Carbohydrate Polymers 103 (2014) 355–358

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Outer shell, inner blocklets, and granule architecture of potato starch

Junrong Huang*, Ningguo Wei, Hongliang Li, Shuxing Liu, Daqing Yang

College of Life Science and Engineering, Shaanxi University of Science and Technology, Weiyang District, 710021 Xi'an, Shaanxi Province, China

ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form
18 December 2013
Accepted 19 December 2013
Available online 25 December 2013

Keywords:
Potato starch
Outer shell
Blocklets
Granule architecture
SEM

ABSTRACT

Potato starch was heated in distilled water and acetic acid solution (pH = 1.5), respectively, then washed with anhydrous ethanol and dried. After cooked in water with certain program, high-resolution scanning electron micrographs (SEM) of broken granules showed isolated outer shells. After treatment with acetic acid, SEM images of dissilient granules exhibited outer shells with randomly assembled spherical inner blocklets. A refined blocklet model was proposed for potato starch granule, the smaller blocklets were compacted densely to form the stiffer outer shell, and the larger blocklets were packed loosely to form the inner shells.

1. Introduction

The starch blocklet model was suggested by Gallant, Bouchet, and Baldwin (1997) and refined by Tang, Mitsunaga, and Kawamura (2006). This concept is strongly supported by atomic force microscopy (AFM) studies on the surfaces (Baldwin, Adler, Davies, & Melia, 1998; Huang et al., 2013; Jiranuntakul et al., 2013; Sujka & Jamroz, 2009; Szymonska & Krok, 2003) and the internal structures (Baker, Miles, & Helbert, 2001; Ridout, Gunning, Parker, Wilson, & Morris, 2002; Ridout, Paker, Hedley, Bogracheva, & Morris, 2003) of starch granules.

SEM has been used to investigate the shell structure of starch granule after α-amylase hydrolysis (Gallant, Bouchet, Buléon, & Pérez, 1992; Gallant, Bouchet, & Baldwin, 1997; Pilling & Smith, 2003) or after chemical gelatinization (Huang et al., 2013). But the clear observation of blocklets by using SEM was not often. The occurrence of spherical blocklet-like structures after α-amylolysis of starch granules has been reported by Gallant et al. (1992). In this paper, we employ SEM to enable high resolution characterization of the outer shells and the inner blocklets of potato starch granules.

2. Materials and methods

2.1. Materials

Potato starch was supplied by Dingbian Yuanze Company (Yulin, Shaanxi, China). The mean particle size was 28.8 μm and the amylose content was 24.2%. Distilled water was used and the chemicals were all analytical grade.

2.2. Preparation of granules for SEM

Starch granules were treated with 3 different programs for exhibiting the structural features of swollen granules, isolated outer shells, and dissilient granules, respectively:

(1) Program-1: 50 mL starch suspension (2%, w/w, db, in water) was heated on a magnetic stirrer from room temperature to 67 °C in 8 min, and then from 67 °C to 77 °C in 15 min at 300 rpm.
(2) Program-2: 28 g starch suspension (2%, w/w, db, in water) was heated by using a RVA (RVA-TecMaster, Perten Ltd., Sweden) in a program: It was stirred for 10 s at 960 rpm to mix and then for 50 s at 50 °C at 160 rpm, heated from 50 °C to 95 °C in 3.7 min, held at 95 °C for 20 min, cooled to 50 °C in 3.3 min, and held at 50 °C for 2.0 min.
(3) Program-3: 28 g starch suspension (5%, w/w, db, in acetic acid solution, pH 1.5) was heated by using the same RVA in a revised program with some modifications: It was held at 95 °C for 16.5 min, and cooled to 50 °C in 3.8 min.

The above mentioned suspensions were poured into anhydrous ethanol (400 mL, 224 mL, and 224 mL, respectively), while stirred at 500 rpm. The mixtures were gently stirred and settled, the supernatant was drained off, and then the sediment was washed with anhydrous ethanol (50 mL) for 3 times and dried at 40 °C.

* Corresponding author. Tel.: +86 29 86168663; fax: +86 29 86168293.
E-mail addresses: huangjunrong@sust.edu.cn, kathyhjr@yahoo.com (J. Huang).

