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# Salt-Induced Colloidal Destabilization, Separation, Drying, and **Redispersion in Aqueous Phase of Cationic and Anionic Nanochitins**

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Supporting Information

ABSTRACT: This study is aimed at facilitating the use of ocean biomass for the isolation and use of derived nanostructures. Specifically, cationic and anionic nanochitins were produced from never-dried crab shells that underwent partial deacetylation (PD-NCh) or TEMPO-oxidization (TO-NCh). The effects of different electrolyte types (NaCl, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, CaCl<sub>2</sub>,  $AlCl_{3}$ , and  $NH_{4}Cl$ ) were investigated with regards to fractionation (via colloidal destabilization and precipitation), drying, and ultimate redispersion of the nanochitins. Sodium carbonate was most effective in the case of PD-NCh processing, whereas no significant effect of salt type was noted for TO-NCh. The results are rationalized in terms of the dispersion stability that resulted from specific counterion adsorption and nanoparticle association as well as electrostatic-charge development at a given solution pH. These effects were used to limit hydrogen bonding and nonspecific interactions upon drying of the nanochitins. The weak interactions between nanochitin and monovalent Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup> explain the experimental observations. Aqueous dispersions reconstituted from dried PD-NCh and TO-NCh were colloidally stable and yielded highly viscous, gel-like nanochitin dispersions at mass concentrations as low as 1.5 and 3.0%, respectively. Our findings are expected to greatly facilitate green processing of nanochitin, an emerging type of biobased nanomaterial.

**KEYWORDS:** nanochitin, salt, destabilization, drying, redispersion

## ■ INTRODUCTION

Chitin is a biopolymer present in the exoskeletons of crustaceans and insects and in the cell walls of fungi. It is a nitrogen-containing polysaccharide composed of N-acetyl-Dglucosamine (GlcNAc) residues linked by  $\beta$ -1,4 glycosides.<sup>1,2</sup> Nanochitin, referred to herein as NCh, comprises high-aspect ratio nanoparticles (nanofibrils and nanorods) that can be prepared readily in aqueous media and have a wide range of applications in drug delivery, food packing, grain-yield enhancement, and so on.<sup>3-6</sup> Like nanocelluloses, however, there is still a major need to control the dispersion state of NCh for appropriate processing, fractionation, concentration, drying, and redispersion, as well as for their deployment in actual applications.

In general, the mass concentrations of freshly prepared NCh dispersions are relatively low (usually <0.5 wt %), and therefore water removal is required for concentration. However, the high viscosity of colloidal dispersions of hydrophilic NCh is also a limitation during handling, heating, and water evaporation. Moreover, several additional drawbacks exist, such as transportation, processing time, and energy consumption.<sup>7</sup> Considering the need for water, effective redispersion or reconstitution of dried or concentrated NCh is critical for the success of any application. The unique properties of NCh depends on its dimensions and surface characteristics (e.g., the nature of its reactive groups and surface chemistry). Irreversible aggregation often occurs when

NCh is concentrated or dried, often leading to property and function losses, similar to the case of nanocelluloses (cellulose nanofibers and nanocrystals, CNF and CNC, respectively).<sup>8,9</sup>

Generic methods for separation, concentration, drying, and later redispersion are necessary to make practical any application of NCh, but these are topics that so far have not been addressed. It is perhaps most useful, therefore, to learn from the current knowledge that exists in this regard in the case of nanocelluloses. Indeed, Fukuzumi et al. reported on the influence of NaCl on the aqueous-dispersion stability of TEMPO-oxidized cellulose nanofibers (TOCNF). They found that TOCNF remained homogeneous up to a NaCl concentration of 50 mM, whereas aggregated gel particles formed at salt concentrations >100 mM, leading to phase separation (gel and supernatant) above 200 mM NaCl.<sup>10</sup> Beck et al. reported that after extensive counterion exchange (>94%) of CNC (from the protonated to the Na<sup>+</sup> form), the obtained neutral CNC could be fully redispersed in water after drying under ambient conditions.<sup>9</sup> Other authors also reported waterredispersible CNF by the addition of NaCl to the dispersion before drying (freeze-drying).<sup>11</sup> Besides salt treatment, surfactants such as cetyltrimethylammonium bromide, CTAB,

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**Figure 1.** (a,b) Photographs of PD-NCh (a) and TO-NCh (b) separated from aqueous dispersions by the addition of different salts (NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, and NH<sub>4</sub>Cl) and centrifugation (10 000 rpm). (c,d) Aggregate sizes (*Z*-averages) and  $\zeta$ -potentials of PD-NCh (c) and TO-NCh (d) after the addition of the given salts at 0.6 and 0.1 M concentrations, respectively.

have been used to facilitate the drying and redispersion in water of nanocelluloses at suitable nanoscales. The effects of salt and surfactants have been rationalized in terms of weakening the intercellulosic hydrogen bonds upon drying.<sup>12</sup> Nanocellulose-based composites obtained from Pickering emulsions stabilized by cellulose nanocrystals and dried were found to be redispersible in water.<sup>13</sup> Microemulsions containing urea or ethylenediamine/NaOH/NaCl were found to be successful in disrupting interfibril hydrogen bonding and resulted in reduced energy demands during deconstruction of the precursor fibers suspended in aqueous media.<sup>14</sup> Could these findings be translated for efficient and cost-effective processing of nanochitin? Testing of this possibility is so far lacking. Although there are differences in their natures and charged groups, both nanocellulose and nanochitin adoption calls for novel approaches for drying and redispersion. However, the knowledge gained from one nanomaterial cannot strictly translate to the other.

