7, 57.8 (2 × CH₂N), 65.4 (CHN), 122.7, 128.9, 131.5, 150.4 (Ar), 170.7 (C = O).

X-Ray crystal structure analysis

TPC (10.0 mmol) was dissolved in 0.01 M HCl solution (*ca.* 20 mL). After stirring for a few minutes, the solution was filtrated, and the filtrate was left to evaporate for about one week. Once of the colorless crystals which formed were collected along with its mother liquor for the X-ray crystallographic analyses. The X-ray intensity data for the crystal of TPC were collected on a Rigaku MM-007 rotating anode diffractometer equipped with a Saturn CCD Area Detector System using monochromated Mo-K radiation at $T = 113(2)$ K. Data collection and reduction were performed by program of Crystalclear [CrystalStructure 3.7.0 and Crystalclear 1.36: Crystal Structure Analysis Package, Rigaku and Rigaku/MS (2000–2005). 9009 New Trails Dr The Woodlands TX 77381 USA.]. All the structures were solved by using direct method and refined, employing full-matrix least

squares on F2 (Siemens, SHELXTL-97) [SHELX97: Sheldrick, G.M. (1997), University of Göttingen, Germany.]. These data (CCDC 793843) can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk). Some data was not good enough due to the poor quality of crystals obtained.

Crystal data for TPC: C₅₂H₈₂N₄O₂₃, $M_r = 1131.22$, monoclinic, space group $C 1 2 1$, $a = 19.723(4)$ Å, $b = 18.459(4)$ Å, $c = 17.842(3)$ Å, $\beta = 112.25(1)^\circ$, $V = 6012.23(1490)$ Å³, $F(000) = 2424$, $Z = 4$, $\rho_{\text{calcd}} = 1.250$ g/cm³, $\mu = 0.098$ mm⁻¹, approximate crystal dimensions $0.22 \times 0.20 \times 0.18$ mm³, θ range = 1.57–25.00°, 22943 measured reflections, of which 9498 ($R_{\text{int}} = 0.0545$) were unique, final R indices [$I > 2\sigma(I)$]: $R_1 = 0.1233$, $wR_2 = 0.3280$, R indices (all data): $R_1 = 0.1449$, $wR_2 = 0.3516$, GOF = 1.221.

Preparation of hydrogels

In a typical procedure, TPC and arginine were dissolved in deionized water. The solution was acidified to pH 3.0 by HCl. Sealed and then immersed into a constant-temperature water bath at 70 °C for 20 min to afford a clear homogeneous solution. When the solution was cooled slowly to room temperature, a stable hydrogel was obtained. All the other hydrogels were prepared following the same procedure.

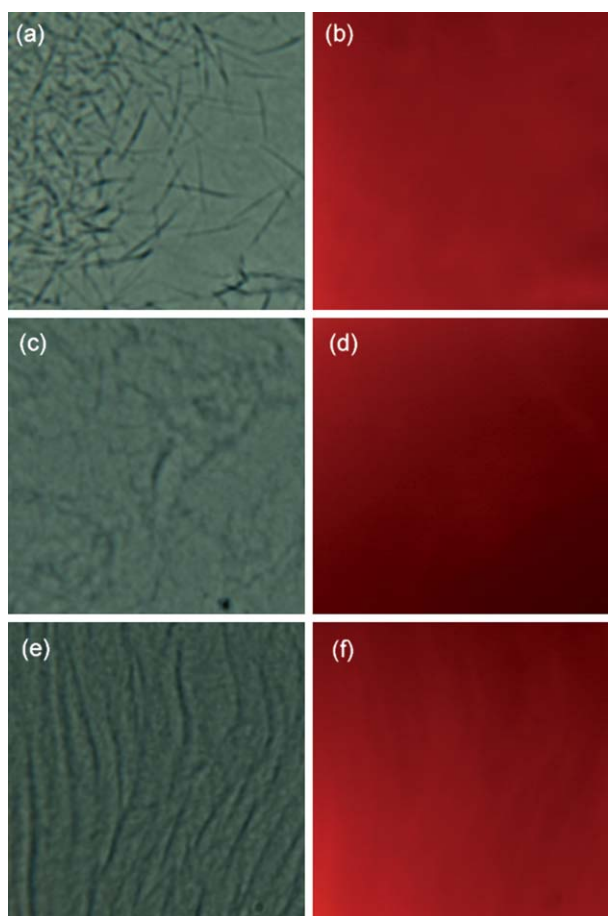


Fig. 5 Optical (left) and fluorescence (right) microscopy images of LMWHs loaded with 1 mM doxorubicin. (a) and (b) TPC/arginine LMWH, (c) and (d) TPC/histidine LMWH, (e) and (f) TPC/lysine LMWH.

