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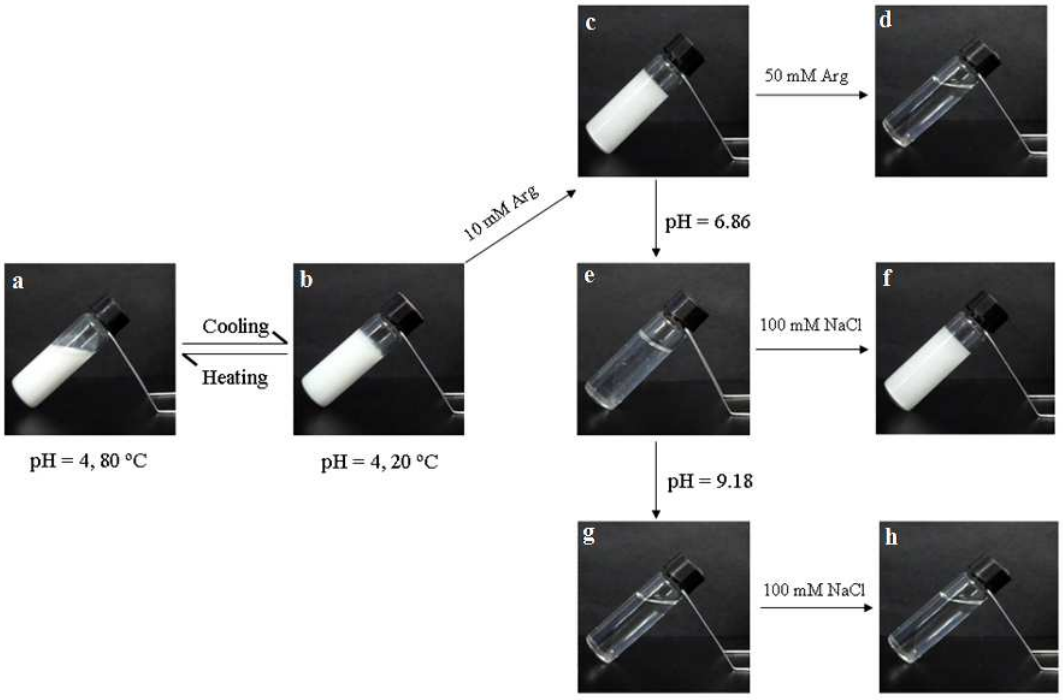


Multiple stimuli-responsiveness of NaDC/amino acid/NaCl mixed systems.

*Author to whom correspondence should be addressed, E-mail: xinx@sdu.edu.cn.

Phone: +86-531-88363597. Fax: +86-531-88361008

Biodegradable, Multiple stimuli-responsive Sodium Deoxycholate/Amino Acids/NaCl Mixed Systems for Dye Delivery

Yongjie Zhang [a, b, c], Xia Xin [a, b *], Jinglin Shen [b], Weiyue Tang [c], Yingjie Ren [c], Lin Wang [b]

[a] *National Engineering Technology Research Center for Colloidal Materials, Shandong University, Jinan, 250100, P. R. China*

[b] *Key Laboratory of Colloid and Interface Chemistry (Shandong University), Ministry of Education, Jinan, 250100, P. R. China*

[c] *China Research Institute of Daily Chemical Industry, Taiyuan Shanxi, 030001, P. R., China*

[*]Author to whom correspondence should be addressed, E-mail: xinx@sdu.edu.cn.

Phone: +86-531-88363597. Fax: +86-531-88361008

Abstract: Supramolecular hydrogels were prepared in the mixtures of biological surfactant sodium deoxycholate (NaDC) and amino acid (glycine (Gly), alanine (Ala), lysine (Lys) and arginine (Arg)) in different pH buffer solutions. We characterized this performance through phase behavior observation, transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray powder diffraction (XRD), Fourier transform infrared (FT-IR) spectra and rheological measurements. The results demonstrate that the presence of Gly and Ala can enhance the formation of the gels while the addition of Lys and Arg could make the breakage of the hydrogen bonds and weaken the formation of the gels. The formation of hydrogels with different gelling kinetics and mechanical properties or the behavior of sol-gel transformation of the systems may be obtained by finely modulating pH. Moreover, the addition of the halide salts (NaCl) can enhance the mechanical strength of the gels. Because of their unique responsiveness to the multi-stimuli environment, these biodegradable and pH-sensitive hydrogels hold great promise as versatile vehicles for dye (or drug) delivery.

Keywords: Hydrogels, sodium deoxycholate, amino acid, pH-responsive, hydrogen bonds.

1. Introduction

Supramolecular gels as a kind of soft materials have attracted great attention due to their unique functionality and potential applications such as high water content and solid shape, which endow them potential applications in tissue engineering.[1-4] Some of the supramolecular gels are constructed through supramolecular 3D networks formed by nanofiber assemblies which are made of amphiphilic molecules.[5-11] Noncovalent interactions such as hydrogen bonding, van der Waals, π–π, and electrostatic interactions drive the formation of these supramolecular nanofiber assemblies.[9–13] In particular, many biomacromolecules in nature such as bile salts, peptides and amino acids can self-assemble into this kind of functional nanostructures.[14-20] Among them, bile acids or their salts, which belong to cholic acid derivatives, possess an amphiphilic structure with a steroidal backbone that is more complex than alkane surfactants and they have a number of biological functions such as solubilization of cholesterol, absorption of dietary fats and fat soluble vitamins.[21-24] Moreover, amino acids are essential components of biological functional protein. They play an important role in constructing human organs and blood and enzyme and other essential parts of body and they are of paramount importance for human physiological function such as metabolism [25, 26]. It is well-known that the biocompatibility of gel materials is crucial for biologically directed applications and environmental considerations [27]. Therefore, the hydrogels formed by bile salts and amino acids may have important potential application in the area of drug release and biological sensor.

Generally, the properties of hydrogels are affected by various external factors, such as pH, temperature, concentration and ionic strength. Especially, the control of the pH plays a key role in tissue engineering and drug delivery.[28-30] For example, for drug delivery applications, the

modulation of the pH of gel forming agent solutions may provide the binding of specific drugs, an optimized release profile and the activation/inactivation of the drugs.[31, 32] In the case of tissue engineering, maintaining the pH in the physiological range is required for the production of cytocompatible injectable systems.[33] Thus, considering that pH, temperature or concentration of the hydrogel directly affects the electrostatic interactions between all of the charged residues in the system, study of its effect on the self-assembly of biodegradable surfactants/amino acids mixed systems is also of considerable importance [34, 35]

In this paper, supramolecular hydrogels were prepared in the mixtures of biological surfactant sodium deoxycholate (NaDC) and amino acid (glycine (Gly), alanine (Ala), lysine (Lys) and arginine (Arg)) in different NaCl concentration and pH buffer solutions. The properties of our systems have been fully characterized by a variety of techniques including phase behavior observation, transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray powder diffraction (XRD), Fourier transform infrared (FT-IR) spectra, and rheological measurements. The responsiveness of NaDC hydrogels on amino acids and NaCl which can control the release of dyes entrapped in hydrogels are also investigated. The purpose of this paper is to study the multiple stimuli-response NaDC/amino acid/NaCl hydrogels and its potential application on drug release. We hope our results will provide useful information in applications in biological and materials science areas.