As a starting point, here we propose a method that uses electrolytes for the fractionation, dispersion, drying, and redispersion in aqueous phase of positively charged, partially deacetylated nanochitins as well as negatively charged, TEMPO-oxidized nanochitins. For this purpose, the effects of salt type and concentration and pH were systematically studied.

### RESULTS AND DISCUSSION

PD-NCh and TO-NCh obtained from crab shells (raw nanochitins) are shown in Figure S1. Partial deacetylation

and TEMPO oxidization was applied to introduce different active groups, amino groups, and carboxyl groups in order to bring the corresponding positive and negative surface charges into nanochitin. When the partially deacetylated chitin was disintegrated under weak-acid aqueous conditions, the surface cationic (positively charged) amino groups ensured that the nanofibrillated PD-NCh remained stably dispersed in water as a result of electrostatic repulsion. On the basis of the same basic theory, the surface anionic (negatively charged) carboxyl groups of TEMPO-oxidized chitin ensured that the TO-NCh was stably dispersed in water under weak alkali conditions. Apart from the differently charged natures of PD-NCh and TO-NCh, obvious morphological differences are observed. Compared with TO-NCh, PD-NCh has a much longer fibril length and aspect ratio.

The differences in electrostatic charge and morphology of PD-NCh and TO-NCh are expected to influence their processability and greatly expand the applications of nano-chitin in diverse fields.

**Fractionation and Concentration of PD-NCh and TO-NCh.** Although many applications based on nanochitin have been reported in recent years, aspects related to their fractionation, water removal (concentration and drying) and redispersion have been largely lacking. However, such steps are critically important in any practical application.<sup>15</sup> The fractionation of PD-NCh and TO-NCh was first facilitated by the addition of salt, where PD-NCh and TO-NCh were readily separated from aqueous dispersions by the addition of NaCl (0.6 M) and centrifugation (~10 000 rpm, 3 min). This

is expected given the effect of electrolytes in colloidal destabilization. However, more interesting is the effect of specific counterion binding, which was investigated with six different salts (NaCl, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, CaCl<sub>2</sub>, AlCl<sub>3</sub>, and NH<sub>4</sub>Cl; Figure 1).

In the absence of salt, PD-NCh and TO-NCh remained stable even after centrifugation for 3 min at 10 000 rpm, revealing their high colloidal stability resulting from the inherent electrostatic charges. This stability is overcome by the addition of salts: the fractionated nanochitin after salt addition and centrifugation (Figure 1a,b) shows that all the chosen salts were suitable for the separation of PD-NCh and TO-NCh. The aggregate sizes and  $\zeta$ -potentials of the respective dispersions after the addition of salts are shown in Figure 1c,d. Accurately, the aggregate size was accessed by the diffusion coefficient determined by dynamic light scattering and reported as the apparent hydrodynamic radius from the Stokes-Einstein equation. Hence, the sizing results should be taken only as an approximation and for comparison on a relative basis. For PD-NCh, the aggregate sizes were 1556  $\pm$  742, 2071  $\pm$  500, 6685 ± 1521, 1962 ± 227, 2970 ± 531, and 1934 ± 266 nm in the corresponding salts at 0.6 M concentrations (NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, and NH<sub>4</sub>Cl, respectively). By comparing these values with the value for the original PD-NCh in the absence of salt (i.e.,  $1326 \pm 137$  nm), the effect of the electrolyte is apparent. Among the salts tested, AlCl<sub>3</sub> induced the largest aggregation. It is believed that the aggregation behavior was induced by chelation between the cations (Na<sup>+</sup>,  $Ca^{2+}$ , or  $Al^{3+}$ ) and the amino groups of PD-NCh, similar to the adsorption of metal ions onto chitosan.<sup>16</sup> For these three cations, the ionic strength for the given salt concentration follows the order  $Al^{3+} > Ca^{2+} > Na^+$ . As the ionic strength decreased, aggregate size decreased a lot. However, an interesting phenomenon is observed for Na2CO3 as a source of Na<sup>+</sup>, the only monovalent cation in the series: at low ionic strength, it produced a distinctively large aggregate size (2970  $\pm$  532 nm). This points to the possible effect of salt addition on the dispersion stability. For PD-NCh, the  $\zeta$ -potential in the presence of salt decreased sharply from  $71 \pm 0.3$  mV in the original dispersion to  $15 \pm 1.2$ ,  $13 \pm 1$ ,  $17 \pm 3$ ,  $13 \pm 0.8$ , and  $-22 \pm 2$  mV with NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, and  $Na_2CO_3$ , respectively. The much lower  $\zeta$ -potentials determined in the presence of salt indicate the possibility for dispersion destabilization, which is expected to correlate with the propensity for the nanochitin to separate out from the dispersion. Most interestingly, Na<sub>2</sub>CO<sub>3</sub> reversed the sign of the  $\zeta$ -potential of PD-NCh to -22 mV, possibly as a result of the corresponding change in pH upon the addition of this salt. Freshly prepared PD-NCh was well dispersed in acetic acid solution, whereby NH<sub>2</sub> groups are protonated into NH<sub>3</sub><sup>+</sup>. As  $Na_2CO_3$  was added,  $CO_3^{2-}$  reacted with acetic acid, consuming H<sup>+</sup> in the dispersion and leading to electrostatic neutralization of PD-NCh. The alkaline nature of Na<sub>2</sub>CO<sub>3</sub> is shown in PD-NCh dispersions that underwent increases in pH values (pH = 10, Table 1). This influences the  $\zeta$ -potential, leading to the negative value indicated before. All other salts induced PD-NCh dispersions that remained acidic in nature and kept positive  $\zeta$ -potential values.

