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Exploration for the Nature of a Unique Natural Polymer-Based Thermosensitive Hydrogel

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Chitosan (CS)/β-glycerol phosphate (GP) system is a heat induced gelling system with a promising potential application, such as an injectable biomedical material. Unlike most thermosensitive gelling systems, CS/GP system is only partially reversible. That is once the hydrogel is fully matured, it only softens but cannot go back to its initial liquid state when cooled down. Here, we perform both small and large amplitude oscillatory shear (SAOS and LAOS) tests on the fully matured CS/GP hydrogel samples at a variety of temperatures within the cooling process. The purpose of such tests is to investigate the structural change of the hydrogel network and thus to understand the possible gelation mechanism of this unique thermosensitive hydrogel. From the LAOS results and the further analysis with Chebyshev expansion method, it shows that CS/GP hydrogel is composed of a colloidal network dominated by hydrophobic interactions at high temperature, and gradually turns into a flexible network dominated by hydrogen bonding when the temperature goes down. Therefore, we may conclude that LOAS is a powerful tool to study the nonlinear behaviour of a polymer system that closely related to its structure, and as a practical example, we achieve a clearer vision on the gelation mechanism of the unique CS/GP thermosensitive hydrogel on the basis of considerable previous studies and assumptions in this laboratory and other research groups.

Introduction

Chitosan (CS) is a linear cationic polysaccharide derived from partial alkaline deacetylation of chitin, a cellulose-like polymer that is extracted from fungal cell walls and arthropod exoskeleton. CS offers several advantages including non-toxicity, biodegradability, biocompatibility and mucoadhesivity, so it has been widely applied in drug delivery,[1] gene delivery,[2] tissue scaffold,[3, 4] biosensor,[5] *etc.* Normally, CS only dissolves in acidic media, and forms gel-like precipitate when pH reaches 6.2. Interestingly, acidic CS solution can be neutralized with a weak base, β-glycerol phosphate (GP), without precipitation or phase separation. Such a CS/GP mixture maintains stably in the liquid state at room temperature and transforms to the hydrogel form when heated to a physiological temperature. This unique thermosensitive hydrogel was first reported by Chenite *et al.* in 2000,[6] and after then, its potential application in drug delivery[7-9] and regenerative medical, especially regeneration of articular cartilage[10-14] has been extensively investigated.

Great effort has been made to understand the gelation mechanism of this interesting CS/GP system at molecular level,[15-19] but unfortunately, no complete conclusion has been reached yet. However, the researchers tend to accept that the numerous interactions involving in this process can be divided into two classes. One is to keep CS soluble, including repulsion between ionized CS molecules and protective hydration effect from glycerol moieties of GP. Another is to induce CS to precipitate, including screening effect of negative charges on GP molecules, and hydrogen bonding as well as hydrophobic interactions between CS molecules. The competition of these two types of interactions leads to the gelation in CS/GP system. The further investigation reveals an important fact that the sol/gel transition of CS/GP is only partially thermal reversible. It was found once the CS/GP system maturely gelated, the "de-gelate" process cannot proceed back to the initial state completely when the temperature decreased.[20] This phenomenon has been mentioned several times in the literatures, however, such a seemingly uneventful process has not been further investigated so far.

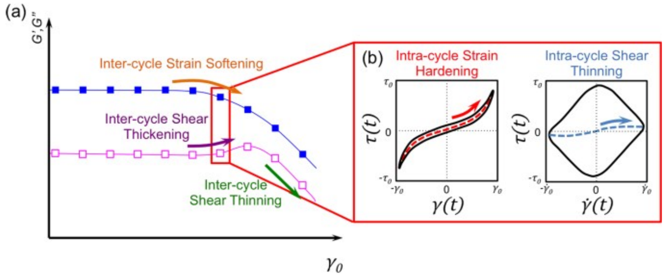
Dynamic oscillatory shear measurement is one of the most common rheological methods, which is considered to be a powerful tool for the analysis of soft materials, ranging from polymer melts, solutions, suspensions, emulsions, colloidal glasses, to hydrogels and beyond. It is categorized into two regimes: small amplitude oscillatory shear (SAOS, where strain amplitude is sufficiently small and only evokes linear viscoelastic response) and large amplitude oscillatory shear (LAOS), which is defined by measurable nonlinear response. SAOS measurement has been thoroughly investigated and is considered as a canonical method for characterizing inner structure of soft materials. However, materials that exhibit similar linear response sometimes presents different nonlinear response. Thus, the new insight provided by LAOS measurements is thought to be quite valuable for the further understanding on the material behaviours.[21]

