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Effects of Ultrasonic Dispersion in Dilute Polysaccharide Solution

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ABSTRACT: In order to evaluate the relationship between dispersion and ultrasonic time, dynamic light scattering experiment was carried out on both 0.20 g/L xyloglucan solution and 0.20 g/L β-galactosidase-degraded xyloglucan solution with different time intervals. Data showed that the variation trends of particle size in xyloglucan and enzyme-degraded xyloglucan solution were similar: decreased first and then increased with the incremental time. Thus, there should be an optimum time interval during which the smallest particle size can be obtained if experimental conditions were excluded in theoretically. In addition, the optimum time interval varies in different polysaccharides since it may be associated with molecular conformation, based on the results of our work. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40973.

KEYWORDS: dilute solution; polysaccharide; ultrasonic dispersion; xyloglucan

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INTRODUCTION

In current research area of polysaccharide materials, existing polysaccharides with improved properties has attracted great interest due to the economic factors. The material properties of a particular polysaccharide crucially depend on their molecular conformation, which can be altered by chemical or physical methods. Polysaccharide dispersion is of great significance in developing functional materials, as the well dispersed polysaccharides can tap their potential unique and high-property functions. Ultrasound, one of the most efficient and powerful techniques among various equipments used for polysaccharide dispersion, can disperse various polysaccharides with a wide range of particle sizes in dilute solution.[1–4] Moreover, ultrasound is widely used to accelerate the dispersion rate of polysaccharides, and more importantly, to improve their physical and chemical properties such as the particle size, molecular weight, viscosity, and activation energy.[5–7]

The mechanism of ultrasonic dispersion is associated with acoustic cavitation. When high-intensity ultrasound is applied into the polysaccharide solution, cavities grow and reach the critical diameter, leading to their implosion and fragmentation, which further generate high energy. Molecular movements are accelerated and intermolecular collisions occurred driven by enough energy. Several factors that affect the formation of cavity bubbles include viscosity, temperature, and pressure. Recently, some studies indicated that the mechanical properties of nanoparticles are associated with ultrasonic time. Rodgers et al. utilized ultrasonic to disperse SiC nanoparticles for 30 min in their study,[8] while Xu et al. indicated that the mechanical properties of dispersed substances were deteriorated when ultrasonic time lasted over 20 min.[9]

Although these studies indicated the superior efficiencies of ultrasound to improve dispersion effects with different ultrasonic time, they failed to further provide reasonable explanations about how ultrasonic time affects dispersion and how to control ultrasonic time to obtain the best dispersion effects. To fill this blank, we proposed a meaningful question from the microscopic view: what is the relationship between the ultrasonic time and conformation change of dispersed polymers?

Polysaccharides, which widely exist in animals, plants, and microorganisms, play an important role in life. With the development of polysaccharide science and technology, polysaccharide and its derivatives have a prospective application in many fields, especially in biomedicine.[10–12] Xyloglucan is a water-

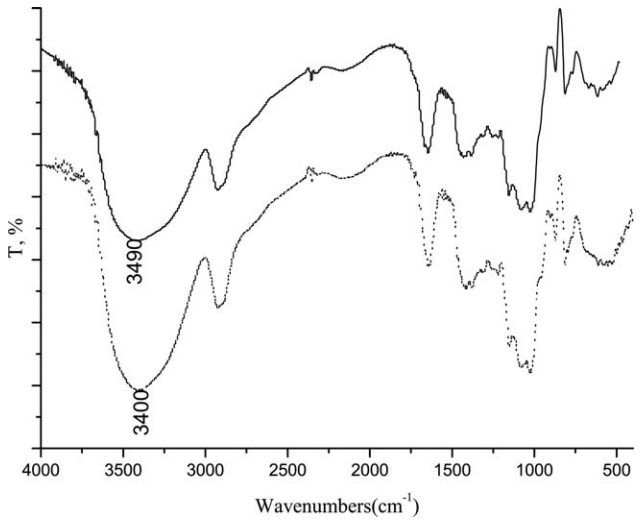

Figure 1. FTIR spectra of xyloglucan (solid line) and enzymatic treated xyloglucan (dashed line).

soluble and biodegradable polysaccharide and is extracted from tamarind seed powder.[13–15] Xyloglucan molecule is composed of β-(1-4)-D-glucan main chain, which is highly branched by substitutes at C-6 by α-xylosyl units. The enzyme-degraded xyloglucan is of great potentials used as drug-delivery carriers.[15–17] Our previous study showed that the dispersion effects of either xyloglucan or enzyme-degraded xyloglucan directly influenced their ability to release drugs.[16]

In the present research, we first hypothesized that ultrasonic time may have significant influence on dispersion effects and then specifically studied the changes of particle size at different times. Finally, a conclusion was made that an optimum time interval existed during ultrasonic dispersion in dilute polysaccharide solution.

