

Efficient, Self-Terminating Isolation of Cellulose Nanocrystals through Periodate Oxidation in Pickering Emulsions

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Many efforts have been made to isolate native nanocrystals from raw materials in the last two decades, such as cellulose nanocrystals (CNCs), but existing methods still suffer from low yields, complicated synthesis processes, and nonuniform sizes of obtained CNCs. This study concerns a facile, self-terminating, and efficient method for the formation of uniform CNCs in high yields during the periodate oxidation process within Pickering emulsions. A biphasic system containing hexane with dissolved hexylamine and an aqueous solution of sodium periodate (NaIO_4) was used as the reaction medium. Regulated by hexylamine, owing to its limited solubility in water, the pH value of the aqueous phase was enhanced to around 9.8, leading to the precipitation of sodium orthoperiodate ($\text{Na}_2\text{H}_3\text{IO}_6$) nanoplates and thus the formation of the initial Pickering emulsions. During the gradual formation of cellulose nanofibers and then CNCs, CNCs were attracted to stabilize the interface of the Pickering emulsions, which prevented further decomposition of CNCs by the oxidizing agent in aqueous suspensions. Thus, this isolation strategy secured the efficient separation of CNCs based on their own particular amphiphilic properties and achieved a high yield of up to 56 wt %.

Native organic nanomaterials have attracted more and more attention throughout the last two decades.^[1–4] One such group of nanomaterials contains native supramolecular crystalline structures of hydrogen-bonded polysaccharide chains, such as nanocellulose and nanochitin with average crystal diameters of 3–10 nm.^[5,6] As the most representative of these nanomaterials, nanocellulose has several advantageous properties, such as easy availability owing to the inexhaustible origin of raw mate-

rials,^[7] excellent mechanical properties with very high E-modules,^[8] well-defined morphology and size, high specific surface area,^[5] high aspect ratio with particular anisotropic properties and therefore the feasibility of forming specific chiral structures in aqueous suspensions,^[5,9] tunable surface chemistry,^[6] and amphiphilicity owing to the presence of both hydrophilic and hydrophobic planes on nanocellulose crystals.^[10] These advantageous characteristics make nanocellulose a promising candidate for a broad range of applications, such as reinforcing fillers,^[8] templates for chiral structures,^[3,11] supports for enantioselective catalysis,^[12] stabilizers of Pickering emulsions based on their amphiphilic properties,^[10] and for the fabrication of biomimetic nanocomposites.^[3]

Several methods have been developed for isolating the smallest native crystalline nanocellulose from raw materials (cellulose nanocrystals or CNCs) with an average diameter of 3–8 nm and an average length of 50–200 nm.^[5] These methods generally include acid hydrolysis,^[13] TEMPO-mediated oxidation,^[14] mechanical treatment,^[15] enzymatic methods,^[16] or combined processes incorporating both pretreatments and mechanical treatments.^[17] The input of chemicals and energy is required to eliminate or break the amorphous region within the raw materials while maintaining the crystalline regions as isolatable nanostructures.^[13,17]

However, even with the precise control of reaction parameters, such as the amount of chemicals or reaction time, the reaction process leading to the formation of nanocellulose is difficult to control. The reasons are primarily that the starting materials are generally a mixture containing both ordered and non-ordered regions and that the nanocellulose formed during isolation could still be further decomposed in the presence of highly aggressive chemicals towards nanocellulose in aqueous solutions.^[17] These drawbacks generally cause additional consumption of more chemicals and energy. At the same time, the separation of CNCs was generally carried out in aqueous solution. Moreover, the synthesis of uniform CNCs is not controllable and the yield is hard to improve. In comparison, biphasic systems forming Pickering emulsions have not been used for the separation of CNCs.