Unfortunately, the response in LAOS regime is distorted from sinusoidal form, therefore, the definitions used in linear regime are no longer valid in this regime. Fourier transform has been one of the most popular methods to quantify nonlinear viscoelasticity. It is mathematical robust and sensitive to nonlinearity, but Fourier transform method is unable to generate a clear physical interpretation for nonlinear behaviour. In the past decades, a few attempts have been made to properly describe the nonlinear viscoelastic response,[22, 23] but there is no satisfying outcome until Ewoldt et al. suggested a new method for analyzing nonlinear signal in 2008.[24] They found by representing the nonlinear response waveform with the Chebyshev polynomials of the first kind, the resulting third-order weighting coefficients, e_3, and v_3, can be directly linked to the intra-cycle behaviour as summarized below:

$$e_3 \begin{cases} > 0 & \text{strain stiffening} \\ = 0 & \text{linear elastic} \\ < 0 & \text{strain softening} \end{cases}$$

$$v_3 \begin{cases} > 0 & \text{shear thickening} \\ = 0 & \text{linear viscous} \\ < 0 & \text{shear thinning} \end{cases}$$

It should be noted that, the terms for nonlinear behaviour in this method is completely different the ones that researchers are familiar with. Usually, when discussing nonlinear behaviour of materials, the terms are based on the change of dynamic moduli or shear stress with strain amplitude.[25] Each of these measurements is the overall results calculated from a whole oscillatory cycle and the term of shear thinning or shear thickening indicates the difference from one cycle to another; therefore these terms should be called “inter-cycle” nonlinear behaviour (Scheme 1a), accordingly. However, the nonlinear behaviour indicated by Chebyshev coefficients is based on the concavity of viscous part and elastic part of instantaneous stress within one oscillatory cycle,[21] *i.e.* “intra-cycle” nonlinear behaviour (Scheme 1b). The intra- and inter-cycle behaviours are not necessarily consistent with each other because they are fundamentally two distinct concepts.[26]



Scheme 1 Inter-cycle (a) and intra-cycle (b) nonlinear behaviour in LAOS measurements.

Based on this method, investigations have been conducted on the LAOS behaviour of a variety of subjects, including colloidal glass,[27] gluten gel,[28] collagen,[29] agarose gel,[30] human skin,[31] and so on. However, only a few of them have made clear correlation between mathematical results and physical behaviours, therefore, it still needs to take plenty of work to fill in the gap between them.

In this study, aiming to the phenomenon we pointed out above, we investigated the structural evolution of fully matured CS/GP hydrogel while cooled down with both SAOS and LAOS rheological measurements. We hope firstly to develop the LAOS in studying the nonlinear behaviour of polymer solutions/hydrogels with Chebyshev expansion method, and secondly to further clarify the gelation mechanism of the unique and interesting CS/GP system.

Experimental

Materials

CS powder was purchased from Jinan Haidebei Marine Biological Product Co. Ltd. (China). GP in the form of pentahydrate, anilinonaphthalene-8-sulfonic acid ammonium salt (ANS), and fluorescein isothiocyanate (FITC) was purchased from Aladdin Reagent Co. Ltd. (China). Acetic acid and urea were purchased from Shanghai Chemical Reagent Co. Ltd. (China). All reagents mentioned above were used without further purification.

Hydrogel Preparation

The preparation of CS/GP was according to our previously work.[17] In briefly, CS solution (3 wt%) was prepared by dissolving CS powder in 1.5 mol/L acetic acid under magnetic stirring at room temperature. 0.3 g GP was dissolved in deionized water before added into 1 mL CS solution dropwise under stirring in an ice bath. The CS/GP mixture was dilute to expected concentration with deionized water or urea solution. The final concentration of CS and GP in the mixture was 1 wt% and 7 wt%, which ensure the pH value of the mixture was 7. The resulting solution was then kept in 37 ℃ water bath for 48 h until to reach the equilibrium. For confocal microscopy, CS/GP mixture with FITC and ANS at concentration of 9 μmol/L and 200 μmol/L was used.

Rheology Measurements

Rheology measurements were performed on an Anton-Paar Physica MCR 301 rheometer using a cone with a diameter of 60 mm and a cone angle of 1° over a Peltier plate. Temperature during measurements was set via the Peltier plate with a water recirculation. A solvent trap (AT-CYL-C/Q1) and silicone oil sealing was employed to diminish dehydration during measurement. Dynamic frequency sweep tests were carried out at small strain amplitude within linear regime (0.5%) and angular frequencies in the range of 0.1 rad/s to 100 rad/s. Dynamic strain sweep were carried out at a constant angular frequency of 1 rad/s and strain amplitude ranging from 0.1% to 10000%. Raw strain-stress data was recorded during all tests.