2. Experimental section

2.1. Chemicals and Materials

Sodium deoxycholate (NaDC), and sodium chloride (NaCl) are analytical reagent grade (A.R.),

while Glycine, L-α-Alanine, L-Lysine and L-Arginine are biochemical reagent grade and all the reagents used in current study were purchased from Sinopharm Chemical Reagent Co. The structures of NaDC and four amino acids are shown in Figure 1. The solutions were prepared in different buffer solutions with pH = 4.00, 6.86, 9.18 (Tianjin Fuyu Fine chemical co., Ltd.) in appropriate amounts. The components of buffer solutions and their concentration are as such: pH=4.00, Potassium hydrogen phthalate, 0.05 mmol L^{-1}; pH=6.86, Mixed phosphate, 0.025 mmol L^{-1}; pH=9.18, Sodium tetraborate, 0.01 mmol L^{-1}. Water used in the experiments was triply distilled by a quartz water purification system. Its conductivity was lower than 1.8 $\mu S \cdot cm^{-1}$ as measured by a DDSJ-308A type conductivity instrument in our laboratory. The samples of hydrogels or solutions had been equilibrated in thermostat at 20.0°C for at least 2 weeks before various kinds of characterization.

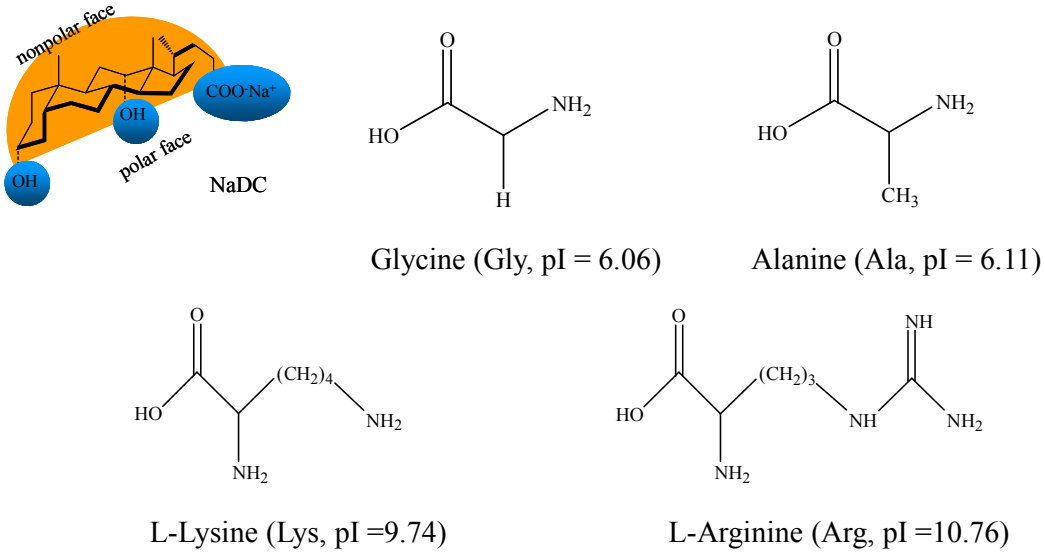


Figure 1. The molecular structures of NaDC and four amino acids investigated.

2.2. Methods and Characterization

TEM observations were carried out on a JEOL JEM-100 CXII (Japan) at an accelerating voltage

of 80 kV. Samples were prepared by directly dipping a Formvar film-coated or ultrathin carbon-coated copper grid into the sample, which was then dried by using an NIR lamp before observations. Field emission scanning electron microscopy (FE-SEM) observations were carried out on a JSM-6700F.

XRD patterns of the freeze-dried samples were measured at room temperature between 1 and 90° in the 2θ scan mode (2.5° min^{-1}) using a Rigaku D/Max 2200-PC diffractometer with Cu Ka radiation (λ = 0.15418 nm) and a graphite monochromator. Fourier transform infrared (FTIR) spectrum was recorded on a VERTEX-70/70v spectrometer (Bruker Optics, Germany).

The rheological measurements were carried out on a HAAKE RS75 rheometer with a cone-plate system (Ti, diameter, 35 mm; cone angle, 1°). In oscillatory measurements, an amplitude sweep at a fixed frequency of 1 Hz was performed prior to the following frequency sweep in order to ensure that the selected stress was in the linear viscoelastic region. The viscoelastic properties of the samples were determined by oscillatory measurements in the frequency range of 0.01-10 Hz. The samples were measured at 20.0 ± 0.1 ℃ with the help of a cyclic oil bath.

3. Results and discussion

3.1 Phase behavior

The macroscopic states of 50 mmol L^{-1} NaDC/Lys and 50 mmol L^{-1} NaDC/Arg systems were observed firstly. It could be seen that pH had significant influence on the formation of hydrogel according to our observation. As pH increased from 4.00 to 6.86, NaDC hydrogel that was formed in the presence of 30 mmol L^{-1} Lys or Arg became to solution state (Figure 2 A, B). And the hydrogel formed by 50 mmol L^{-1} NaDC with the addition of Ala and Gly turned from gel state into

solution state while pH increased from 6.86 to 9.18. Moreover, it also could be observed that as 30 mmol L^{-1} or 50 mmol L^{-1} of Lys and Arg was added into the NaDC hydrogel, the mixed system could not form hydrogel but remain at solution state for a very long period at pH = 6.86, which indicates that Lys and Arg brought breakage of NaDC hydrogel. We suppose that this phenomenon is related with the interactions between amino acids and gelator molecules. Both Lys and Arg have two amino groups, which exist in $-NH_3^+$ form at each pH value investigated. The electrostatic interaction between polar face of NaDC and amino acid as well as the hydrophobic interaction between hydrophobic moieties of NaDC and amino acid are mainly responsible for destroying the self-assembly of nanostructures. Moreover, the steric effect caused by steroidal backbone of NaDC and large size of amino acids are also involved in the breakage of aggregation.