Herein, we report an innovative self-terminating method for efficient isolation of CNCs within Pickering emulsions through periodate oxidation. By using Pickering emulsions starting from a biphasic system (water/hexane), CNCs were enriched at the interface and involved in stabilizing the interface of the Pickering emulsion, together with nanocellulose, during their gradual isolation. Finally, uniform CNCs were obtained in

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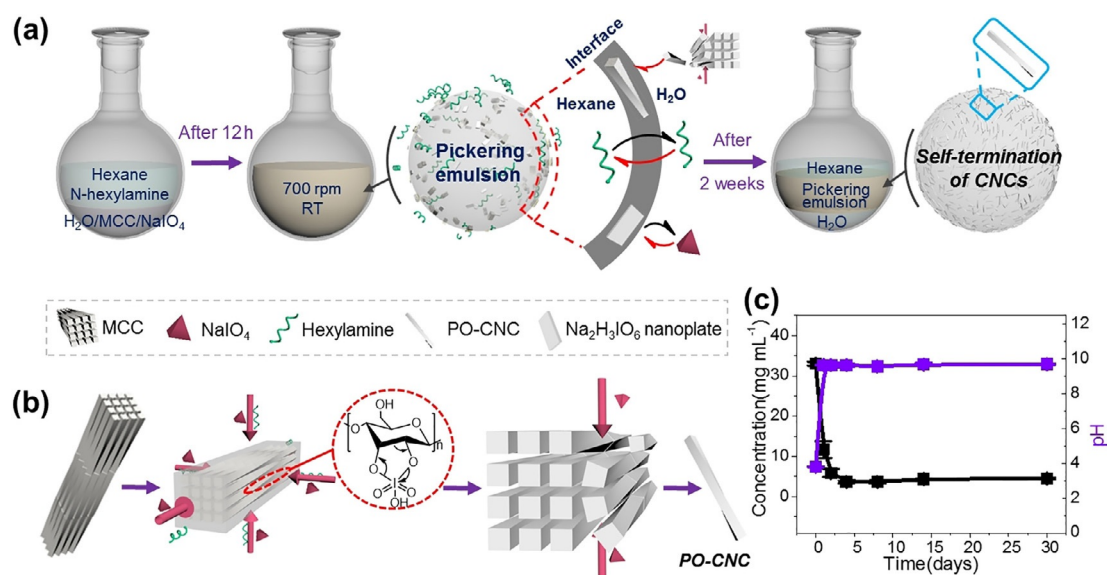


Figure 1. Synthesis of cellulose nanocrystals (PO-CNCs) within Pickering emulsions. a) Reaction process and formation of Pickering emulsion. b) Separation of PO-CNCs from MCC during periodate oxidation in the water phase (non-ordered regions between ordered regions are shown as blank in the image for better visualization). c) Concentrations of dissolved oxidizing agent and pH values in the water phase during the reaction process for up to 30 days.

higher yields through our self-terminating and self-stabilizing processes.

For the synthesis of CNCs, a mixture of aqueous NaIO₄ and hexane containing dissolved hexylamine was used. NaIO₄ was chosen as oxidizing agent for the oxidation of cellulose,^[18–21] whereas the mixture of water and hexane was used for the formation of Pickering emulsions (Figure 1a). By mixing hexane containing hexylamine and aqueous dispersions of microcrystalline cellulose (MCC), Pickering emulsions slowly formed within 12 h under stirring. CNCs were gradually generated during the periodate oxidation, and are hereafter referred to as PO-CNCs (Figure 1a,b). After 2 weeks reaction under ambient conditions in the absence of light, uniform PO-CNCs were obtained with yields of 47–56 wt%. The yields and size distributions of PO-CNCs remained stable in the Pickering emulsion after their formation, even with prolonged reaction times up to 30 days. Thus, the present synthesis route demonstrates a self-terminating method for generating CNCs. It should be noted that the periodate oxidation using the same amount of NaIO₄ and MCC in aqueous solution or in a mixture of hexane and the aqueous dispersion of MCC only led to water-soluble dialdehyde cellulose.^[18–22]

