Stability of partially deacetylated chitin nano-fiber dispersions mediated by protonic acids

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ABSTRACT

Partially deacetylated chitin nano-fiber (DACHN) dispersions were prepared using mechanical treatment of partially deacetylated crab shell α-chitin under acidic conditions mediated by various protonic acids. The representative organic acids had a better efficiency in terms of mediating the nanofibrillation of chitin fibers in comparison to the inorganic acid (HCl). The DACHN dispersed in water at a pH of 3.5 mediated by gluconic acid exhibited the highest yield of nano-fibers (88.6%), followed by succinic, itaconic, and ascorbic acids with a nano-fiber yield of 79.9%, 73.3% and 66.0%, respectively. The pH, conductivity, viscosity, and light transmittance of dispersions were assessed under different storage times. All of the tested DACHN dispersions were stable for at least 15 weeks at 4 °C.

Keywords: partially deacetylated chitin nano-fiber; protonic acid mediation; dispersion stability

1. INTRODUCTION

Nanomaterials derived from natural polymer sources possess extra biological properties. Several renewable and biodegradable polymers, such as cellulose, chitin and starch, are abundant in nature and have the potential to be transformed into nano-whiskers by special processing.¹² In recent years, chitin has become a popular feedstock of natural polysaccharide-based nanomaterial due to its abundance in animals, plants and microorganisms along with its special biodegradable, biocompatible and non-toxic properties.²⁻⁴ Hence, chitin has become an ideal material for applications in the fields of biomedicine, food and cosmetic additives.⁵⁻⁷ Chitin is highly crystalline and known to be structurally similar to cellulose, consisting of acetyl-anhydro-glucosamine and some anhydro-glucosamine units linked with β-(1,4) glucosidic bonds⁸. Furthermore, the high insolubility of chitin is attributed to the polymer chains associated with each other by strong intra- and inter-molecular hydrogen bonds.⁹⁻¹¹

It has been reported that nano-fibers could be prepared from crab/shrimp chitin by traditional acid-hydrolysis with 3 M HCl at a high temperature followed by mechanical dispersing treatment in an aqueous medium. The acid hydrolyzed chitin nano-whiskers were reduced to a width of 10-15 nm and a length of 200-500 nm. However, a substantial loss was caused by the initially extreme conditions in the process.¹²¹³ Subsequently, a new method was established on crab chitin nano-fibers dispersing with positive charges on the surface of the crystalline. The crab chitin was first treated with 33% (w/w) NaOH to partially remove the acetyl groups on the C-2 position. Then, continuous homogenization and ultrasonication were conducted on the partially deacetylated chitin (DACH) in the acetic solution to prepare individual nano-fibers with average width and length of 6 nm and 250 nm respectively.¹⁴ Later, Qi and Fan, et al, selected a different protonic acid to mediate the cationization of chitin and to further improve the nanofibrillation efficiency.¹⁵ Highly transparent dispersions, consisting of mostly individualized partially deacetylated α-chitin nanofibrils (φ = 3.4 ± 1.5 nm), were obtained when ascorbic acid and monovalent acids were used in water at a pH of 3.5. These research results are valuable for a better understanding of nanofibrillation behavior of chitin fibers and further developing applications, but little results have been reported on the cationization and the stability and film formation of chitin nano-fibers.

In this study, nontoxic and environmental friendly acids (gluconic, succinic, malic, itaconic and ascorbic acid) were used to mediate the cationization of partially deacetylated α-chitin and to further prepare chitin nano-fiber dispersions. The stability of chitin nano-fiber dispersed in various acids was evaluated by measuring the pH, the conductivity, the viscosity and the light transmission at different storage times.

2. EXPERIMENTAL

2.1 Materials

Fresh crab (Paralithodes camtschaticus) shells, collected from food waste (Qidong, China), were cut into small flakes and stored in ethanol/water in a refrigerator before purification. All chemicals were reagent grades and used without further purification (Nanjing chemical reagent co.,

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2.2 Purification and deacetylation of chitin

Crab shell flakes were soaked in acetone/water (9/1, v/v) for 5h to extract lipids and pigments. Demineralization, deproteinization and bleaching were carried out with 1 M HCl, 1M NaOH, and 0.3% w/v NaClO₂, respectively. These protocols were conducted twice to prepare purified α-chitin. Subsequently, partial deacetylation was carried out according to a reported procedure. The purified α-chitin slurry was treated at 90°C for 3 h with a 33% (w/v) aqueous NaOH solution, containing a small amount of NaBH₄, to prevent depolymerization. The partially deacetylated chitin (DAC) sample thus obtained was collected in a Buchner funnel, thoroughly washed with distilled water, and stored at 4°C without drying before use. The degree of N-acetylation (DNAc) of DAC (0.67), was calculated from the nitrogen/carbon content ratio obtained by elemental analysis using a Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., USA); this value corresponded to an amine group content of 1.77 mmol/g.

