**Konjac glucomannan nanocrystals prepared by acid hydrolysis**

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**Abstract:** Konjac Glucomannan (KGM) nanocrystals were prepared from the disruption of KGM by acid hydrolysis. Effect of hydrolysis time on the degree of hydrolysis of KGM and the size of nanocrystals were investigated. Results from Infrared spectroscopy (IR) showed that KGM nanocrystals were still composed of mannose and glucose like the raw KGM. Results from X-ray diffractometer (XRD) revealed that the amorphous state of raw KGM had changed into a typical β-type crystalline state of KGM nanocrystals. Results from transmission electron microscopy (TEM) indicated that irregularly oval shape nanocrystals with the mean size of 15-40 nm were observed.

**Introduction**

Nowadays, there is considerable interest in processing polymeric composite materials filled with rigid particles having at least one dimension in the nanometer range. This class of material which attracts both scientist and industrial communities is called “nanocomposites” [1]. Because of the nanometric size effect, these composites display some unique outstanding properties with respect to their conventional microcomposite counterparts. The developments of new nanocomposite materials are restricted by both the limited availability of nanoparticles and their strong tendency to aggregate [2]. What’s more, the widespread use of polymeric materials has resulted in serious environmental pollution problems. The study and exploitation of a kind of nanoparticles from renewable resources has drawn more and more attention in view of their environmental friendliness to the earth [3]. This ecological awareness has led to development of new biodegradable materials, which can be valid alternatives in specific situations when recycling or incineration is difficult or not economically feasible. Compared to the inorganic nanoparticles originated from mineral resources, natural polymer nanoparticles have some interesting advantages, such as, easy accessibility, very common materials, degradability, and high diversities of structures.

The stable nanocrystals structure suspensions can be obtained from polysaccharide of propagation after acid hydrolysis or alkaline hydrolysis after sonication method to dispersion [4-7]. At the present time, the main nanocrystals were rod-like platelets (eg. cellulose [4, 5] and chitin nanocrystals [8]) and platelet-like nanoplatelets (eg. Starch nanocrystals). The natural polysaccharide nanorods and nanoplatelets with high surface area and rigidity structure present interesting reinforcing properties such as inorganic nano-materials.

The nanocrystals structure from polysaccharide can be totally biodegraded in nature.
and has strong tendency to be biologically consistent. Compared to abiotic-nanoparticles, these nanocrystals can avoid accumulation after usage. Therefore this ecological awareness has led to the renewable polymeric matrix usage, which can substitute abiotic-nanoparticles which resulted in serious environmental pollution and harm to our health. For polymeric nanocomposite materials, the most important role is the interface effects between nanoparticles with nanometric size and the polymeric matrix. Compared to abiotic-nanoparticles, these nanocrystals contain hydroxyl groups on the surface which interacted with the polar groups of polymer matrix to form strong bond interface [9]. Presently, the use of natural polysaccharide nanocrystals has been successfully modified to enhance the rubber, starch plastics, PLA plastic, polyvinyl alcohol, polypropylene, PVC, plastics, soy protein, polyurethane, etc [10-16]. In practice the Italian company Novamont and Goodyear Tire and Rubber Company had used the starch nanoparticles to replace the traditional rubber partially to enhanced fillers-carbon black and silica, to develop environment-friendly and low rolling resistance tires. In addition, the cellulose whiskers were also used to modify polyelectrolyte containing lithium-ion, which can be used as the raw material for lithium batteries [17].

Konjac glucomannan (KGM), one of the most abundant and totally biodegradable renewable resources in nature, has similar structure to cellulose and starch. Its main chain consists of β-1,4 linked mannose and glucose units with a low degree of acetyl groups (Figure 1). KGM is a water-soluble polysaccharide extracted from the tubers of konjac.

![Fig. 1. Chemical structure of KGM.](attachment:image)

As a health food which has long been used in China and Japan, KGM has availability in large quantities, renewability, biodegradability, and low cost [18]. The raw and physical or chemical modified KGM had successfully applied in various fields such as pharmaceuticals, food, and chemical engineering [19-22]. The semi-rigid structure and the inter-crystal structure of KGM facilitate preparation of nanocrystals. The Native KGM with crystalline state consists of manna type I (without water molecules in the crystal structure) and manna type II (water molecules in the crystal structure) [22, 23].

In the present work, water-insoluble and highly crystalline residue of KGM nanocrystals with out-of-order sequences containing glucose and mannose were obtained by using sulfuric acid hydrolysis [24, 25]. The nanocrystals suspensions with good stability were treated by alkalescence solution neutralization [26]. The morphology and chemical structure of the resulted nanocrystals were investigated.
Results and discussion

Morphology of KGM nanocrystals

TEM micrographs of negatively stained KGM samples obtained by hydrolysis in 3.2 M H₂SO₄ at 40 °C for various days are shown in Figure 2. After hydrolyzing KGM for several days, micrometersized fragments could be seen in the TEM photographs.