0144-8617/$ – see front matter
http://dx.doi.org/10.1016/j.carbpol.2013.12.064

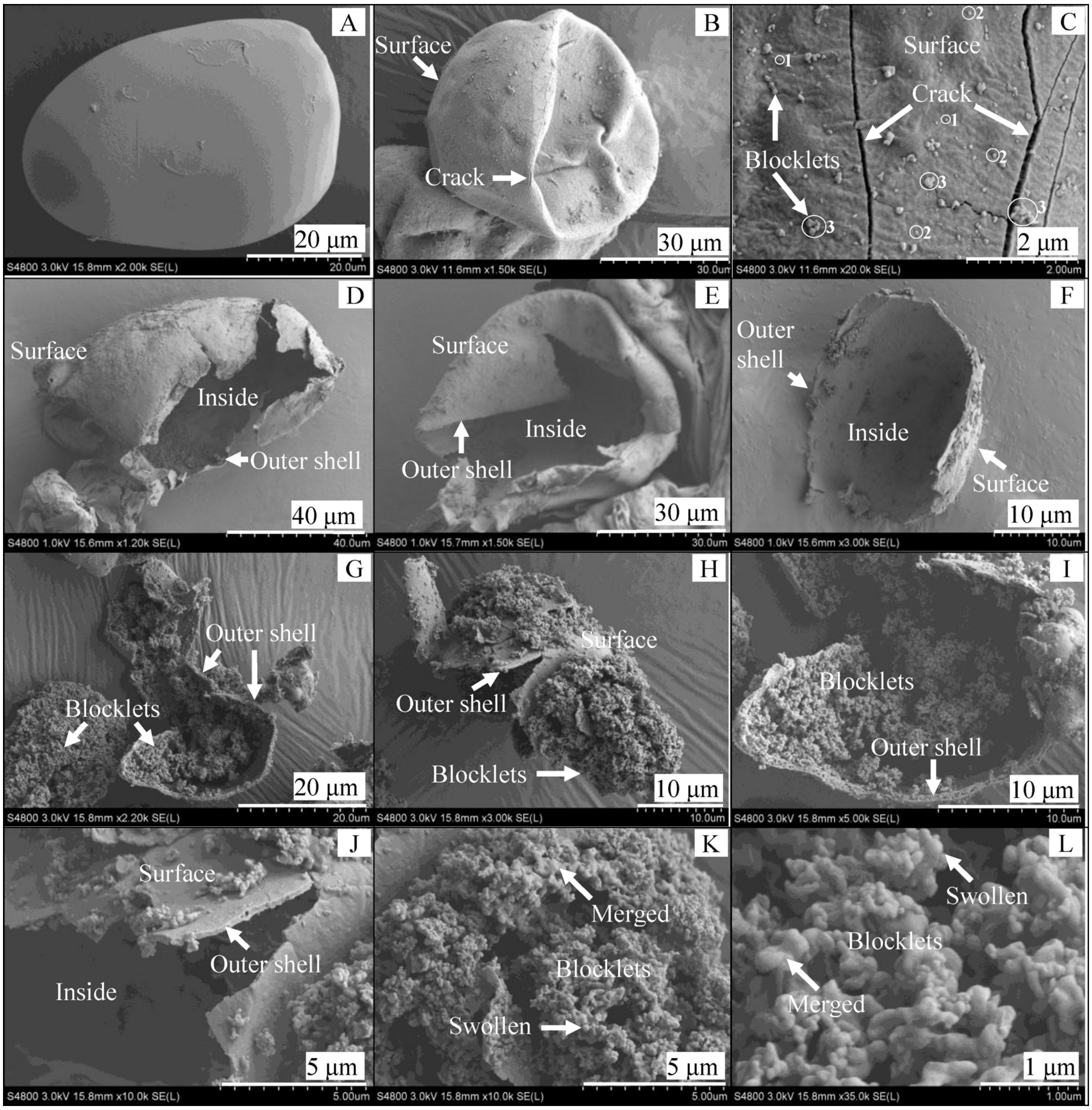

Fig. 1. High-resolution SEM images of the potato starch samples. (A) Native; (B and C) treatment with Program-1; (B) a swollen and distorted granule; (C) part of the granule surface showing cracks and blocklets (3 groups); (D–F) treatment with Program-2; broken granules showing outer shells and almost empty inside; (G–L) treatment with Program-3; (G and H) dissilient granules showing the outer shells and the inner blocklets; (I and J) fracture surface of the outer shells, showing pores or compact structure; (K and L) part of the inner blocklets.