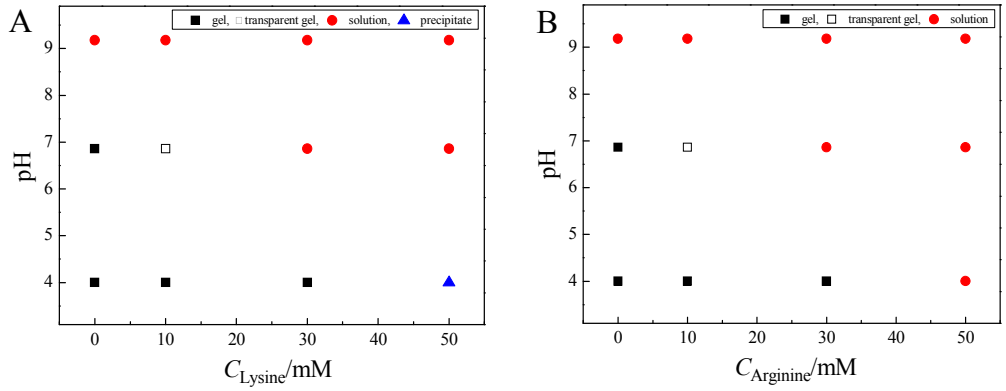


Figure 2 Phase behavior of self-assembled systems of (A) 50 mmol L^{-1} NaDC/Lys and (B) 50 mmol L^{-1} NaDC/Arg at different pH.

The addition of NaCl had impact on the formation of NaDC hydrogel. Transparent gels (that were prepared at pH = 6.86 in the presence of 10 mmol L^{-1} Arg or Lys) turned into white gels when 100 mmol L^{-1} NaCl was added in the process of preparing hydrogels (Figure 3 A, B). Furthermore, Figure 4 vividly exhibits the macroscopic transformation of hydrogels/solutions as a consequence of several kinds of stimuli. It could be seen that NaDC hydrogels exhibited sensitive response to a

series of external stimuli such as temperature, pH, amino acid, and inorganic salt, to realize the reversible gel-sol transition.[36] In details, NaDC solution could self-assemble into white hydrogel (native gel) at pH = 4.00. If the hydrogel was heated to 80°C (Figure 4a), it would transform into sol state and this transformation was reversible for the hydrogel would self-recover if the temperature was cooled back to 20°C (Figure 4b). This white color of the hydrogel became oyster white when 10mmol L^{-1} Arg was added (Figure 4c) and would completely be destroyed and change to solution if the concentration of Arg was further increased to 50mmol L^{-1} (Figure 4d).

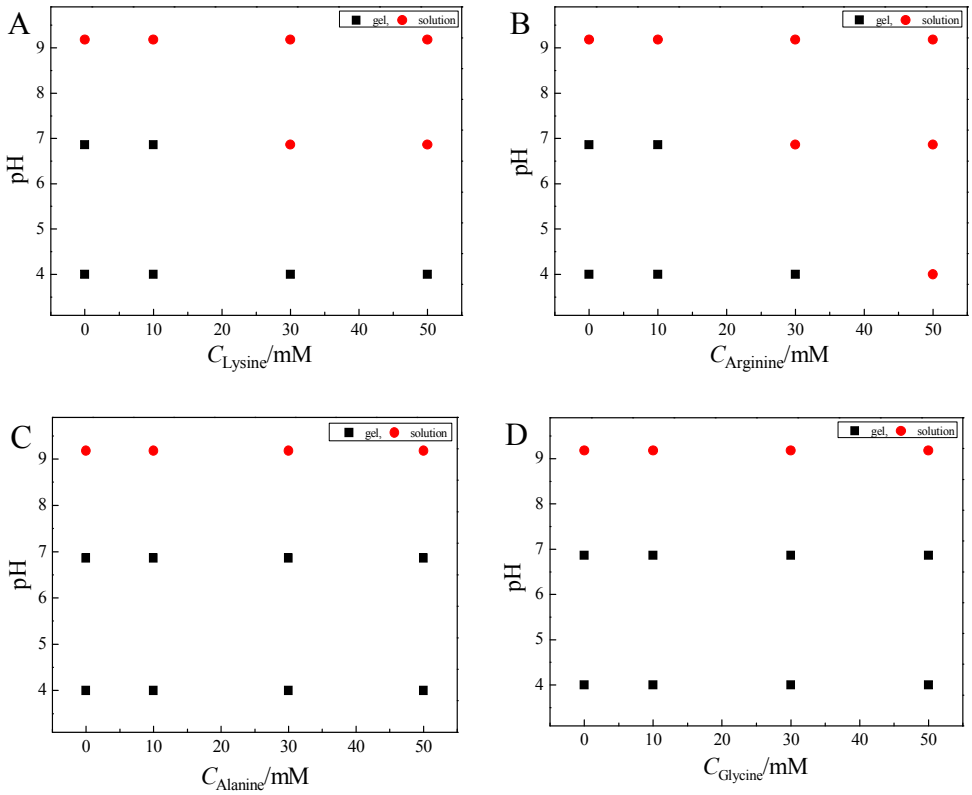


Figure 3 Phase behavior of self-assembled systems of (A) 50 mmol L^{-1} NaDC/Lys; (B) 50 mmol L^{-1} NaDC/Arg; (C) 50 mmol L^{-1} NaDC/Ala; (D) 50 mmol L^{-1} NaDC/Gly in the presence of 100 mmol L^{-1} NaCl at different pH.

Moreover, the colour of the hydrogel with 10 mmol L^{-1} Arg transformed from oyster white into transparent when the pH was tuned from 4.00 to 6.86 (Figure 4e) and the hydrogel further

transformed into a clear solution when pH was further tuned to 9.18 (Figure 4g). The transparent hydrogel of 50 mmol L^{-1} NaDC/10 mmol L^{-1} Arg at pH = 6.86 would turn to oyster white again as a consequence of the addition of 100 mmol L^{-1} NaCl (Figure 4f) while no visible changing occurred to the clear solution of this sample at pH = 9.18 when NaCl was added (Figure 4h).