2.3 Preparation of partially deacetylated chitin nano-fiber (DACN) dispersions

A 0.5% (w/w) DAC suspension was prepared in a centrifuge tube using the ~10% DAC slurry in distilled water. The pH of the DAC suspension was adjusted in the range of 3-4 by controlling the amount of the acid added. The suspension was then treated for 30 seconds to obtain a highly viscous dispersion with a homogenizer (T25, Changzhou guohua electric appliance co., LTD). Then, the dispersion was subjected to three “two minutes sonication cycles” using an ultrasonic generator (VCX-500, SONICS, USA). A homogeneous and viscous dispersion was obtained after centrifugation. The yield (Y) of DACN in the dispersion was calculated from the dry weight of the non-fibrillated fractions (μ) according to the following equation: Y (%) = 100 × (mt – μ)/mt, where mt is the dry weight of total DAC in the dispersion.

2.4 Stability evaluation of the DACN dispersions

The 0.2% (w/w) DACN dispersions were stored in sample bottles under 4°C. The changes in pH, conductivity, viscosity and light transmittance were measured at 0, 1, 3, 6, 10 and 15 weeks, to assess the stability of dispersions.

2.5 Instrumental measurements and characterization

Transmission electron microscopy (TEM) images of chitin nano-fibers were obtained with a JEOL electron microscope (JET, 2100) at an accelerating voltage of 100 kV11. A rotational rheometer (RS6000, HAAKE) was used to determine viscosity of the 0.2% (w/w) DACN dispersions. The pH value and conductivity were tested by an auto-titrator (AUT 701, TOADDK). An ultraviolet spectrophotometer (UV-2100pro) was used to examine the light transmittance of the 0.2% (w/w) DACN dispersions.

3. RESULTS AND DISCUSSION

3.1 Nanofibrillation behaviour of DACN mediated by different protonic acids

The previous studies showed that nanofibrillation of chitin can be achieved through mechanical treatment of positive charged partially deacetylated α-chitin with a small amount of acetic acid. The electrostatic repulsion between the chitin molecular chains brought by the high density positive charges of NH₃⁺ at pH 3-4 was the main driving force for the separation of the chitin nano-crystals. Protonation of the C2-NH₂ of the DAC under acidic conditions was a key factor in the preparation of nano-fibers. Based on this mechanism, it was predicted that different acids, regardless of their organic or inorganic nature, can act as a protonic acid to cationize the C2-NH₂ of partially deacetylated chitin and further lead to the nano-dispersing of chitin fibers. In this study, six kinds of representative protonic acids listed in Table 1 (monovalent acid: acetic acid; polyvalent acid: succinic acid; hydroxyl acid: gluconic acid; vinyl acid: itaconic acid; amino acid: aspartic acid; inorganic acid: HCl) were used to cationize the partially deacetylated α-chitin (degree of N-acetylation: 0.67). The nanofibrillation behaviour of chitin fibers mediated by different protonic acids was estimated from various aspects, including appearance, light transmittance of nano-dispersions, and the nanofibrillation efficiency.

The pH of the DACN suspension was adjusted to 3.5 by controlling the amount of the acid added. Larger amounts of acid had to be added to the dispersion when using weak acids, whereas the opposite was the case for strong acids to reach the target pH (3.5). In either case, when the DACs with DNAs 0.67 were disintegrated in water at a pH of 3.5, they were changed to highly viscous, homogeneous and transparent dispersions (Fig. 1). The dispersions consisted of solid fibrils of partially deacetylated α-chitin disintegrated at nano scale in acidic water. The homogeneous and transparent DACN dispersions can be achieved effectively by all of the protonic acids used in this study as listed in Table 1. In contrast, when the DACs were disintegrated in water at a pH 6-7 without the mediation (or cationization) by any protonic acid, the slurries were not homogeneous. The Birefringence was observed at rest between cross polarizers for the transparent DACN nano-fiber dispersions at a consistency of 0.2 % (Fig. 1). The birefringence indicated that the nano-fibers had optical anisotropy, were highly viscous, and stably present in the acidic aqueous dispersions.

