



CHITIN LIQUID CRYSTAL-DERIVED SPONGE-LIKE AEROGEL

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Abstract. Chitin nanocrystals in anisotropic liquid crystals have been used as a colloidal precursor to fabricate hydrogels and aerogels. Native chitin nanofibrils are deacetylated and hydrolyzed to generate rod-shaped chitin nanocrystals that are dispersible in water to form colloidal aqueous suspensions. Chitin nanocolloids self-organize into anisotropic liquid crystals that can solidify into layered nematic films. Chitin liquid crystals are hydrothermally gelatinized with formaldehyde crosslinkers to form homogeneous chitin hydrogels. The removal of water in the hydrogels by freeze-drying recovers ultralight chitin sponge-like aerogels with morphological retention of layered nematic chitin structure. These biocompatible chitin aerogels hold promise for developing advanced functional materials such as fabrics for antibacterial bandages, tissue engineering, and hydrophobic absorbents for oil/water separation. Potentially, chitin nanocrystals assembled in the aerogels may be functionalized into hydrophobic sponges for oil/water separation or carbonized into nitrogen-doped carbon foams for supercapacitors.

Keywords: chitin nanocrystal, liquid crystal, gelation, hydrogel, sponge-like aerogel

1 Introduction

Over two centuries after Braconnot's discovery of chitin [1], the inspiration of chitin-based materials has paved a sustainable way for biomedical [2], environmental, and energy applications [3]. Chitin is a polymer of *N*-acetyl-D-glucosamine units linked by β -1-4 bonds and is abundant in the shells of crustaceans and the cell walls of fungi [4]. Chitin is considered as a cellulose derivative with acetamide groups at C2 positions, which makes it be more versatile than cellulose for chemical functionalization [5]. Due to the inherent characteristics of biocompatibility, biodegradability, and biodiversity, chitin is a novel carbohydrate for producing sustainable materials [6].

Native chitin often has nanofibril structures with variable sizes and geometries depending on biomass sources [7]. In arthropod exoskeletons, chitin macromolecules aggregate into crystalline nanofibrils that exhibit the structural complexity at different levels of nanofibril

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assemblies. Some arthropods such as jewel beetles and mantis shrimps possess their chitin fibril-based exoskeletons with helicoidal ordered structures analogous to lyotropic liquid crystals [8]. These chiral chitin structures can interact with visible light to appear circularly polarized coloration, presenting an interesting way to exploit biomimetic materials with photonic properties [9].

In 1993, Revol and Marchessault pioneered the discovery of the preparation of chitin nanocrystals by acid-catalyzed hydrolysis of chitin fibrils [10]. Chitin fibrils are hydrolyzed by acid to decompose its amorphous regions and transform into rod-shaped chitin nanocrystals. Chitin liquid crystals can conserve its anisotropic structure on solidification to generate optically active films. Revol and Marchessault's pioneering work remarks an important milestone for later studies of using chitin nanocrystals as a precursor or template to fabricate new materials via self-aggregation [11].

The most previous efforts for the synthesis of chitin-based materials have relied on the evaporation-induced self-assembly of chitin nanocrystals [12]. These successful works have often obtained stacked thin films from the deep aggregation of chitin liquid crystals. However, the preparations of highly porous aerogel-like materials from chitin liquid crystals are not yet reported much. These highly porous biopolymers are anticipated to be lightweight materials that may be sponge-like attractive for sustainable applications.

Herein, we report the fabrication of ultralight sponge-like aerogels from chitin liquid crystals. Chitin nanofibrils were purified from crustacean shells and sequentially hydrolyzed to generate rod-shaped chitin nanocrystals. The positively charged chitin nanocolloids dispersed in water and self-organized into an anisotropic phase to form liquid crystals. Chitin liquid crystals were hydrothermally gelatinized with formaldehyde crosslinkers to form chitin hydrogels. The hydrogels were freeze-dried to generate ultralight chitin sponge-like aerogels that exhibited the structural integrity of the layered chitin nanocrystal assemblies.

2 Experimental

Chemicals

Crustacean shells (*Scylla paramamosain* and *Portunus pelagicus* species) were obtained from discarded sources of seafood processing. NaOH, HCl, H₂O₂ were purchased from Quangzi, China and used without further purification.