For TO-NCh, the salt addition resulted in less notable differences in the particle sizes and dispersion stabilities. The average sizes reached 968  $\pm$  59, 1565  $\pm$  198, 3626  $\pm$  394, 933  $\pm$  5, 1013  $\pm$  187, and 1036  $\pm$  79 nm, whereas the  $\zeta$ -potentials reached  $-28 \pm 1.2$ ,  $-11 \pm 1.3$ ,  $10 \pm 3.5$ ,  $-27 \pm 2.5$ ,  $-25 \pm 5$ ,

Table 1. PH of Dispersions of PD-NCh and TO-NCh at 0.6 and 0.1 M Salt Concentrations, Respectively

	no salt	NaCl	$\operatorname{CaCl}_2$	AlCl <sub>3</sub>	CH <sub>3</sub> COONa	Na <sub>2</sub> CO <sub>3</sub>	NH <sub>4</sub> Cl
PD- NCh	2.7	2.4	2	1.2	4.8	10	2.5
TO- NCh	5.6	4.7	4.3	2.8	7.4	12	5.5

and  $-28 \pm 1.7$  mV in the presence of 0.1 M NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, or NH<sub>4</sub>Cl, respectively. NaCl, CH<sub>3</sub>COONa, and NH<sub>4</sub>Cl addition resulted in no significant differences comparing the characteristic size measured in the electrolyte-free dispersion (964 nm). In contrast, CaCl<sub>2</sub> and AlCl<sub>3</sub> produced remarkable increases in the sizes of the aggregates. A less negative  $\zeta$ -potential was measured upon the addition of salt to the initially colloidally stable TO-NCh (-64mV in the absence of salt). In contrast to the observed protonation of PD-NCh (NH<sub>3</sub><sup>+</sup> development leading to higher electronic repulsion), the COO<sup>-</sup> groups in TO-NCh remained dissociated. COO<sup>-</sup> neutralization with the respective cation reduced electrostatic repulsion and resulted in a lower colloidal stability. At a given electrolyte concentration, the ionic strength increased in the order  $Al^{3+} > Ca^{2+} > Na^+$ , and their effect on TO-NCh translated to the same order of increased aggregate sizes and reduced colloidal stabilities. The results indicate the neutralization of COO<sup>-</sup> in TO-NCh with the counterions (Na<sup>+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup>, and  $NH_4^+$ ), similar to the observations made for TEMPO-oxidized cellulose nanofibers.<sup>10</sup> For TO-NCh, AlCl3 even resulted in charge reversal. Similar to previous observations related to the effect of Na<sub>2</sub>CO<sub>3</sub> on PD-NCh, this also could be explained by the change of pH induced by AlCl<sub>3</sub> (when 0.1 M AlCl<sub>3</sub> was added to the TO-NCh dispersion, the pH was reduced to 2.8; Table 1).

Drying and Redispersion of PD-NCh and TO-NCh. Electrolyte addition to aqueous nanochitin dispersions (either PD-NCh or TO-NCh), followed by centrifugation, facilitated water separation (>90% of the initial water was removed). What is of most significance, though, is that the wet, concentrated PD-NCh or TO-NCh could be readily redispersed after electrolyte removal by ion exchange (see Supporting Information Figure S2). However, in such cases, the low mass concentrations of nanochitin after ion exchange restrict most of the applications. Therefore, full drying was applied, and the effects of electrolytes were studied in relation to the production of stable aqueous dispersions in water (redispersion or reconstitution). Thus, PD-NCh and TO-NCh were completely dried at 60 °C for about 12 h, Figure 2a,b. Sheetlike morphologies were observed for the dried PD-NCh and TO-NCh, which were also white in color, likely as a result of the presence of salt (the dried PD-NCh or TO-NCh solids were translucent after being dried from salt-free dispersions).

For effective redispersion, the residual salt present in the dried PD-NCh or TO-NCh needs to be removed. Hence, acid was applied for this purpose. After being washed in dilute acetic acid or hydrochloric acid for several times, the initial sheetlike structures were redispersed in acetic acid solution (for PD-NCh) or in distilled water (for TO-NCh); this was followed by homogenization and ultrasonication (Figure 2c,d). The extent of redispersion of the nanochitins was studied after very brief homogenization (30 s, 10 000 rpm) and ultrasonication (30 s, 40% power). For PD-NCh, the sample initially treated with  $Na_2CO_3$  was homogeneously and fully



Figure 2. (a,b) Photographs of PD-NCh (a) and TO-NCh (b) after ion exchange and drying overnight at 60 °C and (c,d) followed by redispersion in water, showing the effects of the different salts (NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, and NH<sub>4</sub>Cl) present in the original aqueous dispersion.

