

Architectonics on Fluid Interfaces using Polysaccharide-based Nanofibrils as Building Blocks

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**Architectonics on Fluid Interfaces using
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List of abbreviations

Abs.: Absorbance

ACC: Aqueous counter collision

ACC-BCNF: Bacterial cellulose derived CNF prepared by ACC method

Ac₂O: Acetic anhydride

AFM: Atomic force microscopy

A-PS: Acetylated ACC-CNFs/PS composite

AWCA: Apparent water contact angle

BC: Bacterial cellulose

BN: α -Bromonaphthalene

CAA: Monochloro acetic acid

C8-CNF: Octanoylated (C8) ACC-BCNF

CFW: Calcofluor white

ChNC: Chitin nanocrystal

ChNF: Chitin nanofiber

CI: Crystallinity index

CLSM: Confocal laser scanning microscopy

C8-LB film: LB film fabricated from C8-CNFs

CMF: Cellulose microfibril

CNC: Cellulose nanocrystal

CNF: Cellulose nanofibril

C-PS: ACC-CNFs/PS composite

CtNF: Chitosan nanofiber

DCM: Dichloromethane

DDA: Degree of deacetylation

DIM: Diiodomethane

DMAc: *N, N*-Dimethylacetamide

DMAP: 4-Dimethylaminopyridine

DS: Degree of substitution

EG: Ethylene glycol

FE-SEM: Field emission-scanning electron microscopy

FFT: Fast Fourier Transform

FT-IR: Fourier transform infrared spectroscopy

HA-ACC-CNF: ACC-CNF acetylated in homogeneous dispersion system

HBSP: Hardwood bleached sulfite pulp

h-CNF LB film: Hydrolyzed CNF LB film

IGC: Inverse gas chromatography

JNP: Janus nanoparticles

LB: Langmuir-Blodgett

LFM: Lateral force microscopy

LS: Langmuir-Schaefer

MCC: Microcrystalline cellulose

NFC: Nanofibrillated cellulose

NOC: Nematic ordered cellulose

Nylon66: Polyhexamethylene adipamide

O/W: Oil-in-water

PA-ACC-CNF: ACC-CNF acetylated in Pickering emulsion system

PE: Polyethylene

PET: Polyethylene terephthalate

PMMA: Polymethyl methacrylate

PP: Polypropylene

PS: Polystyrene

SSA: Specific surface area

TBO: Toluidine blue O

TEA: Triethylamine

TEMPO: 2,2,6,6-Tetramethylpiperidine-1-oxyl

TOCNF: TEMPO-oxidized cellulose nanofibril

UV/Vis: Ultraviolet/visible

VAc: Vinyl acetate

VCG: van Oss-Chaudhury-Good

WAXD: Wide-angle X-ray diffraction

WCA: Water contact angle

XPS: X-ray photoelectron spectroscopy

A: Surface area

A_{eff} : Effective surface area of nanofibrils

A_{ow} : Oil-water interfacial area

C: Surface coverage ratio of droplets

d : Film thickness

$D_{3,2}$: Sauter's mean diameter

D_N : Number average droplet diameter

D_v : Volume average droplet diameter

F_{CNF} : Area fraction of CNF

F_s : Area fraction of substrate

g : Gravitational constant

h : Height of nanofibrils

I : Intensity

l : Length of nanofibrils

m : Dry weight of nanofibrils in the system

r : Ratio of actual surface area and apparent surface area

R_0 : Drop radius of curvature at the top of droplet

R_a : Arithmetic average surface roughness

R_{ad} : Adsorption ratio

R_{eo} : Emulsified ratio of oil phase

R_q : Root mean square surface roughness

t : Time

V_0 : Total volume of oil phase

V_{sed} : Sedimentation volume of nanofiber suspension

w : Width of nanofibrils

W_{ad} : Work of adhesion

W^{AB} : Acid-base interaction component of work of adhesion

W^{LW} : Lifshitz-van der Waals interaction component of work of adhesion

β : Bond number

γ : Surface free energy

γ_{12} : Interfacial free energy between phase 1 and phase 2

γ^{AB} : Acid-base interaction component of surface free energy

γ^{LW} : Lifshitz-van der Waals interaction component of surface free energy

γ^+ : Electron acceptor component of γ^{AB}

γ^- : Electron donor component of γ^{AB}

θ : Contact angle (CA)

λ : Wavelength

Π : Surface pressure

π : Circular constant

ρ : Density

φ : Apparent contact angle (ACA)

ψ : Orientation angle of nanofibrils

General introduction

1. Emergence of functions in architectonics of nature

Rapid development in modern technology in the 19-20th centuries has provided abundant inexpensive synthetic materials to result in their mass consumption, but the recent material production system depending on petroleum resources is facing crucial environmental problems.^{1,2)} In contrast, bio-based structural materials are produced from renewable resources, and therefore, implementation of the circular system is aspired for sustainable material production process in the 21st century.