Therefore, the formation of Pickering emulsions during the synthesis of CNCs plays a critical role. Pickering emulsions resulted from the combined effects of hexylamine, precipitated Na₂H₃IO₆ nanoplates (see the Supporting Information, Figure S2) and smaller cellulose fibers or CNCs. During mixing at the beginning, hexylamine in hexane was partly dissolved in the water phase. The dissolution of hexylamine in water increased the pH value in the water phase from 3.9 ± 0.1 to 9.8 ± 0.2 (Figure 1c and Figure S1). The increase in pH caused the precipitation of 65 ± 2 wt% of NaIO₄ from the aqueous solution (Figure 1c and Figure S1e), leading to crystalline Na₂H₃IO₆ nanoplates with an average thickness of around

250 nm (Figure S2). These Na₂H₃IO₆ nanoplates together with hexylamine were able to stabilize the oil-in-water Pickering emulsions. The nanoplates were also the main content of Pickering emulsions from the beginning (Figure S2). Moreover, they could be re-dissolved in the water phase if the concentration of NaIO₄ in the aqueous solution decreased (Figure S1e).

Owing to the limited solubility of hexylamine in water and a hexylamine pool in the hexane phase (Figure S1b,d), the pH value in the aqueous phase was maintained at around 9.8 ± 0.2 during the oxidation process for up to 30 days (Figure 1c). The constant pH value precisely regulated the concentration of NaIO₄ in water phase (Figure 1c). Following the oxidation of cellulose and thus the consumption of oxidizing agent, Na₂H₃IO₆ nanoplates at the interface are re-dissolved into aqueous solution and thus acted as a reservoir for oxidizing agent (Figure S1d,e). Subsequently, periodate oxidation in the water phase was terminated in an alkaline environment with a low and constant concentration of NaIO₄ of 4.5 ± 0.2 mg mL⁻¹ (Figure 1c). Thus, we achieved a controlled system maintaining a constant pH value and a low concentration of oxidizing agent in the water phase (Figure 1a and Figure S1). Under these reaction conditions, the non-ordered regions between crystalline domains and in the outer layers of cellulose microfibrils were preferentially oxidized, degraded, and then removed, which was promoted by the existing hexylamine.^[22–24] Thus, cellulose nanofibers and CNCs were attracted during the mixing to stabilize the Pickering emulsion interface of water and hexane (Figure 1a and 1b). Further oxidation of CNCs was strongly retarded due to the low concentration of NaIO₄ and the relocation of CNCs from the aqueous suspension to the interface of Pickering emulsions.

To reveal details during the periodate oxidation process within Pickering emulsions, the isolation process was tracked by detecting the size of the products after various reaction

times (Figure 2). Under slight mechanical stirring within 2 days, oil-in-water Pickering emulsions gradually formed at the top of the mixture and cellulose microfibrils remain in the bottom suspension (Figure 2, 2 days, and the Supporting Information). In addition, an obvious size reduction of cellulose fibers was detected in comparison to starting MCC. Transmission electron microscopy (TEM) measurements indicated the presence of nanofibers in the Pickering emulsions with diameters of less than 100 nm after 2 days oxidation (Figure 2b, 2 days). After 4 days oxidation, most of the cellulose was transferred to the Pickering emulsion layer and the remainder of the cellulose bundles within the suspension were strongly reduced in diameter to less than 200 nm (Figure 2b, 4 days). After 8 days oxidation, there was almost no visible solid in the aqueous suspension any more (Figure 2b, 8 days). Cellulose nanofibers within the Pickering emulsions generally had diameters of less than 100 nm. Although associated nanocellulose bundles were still present, optically visible large particles around the interface disappeared slowly within the first 8 days in comparison to the oxidation after 2 and 4 days (Figure 2c).

With extended oxidation time to 14 days, PO-CNCs with yields of 47–56 wt% were obtained without harsh post-treatments, such as long-time ultrasonication or homogenization at high power (see the Supporting Information). With even longer oxidation times of up to 30 days, PO-CNCs were obtained with similar yields, which indicates that the generated PO-CNCs did not decompose during extended oxidation in such reaction systems and that formation of PO-CNCs by periodate oxidation was terminated after their formation (Figure 1a–c). This self-terminating process is primarily due to the formation of stable oil-in-water Pickering emulsions by involving PO-CNCs at the interface after their formation. Moreover, as-prepared PO-CNCs, for example, after 30 days oxidation, formed characteristic chiral structures in aqueous dispersions (Figure 2b).