CD spectroscopy

CD data were gathered at 25 °C on a JASCO J-715 instrument, by using a 1 mm path length quartz cuvette. Spectra were collected from 240 to 350 nm and scanned three times for averaging. All spectra were corrected by subtracting the baseline. The TPC samples were prepared in deionized water before testing.

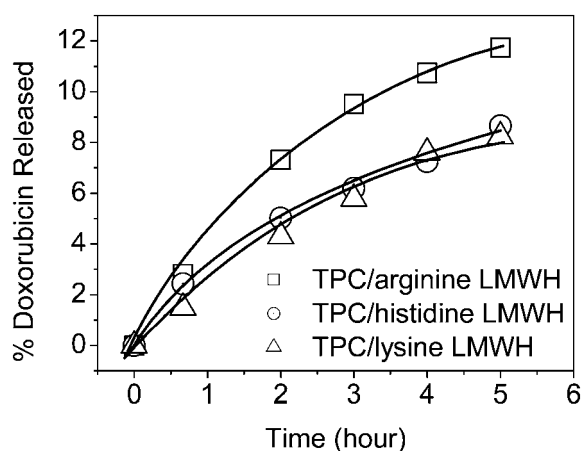


Fig. 6 Model dye molecule release profile of LMWHs.

T_{gel} measurements

T_{gel} was determined by a 'dropping-ball method',²⁶ a small ball (about 100 mg) was placed on top of the hydrogel in a test tube (inner diameter 1.0 cm), which was slowly heated in a waterbath at a rate of 1 °C min⁻¹. The T_{gel} was defined as the average value that the ball begins to fall and falls to the bottom of the test tube. All experiments were repeated three times.

DLS analysis

The sample of TPC was prepared from 5.0 mM solution, which corresponded to the gelation concentration, and was examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (Turbo Corr.) at a scattering angle of 90°.

Zeta potential measurement

The zeta potential of the micellar aggregation of TPC was measured by using ZetaPALS + BI-90 instrument (Brookhaven Co. USA). The concentration of TPC solution is 5.0 mM at pH 3.0.

AFM Studies

AFM was used to obtain the nanostructures of LMWHs. A thin film of the wet gel was placed on a freshly cleaved mica substrate and immediately imaged in tapping mode on a multimode Veeco Nano IIIa atomic force microscope (Camarillo, USA).¹⁵ All of the measurements were performed in ambient air, and height images were recorded with 512- × 512-pixel resolution.

TEM studies

Placing a piece of gel on the carbon-coated copper grid and then allowed to dry under vacuum at 25 °C for 72 h. The TEM images were taken by Philips Tecnai G2 20S-TWIN microscope operating at an accelerating voltage of 200 keV.

SEM studies

The morphologies of the xerogels were investigated using SEM. For the SEM studies, gel materials were frozen in liquid N₂ and then lyophilized. The micrographs were taken using a HITACHI S-3500N SEM.

Thermoreversible experiments

The fluorescence intensity of ANS was followed while the temperature was raised gradually from 25 °C to 70 °C; in the next step, the fluorescence intensity of the hot sample was recorded while its temperature was decreased gradually from 70 °C to 25 °C. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) on a VARIAN CARY Eclipse spectrometer at an excitation wavelength 360 nm.

Optical and fluorescence microscopic images

All imaging experiments were performed on an OLYMPUS BX51 + Mc MP5 fluorescence microscope. The total magnification was 1000×.

Doxorubicin release studies

TPC and amino acids were allowed to form a hydrogel in the absence and in the presence of 1.0 mM doxorubicin solution. Then each set of gels was separately immersed into 1 mL of PBS buffer (10 mM, pH 7.1). At different times, the respective solution was removed from the gels of each set and UV–vis absorption at 490 nm of the solution (containing doxorubicin) was recorded using a blank solution as reference. After recording, previously removed water (1 mL) was added to the respective gels. This cyclic process was continued for 5 h. This approach allows us to quantify the amount of doxorubicin released from the gel. UV–vis spectra were recorded on a Shimadzu UV-2401PC spectrophotometer.²⁵

The diffusion coefficient (D) of doxorubicin in the gel matrix was calculated using equation:

$$M_t/M_\infty = 4(Dt/\pi\lambda^2)^{1/2}$$

M_t is the total amount of molecules released during the measurement, M_∞ is the total amount of doxorubicin that was kept in the matrix, λ is the hydrogel thickness, t is the time of the measurement, and D is the diffusion coefficient of the molecule.

Acknowledgements

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