Preparation of Cellulose Nanocrystals based Core-Shell Particles
with Tunable Component Location

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Abstract: We report a versatile method for preparing a particulate composite based on cellulose nanocrystals (CNCs) and polyethylene glycol (PEG) via the self-organized precipitation method. The particulate composite had a core–shell structure, and depending on the molecular weight of the PEG, two types of particulates could form: one with CNCs as the core and the other with CNCs as the shell.
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Cellulose nanocrystals (CNCs), derived from wood and other natural sources, are anisotropic, rod-shaped nanoparticles[1-3]. CNCs are regarded as one of the most promising sustainable and nontoxic materials, having versatile, attractive characteristics such as high mechanical strength, high gas barrier, and unique optical properties.

Incorporating CNCs with general polymer can exploit the attractive characteristics of CNCs. Especially, particulate composite fabrication of CNCs with polymer would be worth developed. One of the reasons is that the surface of CNCs is so hydrophilic that CNCs cannot be easily dispersed in a non-polar matrix simply by mixing. Thus, stable-composite fabrication using polymers is a practical way of imparting amphiphilic properties to CNCs[4,5]. In addition, the particulate composite can be used as a secondary building block to fabricate higher-order materials with more sophisticated architecture, an application that cannot be achieved with film-based CNC materials.

Among the various forms of CNC–polymer particles, core–shell particles with regulated CNC locations are a target for achieving controlled characteristics of CNCs. CNC particulates coated with other components can be dispersed in various media by controlling the affinity between the polymer component and the matrix. On the other hand, polymer particulates having a CNC crust can yield stable micro-spaces with cellulose walls similar to those of plant cells, resulting in a bio-inspired material with high mechanical strength.

In this study, we propose a fabrication method for the particulate composite of CNCs and polymer with a tunable component location. Confinement of CNCs in particles has been challenged by some groups, who chose to fabricate core–shell particles with CNC core by utilizing microfluidic emulsification[6,7]. Wang et al. also reported the synthesis of core–shell particles with CNC cores by emulsion polymerization, which yielded particles with 30–200 μm diameters[8]. On the other hand, Jativa et al. also reported

CNC capsules, in which CNCs are in the shell of a core–shell particle[9]. However, no previous studies have reported a particulate-composite fabrication method that can create core–shell particles that contain CNCs either in the core or the shell; developing a widely applicable method is a challenge. To widen the selection of possible counterpart components, the fabrication method should be simple, with few restrictions.

To create a core–shell-like composite featuring CNCs with tunable component locations, we used the self-organized precipitation (SORP) method proposed by Yabu[10,11]. Unlike other methods such as dispersion polymerization[12] or emulsion polymerization[13], the SORP method does not require strict polymerization control or additional surfactant. The SORP method is known to be a facile method for preparing particles composed of two components. It usually involves two solutes and two miscible solvents (one solvent is good at dissolving solutes, while the other is poor). When the good solvent is evaporated, the solubility of the solutes decreases, causing core–shell particles to form. The particle architecture, i.e., the component location, is controlled by the difference in affinity between the two solutes and the poor solvent. Thus, by controlling the affinities between the CNCs and polymer components, this method can be applied to produce particles with a tunable location.

In this paper, we report the fabrication of core–shell-like particles via the SORP method. Two types of CNC/polyethylene glycol (PEG) particles with different CNC locations were obtained simply by changing the molecular weight of PEG. In the experiment, CNCs and PEG were used as the two solutes, and water and ethylene glycol (EG) were used as the two miscible solvents. In this
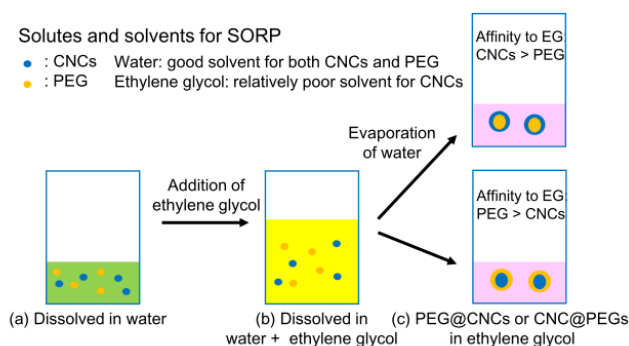

Figure 1 Schematic concept of CNCs/PEG particle fabrication with tunable CNCs locations *via* SORP method.

system, water is a good solvent for both CNCs and PEG, and EG has a higher boiling point than water. We chose PEG as the polymer component because the affinity between PEG and solvent can be varied through the molecular weight[14]. Furthermore, PEG has been widely applied in biomedical materials because of its non-toxicity and bio-degradability, characteristics that could make the combination of CNCs and PEG a sustainable material.