MATERIALS AND METHODS

Materials

Xyloglucan was prepared from tamarind seed powder as the same method mentioned in our previous study.[17] About 2 g xyloglucan powders were dissolved in 198 mL distilled water with intense stirring at 60°C. Then the solution was centrifuged (AVANTI J-26XP, BECKMANCOULTER, USA) with 10,000 rpm for 10 min to remove the water-insoluble fraction. The supernatant was filtrated with 1 μm membrane. The xyloglucan was precipitated with two volumes of 95% ethanol and then lyophilized (FD-1-80, Beijing Boyikang Instruments Co. China) for further experiments.

β-Galactosidase was purchased from Beijing Hongrunbaoshun Co. China. All other chemicals not mentioned above were analytical grade and purified by the standard methods.

Chemical Analyses

Xyloglucan (XG) were hydrolyzed with 72% H_2SO_4 (1 h, at 100°C) and then diluted to 8% (5 h, at 100°C). After removal of acid, the solutions were neutralized with $BaCO_3$, filtered and the filtrate from each hydrolysis reduced with $NaBH_4$, followed by acetylation with pyridine–Ac_2O(1 : 1 vol/vol) for 12 h at 25°C. The obtained alditol acetates were analyzed by gas chromatography-mass spectroscopy (GC-MS) (TRACEMS2000, FINNIGAN) at 220°C with DB-225 capillary column, the carrier gas being N_2.[18]

FTIR Measurements

Infrared spectra were recorded using the Spectrum-2000 spectrometer (PerkinElmer, US). Spectra were recorded by averaging 32 scans with the resolution of 4 cm^{-1} to reduce the noise. If required, the spectra were corrected by a standard spectral subtraction procedure to cancel the contribution of water vapor.

Gel Permeation Chromatography

The molecular weight of XG sample was evaluated by gel permeation chromatography (GPC, Prominence GPC, Shimadzu Co. Japan). The chromatography system was equipped with TSK-G3000 columns (TSK, Japan). RID-10A detector was used to measure the refractive index (RI) signal. The mobile phase (0.7% Na_2SO_4) was delivered at a flow rate of 0.5 mL/min, with an injection volume of 50 μL. The standards were pullulan with molar mass from 5 to 800 kDa (p-82, Shodex, Japan). All data, including the polydispersity index, were acquired and analyzed using Shimadzu CLASS GPC software.

Enzymatic Degradation

About 1 g purified xyloglucan powder was well dissolved in 99 g distilled water and then 20 mg β-galactosidase was added into it. The mixture was kept in 40°C oscillator with gentle stirring for 2 h, and then the enzyme was deactivated by heating at 95°C for 20 min. Then the mixture was centrifuged with 10,000 rpm for 10 min to remove the water-insoluble fraction. The supernatant was filtrated with 1 μm membrane. Enzyme-degraded xyloglucan was precipitated with two volumes of 95% ethanol, then lyophilized and stored for further use.

Ultrasonic Dispersion and Dynamic Light Scattering

Ultrasound (SB5200DT, 250W, Ningbo Scientz Biotechnology Co. China) was launched into 100 mL 0.20 g/L enzyme-degraded xyloglucan solution at 25°C and lasted for 120 min. The particle sizes were measured after 5, 10, 20, 30, 60, and 120 min by using the Malvern's Mastersizer (ZS90) at 25°C. Measurements were repeated for five times for each sample.

For comparison, 100 mL 0.20 g/L xyloglucan solution was treated with ultrasonic dispersion and the samples were analyzed as the same method mentioned above.

Statistical Analysis

Data was expressed as means ± standard deviations (SD) of five replicated determinations.

RESULTS

Compared to xyloglucan, there were no peaks vanishing or reforming after enzymatic treatment in FTIR spectra (Figure 1). After the enzymatic hydrolysis, however, the bands of hydroxyl group near 3490 cm^{-1} became narrow and moved to low wavenumber (3400 cm^{-1}).