Table 2. Redispersibility and Reconstitution in Acetic Acid and Water of PD-NCh and TO-NCh, Respectively, after Treatment with the Different Salts Followed by Drying<sup>a</sup>

	redisper	sibility <sup>b</sup>	Z-average (nm)		ζ-potential (mV)		conductivity (mS cm <sup>-1</sup> )	
salt	PD	ТО	PD	ТО	PD	ТО	PD	ТО
no salt <sup>c</sup>	Ν	Ν	$1326 \pm 137$	$968 \pm 60$	$71 \pm 0.3$	$62 \pm 2.0$	$0.7 \pm 0.01$	$0.1 \pm 0.01$
NaCl	Ν	Y	d	$433 \pm 13$	—	$54 \pm 0.8$	—	$0.04 \pm 0.01$
NH <sub>4</sub> Cl	Ν	Y	—	$522 \pm 11$	—	$50 \pm 0.3$	—	$0.03 \pm 0.01$
CH <sub>3</sub> COONa	Ν	Y	—	$430 \pm 30$	—	$45 \pm 1.0$	—	$0.02 \pm 0.01$
Na <sub>2</sub> CO <sub>3</sub>	Y	Ν	$741 \pm 25$	—	$59 \pm 2.3$	_	$1.29 \pm 0.1$	_

<sup>*a*</sup>The average aggregate sizes,  $\zeta$ -potentials, and conductivities of the reconstituted dispersions are also included. For treatments with CaCl<sub>2</sub> and AlCl<sub>3</sub>, no redispersion was possible for either of the two nanochitins, and therefore data are not presented for such salts. <sup>*b*</sup>Y, yes; N, no. <sup>*c*</sup>The data for the original, never-dried nanochitins. If these samples were dried, no redispersion was possible. <sup>*d*</sup>Lack of data because redispersion was not possible.

dispersed. The PD-NCh treated with the other salts (NaCl,  $CaCl_2$ ,  $AlCl_3$ ,  $CH_3COONa$ , and  $NH_4Cl$ ) and with no salt treatment remained aggregated, unfortunately. For TO-NCh, treatment with NaCl,  $NH_4Cl$ , and  $CH_3COONa$  made TO-NCh fully disperse in water (TO-NCh with no salt treatment and after  $CaCl_2$ ,  $AlCl_3$ , and  $Na_2CO_3$  addition was not fully redispersed in water). The results of redispersion are summarized in Table 2.

The data for the characteristic sizes,  $\zeta$ -potentials, and conductivities of the nanochitin samples that were dried and then redispersed in acetic acid solution or water (PD-NCh and TO-NCh, respectively) are shown in Table 2. Compared with the never-dried PD-NCh (1326  $\pm$  137 nm), the characteristic size for this nanochitin redispersed after Na<sub>2</sub>CO<sub>3</sub> treatment was  $741 \pm 25$  nm. Likewise, for TO-NCh, with an original size of 968  $\pm$  59 nm, treatment with NaCl, NH<sub>4</sub>Cl, and CH<sub>3</sub>COONa yielded sizes of  $433 \pm 13$ ,  $522 \pm 11$ , and 430 $\pm$  30 nm, respectively. It is surprising that all the nanochitins that were successfully redispersed presented a smaller characteristic sizes but much more uniform morphology. The results can be explained in terms of charge screening, as confirmed by the TEM images (Figure 3). Furthermore, the homogenization and ultrasonication used for redispersion are believed to be the reason for the reduction in nanochitin length. After the washing out of the salt in the dried nanochitin, both PD-NCh and TO-NCh kept sheetlike



**Figure 3.** TEM images of redispersed PD-NCh after treatment with  $Na_2CO_3$  (a) and TO-NCh after treatment with NaCl (b),  $NH_4Cl$  (c), and  $CH_3COONa$  (d). The scale bars shown are 500 nm.



Figure 4. SEM images of dried PD-NCh and TO-NCh after treatment with NaCl, CaCl<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, Na<sub>2</sub>CO<sub>3</sub>, and NH<sub>4</sub>Cl, as indicated.

morphologies (Figure S3), which indicated the contribution of hydrogen bonds within the nanochitins. Normally, the biggest challenge in achieving complete individualization of nanochitins from raw materials was the fact that they were tightly bonded to each other through hydrogen bonding.<sup>17</sup> Therefore, mechanical treatment with optional chemical modification must be applied to break such strong interfibrillar hydrogen bonding, allowing gradual disintegration.<sup>18</sup> This rule also applied to dried nanochitins; hydrogen bonds formed between nanochitins in the drying process, hindering successful redispersion. For the successful redispersion of PD-NCh and TO-NCh, homogenization (30 s, 10000 rpm) and ultrasonication (30s, 40% power) were applied for short amounts of time. Although the homogenization and ultrasonication treatments applied here were mild, the redispersed nanochitin here are believed to be easier to break because of physical cross-linking from the drying process and excess mechanical treatment in redispersion, leading to smaller sizes. Interestingly, although redispersed nanochitins showed smaller particle sizes, redispersed PD-NCh and TO-NCh both exhibited much more uniform sizes, which might be useful for special applications, as shown in Figure S4.