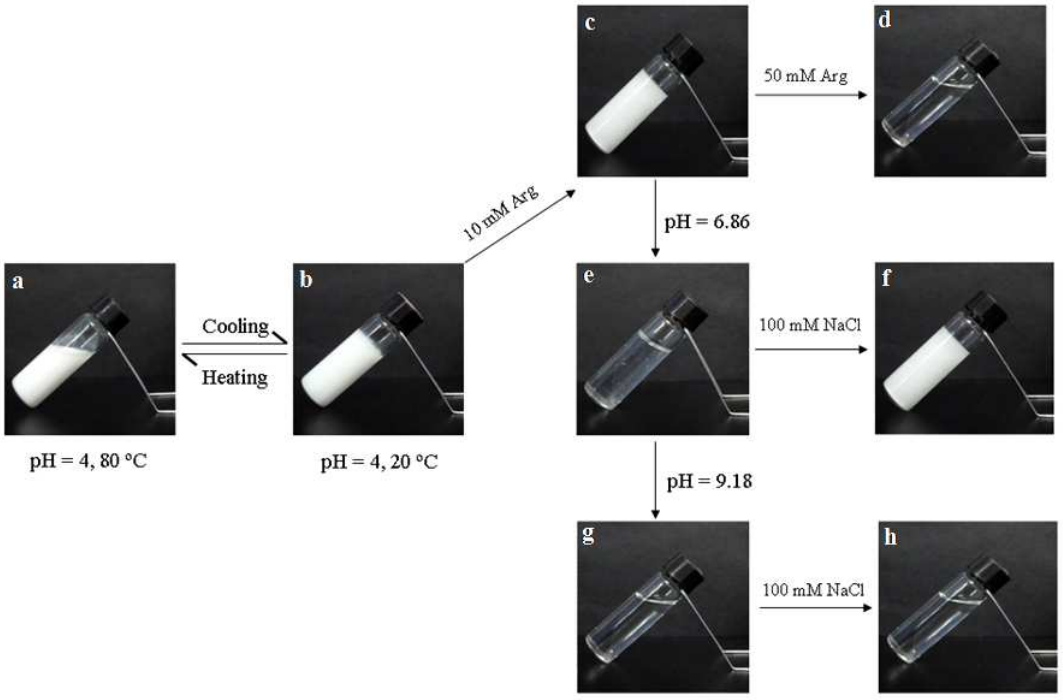


Figure 4 Stimuli-responsiveness of NaDC/amino acid/NaCl mixed systems.

3.2 Microstructures of the self-assembled aggregates

The microstructures of the hydrogels were investigated by TEM and FE-SEM observations. And the microscopic visions of self-assembled nanoarchitectures could be a support for macroscopic properties of hydrogels and provided interpretation for the mechanism of self-assembly.

3.2.1. The effect of pH on the microstructures

Microscopic structures of hydrogels or solutions self-assembled in the presence of Ala and Gly varied in a similar trend when tuning the pH. Networks fabricated by relatively wider nanofibers

and nanorods with low aspect ratio were observed in the TEM images of hydrogels formed at pH = 4.00 (Figure 5 a, d). While tuning the pH from 4.00 to 6.86, nanofibers that formed networks became relatively thinner and gelator molecules were more inclined to self-assemble into nanorods with higher aspect ratio (Figure 5 b, e). When further increasing the pH from 6.86 to 9.18, NaDC could not self-assemble into network of hydrogels but spherical aggregates in the solutions (Figure 5 c, f). Therefore, it could be definitely inferred that pH of the NaDC/amino acid/NaCl system worked as an effective factor to modulate the aggregation behavior of gelator molecules and consequently influence the macroscopic state of the system.

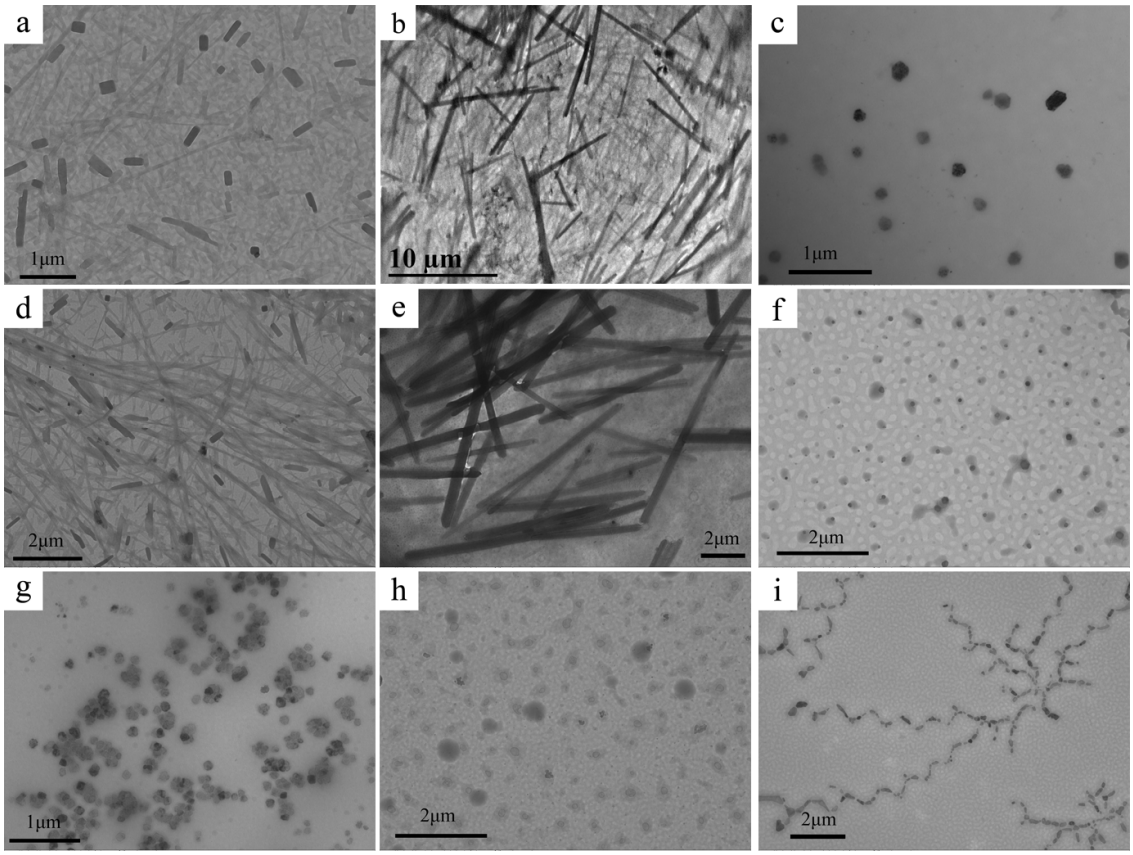


Figure 5 TEM images of 50 mmol L^{-1} NaDC/100 mmol L^{-1} NaCl hydrogels or solutions in the presence of 50 mmol L^{-1} Ala at (a) pH = 4.00, (b) pH = 6.86, (d) pH = 9.18; 50 mmol L^{-1} Gly at (d) pH = 4.00, (e) pH = 6.86, (f) pH = 9.18 and 50 mmol L^{-1} Arg at (g) pH = 4.00, (h) pH = 6.86, (i) pH

= 9.18.

Besides, pH also had an impact on the morphology of aggregates in the 50 mmol L^{-1} NaDC/50 mmol L^{-1} Arg/100 mmol L^{-1} NaCl solutions. There existed both amorphous nanoparticles (which might be aggregated by NaDC and amino acid molecules) and more crystalline ones (which might be attributed to NaCl crystals) in such solution at pH = 4.00 (Figure 5 g). Only amorphous aggregates were observed in the TEM vision of such solution at pH = 6.86 (Figure 5 h). When further increasing the pH of solution to 9.18, the crystalline nanoparticles were predominant and it was interesting to notice that those crystalline particles gathered together to depict special patterns spontaneously (Figure 5 i).