From the data of carbohydrate compositions in Table I, it shows that there are no new carbohydrate derivatives in monosaccharide compositions of the enzymatic treated xyloglucan. After

Table I. Monosaccharide Composition of Xyloglucans (25°C)

	Monosacharides (mol %)		
Sample	Galactose	Xylose	Glucose
XG	16.3	37.3	46.4
Enzyme-degraded xyloglucan	9.7	40.2	50.1

enzymolysis, the contents of galactose, xylose, and glucose were altered. In xyloglucan, resulted from enzyme treatment, the galactose residues decreased from 16.3 to 9.7%, while the xylose residues increased from 37.3 to 40.2% and glucose residues increased from 46.4 to 50.1%.

The molecular weight and polydispersity coefficient of xyloglucan and enzymolyzed xyloglucan measured by GPC were 496 K, 1.07 and 564 K, 1.13, respectively. There are no other peaks in the GPC elution profile of enzymolyzed xyloglucan (Figure 2), indicating that the polymer segment were not produced in the enzymatic hydrolysis of β-galactosidase.

From Figure 3, particle diameter of xyloglucan and enzyme-degraded xyloglucan significantly decreased from 114.0 nm to 23.5 nm, from 93.0 nm to 18.9 nm, respectively, after 5 min, and kept decreasing during the next 5 min and reached 18.5 nm and 7.0 nm, respectively, at 10 min. However, a very interesting phenomenon was that the particle diameter of both xyloglucan and enzyme-degraded xyloglucan respectively increased to 24.8 and 14.2 nm at 20 min; 31.0 nm and 19.9 nm at 30 min. Moreover, an obvious time inflection point (around 10 min) was observed in enzyme-degraded xyloglucan solution, which was distinctive from the wide time interval (5–30 min) in xyloglucan solution. Particle diameter of xyloglucan and enzyme-degraded xyloglucan increased to 233.5 and 153.9 nm, respectively, at 120 min, which were much larger than that at 0 min.

The distribution of different-sized particles in xyloglucan solution is showed in Figure 4. The particle size at 5, 10, 20, and 30 min were much smaller and showed a relatively narrow size scope than that at 0 min, indicating that the xyloglucan molecules were homogeneously dispersed within 30 min, and the smallest-size particles were mainly centralized at 10 min. With the extension of time, particle size increased significantly and showed a much wider size scope at 60 and 120 min.

Figure 5 shows the distribution of different-sized particles in enzyme-degraded xyloglucan solution. It displayed a similar varying trend of particle size, compared with xyloglucan solution. But the size scope at 0 min and 60 min had a relatively large overlap, about 25% enzyme-degraded xyloglucan molecules possessed similar size at 0 min and 60 min, this finding was different from the result in xyloglucan solution.

Figure 6 shows the particle diameter of enzyme-degraded xyloglucan solution and xyloglucan solution at different concentration. The particle diameter of xyloglucan solution is 15.54 nm, 18.52 nm, 17.33 nm, when the concentration is 0.1%, 0.2%, 0.3%, respectively. The particle diameter of enzyme-degraded xyloglucan solution is 6.427 nm, 7.1 nm, 7.006 nm, when the concentration is 0.1%, 0.2%, 0.3%, respectively.

The particle diameter of enzyme-degraded xyloglucan solution and xyloglucan solution differed narrowly, when the concentration is between 0.1% and 0.4%.

DISCUSSION

Because partial galactose residues were removed in enzyme treatment, hydrogen bonding energy changed in the process. It is good evidence that the positions of characteristic absorption peak of hydroxyl group were altered after enzymolysis. However, there were no new carbohydrate derivatives in monosaccharide compositions after enzymolysis, which indicated that the functional groups of XG were not altered in the enzymatic hydrolysis.

The molecular weight of enzyme-degraded XG was similar to that of XG, but galactose residues were degraded from 16.3 in XG to 9.7% in enzyme-degraded XG. Enzyme-degraded xyloglucan solution gelated in 2 wt % and occurred thermo-sensitive hydrogel. It was able to sustained-release drugs and would not harm to animals in our previous studies.[16]

The molecular conformation of xyloglucan differ after degraded by β-galactosidase, and the enzyme-degraded xyloglucan solution turn into thermally reversible gel.[19]

Molecules of polysaccharide in dilute solution mainly exist in the form of isolated coils. These isolated coils begin to aggregate and form linear-group structure when solution concentration reaches critical concentration (C^*).[20] The theoretical value of C^*