The appearance of PD-NCh and TO-NCh dried in the forms of "membranes" are included in Figure 4. The PD-NCh and TO-NCh dried directly from the aqueous dispersion (no salt treatment) included a clear fibrillar structure, whereby the nanochitins were likely bound together via hydrogen bonding. Such fibrillar structures were absent in the cases of PD-NCh and TO-NCh treated with salts; however, these included salt crystals. For dried PD-NCh and TO-NCh without salt addition, strong and abundant intermolecular hydrogen bonds formed upon drying, making redispersion unsuccessful, as is also the case with cellulose nanofibers.<sup>9</sup> Drying after salt treatment hindered hydrogen bonding between the nanochitins, at least for the cases presented in Table 2. However, this is not the only effect at play, because not all the salts resulted in successful redispersion.

We hypothesize that an additional factor exits that led to weak interactions between nanochitins. As described before, a stronger ionic strength led to better charge screening, as expected, and this induced higher complexation density. The strong complexation between salt counterions (e.g., Ca<sup>2+</sup> and Al<sup>3+</sup>) and active groups (e.g., NH<sup>3+</sup> in PD-NCh and COO<sup>-</sup> in TO-NCh) is likely the reason for the irreversible aggregation and limited redispersion ability. Treatment of PD-NCh with NaCl, CH<sub>3</sub>COONa, and NH<sub>4</sub>Cl, which led to irreversible aggregation, may be explained by the effects of the pH values, which according to Table 1 were 2.4, 4.8, and 2.5, respectively. The pH of the PD-NCh dispersion kept its acidic nature after the addition of the three salts. As described before, the PD-NCh dispersion remained stable because of the electrostatic repulsion resulting from the NH<sub>3</sub><sup>+</sup> groups on this nanochitin. The acidic nature is necessary for strong electrostatic repulsion in PD-NCh. Different from strong complexation with salt counterions (such as  $Ca^{2+}$  and  $Al^{3+}$  or  $Na^{+}$  and  $NH_{4}^{+}$ ), the three salts are believed to engage in weaker complexation with PD-NCh. The acid nature of PD-NCh after treatment with these salts is believed to result in protonated amino groups in PD-NCh that lead to further layering between the nanochitin and salts when dried together, which resulted in more hydrogen bonds in the nanochitin and irreversible aggregation of the dried material. However, the treatment of PD-NCh with Na<sub>2</sub>CO<sub>3</sub> led to H<sup>+</sup> consumption, as NH<sub>3</sub><sup>+</sup> was converted to  $NH_2$  by a reaction with  $CO_3^{2-}$ , resulting in (1) the screening of electrostatic repulsions between the nanochitins, which was otherwise needed for redispersion, and (2) reduced and



Figure 5. Summary of the effect of salt treatment for nanochitin separation from acetic acid solution (PD-NCh) or water (TO-NCh) via colloidal destabilization, drying, and redispersion in the original media.



**Figure 6.** Particle sizes (a), dispersion stabilities (b), and pH values (c) of Na<sub>2</sub>CO<sub>3</sub>-induced PD-NCh, and particle sizes (d), dispersion stabilities (e), and pH values (f) of NaCl-, NH<sub>4</sub>Cl-, and CH<sub>3</sub>COONa-induced TO-NCh.

relatively weak hydrogen bonds forming in dried PD-NCh after the treatment with  $Na_2CO_3$ , which resulted in successful redispersion. For  $NH_4Cl$ -induced PD-NCh, there might be another reason for the unsuccessful redispersion: decomposition into HCl and  $NH_3$  and then volatilization when heated. Cracks shown in Figure 4 were strong evidence for the disappearance of  $NH_4Cl$ . As  $NH_4Cl$  decomposed and volatilized in the drying process, the nanochitin became concentrated and unable redisperse.

Similar sheetlike morphology incorporating the salts after the treatment of TO-NCh with NaCl,  $CH_3COONa$ , and  $NH_4Cl$  is also seen in Figure 4, which leads to favorable redispersion. Missoum et al. proposed that ion-dipole interactions played an important role in redispersion of freeze-dried cellulose nanofibers, whereby the number of hydrogen bonds were significantly reduced when NaCl was added to dispersions of cellulose nanofibers.<sup>11</sup> Considering the carboxyl groups in cellulose nanofibers and TO-NCh, the mechanism of redispersion might be similar. For PD-NCh, the chelation reaction was likely to be the main reaction between salts and PD-NCh, whereas the electrostatic affinity between cations (Na<sup>+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup>, and NH<sub>4</sub><sup>+</sup>) and carboxyl groups (COO<sup>-</sup>) was probably the main effect that existed between the salts and TO-NCh.<sup>19</sup> Stronger ionic strengths also limited



Figure 7. Light transmittance of excess solution or supernatant after centrifugation (10 000 rpm, 10 min) of nanochitin dispersions after treatment with the respective salt at different concentrations. (a) PD-NCh treated with Na<sub>2</sub>CO<sub>3</sub>. (b–d) TO-NCh treated with NaCl (b), NH<sub>4</sub>Cl (c), and CH<sub>3</sub>COONa (d). The transmittance of distilled water was used as a control.

redispersion of TO-NCh because of the stronger complexation upon treatment with  $CaCl_2$  and  $AlCl_3$ . The treatment of TO-NCh with monovalent ions (Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup>) led to weak electrostatic attraction, and they were more easily washed out using acid solution, resulting in successful redispersion.