In nature, enzymatically synthesized³⁻⁵⁾ structural units (= “building blocks”) are assembled into hierarchically ordered structures. The elaborate organization process, “self-organization”, is carried out under mild conditions in water media, and thereby, the process is regarded as an essential strategy in development of recent bottom-up nanotechnology. In addition, the ordered assembly of building blocks allows to emerge excellent structure-correlated functions^{6,7)} which are unpredictable from the functions of individual building blocks.

Lipid bilayer^{7,8)} is a well-known example of the structure-function relationships, which enables selective mass transfer⁷⁾ and/or interaction with other cells (Fig. 1). Following phospholipid molecules having surfactant-like amphiphilic structure assemble favorably into the bilayer structure, small domains in the phospholipid layer are reinforced by cholesterol. The rigid domains move around the bilayer structure together with the embedded glycoproteins and transfer proteins which play significant roles as cell markers⁹⁾ and tunnels for transportation,⁷⁾ respectively.

In cell wall architectures near the plant cell membranes, cellulose microfibrils (CMFs) are deposited on the cell membrane while forming oriented CMF layers (Fig. 2).

The cellulose molecules crystallize soon after the biosynthesis on the membrane, thereby self-organizing into a CMF with 3-4 nm in width. The architecture in the CMF with extended chain crystal renders the excellent Young modulus¹⁰⁾ (approx. 150 GPa) and elongation strength¹¹⁾ (2-6 GPa) of a single CMF. Additionally, the microfibril angles of CMF layers on the plant cell membrane relate not only to the mechanical strength of cell walls,¹²⁾ but also direction of cell enlarging.¹³⁾

In recent years, the development of defibrillation techniques has successfully provided fine cellulosic fibers with 3-100 nm in width termed cellulose nanofibrils (CNFs) that own superior characteristics derived from natural nanostructures mentioned above.¹⁴⁾

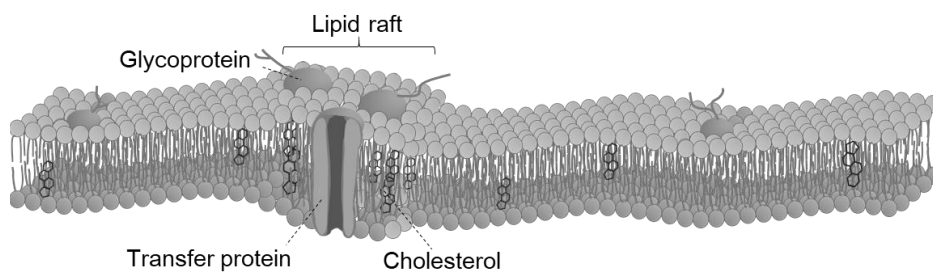


Fig. 1 Assembled structure of lipid bilayer.

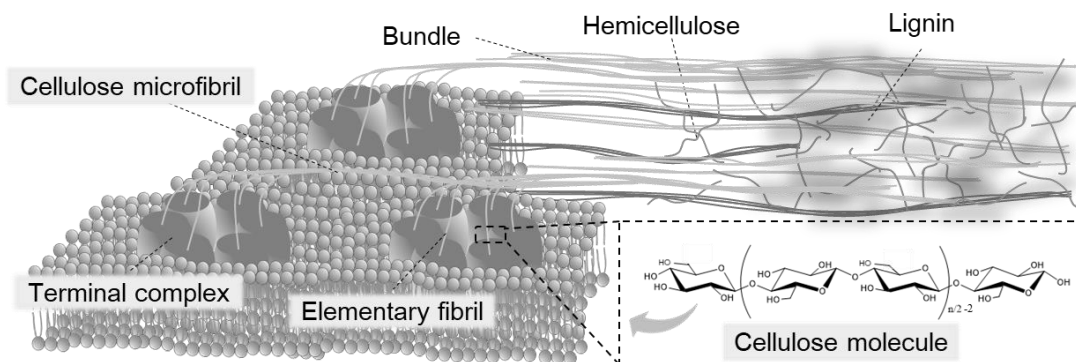


Fig. 2 Self-organization of cellulose on the cell membrane.