Both TEM and atomic force microscopy (AFM) analyses confirmed the 1D nanostructure of obtained PO-CNCs (Figures 2b and 3). Single PO-CNCs prepared after 14 days periodate oxidation generally have diameters between 4 and 8 nm (Figure 3a). The average diameter of PO-CNCs after 14 days oxidation lies at 5.1 ± 0.96 nm and the average length is 120.1 ± 7.9 nm. PO-CNCs after 30 days oxidation are even more uniform with diameters between 4 and 7 nm.

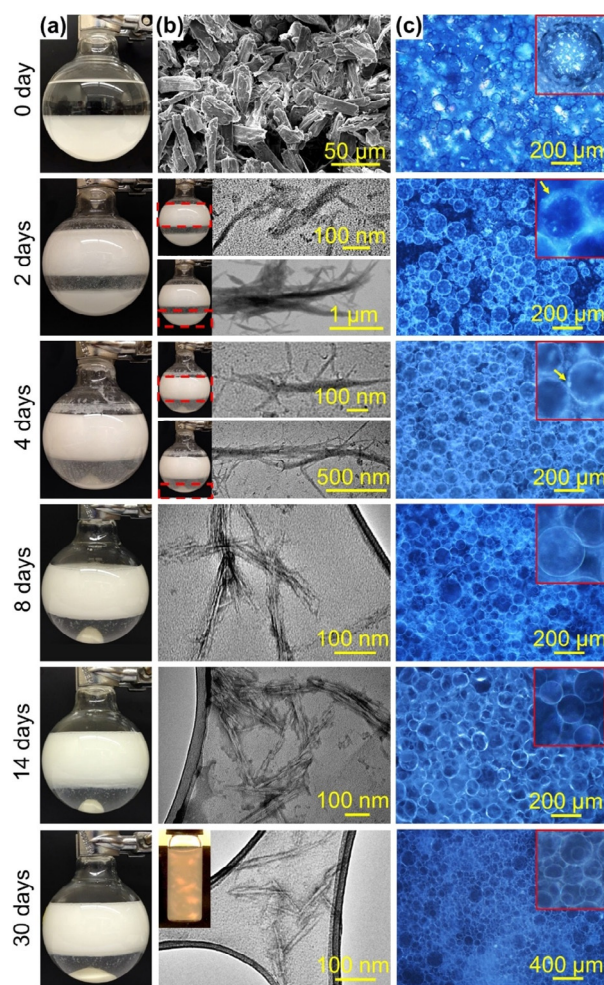


Figure 2. The reaction process during the formation of PO-CNCs and stable Pickering emulsions for up to 30 days. a) Optical images of reaction mixtures after corresponding reaction times of 0–30 days (photos were taken after stopping stirring for 1 h). b) Morphology of purified products ranging from microscale MCC through bundles of nanofibers to PO-CNCs. At the beginning of the reaction, a typical SEM image of MCC is shown. After 2 and 4 days reaction, TEM images of the products in the upper emulsion layers and the bottom suspensions are shown, respectively. After 8, 14, and 30 days reaction, TEM images of the products in the emulsions are displayed. The inset in the TEM image of PO-CNCs after 30 days illustrates a representative optical image of the aqueous suspension of PO-CNCs between crossed polarizers. c) Microscopic images of the reaction mixture (0 days) and the emulsions after 2–30 days oxidation. The insets show selected regions within the images with 2–5 times magnification.