3.2.2. The effect of the concentration of amino acid on the microstructures

The concentration of amino acid (Ala) also affected the microscopic architecture of hydrogels significantly. NaDC tended to self-assemble into relatively thinner and shorter nanorods when forming hydrogel without any amino acid (Figure 6 a). The incorporation of Ala contributed to the growth of nanorods which gained an average length of 8 μm (Figure 6 b, c, e). As the concentration of Ala incorporated into hydrogels increased, the networks became denser, which was in accordance with the result of rheological test (discussed later) that showed the addition of Ala would increase the mechanical strength of NaDC hydrogels. In order to acquire a more authentic vision of the nanorods in NaDC hydrogels, SEM characterization were operated on hydrogels which were prepared in the presence of 30 mmol L^{-1} (Figure 7 a), 50 mmol L^{-1} (Figure 7 b) Ala and 100 mmol L^{-1} NaCl at pH = 6.86. The formation of nanofibers was considered to be driven by the interplay of hydrogen bonding, electrostatic attraction, and hydrophobic interaction, which was supported by XRD and FT-IR spectra below.

3.2.3. The effect of the concentration of NaCl on the microstructures

The effect of NaCl on the microscopic structure was also characterized by TEM. Relatively shorter nanorods with low aspect ratio could be seen from TEM images of hydrogels prepared without NaCl and in the presence of 50 mmol L^{-1} Ala (Figure 6 d). While 100 mmol L^{-1} NaCl was added into the hydrogel, gelator molecules were inclined to self-assemble into longer nanorods with an average length of 8 μm (Figure 6 e). Further increase (200 mmol L^{-1}) of the concentration of NaCl would induce the nanorod aggregates to grow wider in diameter. Therefore, it can be concluded that the presence of NaCl in NaDC/amino acid system could enhance the assembly-driving force between gelators and consequently strengthen the formation of hydrogel. Moreover, it was interesting to find there were spherical aggregates (with diameter of about 200 nm) statistically dispersing in the hydrogel system (Figure 6 f).

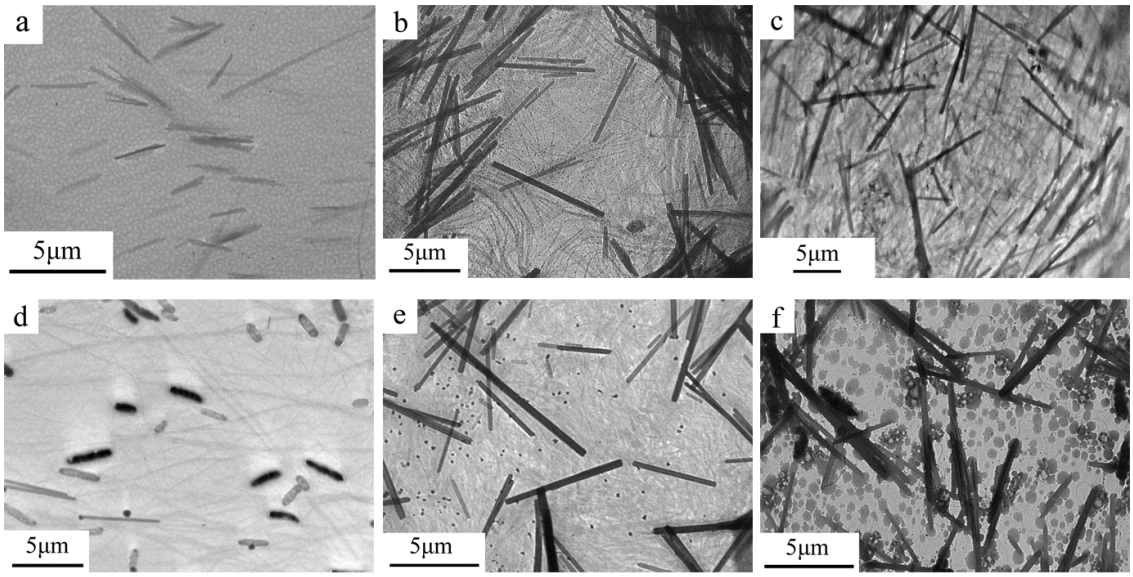


Figure 6 TEM images of NaDC hydrogels formed in the presence of 100mmol L^{-1} NaCl and of (a) no Ala incorporated; (b) 30 mmol L^{-1} Ala; (c) 50 mmol L^{-1} Ala. TEM images of NaDC hydrogels with 50mmol L^{-1} Ala incorporated and with (d) wtihout NaCl; (e) 100 mmol L^{-1} NaCl; (f) 200 mmol

L^{-1} NaCl at pH = 6.86.

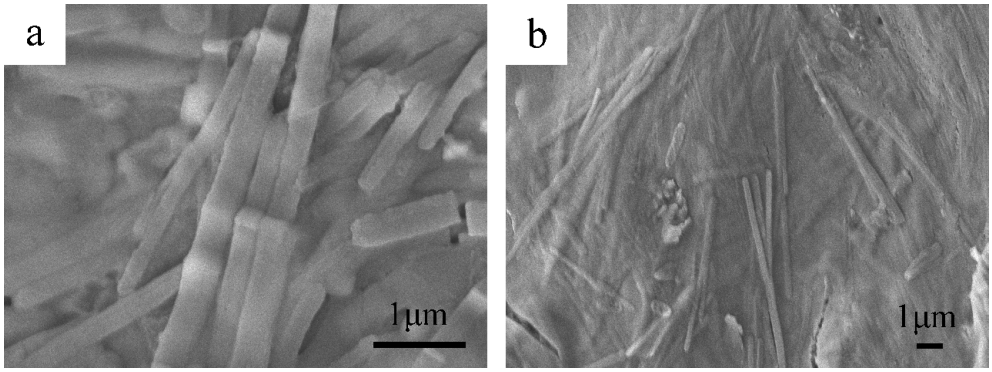


Figure 7 SEM visions of nanorods assembled in (a) 50 mmol L^{-1} NaDC/30 mmol L^{-1} Ala/100 mmol L^{-1} NaCl and (b) 50 mmol L^{-1} NaDC/50 mmol L^{-1} Ala/100 mmol L^{-1} NaCl mixed systems.