Prepare Chitin hydrogels and sponge-like aerogels

Chitin: Crab shells (~50 g) were treated with a NaOH aqueous solution (5 wt%, 1000 mL) at 80 °C for 6 h for protein removal and then treated with an HCl aqueous solution (7 vol.%, 1000 mL) twice at room temperature for 24 h for mineral removal. These deproteinized and decalcified chitin samples were washed with copious water and then treated with an H₂O₂ aqueous solution (5 vol%, 1000 mL) at 90 °C for 3 h to bleach organic pigments. These chemical purifications yielded white chitin nanofibrillar shells with an extraction yield of ~13 wt%.

Chitin nanocrystals: Chitin nanocrystals were prepared via sequential deacetylation and hydrolysis of native chitin nanofibrils. The purified chitin flakes (~5 g) were treated with a NaOH aqueous solution (33 wt%, 80 mL) at 90 °C for 2 h for surface deacetylation of some amino groups. The deacetylated chitin samples were collected and neutralized by washing thoroughly with water, and then treated with an HCl aqueous solution (4 M, 100 mL) at 104 °C for 18 h to hydrolyze nanofibrils into chitin nanocrystals. Because organic pigments were oxidized during acid hydrolysis to form dark-brown reaction mixtures. These dark brown colors in the reaction mixtures were oxidized by H₂O₂ (30 vol.%, 25 mL) at 90°C for 30 min to obtain cloudy chitin solutions. After acid hydrolysis, the reaction mixtures were diluted with 100 mL water and then centrifuged to remove soluble chitin species. This purification was repeated three times to obtain chitin aqueous suspensions. The chitin nanocrystal colloids were adjusted to the concentration of ~6.6 wt% and the pH value to ~4 to obtain a stable chitin aqueous suspension.

Chitin hydrogels and sponge-like aerogels: A homogeneous aqueous mixture was prepared by adding formaldehyde (0.2 mL) to the chitin aqueous suspension (~10 g, ~6.6 wt%, pH~4) under stirring and sonification. These mixed suspensions were then treated hydrothermally at 70 °C for 20 h to form formaldehyde-crosslinked chitin hydrogels. The hydrogels were freeze-dried to generate ~500 mg of ultralight chitin sponge-like aerogels.

3 Results and discussion

Crab shells were used as a starting material to prepare chitin nanocrystals. Crab shells were treated with a hot dilute NaOH solution to remove proteins. The deproteinized shells were treated with a dilute HCl solution at ambient conditions to remove calcium-based minerals. The decalcified shells were then bleached with a hot H₂O₂ solution to oxidize organic pigments. The overall purification process of crab shells yielded white chitin nanofibrillar shells (Figure 1a). Infrared (IR) spectrum (Figure 8b) of the purified chitin samples shows a hydroxyl stretching band at 3480 cm⁻¹, amide stretching bands at 1560–1660 cm⁻¹, and carbonyl stretching band at 1030 cm⁻¹, which are characteristic of α -chitin [13].

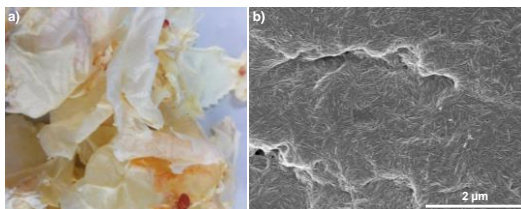


Fig. 1. Natural chitin nanofibrils. Photograph of native chitin nanofibrils prepared from crab shells and (b) SEM image of purified chitin nanofibrils.

Powder X-ray diffraction (PXRD) patterns (Figure 8a) of the purified chitin samples show diffraction peaks at 9.5° , 19° , 23° corresponding to (020), (110), (130) planes characteristic of α -chitin crystallites. No calcite could be observed in the purified chitin samples to verify the removal of calcium carbonate. The diffraction peaks are relatively sharp indicative of high crystallinity of native chitin fibrils in crab shells. Scanning electron microscopy (SEM) images (Figure 1b) show natural nanofibrils in the purified chitin shells. These native chitin nanofibrils with the diameter range of ~ 10 – 20 nm self-organized into stacked layers through shells. These results led to a conclusion that the chitin samples chemically extracted from crab shells are α -chitin crystalline nanofibrils.

We investigated the chemical transformation of native chitin nanofibrils into chitin nanocrystals by sequential deacetylation and hydrolysis. The purified chitin nanofibrils were treated with a hot concentrated NaOH solution to deacetylate some amino groups on chitin fibrils. The resulting nanofibrils were treated with a hot 4 M HCl aqueous solution to hydrolyze into chitin nanocrystals. Visibly, chitin nanofibril solids were hydrolyzed by acid to form a cloudy colloidal solution. The hydrolyzed chitin nanoparticles were purified from the reaction mixture and kept in forms of both liquid and solid to investigate its structural properties.