Figure 1 shows a schematic of the SORP method applied to the CNC–PEG solute in the water–EG system. First, CNC and PEG are dissolved in water (Fig. 1a), and EG is added to the solution (Fig. 1b). Water then evaporates, while EG remains unevaporated (Fig. 1c). In this process, CNCs and PEG tend to deposit because of their decreased solubility in EG. The resultant core–shell particles depend on the relative affinities of CNC and PEG to EG, as shown in Fig. 1c. If the CNC–EG affinity is greater than that of PEG–EG, then CNCs form a shell around the PEG core because the CNC–EG interaction is favored, and vice versa. Using PEG solubility differences, we aimed to fabricate two types of core–shell particles with tunable CNC locations, as shown in Fig. 1c.

We used two types of PEG with average molecular weights of 2,000 and 20,000, denoted hereafter as PEG2,000 and PEG20,000, respectively. CNCs having a length of around 40–100 nm and a width of 2–5 nm CNCs were purchased from CelluForce, Canada. CNC has sulfonic acid groups with sulfate content of ca. 250 mmol kg^{-1}. PEG20,000 and PEG2,000 were purchased from Wako Chemical Industry, Japan, and their molecular weight ranges claimed by the supplier are 15,000~25,000 and 1,800~2,200, respectively. The sample preparation procedure is represented in Fig. 1. CNCs and PEG were separately dispersed in Mill-Q water completely to a concentration of 8 g L^{-1}. For the preparation of the CNC/PEG sample, the CNC dispersion was mixed with the PEG dispersion and water to prepare 4 mL of 2 g L^{-1} of CNCs and PEG. The same volume (4 mL) of ethylene glycol was gradually added to form an aqueous mixture. The solution was stirred for 24 hours to prepare a mixture of CNCs (1 g L^{-1}) and PEG (1 g L^{-1}) in the water/ethylene glycol solvent mixture. The solutions for both systems were transparent at this stage, as shown in Fig. 2a, b.
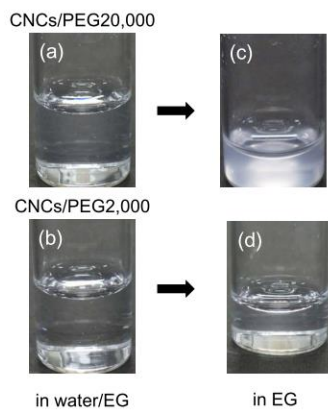

Figure 2 Appearances of CNCs/PEG20,000 ((a),(c)) and CNCs/PEG2,000((b),(d)) solutions in the process of SORP method: Before EG evaporation dispersed in water/EG ((a),(b)) and after the evaporation of EG ((c), (d))

Core–shell particles with CNC shell were obtained when using PEG20,000. The CNC/PEG20,000 solution in water and EG was transparent at room temperature. The mixtures were heated at 45 °C to evaporate to half the volume in order to remove the water and obtain the EG solution. The evaporation of ca. 2 ml of water out of 4 ml mixture solution takes three days. At this stage, the CNC/PEG20,000 solution turned turbid, as shown in Fig. 2c. However, we could not observe the particle formation clearly by optical microscopy; we confirmed the fluctuation in the contrast of the optical microscopy image for the CNC/PEG20,000 solution.

Subsequently, we took small amounts of the dispersions and kept in a 40 °C vacuum oven for two days to dry completely, and characterized the dried state. We observed the dried samples using an optical microscope (Olympus Bx-51, Tokyo, Japan). Figure S1a is a representative microscope image for the CNCs/PEG20,000 sample. The image indicates the formation of numerous particles with irregular shapes. We observed particles ranging in size from 2 μm to several micrometers. In the case of the larger particles, core–shell-like contrast could be observed in the images, with dark, irregular shells surrounding brighter cores. These results suggest that the SORP process formed core–shell particles of CNCs and PEG.

To identify the core–shell components, we observed the dried particles using fluorescence microscopy. Rhodamine 6G (R6G) dye was used as the fluorescent probe. To identify the location of the CNCs and PEGs in the composite, samples containing R6G were also prepared besides CNC/PEG. R6G was added to the CNC/PEG aqueous mixture to a concentration of 2.46×10^{-5} mol L^{-1} before EG addition. The final concentration of R6G in the solvent was 1.23×10^{-5} mol L^{-1}.