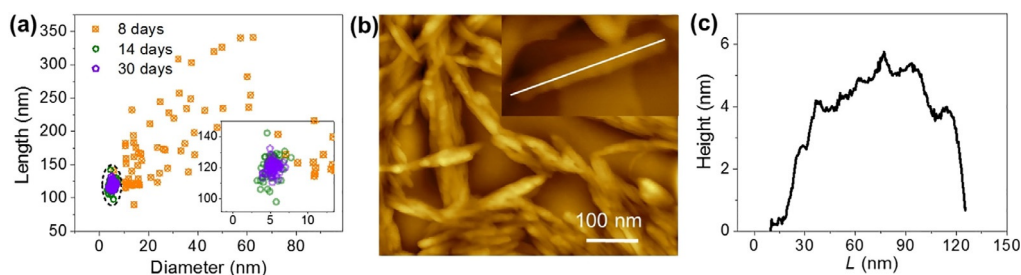


Figure 3. a) Diameter distribution of the products after 8, 14, and 30 days oxidation. Inset: magnified region showing the diameters and lengths of PO-CNCs after 14 and 30 days oxidation. b) Typical AFM image of dried PO-CNCs on wafer. The inset shows one single PO-CNC. c) The height profile of one PO-CNC as shown in the inset in (b).

The surface of the PO-CNCs did not contain significant amounts of aldehyde or carboxyl groups, in contrast to TEMPO-CNCs as shown by solid-state ^{13}C cross-polarization magic-angle spinning (CP/MAS) NMR spectroscopy (Figure 4a).

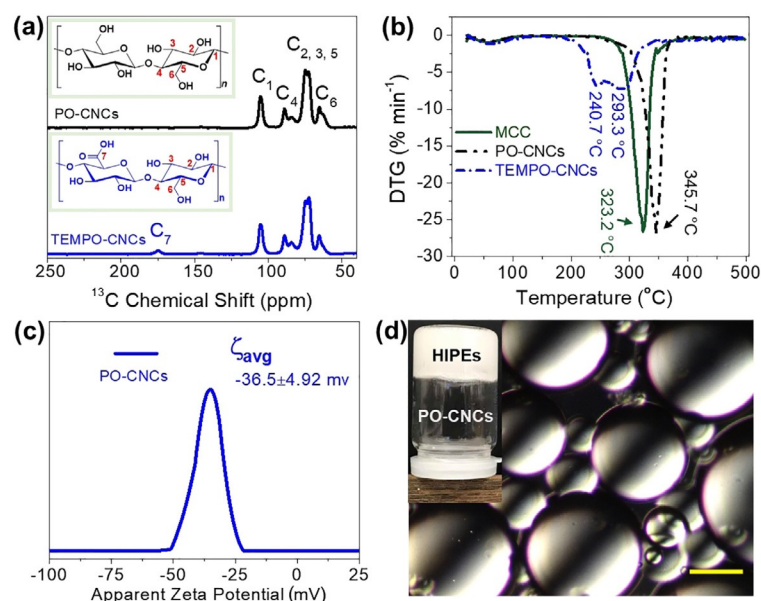


Figure 4. a) Solid-state ^{13}C CP/MAS NMR spectrum of PO-CNCs and TEMPO-CNCs (CNCs prepared by TEMPO-mediated oxidation) at $\delta = 40\text{--}250$ ppm. The insets show the chemical structures of anhydroglucose units of cellulose backbone with and without carboxyl groups. b) TGA curves of MCC, PO-CNCs, and TEMPO-CNCs. c) Zeta potentials of PO-CNCs. d) Representative POM images of the Pickering emulsions stabilized by PO-CNCs (3.5 mg mL^{-1}) with a hexadecane/water ratio of 90:10 v/v (POM images recorded after dilution of the Pickering emulsions in water for better observation.). The scale bars in POM images represent 100 μm . The insets display the optical images of the Pickering emulsions.

According to conductive titration of PO-CNCs, a very low amount of carboxyl groups ($0.14 \pm 0.01 \text{ mmol g}^{-1}$) was present at the surface of the PO-CNCs (titration curves in Figure S4). After further oxidation with sodium chlorite, the amount of carboxyl groups increased only slightly to $0.17 \pm 0.2 \text{ mmol g}^{-1}$, indicating the presence of aldehyde groups at a concentration of around 0.03 mmol g^{-1} . Furthermore, no amines—including hexylamine—could be grafted onto the surface of PO-CNCs by covalent bonds under the reaction conditions for Schiff-base formation, as shown by dynamic nuclear polarization (DNP)-enhanced ^{15}N CP/MAS NMR spectroscopy and elemental analysis (see the Supporting Information, Figure S3). Thermogravimetric analysis (TGA) of the PO-CNCs demonstrated the same hydrolysis trend as for MCC, but the thermal decomposition temperature of PO-CNCs ($355.7 \pm 0.2^{\circ}\text{C}$) is much higher than that of MCC (323.2°C) and of the CNCs prepared by TEMPO-catalyzed oxidation (293.3°C ; Figure 4b and Figure S5).^[25] Thus, the PO-CNCs mostly have hydroxy groups and a very low amount of carboxyl and aldehyde groups at the surface. Moreover, PO-CNCs had a crystallinity of approximately 55% based on solid-state ^{13}C NMR spectroscopy, which is comparable to other CNCs (e.g., TEMPO-CNCs; Figure S6).