Nonlinear Rheological Data Analysis

The analysis of nonlinear data was conducted according to the Chebyshev expansion method.[24] In their study, a few definitions were introduced in order to describe the distorted nonlinear response. For the description of the elastic component, two measures were introduced, the minimum-strain and the large-strain modulus, G'_M and G'_L

$$G'_M \equiv \left.\frac{\mathrm{d}\sigma}{\mathrm{d}\gamma}\right|_{\gamma=0}$$

$$G'_L \equiv \left.\frac{\sigma}{\gamma}\right|_{\gamma=\pm\gamma_0}$$

where γ represents the instantaneous strain value during the oscillatory cycle, σ represents the instantaneous stress response corresponding to such strain value, and γ_0 represents the strain amplitude of the oscillatory cycle.

For the viscosity component, two other measure were proposed, the minimum-rate and the large-rate dynamic viscosity, η'_M and η'_L:

$$\eta'_M \equiv \left.\frac{\mathrm{d}\sigma}{\mathrm{d}\dot{\gamma}}\right|_{\dot{\gamma}=0}$$

$$\eta'_L \equiv \left.\frac{\sigma}{\dot{\gamma}}\right|_{\dot{\gamma}=\pm\dot{\gamma}_0}$$

In this study, G'_M, G'_L, η'_M and η'_L were calculated from raw data with a module provided by Anton-Paar Inc. Then, two dimensionless indexes of nonlinearity, strain-stiffening ratio S and shear-thickening ratio T, were defined as the results following the formula below:

$$S = \frac{G'_L - G'_M}{G'_L} = \frac{4e_3 + \cdots}{e_1 + e_3 + \cdots}$$
$$T = \frac{\eta'_L - \eta'_M}{\eta'_L} = \frac{4v_3 + \cdots}{v_1 + v_3 + \cdots}$$

S and *T* can act as indicators of intra-cycle behaviours as well as Shebyshev coefficients e_3 and v_3. $S = 0$ corresponds to linear elastic response. $S > 0$ indicates intra-cycle strain-stiffening, meaning that the instantaneous modulus increase with instantaneous strain and $S < 0$ indicates intra-cycle strain-thinning, the opposite situation. Similarly, $T = 0$ stands for linear response, $T > 0$ for intra-cycle shear-thickening, which is when the instantaneous viscosity increase with instantaneous shear rate, and $T < 0$ for shear-thinning, on the contrary.

Confocal Laser Scanning Microscopy (CLSM)

100 μL CS/GP solution with florescent probe was pipetted onto a thin bottom dish and sealed, then incubated at 37 ℃ for 2 h before observation. Images were captured by a Leica TCS SP5II CLSM (Leica, German) in florescent mode.

Results and Discussion

Partial Thermal Reversibility of CS/GP Hydrogel

The most attractive property of CS/GP system is it can convert from solution to hydrogel around physiological temperature 37 ℃.[32] Therefore, in this research we first heated CS/GP solution to 37 ℃ and kept 48 h to form a fully matured hydrogel. Then, we cooled down the hydrogel at a rate of 1 ℃/min, and monitored the cooling process with dynamic rheology test. Fig. 1 shows that with the decrease in temperature, the damping factor (ratio of G'' to G') gradually increases during the whole process, which indicates the viscoelastic solid sample turned into a form closer to liquid. In the meantime, the changes of *G'* and *G''* are quite complicated. *G'* first decreases and finally reaches an equilibrium plateau at about 5 ℃, while *G''* decreases at first and then increases when the temperature is lower than 15 ℃.

In order to get a better insight into the structural change of CS/GP hydrogel during the cooling down process, frequency sweeps were performed at a series of temperature points (Fig. 2). It shows that CS/GP starts with a nearly frequency independent *G'*, while *G''* also presents a frequency independent plateau at low frequency (< 1 rad/s) and tips up at high frequency (> 1 rad/s). As temperature goes down, besides the obvious modulus loss, *G'* gradually becomes frequency dependent, and the width of plateaus in *G''* turns more and more narrow. In addition, *G''* also shows more frequency dependent at high frequency with the decrease in temperature. All these indicate the integrity of network in CS/GP samples is compromised during the cooling down process.

Power-law exponents of *G'* and *G''* against angular frequency ω, *m* and *n*, were calculated to describe the evolution of frequency dependency. It is known that for a perfect hydrogel, *m* and *n* should both equal to 0. For an ideal polymer solution, $m = 1$, $n = 2$, and at gel point, $m = n = 0.5$.[33] In this case, we find *m* remains a relatively low value at all temperature (0.02–0.22) and *n* also does not excess the value of critical gel. In addition, both the values of *m* and *n* increase with the decrease in temperature, indicating the CS/GP system drifts away from gel state when cooling down. Therefore, it can be concluded the CS/GP network remains, but loosens and weakens in the cooling process in all experimental temperature range (0 – 37°C).