The optical microscope was equipped with a Bx2-FL-1 fluorescent unit, and the fluorescent optical microscopic (FOM) images were taken with an Olympus U-MSWB-3
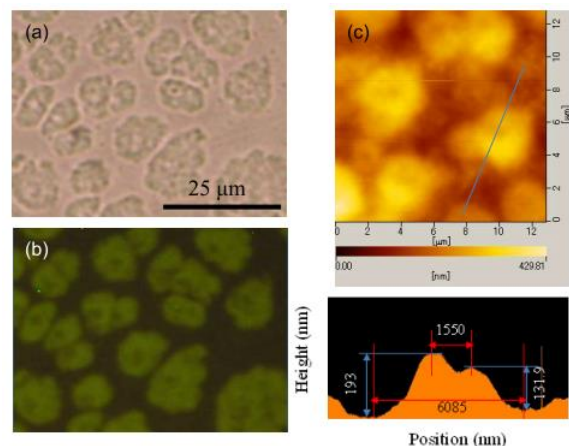

Figure 3 Observed images of dried CNCs/PEG20,000/R6G. (a) Bright field microscope image, (b) fluorescent optical microscope image, and (c) AFM topological image with cross-section.

filter. In a preliminary test using fluorescent measurements, we confirmed that R6G selectively adsorbed to CNCs in water (Fig. S2, S3). Thus, we concluded that we could identify the location of the CNCs using R6G fluorescence and FOM.

We observed the CNCs/PEG20,000 particles containing R6G (CNCs/PEG/R6G). Figures 3a, and b show images of dried CNC/PEG20,000/R6G by bright-field and FOM, respectively. In Fig. 3a, we can see similar images without R6G, as shown in Fig. S1, which indicate that the addition of R6G did not change the morphology of the core–shell particulates. In the fluorescent images (Fig. 3b), we could locate the position on CNCs to which R6G adsorbed. Figure 3b shows bright-green shells (rims) and darker particulate centers. The results of FOM suggest that the CNCs are located in the shell. The core–shell structure can be visualized more clearly in Fig. S4, which shows color-modified images with specific color assignments for each measurement. Thus, we confirmed that in the CNC/PEG20,000 system the structures were core–shell-like particulates with CNC shell components.

To further identify the particulate structure, we performed AFM observation using the Nanonavi IIs station and a Nanocute unit (Hitachi High-Tech Science Corp. Tokyo, Japan) in dynamic force mode with a PRC-DF40P cantilever (SII Nanotechnology, Tokyo, Japan). The location of PEG is confirmed by the AFM images shown in Fig. 3c. AFM topological observations can reveal the exact particle morphology. The particulates observed by AFM were donut-shaped, with a small cavity in the center. This shape also confirms the core–shell-like structure visualized by optical microscopy in Fig. 3a. The particle diameter was found to be about 6 μm, which is comparable to the diameter obtained from the optical microscope image. The cross-sectional view indicates that the height was 100–200 nm. The cavity had a depth of several tens of nanometers, but it had a certain height relative to the background, which suggests that this is not a simple hole but it is filled with a PEG component.

On the other hand, the same procedure using CNC/PEG2,000 yielded core–shell particles with a CNC core and PEG shell, structures that were distinctively different from the CNC/PEG20,000 particles. The difference was confirmed by the appearance of the sample after the water evaporation process represented in Fig. 2d. At this stage, the sample liquid was transparent, while for the CNC/PEG20,000 sample, the liquid was turbid. We also dried and observed this sample. A representative optical microscopy image is shown in Fig. S1. It indicates the formation of numerous monodisperse spherical particles around 1 μm in diameter with clear core–shell contours: the shells were brighter than the cores, which were dark gray. The particle size was an order of magnitude smaller than the CNC/PEG20,000 particles. Therefore, the particles obtained from the CNC/PEG20,000 and CNC/PEG2,000 samples were clearly different: the contrast was inverted, and there was a difference in particle size and shape.