The presence of almost only hydroxy groups and a tiny amount of carboxyl and aldehyde groups at the surface of PO-CNCs should be attributed to the instability of the oxidized cellulose chains.^[26] The periodate oxidation generally starts with

the formation of bonds between NaIO_4 and the diols $[\text{C}2(\text{OH})\text{--}\text{C}3(\text{OH})]$ within anhydroglucose units of outermost cellulose chains of cellulose fibers.^[18–22] The bond between C2 and C3 is cleaved during the oxidation of diols into two aldehyde groups. Following this, the hydrogen bonds related to the hydroxy groups at C2 and C3 are cleaved. This is particularly critical for the OH groups at C3, which are generally involved in the $\text{C}3(\text{OH})\cdots\text{C}5(\text{O})$ hydrogen bonds.^[27] The cleavage of these hydrogen bonds leads to many defect sites within the outermost layers. Further degradation of the oxidized cellulose, which was promoted by the presence of hexylamine,^[23,24] would cooperatively promote the removal of these outermost oxidized cellulose chains.^[27,28] Subsequently, no significant amount of carboxyl or aldehyde groups is present on the surface of PO-CNCs. According to the widely accepted model of CNCs (Figure S7),^[29] the distribution of hydroxy groups on each crystal plane is not homogeneous, which is induced by the crystalline organization of cellulose chains during the formation of hydrogen bonds.^[10,30] Because the peeling-off of the surface chains during oxidation can only happen on the hydrophilic crystal planes with hydroxy groups, the hydrophobic planes without exposed hydroxy groups should remain, leading to PO-CNCs with enhanced amphiphilic properties.

In fact, PO-CNCs very easily stabilized oil-in-water high internal phase Pickering emulsions (HIPEs) with chiral properties by a facile one-step process (Figure 4d and Figure S8). More than 90% of the organic phase (hexadecane) could be stabilized within the Pickering emulsions by using an aqueous suspension of PO-CNCs with a concentration of 3.5 mg mL^{-1} . Only a one-step emulsification process with very low energy input was required, for example, by using a homogenizer at 7000 rpm for less than 10 s (Figure 4d). In comparison, very few HIPEs reported to date have used CNCs as a stabilizer without surface modification.^[10,29] Compared with the CNCs obtained after the hydrochloric acid hydrolysis,^[10,29] only half the amount of PO-CNCs with a surface zeta potential of $-36.5 \pm 4.92 \text{ mV}$ (ca. 0.044 wt % of the whole Pickering emulsion system) was required to stabilize 90% hexadecane within the Pickering emulsions by a one-step emulsification (Figure 4c,d).^[10] This advantage of easily and efficiently stabilizing HIPEs should predominantly be attributed to the enhanced amphiphilic properties of PO-CNCs.

In summary, we have developed an innovative and highly efficient and facile self-terminating method for isolating uniform cellulose nanocrystals (CNCs) with high yields through periodate oxidation (PO) in Pickering emulsions. The obtained PO-CNCs showed similar dimensions of CNCs whereas their surface predominantly contains hydroxy groups. PO-CNCs were highly

thermostable and could easily stabilize surfactant-free high internal phase Pickering emulsions (HIPEs) through a facile one-step emulsification process by using extremely low amounts of PO-CNCs.

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Conflict of interest

The authors declare no conflict of interest.

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