![TEM micrographs of negatively stained samples of KGM nanocrystals hydrolyzed for 4 days (a), 5 days (b), 6 days (c), 7 days (d, e, f), respectively.](image)

In Figure 2a, the lamellar structure of particles protruding out of the contrasting layer was observed. In Figure 2b and 2c, irregular oval platelets were observed, indicating that the amorphous region in KGM molecular chains collapsed after hydrolysis and the crystalline region of hydrolyzed residue was formed. In Figure 2d-f for hydrolyzed residue after 7 days, nearly individual KGM nanocrystals were observed. Despite the variety of shapes, characteristic geometrical features of the nanocrystals with mean size of 15-40 nm were observed.

The experimental data results and the analysis in this study have proven to be very useful for establishing the optimization of the preparation of aqueous suspensions of KGM nanocrystals. Take the results from TEM into consideration, the optimal preparation conditions for KGM nanocrystals were 7 days of hydrolysis time, 3.2 M concentration of sulfuric acid, 40 °C of hydrolysis temperature, and 12.5 wt% of KGM concentration. The yield of nanocrystals was 23.7 wt %.

X-ray analysis

The raw KGM samples consist of two kinds of crystal state: one is α-manna type
(non-crystalline), the other is β-manna type (crystalline) [32]. Figure 3 showed the X-ray diffraction pattern of raw KGM (a) and KGM nanocrystals (b).

![Figure 3. X-ray diffraction pattern of original KGM (a) and KGM nanocrystals hydrolyzed for 7 days (b).](image)

The crystallinities of raw KGM, N-KGM were calculated to be 11.2% and 38%, respectively. For the raw KGM, no obvious diffraction peaks were found, indicating that KGM is in an amorphous state corresponding to α-manna type. For KGM nanocrystals after the hydrolysis for 7 days, one can see two sharp diffraction peaks at around 2θ=20.9° and 2θ=14.7°, respectively, indicating that the crystallinities of KGM nanocrystals increased significantly in contrast to the raw sample. It can be explained that sulfuric acid broke the intermolecular and intramolecular hydrogen bonds in the hydrolysis process, resulting in oligosaccharides with low molecular weight and high degree of crystallinity. In other words, the amorphous sequences of KGM were disrupted and the highly ordered sequences of KGM remained after hydrolysis.

**Structure of KGM nanocrystals**

The IR spectra of raw and nanocrystal of KGM at the range of 4000-500cm\(^{-1}\) are shown in Figure 4. The peaks at 2920 and 2885 cm\(^{-1}\) are attributed to the stretching of methyl groups, and of the peak at 1730 cm\(^{-1}\) is assigned to the carbonyl of acetyl groups in KGM [33]. The characteristic absorption bands of mannose in KGM appeared at 876 and 808 cm\(^{-1}\) [18]. The absorption band at 1653 cm\(^{-1}\) was attributed to the intramolecular hydrogen bonds. The peaks at 1100 and 1022 cm\(^{-1}\) were assigned to the stretching of C-O-C groups. In contrast, the spectrum of KGM nanocrystals is quite similar to that of original KGM and no new peaks appeared, indicating that the chemical structure and molecular linkage of KGM did not change after hydrolysis.

The only difference between the original KGM and KGM nanocrystals is the stretching vibration state of hydroxyl groups. In comparison, the stretching vibration of hydroxyl
groups changed from 3423 cm\(^{-1}\) to 3350 cm\(^{-1}\), and the peak shape of KGM nanocrystals became narrower, indicating the intermolecular hydrogen bonding state of original KGM was interrupted partially.

![IR spectra](image)

**Fig. 4.** IR spectra of original KGM (a) and KGM nanocrystals hydrolyzed for 7 days (b).

**Conclusions**

KGM nanocrystals with mean size about 15-40 nm were prepared by hydrolysis using 3.2 M sulfuric acid at 40 °C for 7 days from original KGM. The chemical structure and molecular linkage of KGM nanocrystals showed no change compared to that of the original KGM. The crystallinity of KGM nanocrystals was significantly improved owing to that the crystal state of KGM had changed from amorphous state (\(\alpha\)-type) to highly crystalline state (\(\beta\)-type), resulting from the disruption of amorphous region and the arrangement of ordered region of KGM by acid hydrolysis. The promising application of biodegradable KGM nanocrystals is expected to attract more attention in the future.

**Experimental**

**Materials**

The raw KGM samples used in this study were purchased from Qiangsen Food Co Ltd. (Wuhan, China) and further purified by mixing with three times weight of 50 wt % and 80 wt % ethanol for 2 h, respectively, and with waterless ethanol for 4 h, and then vacuum-dried at 60 °C for 4 h. The intrinsic viscosity ([\(\eta\)]) of cadoxen solution of raw KGM materials was measured to be 585 cm\(^3\)g\(^{-1}\) by using Ubbelohde viscometer (Ningbo Tianheng Instrument Co. Ltd., China) at 25 ± 0.05 °C and the viscosity-average molecular weight (M\(_v\)) of raw KGM was calculated to be \(1.25\times10^6\) according to the Mark-Houwink equation \([27]\): \([\eta] = (3.55\times10^{-2}) M_v^{0.69}\).
All other chemical reagents were commercially purchased and of analytical grade.