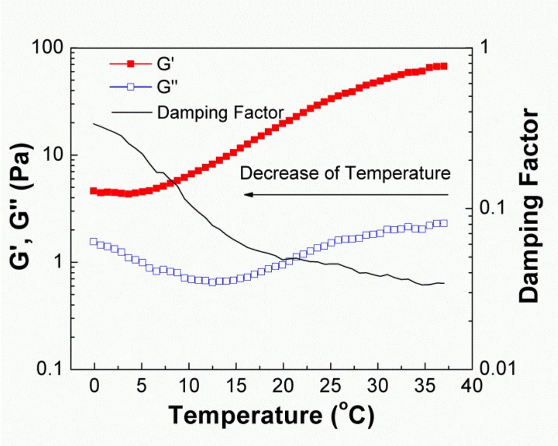


Fig. 1 Changes of storage modulus *G'*, loss modulus *G''* and damping factor at small amplitude (0.5%) during the cooling down process of a matured CS/GP hydrogel.

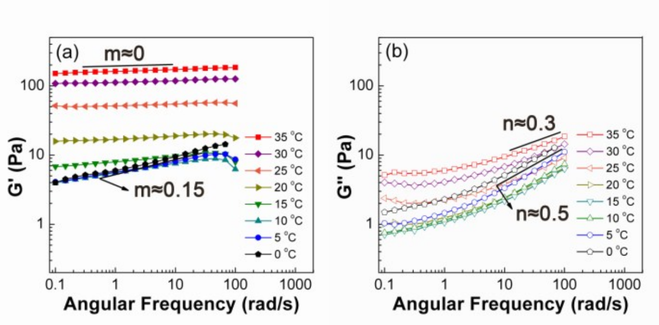


Fig. 2 Storage modulus *G'* (a) and loss modulus *G''* (b) at small amplitude (0.5%) as functions of angular frequency at various temperatures.

LAOS measurements of CS/GP Hydrogel

Fig. 3 shows the results of CS/GP hydrogels subjected to LAOS tests at each temperature point. Fig. 3a and 3b are the original curves, indicating the moduli generally decrease with the decrease in temperature. In order to eliminate the modulus factor and only distinct the difference in the yielding behaviour at different temperature point, we normalize each LAOS curve by the linear plateau modulus, and the results are presented in Fig. 3c and 3d. It can be found that at high temperature (>15 °C), *G'* decreases suddenly after plateau range and *G''* shows an overshoot before a sudden drop. This phenomenon can be classified as Type III, which is usually attributed to the destruction of associated structure, according to the guideline proposed by Ahn et al. in 2003.[25] In addition, the yielding point is around the strain amplitude of 1 and gradually moves to higher strain upon lower temperature, indicating the hydrogel turns to more flexible. However, at low temperature (0–15 ℃), both moduli curves exhibit different behaviour from those at high temperature (>15 °C). They have not a significant plateau range, and both decline before a weak overshoot appeared. This can be classified as a combination of Type I and Type IV. A similar behaviour change was reported in the case of another thermosensitive gel PEO-PPO-PEO,[34] and the weak overshoot was associated with the formation of a shear induced structure.

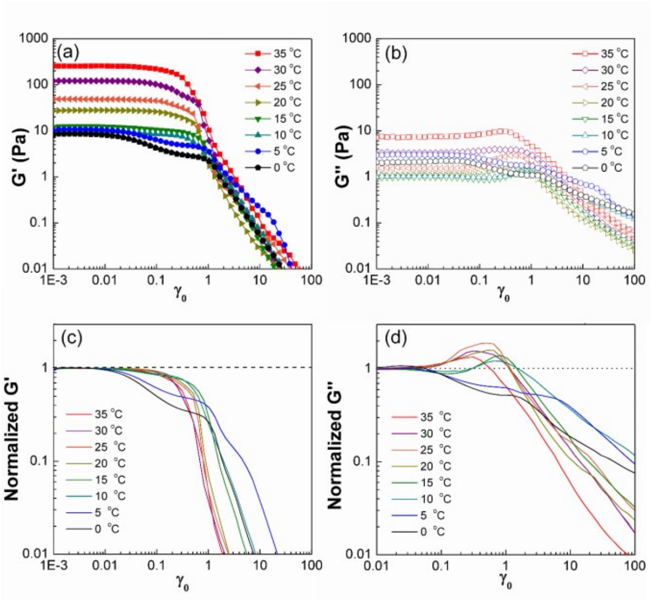


Fig. 3 Storage modulus *G'* (a) and loss modulus *G''* (b) as functions of strain at various temperatures, and the corresponding results after normalized to the plateau modulus (c and d), respectively.

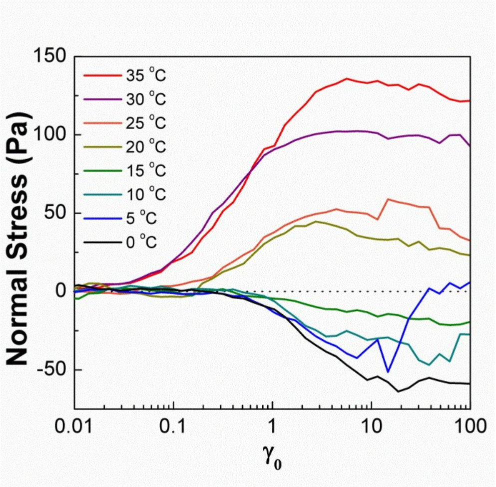


Fig. 4 Normal stress of CS/GP as function of strain at various temperatures.