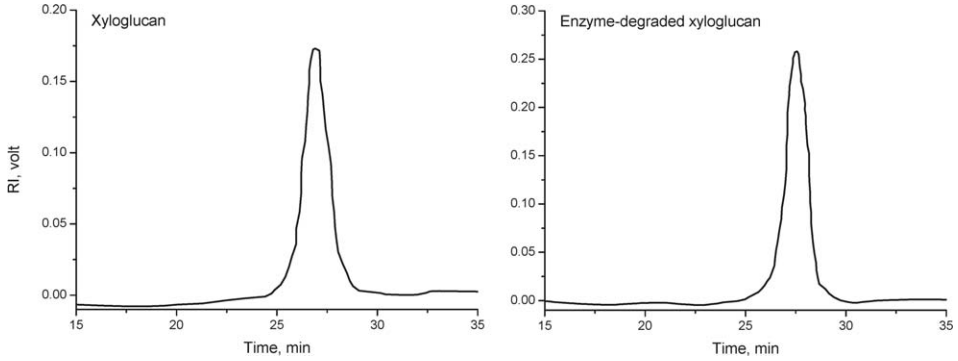

Figure 2. GPC elution profile of xyloglucan (left) and enzyme-degraded xyloglucan (right).

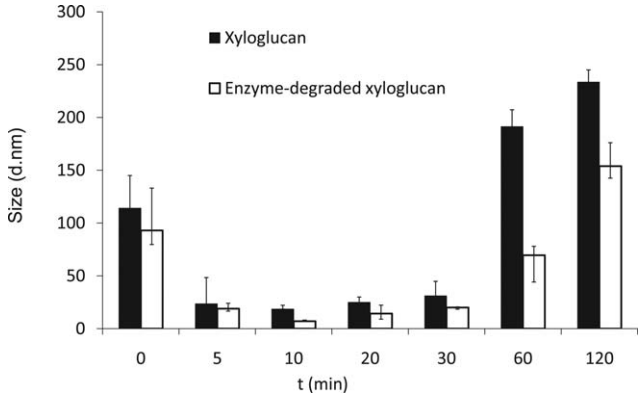

Figure 3. Particle diameter of xyloglucan and enzyme-degraded xyloglucan at different ultrasonic dispersing time (25°C). Data are given as mean ± S.D.

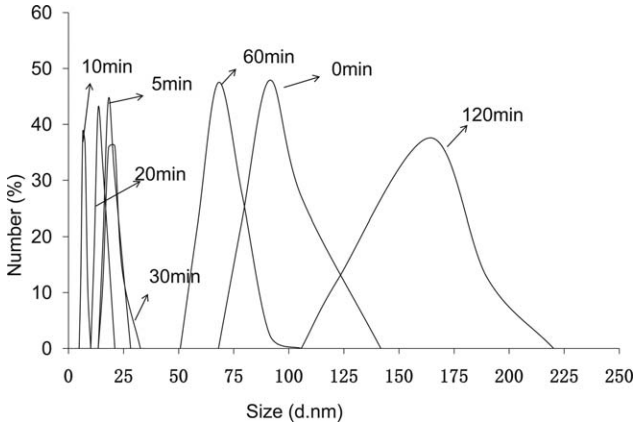

Figure 5. Distribution of particle number of enzyme-degraded xyloglucan solution at different given dispersing time (25°C).

is associated with relative molecular weight (M) and calculated by the following formula[20]:

$$C^* \approx M^{-4/5} \quad (1)$$

Relative molecular weight of xyloglucan and enzyme-degraded xyloglucan were 564 K and 496 K, respectively. Based on these data, C^* of xyloglucan solution and enzyme-degraded solution were 0.025 g/L and 0.028 g/L, respectively. Because of the detection limit of the DLS equipment (Zetasizer Nano), the actual concentration of the dilute solution in this study was 0.20 g/L, about eightfold larger than their C^*. Therefore, both xyloglucan molecules and enzyme-degraded xyloglucan molecules existed mainly in the form of loose linear-group structure and the rest were isolated coils.

During the process of ultrasonic dispersion, large amount of energy was released. After the energy was absorbed by macromolecules, the movements of macromolecules were accelerated. Because the hydrogen bondings between water molecule and macromolecules were tighter than the hydrogen bondings of intermacromolecules. At the first stage, the forces between macromolecules were destroyed. Macromolecules began to convert from the form of loose linear-group structure to the form of isolated coils. At the first stage, the particle diameter decreased. With the further release of energy, the polysaccharide intermolecular hydrogen bondings were opened. At the last stage, the molecular chains swelled and the particle diameter increased.