**Preparation of the Nanocrystals**

The optimal hydrolysis time was predetermined according to the yield of hydrolyzed residue calculated by the weight ratio of the freeze-dried hydrolyzed products to the initial KGM sample and the apparent mean size of hydrolyzed particles measured by laser granulometry. Parameters initial KGM concentration and speed of stirring do not influence hydrolysis kinetic because of its catalytic nature [24, 28]. The concentration of sulfuric acid of 3.2 M and the hydrolysis temperature of 40 °C were chosen according to Dufresne [24] and Cao et al [15]. The effect of hydrolysis time on the yield of hydrolyzed residue and the apparent mean size of hydrolyzed particle was illustrated in Table 1. From 4 to 7 days, the yield of hydrolyzed residue and the apparent mean size of hydrolyzed particle decreased gradually with increasing hydrolysis time.

**Tab. 1.** Effect of hydrolysis time on the yield of hydrolyzed residue and the apparent mean size of hydrolyzed particle.

<table>
<thead>
<tr>
<th>Hydrolysis time / day</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of hydrolyzed residue / wt.%</td>
<td>30.1</td>
<td>28.2</td>
<td>26.7</td>
<td>23.4</td>
<td>22.5</td>
</tr>
<tr>
<td>Apparent mean size of hydrolyzed particle / μm</td>
<td>8.0</td>
<td>4.0</td>
<td>3.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

However, the apparent mean size of hydrolyzed particle after 8 days was abnormally increased. This result is unexpected and interesting, because our goal was to prepare small residues. The similar results had been observed for the preparation of nanocrystals from waxy maize starch [24].

The 37.5 g of KGM water suspension (12.5 wt %) was mixed with 300 ml of 3.2 M H₂SO₄ solution in a 500 ml Erlenmeyer flask. The suspensions were thermo-stated at 40 °C with stirring at 300 rpm and with intermittent shaking. After several days, the suspension was cooled to room temperature and the insoluble particles were filtered and diluted with water. Then the suspension was repeatedly washed with distilled water by successive centrifugations at 6000 rpm until the pH of suspension reached up to about 3. The supernatant containing the agglomerated KGM nanocrystals particles were dialyzed against distilled water for several days until the pH of outside membrane water reached neutrality [26]. However, pH value of the inner suspension was around 5. Then 1.0% (w/w) NH₃ solution was added to the KGM nanocrystals suspension with stirring to adjust the pH value of suspension to about 7.0. The obtained suspension was washed with distilled water by successive centrifugations until neutrality. Finally, the nanoparticles were obtained by freeze-drying the suspension.

**Characterization**

-Transmission Electron Microscopy

Transmission electron microscope (TEM) observations were performed using a Hitachi H7000-FA (Japan) microscope. Specimens for conventional TEM imaging were prepared. After sonication, a drop of dilute KGM nanocrystals suspension was deposited onto a glow discharged copper-coated microscopy grid. Prior to complete drying, a drop of 2% (w/v) uranium acetate negative stain was added. After 1 min, the
liquid in excess was blotted with filter paper and the remaining film was allowed to dry. Unstained specimens for electron diffraction purpose were also left to equilibrate overnight in a 95% relative humidity atmosphere. Once positioned into a Gatan 626 specimen holder, they were quench-frozen in liquid nitrogen, transferred into the microscope, and observed at low temperature (-180°C). All specimens were observed using a Hitachi H7000-FA TEM, operated at 80 kV for imaging and 200 kV for diffraction. Micrographs were recorded on Kodak SO163 films.

-Laser granulometry measurements

Laser granulometry measurements were carried out with a Malvern Mastersizer (DLS, Brookhaven Instruments Co., USA). The suspensions were characterized from the median article size $d_{50}$, which divides the population into two equal halves. The experimental results were affected by the choice of dispersant, the dispersion medium, and the mixing time [29]. So the results from laser granulometry are the aggregate size of the particles, rather than the primary structure size [30].

-X-ray Diffraction

The X-ray diffraction (XRD) patterns of the powder samples were recorded with a Rigaku (Denki, Japan) D/Max-A X-ray diffractometer and used a Cu Ka1 radiation ($\lambda=0.154$ nm) at 30 kV and 50 mA. The diffraction angle ranged in a range of $2\theta=3°$~$60°$ using a fixed time mode with a step interval of 0.028. Additionally, the crystallinity was calculated by the equation [31]:

$$Xc = Fc / (Fc + Fa) \times 100\%$$

where $Xc$ is crystallinity, and $Fc$ and $Fa$ are the areas of crystal and no crystalline regions, respectively.

-IR spectroscopy

IR spectroscopy of the powder samples were recorded with a Fourier transform infrared spectrometer (60SXBM, NICOLET, U.S.A). The test specimens were prepared by the potassium bromide method.

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