Chemical bonding analyses also show distinct IR bands of α -chitin in the hydrolyzed chitin samples (IR, Figure 8b). PXRD patterns (Figure 8a) of the hydrolyzed chitin samples show distinct diffraction peaks with positions and shapes similar those of native chitin nanofibrils. These analyses supported the structural integrity of α -chitin crystallites from the deacetylation and hydrolysis of the fibrils. Transmission electron microscopy (TEM) images (Figure 2b) of the chitin aqueous solutions reveal that chitin crystallites are discrete rod-shaped nanoparticles with diameters of 5–15 nm and lengths of 300–500 nm that are similar to those reported in the literature [15]. These diameter range sizes are considerably smaller than those of native chitin nanofibrils (~ 10 – 20 nm diameter and ~ 500 – 1000 nm length), suggesting the chemical decomposition of amorphous disordered regions of nanofibrils to generate smaller rod-shaped

chitin nanocrystals. These results confirmed that the reacted products prepared from crustacean shells are rod-shaped α -chitin nanocrystals.

Chitin nanocrystals were dispersed in water to form a colloidal solution. In acidic media, chitin nanoparticles formed a stable colloidal suspension. The protonation of amino groups provided positively charged surfaces to chitin nanorods to make these nanocolloids suspended in water, owing to repulsive electrostatic forces between nanoparticles. We found that chitin aqueous suspensions become more homogeneous and stable at the critical concentration of 6.6 wt% and the acidic pH of ~ 4 (Figure 2a). The chitin aqueous suspensions were dried under ambient conditions to form solidified films (Figure 3a). These chitin films are large, crack-free, and flexible with dimensions up to several centimeters. Scanning electron microscopy (SEM) images (Figures 3b,c) of the assembled chitin films show a layered structure with the long-range alignment of nanorods within each layer. This proves that the chitin aqueous suspension is an anisotropic liquid crystal. Chitin nanorods dispersed in water to form a stable aqueous suspension. Above the critical concentration, chitin nanorods self-organized into a nematic liquid crystalline structure that was preserved during evaporation-induced self-assembly to form layered films.

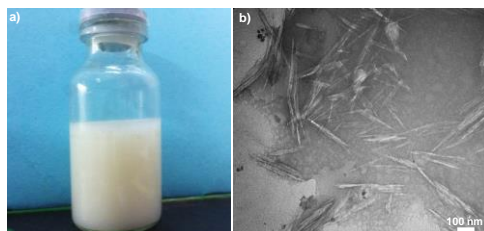


Fig. 2. Chitin liquid crystals. (a) Photograph of chitin liquid crystals (6.6 wt%, pH ~ 4) and (b) TEM image of rod-shaped chitin nanocrystals.

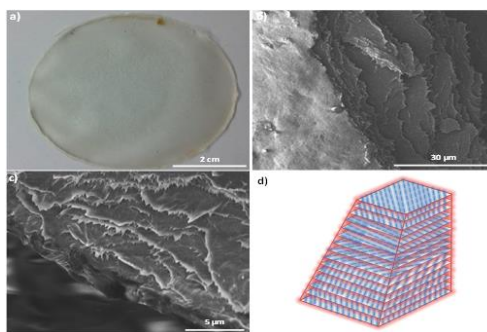


Fig. 3. Layered anisotropic structure of chitin liquid crystals. (a) Photograph of chitin films cast from chitin liquid crystals, (b,c) SEM images viewed along fractured edges of assembled chitin films at different magnifications, and (d) An illustration of layered nematic structure of chitin nanocrystals.

Biopolymer hydrogels are of great interest for biomedical applications in self-healing, drug delivery, and tissue engineering [14]. Liquid crystals are a novel substance to study the gelation of its anisotropically organized networks into soft hydrogels. We, hereby, fabricated chitin hydrogels from chitin liquid crystals. We investigated hydrothermally crosslinked gelation of chitin aqueous suspensions to prepare chitin hydrogels.

Recent works have shown the preparation of biopolymeric liquid crystals-derived hydrogels using metal ionic crosslinkers or via hydrothermal treatment [15]. We first attempted to apply these reaction conditions for chitin liquid crystals. Obviously, the addition of metal ions (e.g., Fe^{3+} or Cu^{2+}) to chitin aqueous suspensions formed relatively gelled mixtures that turned to be solids on solidification. Initial electron microscope examinations showed poor homogeneous structures of stacked fibril assemblies in these solidified composites, indicating the disruption of the nematic organization of chitin nanocrystals. We also realized that the gelation of pure chitin liquid crystals could not occur by hydrothermal treatment. These failure experiments were later addressed to prepare chitin liquid crystal-derived hydrogels.