The tested enzyme-degraded xyloglucan possessed a relatively small polydispersity coefficient of 1.07, and thus showed an obvious monodispersity in particle size. This explained why an apparent time inflection point at 10 min was observed in enzyme-degraded xyloglucan during ultrasonic dispersion.

In our study, the concentration of enzyme-degraded xyloglucan solution was 0.20 g/L, and the force between water molecular and macromolecule was lower than the intramolecular force of polysaccharide. So the chains contracted due to the attractive interaction among the monomers, and water served as a poor solvent. After treated by ultrasonic dispersion more than 30 min, macromolecules chains swelled to the ideal state. So the water nearly served as a good solvent. In dilute solution, the particle diameter changing followed with the time of ultrasonic could reflect the property of solvent. This is one of the reasons of forming gel.

On the contrary, xyloglucan had a larger polydispersity coefficient of 1.13 and its dispersion process was very complicated. Long molecular chains began to disperse before the termination

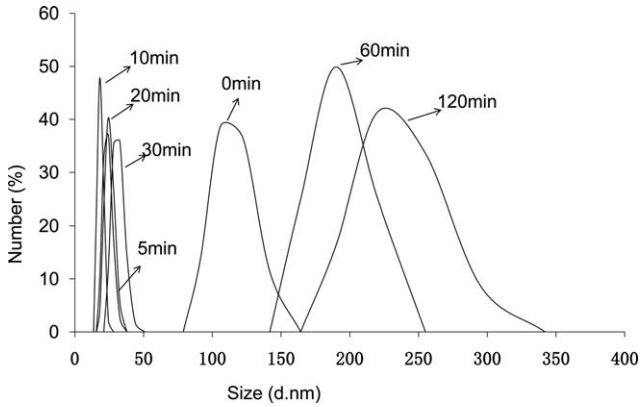

Figure 4. Distribution of particle number of xyloglucan solution at different given dispersing time (25°C).

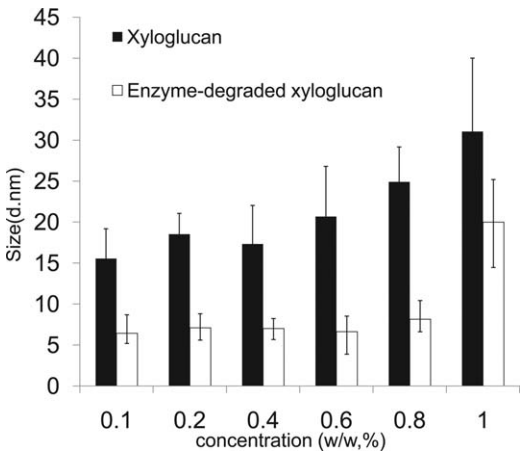

Figure 6. The particle diameter of enzyme-degraded xyloglucan solution and xyloglucan solution at different concentration (25°C).

of dispersion of short molecular chains due to the large amount of side chains in xyloglucan. Therefore, no obvious changes of particle size were observed during 5–30 min in xyloglucan solution. And with the extension of ultrasonic time, cavitation enhanced surface activity of small particles. As a result, dispersed chains reaggregated under the driving force of increasing surface activation energy, and this was the reason why particle size began to increase after a certain time interval.

In addition, galactose residues could attract hydrous ions due to their large electronegativity, forming into large-sized hydrous group. Enzymatic treatment removed partial galactose residues and thus weakened electronegativity of the whole molecule. This explained why the particle size of enzyme-degraded xyloglucan became smaller from the view of ability to combine hydrous ions. However, both xyloglucan and enzyme-degraded xyloglucan showed similar varying trend of particle size: decreased at first and then increased after a certain time, indicating that the difference in side chains amount had no obvious influence on dispersion effects.

The particle sizes of xyloglucan at 5, 10, 20, and 30 min are similar, and perhaps this is the reason why most researchers use ultrasound to disperse polysaccharides within a short time, usually 5–30 min. But unfortunately, they may have neglected the influence of ultrasonic time on molecular conformation in polysaccharide solution. Our research is of great interest since we have filled up this blank by proposing a general relationship of ultrasonic time and molecular conformation, which essentially determine the dispersion effects of polysaccharide solution.