3.3. XRD analysis

To get more detailed information about our observed hydrogels, we used XRD spectra to detect the self - assembly model of the hydrogels.[37, 38] It can be seen from Figure 8A that the peaks detected at a 2θ value between 10-25° which correspond to a short-range spacing might be ascribed to the combination of several NaDC and amino acids molecules through hydrophobic and hydrogen bond interaction in the unit of the arrays. The differences between these three curves are probably because of the pH of buffer solutions regulating the arrangement of molecules. Moreover, the six mean diffraction peaks at 27.334, 31.692, 45.449, 56.477, 66.227, and 75.302° originate from NaCl nanocrystals, which correspond to the (111), (200), (220), (222), (400), and (420) planes and appear in all three curves. In general, the size of the nanocrystal can be estimated from Scherrer formula (eq 1):

$$D_{hkl} = K\lambda/(\beta\cos\theta) \quad (1)$$

Where λ is the X-ray wavelength (0.154 nm); β is the full width at half-maximum; θ is the

diffraction angle; K is a constant (0.89) and D_{hkl} is the size along the (hkl) direction. According to Scherrer formula, the averaged size of the NaCl nanocrystals is calculated to be 2.821 Å based on the diffraction peak of the (200) plane.

Then, we fixed pH at 4 and investigated the influence of concentration of NaCl on the self assembly model of the hydrogels (Figure 8B). It can be seen that with the increase of the concentration of NaCl, the intensity of the diffraction peak of NaCl significantly increases, indicating that NaCl has significant influence on the self-assembled structure of the aggregates.

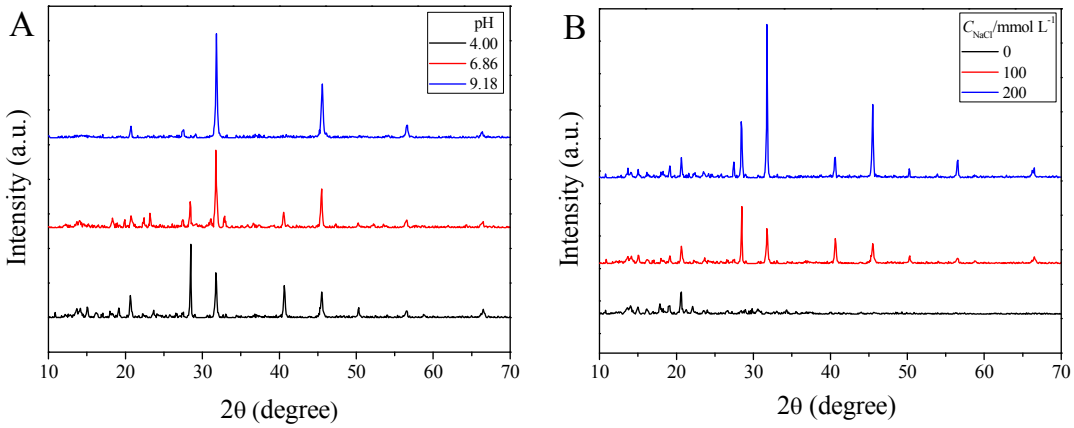


Figure 8 XRD patterns of NaDC xerogels (A) 50 mmol L^{-1} NaDC/50 mmol L^{-1} Ala/100 mmol L^{-1} NaCl at three different pH; (B) 50 mmol L^{-1} NaDC/50 mmol L^{-1} Ala in the presence of different concentration of NaCl at pH = 4.

3.4. Rheological properties

In order to acquire an accurate understanding of the alteration in strength of hydrogels influenced by variations investigated (pH, concentration of amino acids/NaCl), The rheological properties of the hydrogels were studied by means of yield stress and oscillatory experiments.[39] The oscillatory rheological measurements show that the storage modulus (G´) is higher than G″ over the studied frequency range and exhibit an elastic dominant property, demonstrating the "solid-like"

rheological property (Figure 9A), which is general in gels.[23] The decrease of pH could significantly increase the mechanical strength of NaDC hydrogels. G′ of hydrogel at pH = 4.00 was about 3-4 orders of magnitude higher than that at pH = 6.86 in the presence of 50 mmol L^{-1} Ala and 100 mmol L^{-1} NaCl (Figure 9 A). We propose that pH influences the strength of NaDC hydrogel mainly through its impact on hydrogen bonding and electrostatic interaction between NaDC molecules. Low pH (4.00) is beneficial for neutralizing the carboxylate group on the polar face of NaDC molecules which consequently strengthens the hydrogen bonding and weakens the electrostatic repulsion and causes more entanglements and denser networks fabricated by relatively wider nanofibers. As pH increases to 6.86, -COOH groups are more vulnerable to disassociation, which causes weakened hydrogen bonding and stronger electrostatic repulsion. As a consequence, the networks fabricated by relatively thinner nanofibers become sparse which makes weakened strength of hydrogel in rheological test.

The addition of Ala was in favor of strengthening the NaDC hydrogel. At the fixed NaCl concentration of 100 mmol L^{-1}, both G′ and G″ increased with the increase of Ala concentration (Figure 9 B), which should be ascribed to the more rigid networks of the hydrogels caused by more entanglements with increasing fibril density.[36] Ala molecules are inclined to incorporate into the intervals between polar faces of NaDC molecules because of its small size and hydrophilicity. This behavior provides Ala molecules possibility to create more linkages between NaDC molecules through hydrogen bonding and Van der waals interaction, which play an important role in aggregation of nanofibers.

NaCl also contributed to the increasing mechanical strength of hydrogels. The G’ of hydrogel with 100 mmol L^{-1} NaCl was about 1 order of magnitude higher than that with no NaCl added at pH

4.00 while the G' of hydrogel with 100 mmol L^{-1} NaCl was also higher than that with no NaCl added at pH6.86 (Figure 9 A). We speculate that the addition of Na^+ compresses the electric double layer around the polar face of NaDC molecule, thus reducing the electrostatic repulsion. In addition, Na^+ might be able to connect the polar face of NaDC molecules through weak coordination bonds between carboxylate groups and itself.