We eventually figured out that chitin hydrogels could be prepared by hydrothermal treatment of chitin aqueous suspensions (6.6 wt%, pH~4) in the presence of mono/di-aldehyde molecules as a crosslinker. Our experimental attempts indicated that formaldehyde/chitin aqueous mixtures were hydrothermally treated at 70 °C for 10 h for the gelation of chitin nanocrystals to obtain homogeneous hydrogels (Figure 4b). Remarkably, soft chitin hydrogels exhibited the structural integrity of the aqueous suspensions. The chitin hydrogels were stable at ambient conditions as the hydrogel networks could be retained for several weeks. The chitin hydrogels became more rigid upon solvent exchange with alcohol. These results assumed that intermolecular interactions between amino groups of chitin and carbonyl groups of formaldehyde led nanorods to be chemically crosslinked with each other to form chitin hydrogel networks. We observed that chitin hydrogels are highly gelly as their separated fragments can fuse together to recover intact chitin hydrogels. This behavior makes chitin hydrogels interesting for investigating biomedical applications, particular in self-healing and drug delivery [16].

Aerogels are ultralight materials derived from gels where the liquid is replaced by air on solidification and their porous networks are maintained in solid [17]. High porosity and low density make aerogels interesting for a variety of applications in filters, adsorbents, and insulators [18].

From the viewpoints above, we investigated the preparation of chitin aerogels from the resulting hydrogels. Interestingly, the chitin hydrogels were freeze-dried to generate solidified

aerogels (Figure 5a). Figure 7 shows a synthetic method to prepare layered nematic chitin aerogels from hydrothermally crosslinked gelation of chitin liquid crystals. The chitin aerogels mostly retained the shape features of the hydrogels with minor shrinkage. The white chitin aerogels had a relatively rigid structure, which did not easily lose or break the shape upon slightly compressing by hand. The aerogels were stable in air without an observable change in shape or color after extended periods of time of storage in air. Visibly, the aerogels were made up of chitin fibrils interconnected within macroporous networks. The open networks and low-density biofibrils led chitin aerogels to be an ultralight material that is sponge-like (Figure 5b). The yield of the aerogels was approximately 500 mg of solid products obtained from ~10 g of 6.6 wt% chitin aqueous suspensions. These results presented a reliable way of using chitin nanocrystals to prepare novel sponge-like fibers.

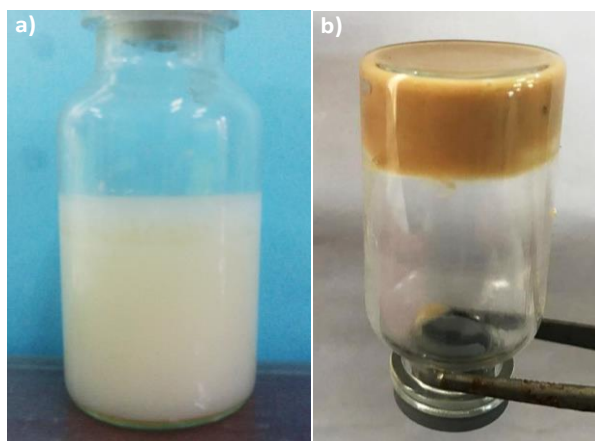


Fig. 4. Gelation of chitin liquid crystals into chitin aerogels. (a) Photographs of formaldehyde/chitin aqueous suspension and (b) formaldehyde-crosslinked chitin hydrogels.

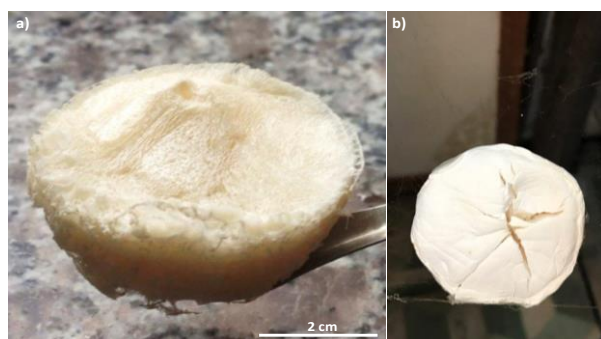


Fig. 5. Chitin nanocrystals-derived aerogels. (a) Photograph of formaldehyde-crosslinked chitin aerogels and (b) Photograph of chitin aerogels mounted on a natural spider web showing its lightweight characteristics.