2. Surface free energy and anisotropy in colloidal assembly

“Colloid” is a sense of the system composed of dispersion media and particles with 10^{-9} - 10^{-6} m in diameter. In this colloidal dimension, particles have large unstable interfaces or surfaces compared with those of bulk materials. The instability is due to the high potential of molecules located near the material surface, and the excess Gibbs free energy per unit area, which is termed “surface free energy”, affects strongly assembly/disassembly process of colloids, diffusion behavior, and sintering process of fine particles.

Recently, organization of colloidal particles have been investigated for photonic materials,¹⁵⁻¹⁷⁾ cell-like reactors,¹⁸⁾ and sensing materials.¹⁶⁾ Fig. 3 shows the assembly of isotropic and anisotropic colloidal particles. The uniform colloidal building blocks assemble into colloidal crystals, which are highly ordered 3D packing states.¹⁷⁾ Alternatively, assembled states of “geometrically¹⁹⁻²¹⁾ or chemically anisotropic particles”²¹⁻²⁴⁾ have various structural patterns. Rod-,¹⁹⁾ plate-,²⁰⁾ snowman-²¹⁾ like particles are categorized as geometrically anisotropic particles, and their structural factors are sensitive to the shape of assembled states.²¹⁾

Chemical heterogeneity is another sense of anisotropy. In colloidal particles, localization of functional groups on the surface has significant meanings. In particular, particles with chemically different two planes are called “Janus particles” named after the ancient Roman god, “Janus” having two faces.²²⁾ Such a chemical anisotropy is given by chemical modification,^{22,24)} phase separation,^{22,23)} and self-assembly.²²⁾

On the other hand, the natural cellulose fiber inherently has hydrophilic hydroxy (OH) surfaces and less-hydrophilic glucan sheet (CH) planes mostly located inside of the fiber.²⁵⁾ Recently, it was clarified that the CNFs prepared by aqueous counter

collision^{14,26,27)} (ACC-CNFs) possess both OH planes and less-hydrophilic CH planes on its single fiber surface,²⁸⁾ inducing favorable adsorption onto the hydrophobic oils or resins in an aqueous media.¹⁴⁾ Hence, the amphiphilic-like ACC-CNF can be regarded as a unique class in colloidal building block having chemically anisotropic surface and geometrical anisotropy by means of the fibrous shape.

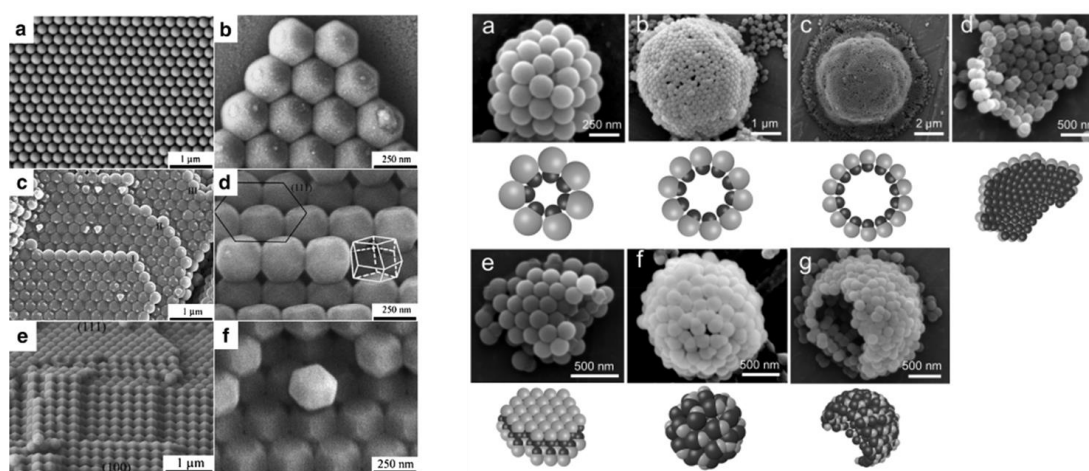


Fig. 3 Left; Colloidal crystals composed from isotropic non-spherical carboxyl-modified polystyrene particles via hot pressing. Reprinted from 15) © 2005 from ACS publications. Right; Assembled structures of the anisotropic snowman-like Janus particles with various shapes. Reprinted from 21) © 2018 from ACS publications.