Another piece of information is provided by the normal stress data recorded during the LAOS tests (Fig. 4). At high temperature (>15 °C), CS/GP samples give out a positive normal stress, which begin to increase at the same strain amplitude where *G'* starts declining (around 0.1). With the decrease in temperature, the maximum normal stress gradually weakens. However, when the temperature decreases to 15 °C, an unusual negative signal appears at the strain amplitude around 1. Normally, most materials expand in the direction perpendicular to the applied shear stress when they subjected to shear, therefore generating a positive normal stress.[35] Only recently, the phenomenon of steady negative normal stress was reported in a few examples that contain semi-flexible network, and it only occurs when the material exhibits an inter-cycle strain-hardening.[36] In our case, the negative normal stress signals are found in the same strain range as the weak overshoot appears, thus we may presume that a shear-induced semi-flexible network is formed in CS/GP system when the temperature is lower than 15 ℃.

Analysis of LAOS Behaviour of CS/GP Hydrogel

As mentioned in the Introduction part, Chebyshev expansion method is capable of obtaining nonlinear behaviour that materials exhibit in LAOS tests, so we try to sort through the chaotic appearance of LAOS results of CS/GP hydrogel with this method. The results are shown in Fig. 5, in which we can divide the strain range into three regions. When the strain amplitude is small enough (<0.02), both *S* and *T* are close to 0, indicating linear behaviour (Region 0). When the strain is larger than 1 (Region II), *S* starts to fluctuate and *T* reaches a negative plateau. The value of *T* gradually moves closer to zero as temperature goes down. In addition to the fact we shown in Fig.3 that both *G'* and *G''* decrease within this strain range, we have reason to assume that in this region, the networks in CS/GP hydrogels are broken and shredded by shear field. Of course, the stronger the sample network is, the stronger the intra-cycle shear-thinning exhibits.

Between these two relatively simple regions, Region I where the strain amplitude is between 0.02 and 1 is most complicated. It corresponds to the nonlinear response of CS/GP hydrogel while subjects to shear but still remains integrated. In this region, a sharp increase of *S* with the strain amplitude is observed at all temperature points. Such a steady rise of intra-cycle strain-hardening suggests that stress is built up and deformation is taken place in the hydrogel network. According to the behaviour of *T*, we divide the temperature points into two groups. In Group 1 (Fig 5a), where temperature is higher than 15 ℃, *T* presents positive value, standing for a shear-thickening behaviour. In the meantime, *S* exhibits a relatively strong positive peak of ~0.7. This intra-cycle strain-hardening/shear-thickening pattern can be associated with the plastic rearrangement within colloidal gel network.[27, 37]

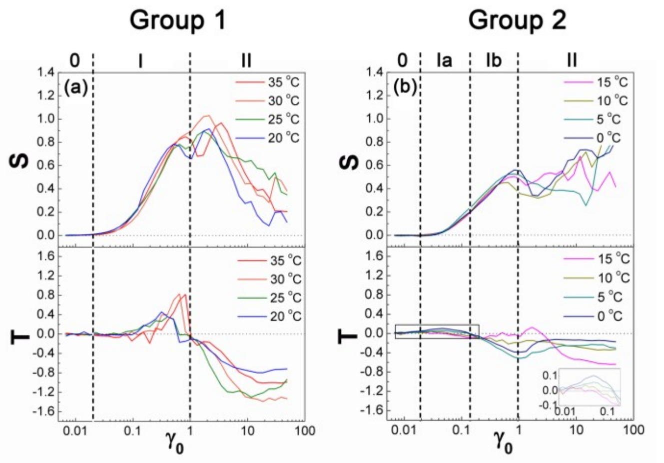


Fig. 5 Strain-hardening and shear-thickening ratio of CS/GP hydrogel as function of strain at different temperature.

At lower temperature (0–15 ℃, Group 2), *S* also exhibits a positive signal, however, it is weaker than the ones in Group 1 with a strength of about 0.4, suggesting the network turns into a weaker form and the tension it can endure is also weaker in this temperature range. We notice that *T* presents two behaviours in Region I, therefore we further divide Region I into two parts, Region Ia and Region Ib. In Region Ia ($0.02<\gamma_0<0.15$), a minor positive peak emerges (Fig. 5, inset), and in Region Ib $(0.15<\gamma_0<1)$, *T* turns negative, which is exactly where Group 1 presents the positive signal. Such a combination of nonlinear behaviour has not been reported before, but is consistent with our observation from a semidilute polyacrylamide solution. It is a common phenomenon found in an associated polymer network (unpublished work), and is usually considered caused by the enhancement of inter-molecular interaction due to the slight shear induced alignment and followed by disentanglement[38].