The gelation and solidification of chitin nanocrystal assemblies maintain the original structure of chitin in the aerogels (PXRD, Figure 8a). IR spectrum (Figure 8b) of the aerogels also shows amide stretching bands at $\sim 1560\text{--}1660\text{ cm}^{-1}$ and a carbonyl stretching band at 1030 cm^{-1} of chitin. TGA analyses (Figure 8c) showed that chitin aerogels were thermally stable up to $\sim 380\text{ }^{\circ}\text{C}$, followed by a gradual loss of weight at a higher temperature. This thermal stability was a little higher than that of chitin nanocrystals ($\sim 350\text{ }^{\circ}\text{C}$), possibly due to crosslinking of chitin with formaldehyde. Nitrogen sorption measurements (Figure 8d) showed that the chitin aerogels have a mesoporous structure in macroporous networks with an average surface area of $\sim 150\text{ m}^2\cdot\text{g}^{-1}$ and broad pore size distribution centered at $\sim 25\text{ nm}$. These macro/mesopores arose from spaces between chitin nanorods highly interconnected within the aerogels.

The structural organization of chitin nanocrystals in the aerogels was assessed using SEM as shown in Figure 6. Overall, the chitin aerogels possessed open macroporous networks of nanorod assemblies. At low magnifications, we saw cracked flakes randomly interconnected with each other into open networks with interspaces of $\sim 2\text{--}6\text{ }\mu\text{m}$. Looking at the cross sections of these $\sim 300\text{ nm}$ -thick flakes, we saw the layered structures of the chitin nanofibrils assembled throughout their entire thickness. From the top views of these layered flakes at higher magnifications, we saw chitin nanorods interconnected with each other to form highly open networks in the assembled flakes. This assumes the anisotropic structure of chitin liquid crystals to be blocked by hydrothermally crosslinked gelation. Under freeze-drying, the frozen water in the chitin hydrogels sublimated directly from the solid phase to the gas phase to open macro/mesopores in the fibrillar networks, producing chitin aerogels.

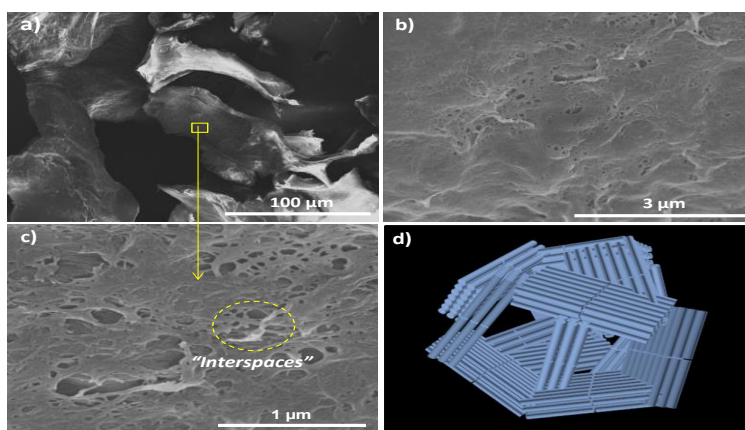


Fig. 6. (a,b,c) SEM images of chitin aerogels viewed at three different magnifications showing open networks of chitin nanorods interconnected within the aerogels and (d) an illustration of layered chitin aerogels.

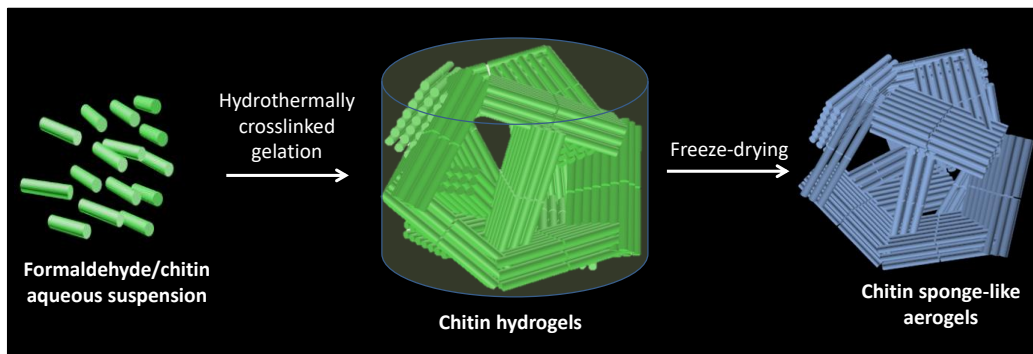


Fig. 7. Schematic of hydrothermally crosslinked gelation of chitin liquid crystals into chitin aerogels with layered nematic structure.