2.3. Observation of granules by SEM

SEM images were obtained with a high-resolution scanning electron microscope (HITACHI S-4800, Hitachi, Japan). The dry starch samples were mounted on a circular metal SEM stub with double-sided sticky tape, coated with platinum, and imaged at an accelerating voltage of 3.0 kV in secondary electron imaging mode.

3. Results and discussion

The size and the amylose content of potato starch used in this study were within the ranges (5.5–72.2 μm and 11.4–31.2%) presented by others (Chen, Schols, & Voragen, 2003; Yusuph, Tester, Ansell, & Snape, 2003; Zaidul et al., 2007). SEM image of native potato starch showed the pristine surface of the granule (Fig. 1A). There were no pores on the surface as pointed out by Fannon and BeMiller (1992).

3.1. Outer shells of potato starch granules

3.1.1. Granules after treatments with Program-1 and 2

After treatment with Program-1, the granules were swollen and distorted, like a deflated basketball with rougher surfaces (Fig. 1B). There were cracks with width of around 10–100 nm on the surface (Fig. 1C). The highly swollen granules with membrane-like envelopes have been shown by light micrograph of normal maize starch after cooking (Obanni & BeMiller, 1995). The granules swelled enormously when cooked in water, due to the uptake of heat and hydration of starch molecules (Fannon & BeMiller, 1992), and the outer shells became fragile toward shear.

After treatment with Program-2, the SEM images showed the broken granules with distinct leathery outer shells (Fig. 1D–F) with almost empty inside, indicating that the outer shells were quite flexible and had the higher resistance to dissolution by heat or shear than the inner shells. The almost completely isolated outer shells provided the possibility of separating the outer shells from the inner blocklets. The results corresponded well with the idea that the periphery was different in structure from the central region of the granule (Fannon & BeMiller, 1992; Fannon, Gray, Gunawan, Huber, & BeMiller, 2004; Gray & BeMiller, 2004; Huber & BeMiller, 2001; Ridout, Parker, Hedley, Bogracheva, & Morris, 2004; Seguchi, Yasui, Hosomi, & Imai, 2000; Ziegler, Creek, & Runt, 2005).

The outer shells covered and protected the inside blocklets, and made each granule independent from each other. It is highly possible that the peak viscosity was linked to the degree of deformability of the outer shells, since peak viscosity occurred at the time point of maximally swelled granules (Blennow, Bay-Smidt, & Bauer, 2001).

3.1.2. Granules after treatment with Program-3

After treatment with Program-3, most granules were broken, showing the distinguished outer shells (Fig. 1G–J). There were no visible inner shells but randomly assembled blocklets. As shown in Fig. 1G, a dissilient granule with the outer shell was like a vessel containing blocklets inside, and resembled a distorted cut pomegranate. The isolated outer shells indicated that the inner blocklets swelled less than the outer shells of potato starch granules, similar to what was found about pea starch granules by Ridout et al. (2004). Some inner blocklets were moved by mechanical force, upturned and attached to the surface of the outer shells (Fig. 1H).

The fracture surface of the outer shells showed the small multipore (Fig. 1I), or compact with the single large pore structure (Fig. 1J). The imperfect or missing blocklets might correspond to these pore-like defects. The thicknesses of the outer shells were between 150 and 500 nm, close to what described about most starch granules that the alternating amorphous and crystalline shells were between 100 and 400 nm thick (Perez, Baldwin, & Gallant, 2009).

The continued shells from inside to the surface (Pilling & Smith, 2003), and the inner shells (Huang et al., 2013) of potato starch granules were observed. The inner shells were destroyed while the outer shells survived after acetic acid treatment, suggesting their different structures. It was suggested that a matrix surrounding blocklets existed (Ridout et al., 2003), and that amylose might serve to connect the blocklets (Tang et al., 2006). This matrix concept might be especially important for the outer shells, since there was an enrichment of amylose toward the periphery of the potato granule (Jane & Shen, 1993), and the starch granules with lower amylose contents were easily deteriorated physically compared with the normal starch granules (Ridout et al., 2003).