According to the analysis by Chebyshev expansion method, we may conclude that fully matured CS/GP hydrogel network at 37 ℃ is composed of colloidal particles, and when the temperature decreases, it gradually turns into one that composed of associate flexible chains. The apparent transition temperature of such a structural change is around 15 ℃.

To confirm this assumption and get a more intuitive view of such a change, CLSM images of FITC-labelled CS/GP hydrogel at different temperature were taken (Fig. 6a-c). FITC dye is covalently attached to CS chain, so the bright spots in Fig. 6a-c indicate the existence of CS molecules. It can be seen that at 37 ℃, CS/GP sample is composed of microparticles with clear edges. When the temperature goes down to 20 ℃, the size of these microparticles expands and the edge blurs, pointing to a partial "dissolution". With the further decrease of temperature to 0 ℃, we can only see a homogenous pattern without any distinguishable microparticles.

In the meantime, we also used a fluorescent hydrophobic probe ANS to monitor the change of hydrophobic area in CS/GP hydrogel (Fig. 6d-f). ANS is known to be without fluorescence in aqueous solution and only become fluorescent when binding to hydrophobic area, thus the bright spots in Fig. 6d-f indicate the hydrophobic regions. At 37 ℃, ANS image shows bright spots (hydrophobic areas) as well as distinctly dark boundaries. These bright spots get dimmer and the size of them become smaller when cooled down, suggesting the hydrophobic areas are reduced. However, even at 0 ℃, there still bright areas can be observed in ANS image, when no microparticles can be distinguished from FITC one. This implies even when CS microparticles collapse and become semidilute associate solution, there is still some hydrophobic areas remained to maintain the network, similar to the "soft gel" state of PEO-PPO-PEO,[34] which also explains the appearance of Type I + Type IV behaviour discussed before.

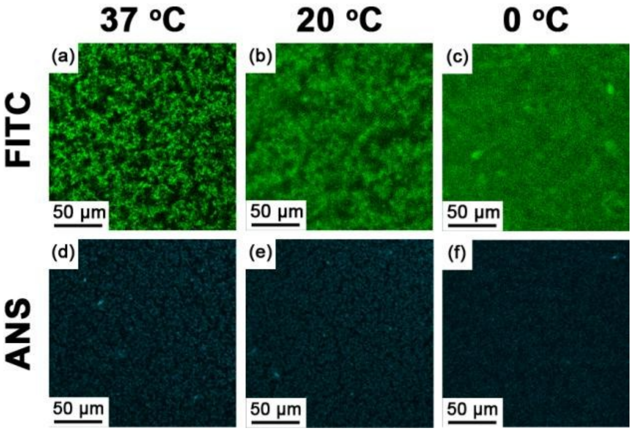


Fig. 6 CLSM images of FITC (a-c) and ANS (d-f) tagged CS/GP samples at various temperatures.

Influence of urea to the rheological behaviour of CS/GP hydrogels

In previous works, the researchers mainly agree that hydrogen bonding is another major interaction besides hydrophobic interaction in CS/GP system.[15] Therefore, we introduce a hydrogen bond disrupting agent, urea into CS/GP system to see its influence on the structure of CS/GP hydrogel during the cooling process. Fig. 7a shows that at high temperature (35 °C), the linear behaviour of CS/GP hydrogel with and without urea is almost identical, indicating under this condition, the interaction responsible for holding the network together is not hydrogen bonding but hydrophobic interaction, as we discussed in the last part. However, at lower temperature (Fig. 7b-d), urea starts to show the effect on weakening both *G′* and *G″* as well as making them more dependent on frequency. We find the lower the temperature is, the more significant this effect shows, so we can come to the conclusion that as temperature goes down, hydrophobic interaction gradually diminishes and hydrogen bonding becomes the dominant one to maintain the network in CS/GP system.

On the nonlinear side, urea shows the ability to enhance the positive T peak at temperature points in Group 1 (Fig. 8a-b), suggesting the rearrangement of the molecular chains is enhanced. We assume that it is because urea acts as a lubricant by disrupting hydrogen bond between colloidal particles. However, at temperature points in Group 2 (Fig. 8c-d), the addition of urea weakens all the nonlinear behaviours, indicating the CS/GP hydrogels become weaker after the urea addition. It is probably because in this situation hydrogen bonding is responsible for holding up the network, so if the hydrogen bond is disrupted by urea, the network is weakened in general, thereby all yielding behaviours presented as nonlinearity are weakened correspondingly.