As can be inferred from Figure 5, the treatment with salts that led to redispersible nanochitin produced two effects: (1) it screened the surface electrostatic charges of nanochitin that allowed colloidal destabilization and effective separation via precipitation, and (2) it prevented the formation of hydrogen bonds in the nanochitin upon drying, enabling redispersion in water.

**Optimum Conditions Favoring Nanochitin Redispersion.** This section deals with the optimization of the conditions for the isolation of redispersible PD-NCh and TO-NCh. For this, treatment of PD-NCh with  $Na_2CO_3$  was considered. Figure 6a–c shows the results for the apparent nanochitin-aggregate size, dispersion, stability, and pH. Likewise, for TO-NCh, the salts tested were NaCl, NH<sub>4</sub>Cl, and CH<sub>3</sub>COONa, and the results are summarized in Figure 6d–f.

PD-NCh aggregated into very large particles after treatment with Na<sub>2</sub>CO<sub>3</sub>, which were apparent even with the naked eye (Figure S5 of the Supporting Information). The tendency observed in the characteristic size indicated the increased colloidal destabilization with salt addition. This was accompanied by an increased pH of the dispersion. Both effects facilitated PD-NCh separation upon centrifugation. For TO-NCh, the salts (NaCl, CH<sub>3</sub>COONa, and NH<sub>4</sub>Cl) induced similar effects. The absolute value of the  $\zeta$ -potential decreased, indicating lower dispersion stability as the salt concentration increased.

The turbidity profiles of the supernatant after separation of the nanochitins (PD-NCh and TO-NCh) from the original dispersion upon salt addition are shown in Figure 7. From the results, it can be concluded that  $Na_2CO_3$  treatment of PD-NCh produced the most efficient colloidal destabilization (Figure 7a). Addition of 0.05 M  $Na_2CO_3$  resulted in high transmittance of the supernatant after centrifugation, indicating complete separation of PD-NCh from the dispersion. Treatment of TO-NCh with NaCl, NH<sub>4</sub>Cl, and CH<sub>3</sub>COONa increased supernatant light transmittance with increasing salt concentrations (Figure 7b-d, respectively), indicating improved separation efficiency, but complete separation was still not achieved even after 0.8 M salt was added.

Effect of pH in TO-NCh Redispersion. To inquire further into the pH-shift effect induced by  $Na_2CO_3$  in PD-NCh dispersions and improve the extraction efficiency of NaCl-, NH<sub>4</sub>Cl-, and CH<sub>3</sub>COONa-induced TO-NCh, we measured the particle sizes, dispersion stabilities, and separation efficiencies of TO-NCh after treatment with NaCl, NH<sub>4</sub>Cl, and CH<sub>3</sub>COONa as functions of pH at salt concentrations fixed at 0.1 M (see Figure S6). The decrease in pH resulted in obvious higher extraction efficiencies for the three salts. When the pH reached 3, almost all the TO-NCh could be separated out from the dispersion according to the high light transmittance of the supernatant after centrifugation. Although TO-



Figure 8. Photographs of redispersed TO-NCh after treatment with NaCl,  $NH_4Cl$ , and  $CH_3COONa$ ; drying at 60 °C; and ion-exchange pretreatment at different pH values.

NCh could be separated more easily under reduced pH, the possibility of redispersion was most relevant. After centrifugation, the separated TO-NCh was also dried at 60 °C and redispersed after the washing out of the salts (Figure 8). It was observed that treatment of TO-NCh with salt in acidic medium could also lead to redispersal into a fully homogeneous dispersion, proving the feasibility of the acid-enhanced salt-induced redispersion of TO-NCh.

The extraction efficiency is one of the key factors impacting the practical applications of redispersed PD-NCh and TO-NCh. As such, the extraction efficiencies of PD-NCh and TO-NCh after the ion-exchange process are included in Figure S7 along with photographs. The extraction efficiencies of PD-NCh and TO-NCh were determined to be 78 and 88%, respectively. The TO-NCh underwent higher separation efficiency compared with PD-NCh. This was likely caused by the natures of the charged groups (i.e., amino groups in PD-NCh versus carboxyl groups in TO-NCh). As described before, amino groups are activated by H<sup>+</sup>, resulting in electrostatic repulsion in PD-NCh and increased colloidal stability. During the ion-exchange process, protons were used to replace Na<sup>+</sup> in the dried PD-NCh. However, partial activation of amino groups is believed to occur during this process, which results in partial dispersion of PD-NCh in the acid solution, resulting in a small mass loss. For TO-NCh, carboxyl groups were much more stable in acid solution and distilled water because of their negative charges. Photographs of dried PD-NCh after treatment with Na2CO3 and of TO-NCh after treatment with CH<sub>3</sub>COONa, before and after ion exchange, are included in Figure S4.