3.2. Inner blocklets of potato starch granules

The blocklets appearing on the surface of swollen granule, after treatment with Program-1, could be divided into 3 groups (Fig. 1C): Group-1, single and small (10–50 nm); Group-2, single and large (50–100 nm); Group-3, the agglomerates of several blocklets (100–500 nm). These blocklets might not be in their 'native' size. They were released from the inside of the granule through cracks to the surface after the dismissal of the inner shells, and were obviously different from those revealed by AFM studies on the granule surfaces: densely packing with size of 20–50 nm (Baldwin et al., 1998; Jiranuntakul et al., 2013; Park, Xu, & Seetharaman, 2011; Sujka & Jamroz, 2009; Szymonska & Krok, 2003). The non-uniform deformability of the blocklets suggested their diversity of the structure.

After treatment with Program-2, most inner part of the granules was moved away and only small amount of blocklets were left on the surfaces of the outer shells (Fig. 1D–F). How to recover the inner blocklets successfully remains a question. After treatment with Program-3, the granules were collapsed, and the inner blocklets were exposed (Fig. 1G and H). The agglomeration of the inner blocklets was random and loose (Fig. 1G–L), and different from those observed on the internal structures by AFM (Ridout et al., 2002). The blocklets were most spherical and seemed to be independent from each other, while some of them were expanded and merged (Fig. 1K and L) due to molecular hydration.

The blocklets were thought to be formed by amylopectin side chains (Gallant et al., 1997) or amylopectin superhelices (Oostergetel & van Bruggen, 1993). Nevertheless, it was difficult to estimate the relationship between amylopectin and blocklets, since the published data were not in agreement with each other. The average diameter of amylopectin clusters was 10 nm (Gallant et al., 1997), while that of each amylopectin superhelix was 18 nm (Vandeputte & Delcour, 2004).

The evidence of independent 10 nm blocklets did not support this concept of amylopectin blocklets, since the clusters or the superhelices should be closely connected to each other due to the covalent bonds.

Blocklets are said to be formed by amylose in spherulites, and blocklets observed in amylose spherulites were smaller than those in amylopectin spherulites (Ma, Floros, & Ziegler, 2011).

Based on this point, it was supposed that the amylose chains were packed into the small blocklets, and that amylopectin was a component of the large blocklets. There possibly existed the blocklets composed of both amylopectin and amylose. Since a complete method of isolating the blocklets from starch granules has not been established, it is still very difficult to get information from the discrete blocklets.

3.3. Scheme of a potato starch granule architecture

In association with other isolated evidences in the literature, such as the inner blocklets were larger than the surface blocklets (Huang et al., 2013), a refined model of a potato starch granule architecture was represented schematically in Fig. 2. The layers of blocklets were surrounding the hilum, and were bundled into spheroidal shells packed in the granule. The larger blocklets were loosely assembled inside, but compactly packed of smaller ones at the outer shell of the granule.

The blocklets were similar in shape (spherical) but differed in size (10–100 nm). The pore-like defects observed accidentally in

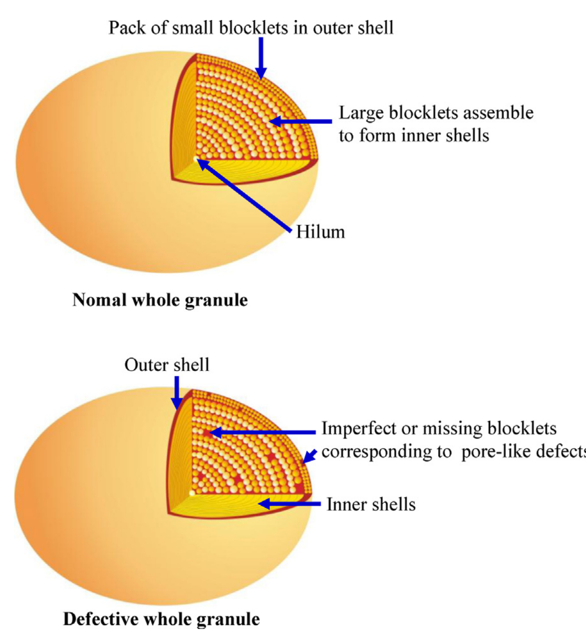

Fig. 2. A schematic diagram of a potato starch granule architecture.

the shells at the core or at the periphery were presented as darker regions in Fig. 2.