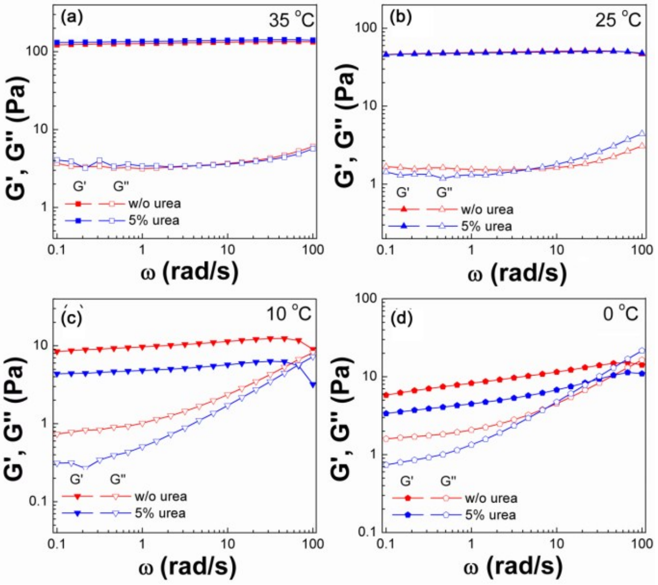


Fig. 7 Effect of urea on linear behaviours of CS/GP at 35 ℃ (a), 25 ℃ (b), 10 ℃ (c) and 0 ℃ (d).

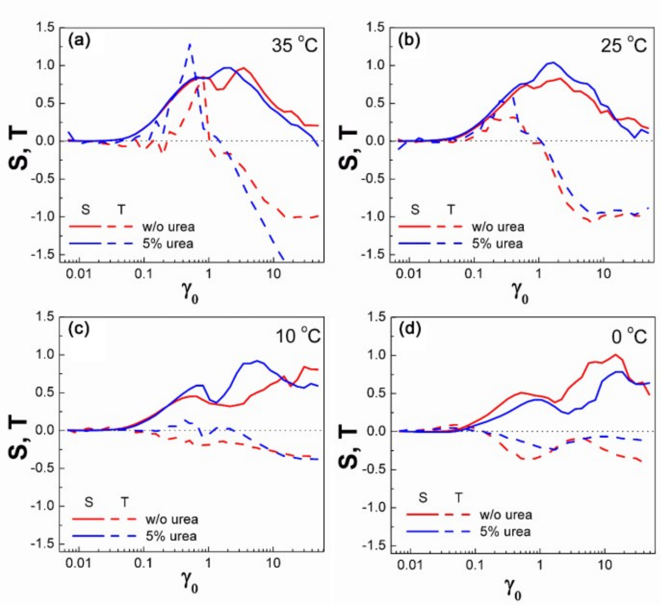


Fig. 8 Effect of urea on nonlinear behaviours of CS/GP at 35 ℃ (a), 25 ℃ (b), 10 ℃ (c) and 0 ℃ (d).

Possible Structure of CS/GP hydrogel and the Formation Mechanism

Based on the information shown above, we propose possible structure and yielding mechanism of CS/GP system at high temperature (35 ℃) and low temperature (0 ℃), shown in Fig. 9. At high temperature, CS/GP system forms a colloidal network that is mainly dominated by hydrophobic interaction. Therefore, when the strain amplitude exceeds linear region (Region 0), it presents an inter-cycle and intra-cycle shear-thickening dissipation peak (Region I) before the final disintegration of network (Region II) (Fig. 9a). However, when the temperature goes down, the hydrophobic areas partially disappear, and the CS/GP sample turns into a flexible network mainly dominated by hydrogen bonding. Thus, when the strain in such a network exceeds linear region (Region 0), chitosan molecule chains start to align, resulting in firstly an increase of energy dissipation and then a shear induced semiflexible network is formed (Fig. 9b).

At last, we may deduce the formation mechanism of CS/GP hydrogel by backward reasoning. We do not consider the electrostatic interactions between CS and GP as an effective role in the gelation process here. As in our previous studies, we found that the electrostatic interactions between CS and GP only prevent CS from aggregation when the pH value of the whole system is around 7. In another word, the role of GP in CS/GP system is to act as a protector, keeping CS molecules from precipitation. We proved that GP molecules do not involved in the final network of CS/GP hydrogel with ^{31}PNMR.[17,39] In 2013, Fillion et al. also found out that the diffusion profile of GP out of CS/GP hydrogel is consistent with free diffusion model, and the fixed charge on CS molecules is the same with free CS in solution, proving that GP molecules do not act as electrostatic crosslinker.[18] Therefore, we suggest when CS aqueous solution is mixed with GP aqueous solution, the hydrogen bonding between CS and GP prevents aggregation of CS molecular chains, so at room temperature, CS/GP system still exhibits a solution state. With the increase of temperature, the hydrogen bonding between CS and GP is weakened, therefore the hydrophobic interactions between CS molecular chains become dominate in the system, leading the gelation of the whole system. The competition of the hydrogen bonding and hydrophobic interaction in CS/GP system decides the gelation temperature of such a thermosensitive hydrogel with different CS/GP composition. In a certain composition, the gelation temperature is able to reach the physiological temperature 37 °C. As the hydrophobic interaction between CS molecular chains once forms, it is hard to break totally, so the CS/GP hydrogel is only partially thermal reversible.