The mass concentrations of the redispersions are extremely important factors in furthering the application of PD-NCh and TO-NCh. Low mass concentrations of nanochitin dispersions inevitably lead to higher costs in concentration processes. Therefore, we tested the mass concentrations of PD-NCh and TO-NCh redispersed directly from dried PD-NCh and TO-NCh after ion exchange (Figure 9). Both PD-NCh and TO-NCh yielded highly viscous dispersions at mass concentrations as high as 1.5 and 3.0%, respectively. Although higher mass



Figure 9. Photographs of redispersed PD-NCh with mass concentrations of 0.3 (a) and 1.5% (b) and redispersed TO-NCh with mass concentrations of 0.7 (c) and 3.0% (d).

concentrations can also be achieved, in such cases the higher viscosity becomes a factor that lowers the efficiency of the ultrasonication.<sup>9</sup> However, in this article, the prepared viscous redispersed PD-NCh and TO-NCh were already good enough for many applications. In sum, we successfully redispersed PD-NCh and TO-NCh after complete drying, facilitating processing and allowing practical and upscale applications of nanochitin.

**Conclusions.** Ultralow mass concentrations (0.1%) of aqueous dispersions of partially deacetylated and TEMPO-oxidized nanochitins (PD-NCh and TO-NCh) were successfully separated via colloidal destabilization and centrifugation. Drying and redispersion were facilitated in the presence of electrolytes. Four key factors were involved in such processes: (1) decreased dispersion stability resulting from counterion adsorption or change of dispersion pH, (2) increased characteristic size by colloidal destabilization or aggregation

by electrostatic screening, (3) limited hydrogen bonding between nanochitins in the presence of salt upon drying, and (4) reduced interaction between nanochitin and monovalent Na<sup>+</sup> or  $NH_4^{+}$ .

For PD-NCh, Na<sub>2</sub>CO<sub>3</sub> was the best salt for separation, drying, and redispersion. The addition of Na<sub>2</sub>CO<sub>3</sub> led to proton  $(H^+)$  consumption in PD-NCh dispersions, transforming the NH<sub>3</sub><sup>+</sup> groups in PD-NCh into NH<sub>2</sub>. The disappearance of electrostatic repulsion in PD-NCh and the change in pH resulted in reduced dispersion stability accompanied by aggregation.

NaCl, NH<sub>4</sub>Cl, and CH<sub>3</sub>COONa were all effective for TO-NCh in terms of separation, drying, and redispersion. The interactions between Na<sup>+</sup> or NH<sub>4</sub><sup>+</sup> and the COO<sup>-</sup> groups of TO-NCh screened the electrostatic repulsion and produced colloidal destabilization and TO-NCh aggregation. Moreover, at a given salt concentration (0.1 M), the addition of H<sup>+</sup> had an effect similar to that of the addition of Na<sup>+</sup> or NH<sub>4</sub><sup>+</sup>.

After separation by centrifugation, most of the water could be removed from PD-NCh and TO-NCh. Heating at 60 °C was effective at drying the separated PD-NCh and TO-NCh; monovalent Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup> led to lower hydrogen bonding between nanochitins and to weak interactions between Na<sup>+</sup> or NH<sub>4</sub><sup>+</sup> and PD-NCh or TO-NCh.

Finally, the facile replacement of monovalent Na<sup>+</sup> or NH<sub>4</sub><sup>+</sup> from dried PD-NCh or TO-NCh by using a dilute acid solution facilitated the redispersion of PD-NCh or TO-NCh. For further applications of redispersed PD-NCh and TO-NCh, highly viscous redispersed PD-NCh (1.5%) and TO-NCh (3.0%) could be prepared directly from the dried nanochitins (after salt washing), leading to green and effective concentration and energy reduction.

### EXPERIMENTAL PROCEDURES

Materials.  $\alpha$ -Chitin was purified from fresh crabs (Callinectes sapidus) acquired at the local market of Helsinki harbor, Finland. Before purification, the crabs were cooked, and their shells was collected as the sources of chitin. The purification steps are briefly summarized as follows: Crab-shell waste was soaked in 1 M HCl for 12 h; this was followed by treatment with 1 M NaOH for 12 h. These two steps were repeated at least three times. The obtained residual solid was decolorized by immersion in 0.5% (w/w) NaClO<sub>2</sub>, and the pH was adjusted to 5 using acetic acid; this was followed by heating for at least 2 h at 70 °C. The purified-chitin solid residues were obtained after washing with distilled water.<sup>20-22</sup> Finally, the sheetlike chitin solids were crushed by using a household blender and stored at 4 °C for further use. Analytical-grade sodium chloride, anhydrous calcium chloride, sodium salt trihydrate, anhydrous sodium carbonate, and ammonium chloride were purchased from VWR Chemicals. Aluminum chloride hexahydrate was purchased from Fluka Analytical and used without further purification.