For example, with respect to the calculation, as for a granule with mean particle size of 30 μm, let us assume that the granule was a sphere, and there were uniform blocklets with size of 50 nm and shells with thickness of 400 nm, respectively. Thus, there were approximate 10^8 blocklets in one granule; 8 layers of blocklets in one shell; 40 layers of shells in one granule; 300 blocklets aline along the radius of the granule; and the volume and the surface area of the blocklet were 60,000 nm^3 and 8000 nm^2, respectively.

In the former proposed blocklet models of the starch granule, the granule was made up of crystalline and amorphous layers, and the blocklets were similar in shape (elongated) but differed in size according to the botanic source (20–400 nm) (Gallant et al., 1997; Tang et al., 2006). The amylose, amylopectin molecules and other components, such as protein and lipid, were not considered in our model, since their location in blocklet or granule had not been elucidated.

4. Conclusions

The observation of the broken and the dissilient potato starch granules by using SEM after cooked in distilled water and acetic acid solution strongly supports the starch blocklet concept. The distinguished outer shells appeared isolated from the inner blocklets. Such evidence draws a new interpretation of the starch granule structure. It is highly possible that the deformability of the outer shells have the significant effects on the peak viscosity of starch. A refined blocklet model of a potato starch granule with outer shells is proposed. The exact location of amylose in relation to the blocklet organization requires further investigation.

Acknowledgements

The financial supports from the National Natural Science Foundation of China (31071562) and Shaanxi Science and Technology Projects (2011KW-26 and 2011K01-17) are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.12.064.

References

Baker, A. A., Miles, M. J., & Helbert, W. (2001). Internal structure of the starch granule revealed by AFM. *Carbohydrate Research*, *330*, 249–256.

Baldwin, P. M., Adler, J., Davies, M. C., & Melia, C. D. (1998). High resolution imaging of starch granule surfaces by atomic force microscopy. *Journal of Cereal Science*, *27*, 255–265.

Blennow, A., Bay-Smidt, A. M., & Bauer, R. (2001). Amylopectin aggregation as a function of starch phosphate content studied by size exclusion chromatography and on-line refractive index and light scattering. *International Journal of Biological Macromolecules*, *28*, 409–420.

Chen, Z., Schols, H. A., & Voragen, A. G. J. (2003). Physicochemical properties of starches obtained from three different varieties of Chinese sweet potatoes. *Journal of Food Science*, *68*, 431–437.

Fannon, J. E., & BeMiller, J. N. (1992). Structure of corn starch paste and granule remnants revealed by low-temperature scanning electron microscopy after cryopreparation. *Cereal Chemistry*, *69*, 456–460.

Fannon, J. E., Gray, J. A., Gunawan, N., Huber, K. C., & BeMiller, J. N. (2004). Heterogeneity of starch granules and the effect of granule channelization on starch modification. *Cellulose*, *11*, 247–254.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers*, *32*, 177–191.

Gallant, D. J., Bouchet, B., Buléon, A., & Pérez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition*, *46*, S3–S16.

Gray, J. A., & BeMiller, J. N. (2004). Development and utilization of reflectance confocal laser scanning microscopy to locate reaction sites in modified starch granules. *Cereal Chemistry*, *81*, 278–286.

Huang, J., Chen, Z., Xu, Y., Li, H., Liu, S., Yang, D., & Schols, H. A. (2013). Comparison of waxy and normal potato starch remaining granules after chemical surface gelatinization: Pasting behavior and surface morphology. *Carbohydrate Polymers*, http://dx.doi.org/10.1016/j.carbpol.2013.07.086

Huber, K. C., & BeMiller, J. N. (2001). Location of sites of reaction within starch granules. *Cereal Chemistry*, *78*, 173–180.