The yield and the light transmittance of DACN dispersions mediated with different protonic acids at 600 nm were shown in Figure 2. The yield calculated by the ratio of chitin nanofibers in the dispersion to

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LTD, China and Sinopharm Chemical reagent co., LTD, China).
Table 1 The representative protonic acids and the abbreviation of chitin nano-fibers

<table>
<thead>
<tr>
<th>Description</th>
<th>Name</th>
<th>Structural formula</th>
<th>The abbreviation of partially deacetylated chitin nano-fiber mediated by various protonic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent acid</td>
<td>Acetic acid</td>
<td><img src="image" alt="Acetic acid" /></td>
<td>DAChN-Ac</td>
</tr>
<tr>
<td>Polyvalent acid</td>
<td>Succinic acid</td>
<td><img src="image" alt="Succinic acid" /></td>
<td>DAChN-Su</td>
</tr>
<tr>
<td>Hydroxyl acid</td>
<td>Gluconic acid</td>
<td><img src="image" alt="Gluconic acid" /></td>
<td>DAChN-Gl</td>
</tr>
<tr>
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<td>Itaconic acid</td>
<td><img src="image" alt="Itaconic acid" /></td>
<td>DAChN-It</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Aspartic acid</td>
<td><img src="image" alt="Aspartic acid" /></td>
<td>DAChN-As</td>
</tr>
<tr>
<td>Inorganic acid</td>
<td>Hydrochloric acid</td>
<td>HCl</td>
<td>DAChN-Hy</td>
</tr>
</tbody>
</table>

Fig. 1 Digital photos and Birefringence of DACHN dispersions mediated with different protonic acids at pH 3.5 (a: acetic acid, b: succinic acid, c: gluconic acid, d: itaconic acid, e: aspartic acid, f: HCl).

non-fibrillated ones reflected the nanofibrillation efficiency to some degree. In this study, nanofibrillation efficiency was compared at different amounts of protonic acid. The yield of DACHN-Gl mediated with gluconic acid achieved the highest yield (88.6%), which was two times higher than that of DACHN-Ac (39.8%) mediated with acetic acid. The pKa of acetic acid and gluconic acid were 4.76 and 3.86 respectively. Hence, the stronger H⁺ dissociation of gluconic acid in comparison to that of acetic acid, leads to a higher nanofibrillation efficiency (higher yield of DACHN) using the same amount of acid. Further increments in the amount of acetic acid used, can also increase the DACHN yield to over 80% (data not shown here). All of the dispersions mediated with organic acids had a higher yield of chitin nano-fibers as to DACHN-Hy (37.6%) mediated with the inorganic acid (HCl). Because most organic acids are weak acids, the H⁺ were gradually dissociated from the organic acid to continuously mediate the cationization of C2-NH₂ groups of chitin and to further promote nano-fibrillation under electrostatic repulsion along with mechanical processing. In contrast, for inorganic acids, almost all the H⁺ was dissociated at the initial stage, and there is no more H⁺ left to be produced in the later stage for electrostatic repulsion in the mechanical treatment process.

Accordingly, the DACHN dispersions showed different grades of transparency. Transparency and viscosity were affected by the size of the fibers, aspect ratio, solid content of fibers and other factors. Therefore, the transparency parameter could reflect fiber dispersion state. Using gluconic acid to mediate the cationization and dispersing of DACHN in water at pH 3.5, the highest light transmittance was obtained (up to 94.6%), whereas acid hydrolyzed α-chitin12 and TEMPO-oxidized α-chitin13 mediated by acetic acid had a transmittance below 80%. Thus, the individualization of α-chitin nano-fibers in the gluconic acid mediated in aqueous dispersions achieved in this study can be regarded as the highest level of fiber disintegration. The light transmittance of the DACHN dispersions mediated with succinic acid, itaconic acid, L-aspartic acid and HCl were higher than those mediated with acetic acid. These results supported that complete nano-dispersion can be achieved by mechanical disintegration at acidic conditions mediated by the aforementioned protonic acid. Once more, this proved that the introduction of high-density cationic charges on the crystalline fibril surfaces had brought about such transparent dispersions consisting of mostly individualized α-chitin nano-fibers.

TEM observations revealed that the transparent dispersions consisted of rod-like nano-fibers, showing that individualization of α-chitin fibrils was achieved by partial deacetylation with 33% NaOH at 90 °C for 3 h and...
subsequent mechanical disintegration in water at a pH of 3.5 mediated with gluconic acid (Fig. 3). This is in full agreement with the previous results related to the mediation induced by acetic acid. An obvious fiber mesh structure was observed. The individual fibrils had a length greater than 200 nm and an average width between 10-20 nm. The TEM images of DACHN confirmed that the transparent dispersions consisted of solid fibrils of partially deacetylated α-chitin dispersed at the nano level.