**Nanofibrillation of Chitin.** PD-NCh (positively charged, partially deacetylated nanochitin) was prepared according to a method described before by us.<sup>22</sup> In short, purified chitin was deacetylated in a 33% (w/w) NaOH solution at 90 °C for 4 h. Then, the partially deacetylated chitin was washed with deionized water and stored at 4 °C until use. Finally, the partially deacetylated chitin was dispersed in distilled water at a concentration of 0.3% (w/v), and drops of acetic acid were added to adjust the pH to ~3 under constant stirring. The obtained suspension was homogenized (5 min, 10 000 rpm using IKA T-25 ULTRA-TURRAX Digital Homogenizer) and ultrasonicated (10 min, 40% power using Branson Digital Sonifier 450, 400 W) to yield partially deacetylated nanochitin. The final aqueous PD-NCh dispersion was obtained after centrifugation (10 000 rpm).

Negatively charged TEMPO-oxidized nanochitin, TO-NCh, was prepared as follows: Chitin (1 g) was suspended in water (100 mL) containing TEMPO reagent (0.016 g, 0.1 mmol) and sodium bromide (0.1 g, 1 mmol). Chitin oxidation was performed in NaClO (5 mL, 7 mmol). The pH of the slurry was maintained at 10 at room temperature by continuous addition of 0.1 M NaOH using a pH-Stat titration system. When no further consumption of the alkali was observed, the oxidation was quenched by adding a small amount of ethanol. After the pH was adjusted to 7 with 0.5 M HCl, the mixture was washed with deionized water and repeated centrifugation. The water-insoluble fraction was obtained and stored at 4 °C. For the preparation of TO-NCh, the oxidized chitin was suspended in deionized water at a concentration of 0.3% (w/v); this was followed by homogenization (5 min, 10 000 rpm), ultrasonication (10 min, 40% power), and centrifugation (10 000 rpm).<sup>21</sup>

Processing (Dispersion, Drying, and Reconstitution) of PD-NCh and TO-NCh. The mass concentrations of the PD-NCh and TO-NCh dispersions were first adjusted to 0.1 wt %; all the experiments were conducted with this original mass concentration. For both PD-NCh and TO-NCh, salt was added to the dispersion, which was shaken by hand or treated with bath sonication at room temperature until the salt was fully dissolved. After keeping the respective dispersion still at room temperature for about 10 min, it was centrifuged at 10 000 rpm for the collection of the precipitate as the concentrated nanochitin. To further remove residual water in the concentrated PD-NCh or TO-NCh dispersion, the samples were dried at 60 °C for 12 h. For the successful redispersion of dried PD-NCh and TO-NCh, they were first ion-exchanged to remove salt: PD-NCh and TO-NCh were washed with dilute acetic and hydrochloric acid, respectively. The dried TO-NCh was further washed with distilled water to adjust the pH to above 5. After ion exchange, the dried PD-NCh and TO-NCh were soaked in an acetic acid solution  $(pH \sim 3)$  and distilled water, respectively; this was followed with homogenization (30s, 10 000 rpm) and ultrasonication (30s, 40% power).

**Colloidal Characterization.** *Turbidity and Particle Sizing.* Light-transmittance measurements (400–800 nm) were conducted with a UV 2550 spectrophotometer (Shimadzu Ltd.) at 25 °C. Dynamic-light-scattering (DLS) measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments Ltd.) at an angle of 90°. The data were analyzed using the built-in software to determine the apparent average particle sizes of PD-NCh and TO-NCh as functions of salt addition.

Dispersion Stability.  $\zeta$ -Potential measurements were conducted to access the dispersion stabilities of PD-NCh and TO-NCh as functions of salt addition with a Zeta-sizer ZS90 (Malvern Instruments Ltd.) at 25 °C. The concentrations of all samples were the same (0.1%).

Morphology of Dispersed Nanochitins. Transmission electron microscopy (TEM) was used for the determination of the morphologies of PD-NCh and TO-NCh (FEI Tecnai 12 Bio-Twin, FEI). Drops of the suspensions (0.05 wt %) were deposited on electron-microscope grids coated with a carbon-reinforced Formvar film and allowed to dry; this was followed by observation at an acceleration voltage of 120 kV. In the case of TO-NCh, the samples were soaked in 0.1% uranyl acetate (about 30 s), washed with distilled water, and dried at room temperature before observation.

Morphology of Dried PD-NCh and TO-NCh. Scanning electron microscopy (SEM) was performed with a Sigma VP (ZEISS) unit for morphology analysis of PD-NCh and TO-NCh after drying. The samples were coated with Pt before examination.

Mass Concentrations in PD-NCh and TO-NCh Aqueous Dispersions. The mass concentrations of the PD-NCh and TO-NCh dispersions were determined gravimetrically following eq 1:

mass concentration = 
$$\frac{W_{\rm d}}{W_{\rm c}} \times 100\%$$
 (1)

where  $W_d$  is the weight of concentrated PD-NCh or TO-NCh after residual water and salt were removed, and  $W_c$  is the weight of the concentrated PD-NCh or TO-NCh dispersion after centrifugation.

### ASSOCIATED CONTENT

#### **S** Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b02062.

Characterization of nanochitin; TEM images; FTIR spectra; TGA analysis; and photographs of nanochitin before and after the addition of salt, drying, and solvent exchange (PDF)

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#### Notes

The authors declare no competing financial interest.

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