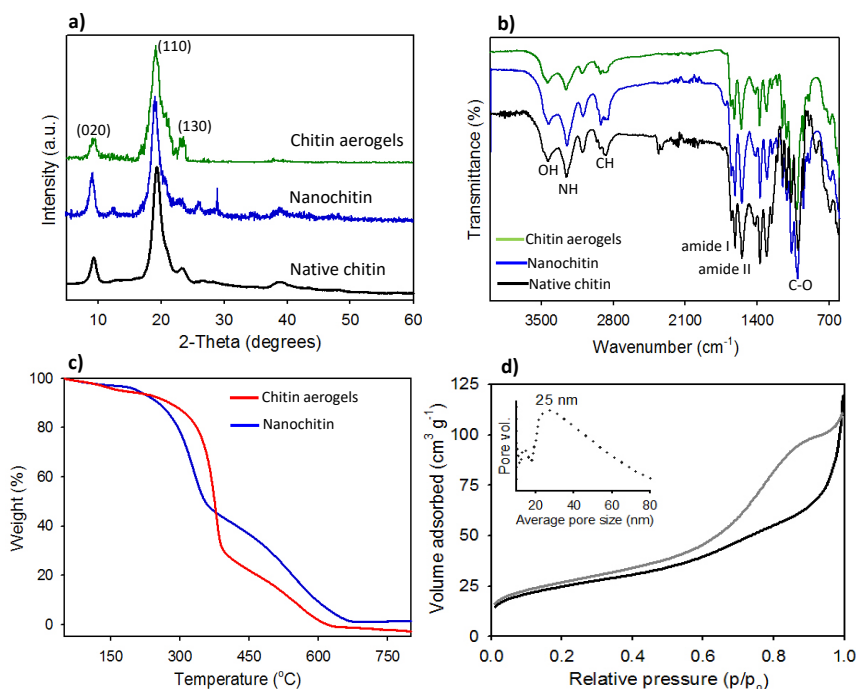


Fig. 8. Structural analyses of chitin-based materials. (a) PXRD patterns of chitin aerogels, chitin nanocrystals, and native chitin nanofibrils, (b) FTIR spectra of chitin aerogels, chitin nanocrystals, and native chitin nanofibrils, (c) TGA curves of chitin aerogels and chitin nanocrystals, and (d) Nitrogen adsorption and desorption isotherms of chitin aerogel.

Since many papers have reported to the preparation of chitin aerogels using different structural forms of chitin precursors [19]. It highlights that this is a new synthetic approach to chitin aerogels from liquid crystals. These chitin aerogel materials may be useful as a

biocompatible substance for adsorption [20], tissue engineering, filtration, and insulation. These chitin aerogels also promise to be a novel precursor for antimicrobial fabrics and for supercapacitor carbonized aerogels or an aerogel template for porous materials. Due to their high porosity, nanoscale, and lightweight network, these chitin aerogels may also be modified with functional additives to form superhydrophobic aerogels that have recently been demonstrated their great potential for oil/water separation [21].

4 Conclusions

In summary, we have shown the experimental evidence of the structural transformation of chitin liquid crystals into ultralight chitin sponge-like aerogels. Chitin shells underwent deacetylation and hydrolysis to fragment native nanofibrils into rod-shaped chitin nanocrystals. The protonation of the exposed amino groups provided a negatively charged surface to chitin nanocrystals, which make them exhibiting repulsive forces from charged neighboring particles to form a stable aqueous dispersion. The colloidal stability of chitin nanorods in acidic media led to the transformation of the aqueous suspensions into anisotropic liquid crystals at the critical concentration. The gelation of liquid crystals was performed by hydrothermal crosslinking of chitin nanocrystals with formaldehyde to generate chitin hydrogels. The solidification of the hydrogels by lyophilization recovered intact chitin aerogels. The most interesting feature of the aerogels is the layered nematic structure of chitin nanorods to be solidified in highly porous fibrillar networks, producing ultralight sponge-like materials. The successful preparation of chitin liquid crystal-derived hydrogels and aerogels has paved an exciting way of investigating their sustainable applications. These chitin nanostructured aerogels may be useful as a novel template for fabricating functional porous materials.

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