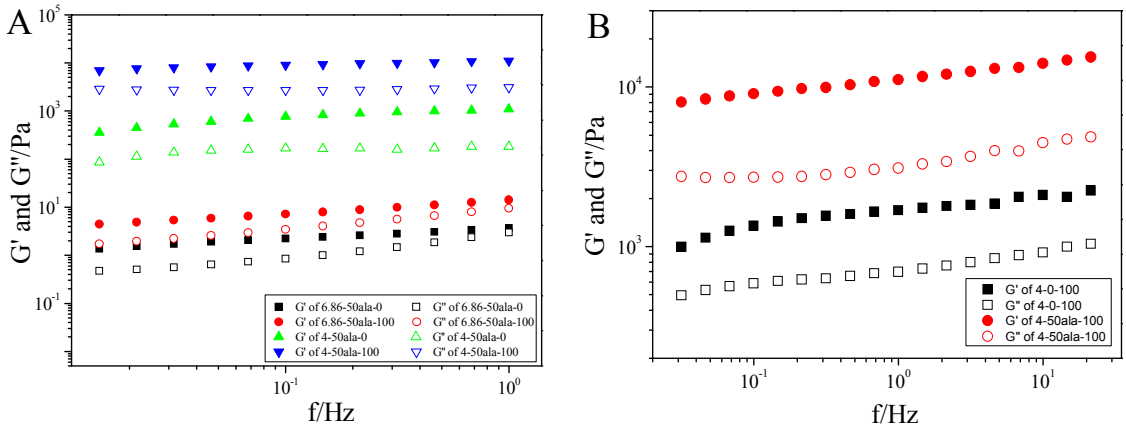
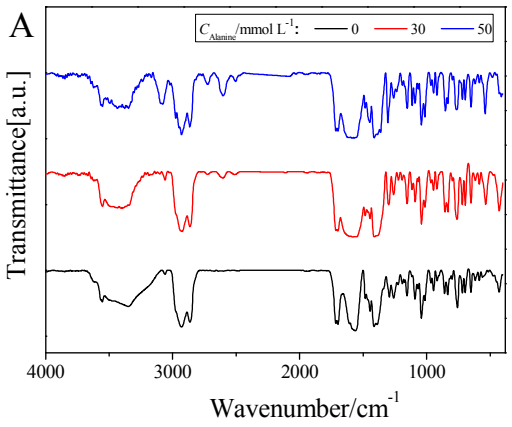


Figure 9 (A) G' and G'' profiles of 50 mmol L^{-1} NaDC/50 mmol L^{-1} Ala versus frequency as a function of pH or of NaCl; (B) G' and G'' profiles of 50 mmol L^{-1} NaDC/100 mmol L^{-1} NaCl versus frequency as a function of Ala concentration at pH = 4.0. Shear stress fixed at 1 Pa.

3.5. FT-IR results

To further study the formation mechanism of nanorods or aggregates, FT-IR as is shown in Figure 10 (A-C), were recorded. The peaks at 3406 cm^{-1} were detected, which were assigned to the asymmetric stretching vibration of –OH group. It can also be seen that in all three Figures, the asymmetric and symmetric methylene stretching bands are located at 2933 and 2859 cm^{-1}, respectively. The weak peak at 1558 cm^{-1} was correlated with the N–H vibration. As the concentration of Ala increases, the characteristic peaks at 2600 cm^{-1} which belong to stretching vibration of methyl of Ala are more and more obvious (Figure 10 A). When pH = 4.00 and 6.86, a

single peak at 1704 cm^{-1} was ascribed to the C=O stretching mode of –COOH, and in this circumstance, hydrogen bonds between carboxyl groups of gelator molecules were more vulnerable to occurrence. When -COOH was converted into $–COO^-$ at pH = 9.18, the single peak disappeared and produced two peaks at 1646 and 1549 cm^{-1}, which were related to the asymmetric and symmetric stretching modes, respectively (Figure 10 B). In this case, the formation of hydrogen bond was hindered. This analysis could be an evidence for the theory that in addition to electrostatic interaction, hydrogen bonds could be the main driving force between the -C=O groups of NaDC molecules and the N-H groups of amino acid molecules. Moreover, it can be seen that for the sample of 50 mmol L^{-1} NaDC/100 mmol L^{-1} NaCl in the presence of 50 mmol L^{-1} of different kinds of amino acids at pH = 6.86, the differences mainly occurs in the range of 2400-3200 cm^{-1} which are ascribed to the different functional groups of amino acids affecting the self-assembly behavior of the system (Figure 10 C).



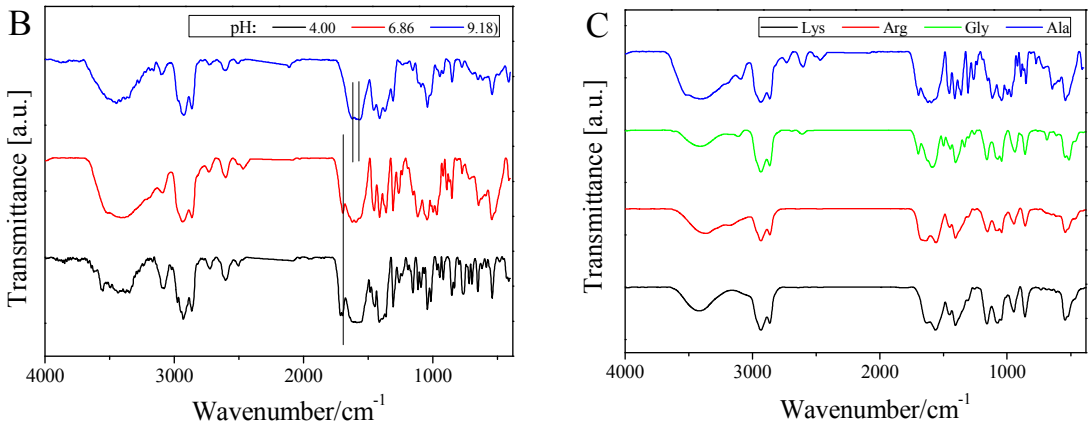


Figure 10 FT-IR spectra of NaDC hydrogels (A) 50 mmol L^{-1} NaDC/100 mmol L^{-1} NaCl in the presence of different concentration of Ala at pH = 4; (B) 50 mmol L^{-1} NaDC/50 mmol L^{-1} Ala/100 mmol L^{-1} NaCl at three different pH; (C) 50 mmol L^{-1} NaDC/100 mmol L^{-1} NaCl in the presence of 50 mmol L^{-1} of different kinds of amino acids at pH = 6.86.

3.6. Application in Controlled Release of Dye

The stimuli-response of NaDC hydrogels to amino acids (Lys and Arg) has a potential in application of controlled release of drugs. In our experiment, dye methylene blue was used as model drug and was incorporated into NaDC hydrogels with (or without) 100 mmol L^{-1} NaCl. Amino acid solutions (200 mmol L^{-1}) of the same pH of hydrogel were added on the top of hydrogel to induce the release of dye. Volume ratio of hydrogel and amino acid solution was 1:3. The process of release was recorded by photograph (Figure 11). When comparing the hydrogels that were operated with different amino acids (Lys and Arg) at pH = 6.86, we noticed a remarkable difference between the capacity of these two amino acids (Figure 11 A and B). Arg could release the dye much faster (44 min) than Lys (56 min) while the buffer solution without any amino acid could hardly release the dye even after several weeks. This was because Arg was more capable of breaking down the NaDC hydrogel and contributed to the diffusion of dye from gel phase to solution, which indicated the

similar phenomenon with phase behavior observation. The UV-vis spectra of methylene blue solution with time for the NaDC hydrogel with the addition of Arg (Figure 11 F) and Arg/100 mmol L^{-1} NaCl (Figure 11 G) at pH = 6.86 and the comparition of the dye concentration as a function of time in these two different systems (Figure 11 H) also revealed the same results.