![Fig. 3 TEM images of DACHN-Gl dispersion mediated with gluconic acid.](image)

![Fig. 4 The changes of conductivity and pH of the DACHN dispersions mediated with various acids at different storage times (a: acetic acid, b: succinic acid, c: gluconic acid, d: itaconic acid, e: aspartic acid, f: HCl).](image)
3.2 Stability of DACHN dispersions

The stability of DACHN dispersions is one of the many important properties for its applications. The high UV–vis transmittance was unchanged for at least two months after preparation of the DACHN dispersion mediated with acetic acid. Thus, the DACHN nano-whiskers can maintain the stable dispersion state without peeling-off of the DACH molecules from the surface of the crystallites in water at pH 3–4 for at least two months. However, some unstable DACHN dispersions were found when mediating the DACHN with boric acid. Crystallization (distinct white sediments) occurred under refrigeration due to the natural physico-chemical properties of boric acid. Therefore, no further data on the unstable dispersions was necessary.

Changes in pH value, conductivity, viscosity and transparency of DACHN dispersions during storage times of with 0, 1, 3, 6, 10 and 15 weeks at 4 °C were then investigated for the relatively stable system.

The changes of conductivity and pH value of dispersions mediated with various acids at different storage times are shown in Figure 4. The results showed that the changes in pH and conductivity of DACHN dispersions were insignificant for most of the protonic acids used in this study. The changes of pH and conductivity of DACHN dispersion mediated with itaconic acid (DACHN-It) were very small when compared with other acids (Figure 4d) although this mediation represented the highest durability and stability.

The DACHN dispersion mediated by gluconic acid (DACHN-Gl) appeared to have a slight decrease in pH and
an increase in conductivity after 6 weeks, and had pronounced changes when the storage time reached 10 weeks (Figure 4c) with no significant changes once exceeded. This was probably due to the self-change of gluconic acid aqueous solution with long-time storage caused by dissociating of H⁺ from the weak acid due to its strongest acid value among the monovalent organic acids. However, the viscosity and transmittance of the DACHN-Gl dispersion remained relatively stable within the 15 weeks of storage. In this study, the boric acid was also used as a protonic acid to mediate the DACHN dispersing. Although the boric acid was effective in preparing nano-fibers, sediment of borate crystals appeared when the dispersion was stored at low temperatures, indicating that the DACHN dispersion prepared with boric acid mediation was evidently unstable.

The viscosity of DACHN dispersions mediated with various acids at different storage times are shown in Figure 5. The DACHN dispersions at the same concentration of 0.2 % showed shear-thinning gel-like property when the shearing rate increased from 1 to 2000 1/s. The viscosity of a polymer solution is the characteristics of its intermolecular interactions between polymer chains. As polymer molecular structure existing in DACHN dispersions, the polymer molecule chain of chitin unwrapped or stretched along the shearing direction. The viscosity values of the dispersions decreased as shearing rate increased. The viscosity gradually became constant when the shearing rate was over 1000 1/s. The viscosity of the dispersions remained almost constant during the 15-week storage time. These results indicated that the DACHN was maintained at the nano-scale without assembling or aggregation and can remain dispersed at least up to 15 weeks of storage time.

**Fig. 6** Transmittance of DACHN dispersions mediated with various acids at different storage times. (a: acetic acid, b: succinic acid, c: gluconic acid d: itaconic acid, e: aspartic acid, f: HCl)
The changes of transmittance of the DACHN dispersion are shown in Figure 6. Most of the DACHN dispersions mediated with various acids at different storage times had good transparency. Originally, the DACHN was dispersed in water at pH 3.5 to form stable and transparent aqueous nano-fiber dispersions. Subsequently, most of the dispersions were stable, and transparent with high light transmittance without any precipitation even after 15 weeks of storage. The constant light transmittance of the dispersion indicated that there was no aggregation formed within 15 weeks storage time under the tested conditions. For comparison, our previous results have shown that the DACHN dispersions mediated with ascorbic acid had unstable transmittance during the storage time.

The pH value, conductivity, viscosity and light transmittance of the DACHN dispersions mediated by any of the protonic acids used in this study except boric and ascorbic acids were stable in storage up to 15 weeks at 4°C.

4. CONCLUSIONS

The nanofibrillation of chitin fibers were obtained through mechanical treatment of partially deacetylated a-chitin in water under acidic conditions mediated by a protonic acid regardless of organic or inorganic nature. The nanofibrillation mediated by different acids showed similarities in the transparency, homogenous appearance, and light transmittance of the resulting nano dispersion. However, the nanofibrillation efficiency (yield of nano fibers) was different, and so was the stability of the nano dispersion. The DACHN-Gl dispersed in water at pH 3.5 mediated by gluconic acid showed the highest yield of nano-fibers, about 88.6%. The DACHN dispersions (except those mediated with boric acid and ascorbic acid) showed good durability and stability for at least 15 weeks. As to the DACHN-Gl dispersion, the pH and conductivity had some fluctuation after 6 weeks due to self-dissociation of gluconic acid, but the viscosity and transmittance remained stable for a minimum of 15 weeks.

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