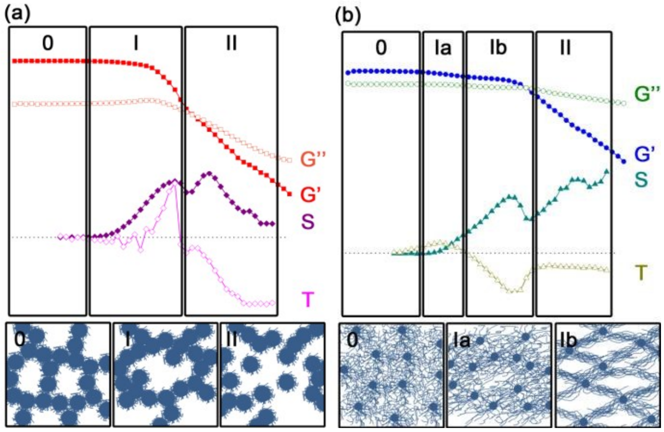


Fig. 9 Yielding process of CS/GP hydrogel under shear at high temperature (35 ℃, a) and low temperature (0 ℃, b).

Conclusions

In this study, the structural change of matured CS/GP hydrogel from 35 °C to 0 °C is monitored with both linear and nonlinear rheological measurements. The nonlinear rheological measurement is performed with a newly developed LAOS technique and data is analyzed by Chebyshev expansion method. According to our result, CS/GP system shows a similar behaviour to another colloidal thermosensitive material, PEO-PPO-PEO. That is, CS/GP hydrogel is composed of a colloidal network dominated by hydrophobic interactions when it forms at high temperature, but gradually turns into a flexible network dominated by hydrogen bonding when the temperature goes down.

LAOS method is able to reveal rich information about architecture and composition within soft materials that SAOS method cannot touch. It would provide a nice complement to the rheological description we have now. However, it should be admitted that, although a series of phenomenological deduction was made based on nonlinear behaviour and an attempt to elucidate the molecular mechanism behind those rheological phenomenon, more *in situ* observation of sample while being sheared is necessary for the further confirmation of such a novelty analytical method. Therefore, another contribution of this work is we successfully correlate the LAOS results and structure of CS/GP hydrogel based on the previous studies and assumptions in this laboratory and other research groups, and propose a more reasonable gelation mechanism of this unique partially reversible thermosensitive biomass-based hydrogel.

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Notes and references

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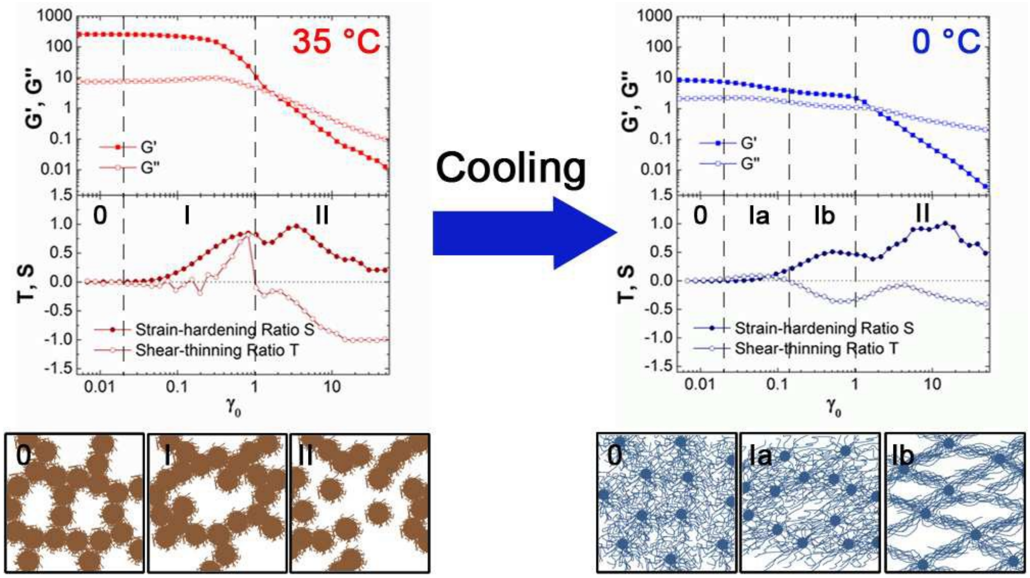

The research not only understands the unresolved CS/GP gelation mechanism, but also helps to interpret the complicated LAOS results.