The addition of NaCl in hydrogel could remarkablely slow the release rate of dye. When 100 mmol L^{-1} NaCl was incorporated into the hydrogels at pH = 6.86, Arg and Lys could release the dye completely in 210 minutes and 260 minutes (Figure 11 C and D), respectively, which were much slower than that of dyes releasing in the absence of NaCl. This result might be attributed to the addition of NaCl inducing the nanofibers to grow stronger and the networks to grow thicker, which is consistent with the rheological results.

The impact of pH on the release progress was also investigated. It can be seen that Lys could release the dye in hydrogel (with NaCl) much slower at pH = 4.00 (600 min, Figure 11 E) than at pH = 6.86 (260 min, Figure 11 D). This is also consistent with the visoelastic properties of the hydrogels in the rheological results. We suppose that lower pH contributes to stronger interactions between gelator molecules by strengthening hydrogen bonding and weakening electrostatic force. Thus it makes the polar face of NaDC to combine more firmly, which can induce the networks to become thicker, so that it would be more difficult for Lys to penetrate into the intervals of gelator molecules to destroy the hydrogels.

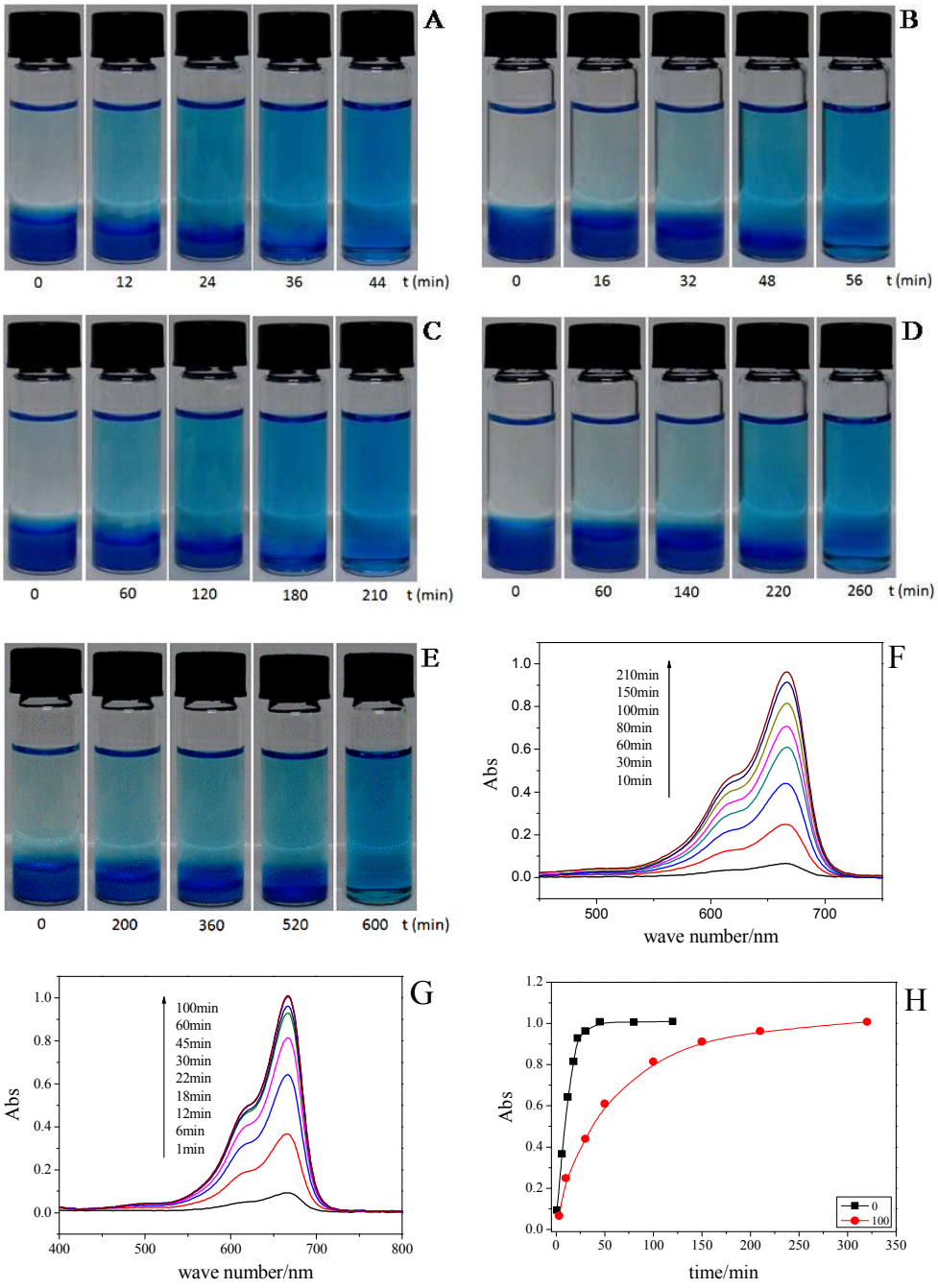


Figure 11 Release of dye (methylene blue) in hydrogels containing (A) Arg; (B) Lys; (C) Arg/100 mmol L^{-1} NaCl; (D) Lys/100 mmol L^{-1} NaCl at pH = 6.86 and (E) Release of dye methylene blue in hydrogels containing Lys/100 mmol L^{-1} NaCl at pH = 4.00. UV-vis spectra of methylene blue solution with time for the NaDC hydrogel with the addition of Arg (F) and Arg/100 mmol L^{-1} NaCl (G) at pH = 6.86 and the comparition of the dye concentration as a function of time in these two

different systems (H).

4. Conclusion

Supramolecular NaDC hydrogels incorporated by four kinds of amino acids and varied concentrations of NaCl at different pH were prepared and the effect of pH as well as amino acids and NaCl on the macroscopic properties and microscopic structures of NaDC hydrogels/solutions were investigated. The decrease of pH would increase the mechanical strength of the hydrogel through influencing the neutralization of carboxyl groups and consequently strengthening the hydrogen bonding and weakening the electrostatic repulsion between the polar face of NaDC molecules. The addition of Ala and Gly would strengthen the NaDC hydrogel for its small size and hydrophilicity which endow them probability of creating more linkages between NaDC molecules. Incorporation of NaCl into the NaDC systems would induce the aggregation of gelator molecules and thus contributed to wider and denser nanofibers that fabricated networks in hydrogel. The responsiveness of NaDC hydrogels on amino acids and NaCl holds promise in controlling release of dyes entrapped in hydrogels. This simulative release test could be further developed into application in pharmaceutical formulation and controlled release of bioactive drugs.

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