CONCLUSION

In ultrasonic dispersion process of xyloglucan solutions, there should be an appropriate time of treatment. More interestingly, the movements of molecular chains affected the dispersivity of xyloglucan in dilute solution, but the molecular side chains were not obviously influenced by the process from our experimental data. Therefore, the control of the ultrasound time should be paid much attention for the polymer dispersion in dilute solution, since the polymers with the difference chain structures have respective dispersion time.

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AUTHOR CONTRIBUTIONS

Prof. Cao supervised the research. Dr. Chen performed almost all of the experiment with the help of Mengting Lian. Miss Liu wrote and rearranged the manuscript. Sha Chen and Pei Guo corrected same errors of grammar and tenses. Xueying Huang, Chao Qi and Renqiang Sun provided technical assistance.

REFERENCES

1. de Azevedo, W. M.; de Oliveira Luna, A. J. H.; Silva, E.; Silva, R. O. *Ultrason. Sonochem.* **2006**, *13*, 433.
2. Koda, S.; Taguchi, K.; Futamura, K. *Ultrason. Sonochem.* **2011**, *18*, 276.
3. Taghizadeh, M. T.; Asadpour, T. *Ultrason. Sonochem.* **2009**, *16*, 280.
4. Zhou, X.-D.; Lin, Q.-F.; Dai, G.-C.; Ji, F.-X. *Degrad. Stab.* **1998**, *60*, 409.
5. Bang, J. H.; Suslick, K. S. *J. Am. Chem. Soc.* **2007**, *129*, 2242.
6. Boldyrev, V. V. *Ultrason. Sonochem.* **1995**, *2*, S143.
7. Camino, N. A.; Perez, O. E.; Pilosof, A. M. R. *Food Hydrocolloids* **2009**, *23*, 1089.
8. Rodgers, R. M.; Mahfuz, H.; Rangari, V. K.; Chisholm, N.; Jeelani, S. *Macromol. Mater. Eng.* **2005**, *290*, 423.
9. Xu, L. R.; Bhamidipati, V.; Zhong, W.-H.; Li, J.; Lukehart, C. M.; Lara-Curzio, E.; Liu, K. C.; Lance, M. J. *J. Compos. Mater.* **2004**, *38*, 1563.
10. Coviello, T.; Matricard, P.; Alhaique, F. *Expert Opin. Drug Deliv.* **2006**, *3*, 395.
11. Dumitriu, S. Polysaccharides: Structural Diversity and Functional Versatility; Marcel Dekker: New York, **2005**.
12. Shelke, N. B.; James, R.; Laurencin, C. T.; Kumbar, S. G. *Polym. Adv. Technol.* **2014**, *25*, 448.
13. Nishinari, K.; Takemasa, M.; Yamatoya, K.; Shirakawa, K. Handbook of Hydrocolloids; Osaka City University and Dainippon-Sumitomo Pharma Co. Ltd: Japan, **2009**; Vol. *19*, p 535.
14. Lucyszyn, N.; Lubambo, A. F.; Ono, L.; Jo, T. A.; de Souza, C. F.; Sierakowski, M. R. *Food Hydrocolloids* **2011**, *25*, 1242.
15. Mishra, A.; Malhotra, A. V. *J. Mater. Chem.* **2009**, *19*, 8528.
16. Chen, D.; Guo, P.; Chen, S.; Cao, Y.; Ji, W.; Lei, X.; Liu, L.; Zhao, P.; Wang, R.; Qi, C.; Liu, Y.; He, H. *J. Mater. Sci. Mater. Med.* **2012**, *23*, 955.
17. Cao, Y.; Gu, Y.; Ma, H.; Bai, J.; Liu, L.; Zhao, P.; He, H. *Int. J. Biol. Macromol.* **2010**, *46*, 245.
18. Freitas, R. A.; Martin, S.; Santos, G. L.; Valenga, F.; Buckeridge, M. S.; Reicher, F.; Sierakowsk, M.-R. *Carbohydr. Polym.* **2005**, *4*, 507.
19. Brun-Graeppi, A. K. A. S.; Richard, C.; Bessodes, M.; Scherman, D.; Narita, T.; Ducouret, G.; Merten, O.-W. *Carbohydr. Polym.* **2010**, *80*, 555.
20. He, M.; Zhang, H.; Chen, W.; Dong, X. Polymer Physics, 3rd ed.; Fudan University Press: China, **2007**; p 73.