Our observations of the CNC/PEG2,000 sample are summarized in Fig. 4. For the samples with added R6G (CNC/PEG2,000/R6G), bright-field optical microscopy (Fig. 4a) and FOM (Fig. 4b) images are shown. Fig. 4a displays core–shell particulates similar to those observed without R6G, shown in Fig. S1b, which shows core–shell particulates with darker centers and brighter surrounding rims. In Fig. 4b, numerous small bright-green dots corresponding to the cores are visible; this is also supported by Fig. S4. This indicates that the cores of the CNC/PEG2,000 particulates consist of CNCs, i.e., the CNC locations are different from those in particulates made from CNC/PEG20,000.
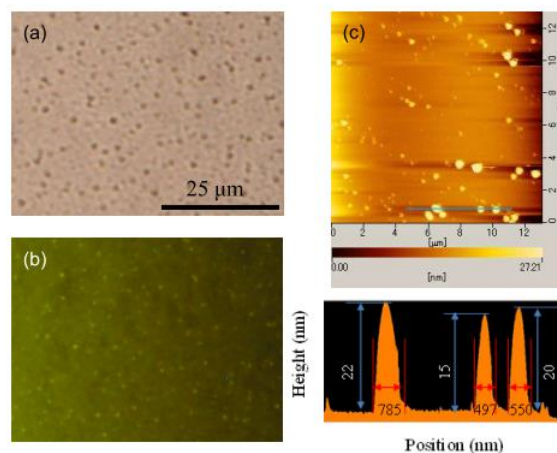

Figure 4 Observed images of dried CNCs/PEG2,000/R6G. (a) Bright field microscope image, (b) fluorescent optical microscope image, and (c) AFM topological image with cross-section.

In the AFM image (Fig. 4c), the particulates with diameters of ca. 500 nm and heights of ca. 20 nm were observed. We calculated particulate diameters from the AFM images that are smaller than from optical microscopy; this can be attributed to the higher resolution in detection by AFM. As discussed above, CNC shells for CNC/PEG20,000 were detected to be higher than the centers. We can conclude that CNC is the higher part because of their stiffness in their dried state. However, in the AFM images for the CNC/PEG2,000 system, smaller dots were detected. In this case, only the CNC core could be visualized, since the PEG2,000 shell could be detected as a lower height. Furthermore, shells made of PEG2,000 could diffuse to the exterior because of the smaller molecular weight of PEG and because of the absence of constraints to PEG diffusion in the shell. This interpretation can explain the smaller diameters for CNC/PEG2,000 in AFM, although further structural investigation, including an investigation in the wet state, should be performed.

In order to confirm the effect of small content of water which might have been left after 50% evaporation of solvent from the initial mixed solution, we examined and compared the results obtained from usual 50% evaporation (water) and ca. 60% evaporation (water+EG). The obtained particle morphology is quite similar, thus the small content of water in the half volume evaporation process does not affect the final morphology.

As discussed above, we have successfully fabricated core–shell particulates with tunable CNC locations by changing the PEG molecular weight. We hypothesized that the placement differences are due to slight differences in affinity between CNC-EG and PEG-EG. All the components are soluble in water, while the solubility of PEG in EG is slightly different, depending on the molecular weight. PEG20,000 is less soluble in EG as compared with PEG2,000. This is evidenced by the appearance of EG solutions of PEG2,000 and PEG20,000 (Figure S5). In the CNC/PEG20,000 system, the PEG component is not completely soluble in EG, and CNC has a higher affinity to EG as compared with PEG20,000. In EG, this results in the formation of a core–shell structure, with CNC forming the shell. By contrast, PEG2,000 has a higher affinity to EG than CNCs, which results in a core–shell structure with a PEG2,000 shell. The difference in affinity to EG has a significant effect on the morphology. The affinity between the polymer and solvent can change drastically ~~depending on~~ by the molecular weight as evidenced by Fig.S5. These polymer characteristics can be applied to control the morphology without changing the component itself.

In summary, we have reported a simple approach for preparing CNC-based core–shell particles via modified SORP method in which particle formation was carried out by simply evaporation of solvent containing solutes with different solubility. In this study, we showed that the molar weight of the added PEG (2,000 vs 20,000) changes the resultant particulate's morphology, CNC location, and shape and size. On the basis of these findings, we expect that core–shell particles with similar compositions can be designed to include CNCs in different locations, which can enrich the applications of this particulate composite system.

Supporting Information is available on http://dx.doi.org/10.1246/cl.******.

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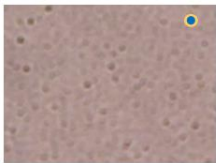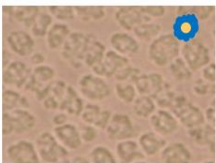
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Graphical Abstract
Textual Information

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Title(required)	Preparation of Cellulose Nanocrystals based Core-Shell Particles with Tunable Component Location
Authors' Names(required)	Jie Zhang, Hinako Furushima, and Teruyuki Nakato, Emiko Mouri

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CNCs @PEG   PEG@CNCs

Tunable Core-Shell Particles