Jane, J.-L, & Shen, J. L. (1993). Internal structure of the potato starch granule revealed by chemical gelatinization. *Carbohydrate Research*, *247*, 279–290.

Jiranuntakul, W., Sugiyama, S., Tsukamoto, K., Puttanlek, C., Rungsardthong, V., Puncha-arnon, S., et al. (2013). Nano-structure of heat–moisture treated waxy and normal starches. *Carbohydrate Polymers*, *97*, 1–8.

Ma, U. V. L., Floros, J. D., & Ziegler, G. R. (2011). Effect of starch fractions on spherulite formation and microstructure. *Carbohydrate Polymers*, *83*, 1757–1765.

Obanni, M., & BeMiller, J. N. (1995). Identification of starch from various endosperm mutants via ghost structures. *Cereal Chemistry*, *72*, 436–442.

Oostergetel, G. T., & van Bruggen, E. F. J. (1993). The crystalline domains in potato starch are arranged in a helical fashion. *Carbohydrate Polymers*, *21*, 7–12.

Park, H., Xu, S., & Seetharaman, K. (2011). A novel in situ atomic force microscopy imaging technique to probe surface morphological features of starch granules. *Carbohydrate Research*, *346*, 847–853.

Perez, S., Baldwin, P. M., & Gallant, D. J. (2009). Structure features of starch granules I. In J. N. BeMiller, & R. L. Whistler (Eds.), *Starch: Chemistry and technology* (3rd ed., pp. 149–192). New York: Academic Press/Elsevier.

Pilling, E., & Smith, A. M. (2003). Growth ring formation in the starch granules of potato tubers. *Plant Physiology*, *132*, 365–371.

Ridout, M. J., Gunning, A. P., Parker, M. L., Wilson, R. H., & Morris, V. J. (2002). Using AFM to image the internal structure of starch granules. *Carbohydrate Polymers*, *50*, 123–132.

Ridout, M. J., Parker, M. L., Hedley, C. L., Bogracheva, T. Y., & Morris, V. J. (2003). Atomic force microscopy of pea starch granules: Granule architecture of wild-type parent, *r* and *rb* single mutants, and the *rrb* double mutant. *Carbohydrate Research*, *338*, 2135–2147.

Ridout, M. J., Parker, M. L., Hedley, C. L., Bogracheva, T. Y., & Morris, V. J. (2004). Atomic force microscopy of pea starch: Origins of image contrast. *Biomacromolecules*, *5*, 1519–1527.

Seguchi, M., Yasui, T., Hosomi, K., & Imai, T. (2000). Study of internal structure of waxy wheat starch granules by KI/I_2 solution. *Cereal Chemistry*, *77*, 339–342.

Sujka, M., & Jamroz, J. (2009). α-Amylolysis of native potato and corn starches – SEM, AFM, nitrogen and iodine sorption investigations. *LWT-Food Science and Technology*, *42*, 1219–1224.

Szymonska, J., & Krok, F. (2003). Potato starch granule nanostructure studied by high resolution non-contact AFM. *International Journal of Biological Macromolecules*, *33*, 1–7.

Tang, H., Mitsunaga, T., & Kawamura, Y. (2006). Molecular arrangement in blocklets and starch granule architecture. *Carbohydrate Polymers*, *63*, 555–560.

Vandeputte, G. E., & Delcour, J. A. (2004). From sucrose to starch granule to starch physical behaviour: A focus on rice starch. *Carbohydrate Polymers*, *58*, 245–266.

Yusuph, M., Tester, R. F., Ansell, R., & Snape, C. E. (2003). Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions. *Food Chemistry*, *82*, 283–289.

Zaidul, I. S. M., Yamauchi, H., Takigawa, S., Matsuura-Endo, C., Suzuki, T., & Noda, T. (2007). Correlation between the compositional and pasting properties of various potato starches. *Food Chemistry*, *105*, 164–172.

Ziegler, G. R., Creek, J. A., & Runt, J. (2005). Spherulitic crystallization in starch as a model for starch granule initiation. *Biomacromolecules*, *6*, 1547–1554.