3. Nanopolysaccharides and their self-organization

Nanocelluloses, one of the building blocks in the plant cell walls, are classified as cellulose nanocrystals^{29,30)} (CNCs) and CNFs.^{26,27,31)} Rod-like CNCs and chitin nanocrystals (ChNCs) are conventionally obtained by removal of non-crystalline regions with sulfuric acid hydrolysis.^{29,30)} Here, it is noted that the left-handed twist in C(h)NCs and sulfate half ester groups, which are introduced during sulfuric acid

hydrolysis process, play key roles for self-ordering of CNCs suspensions, resulting in forming chiral nematic liquid crystals.^{30,32,33)} As represented by sulfate half ester groups, surface modification of C(h)NCs is a strong tool for inducing assembling ordered structures. In fact, the star-³⁴⁾ or pillar-like³⁵⁾ CNC assembly was developed by the selective functionalization of reducing ends.^{34,35)}

CNFs^{14,26,27,31,36-38)} and chitin nanofibers³⁹⁾ (ChNFs) with a high aspect ratio (≥ 100) are produced by various methods such as mechanical defibrillation,³⁶⁾ combined method using chemical treatments (e.g. TEMPO-mediated oxidation,³¹⁾ phosphorylation,³⁷⁾ and succinic acid modification³⁸⁾) with mechanical disintegration, and physicochemical process such as ACC.^{14,26,27)} Similarly to the C(h)NCs, assembly of the C(h)NFs was extensively investigated for improvement of mechanical strength⁴⁰⁾ and cell responses.^{41,42)} Although the C(h)NFs with high aspect ratios effectively improve mechanical strength of nanocomposites⁴³⁾ and stability of Pickering emulsions comparing with rod-like short C(h)NCs,⁴⁴⁾ the self-organization of the C(h)NFs is restricted by entanglement of fibers.^{40,45)} Hence, artificial fabrication of uniaxially oriented C(h)NF structure needs impression of external forces.⁴⁰⁾ In contrast, living plant cells force organization of cellulose molecules into oriented CMFs layers at the “interface” between the aqueous phase and liquid crystalline lipid bilayer phase.

4. Architectures using fluid interfaces

The initial studies about molecular assembly on the air-water interface began at the late 19th century, which was achieved by Rayleigh (1842-1919) and Pockels (1862-1935).⁴⁶⁾ The works were further developed into a fabrication method of substrate-supported molecular films by Langmuir (1881-1957) and coworkers, which is presently

known as Langmuir-Blodgett/Schaefer (LB/LS) technique,^{47,48)} and these works initiated today's basis of surface science.

Heterogeneity in the air-water interface of the LB trough induces unique molecular assemblies. Long alkyl chains of fatty acids or side chains of cellulose derivatives are repelled from the water surface, then stand up towards the air side to decrease interfacial free energy.⁴⁹⁻⁵¹⁾ Concerning the LB process of cellulose derivatives, the 2,3-di-*O*-octadecylcellulose, the molecular backbone was also altered from 2₁ screw of cellulose backbone into onefold axis during compression at the air-water interface.^{50,51)} In addition, the compression and flow direction in LB process induce uniaxial ordering of CNCs on the air-water interface, then the aligned CNC LB film shows anisotropic surface functions.⁵²⁾

Pickering emulsions reported by Ramsden⁵³⁾ and Pickering⁵⁴⁾ in early 20th century is another assembling system at fluidic interfaces, which are stabilized by adsorption of solid particles. In Pickering emulsions, the energetically unstable oil-water interface is firstly stabilized by adsorption of solid particles, then these adsorbed particles are rearranged to energetically favorable location at the oil-water interface to decrease interfacial free energy of the fluids by dense packing of particles.⁵⁵⁾ The motion and assembled state of the solid particles at oil-water interface are greatly influenced by salt conditions and geometry of solid particles.⁵⁶⁾ The organized structures of solid particles surrounding droplets are also used as templates for preparation of ordered structures such as core-shell/hollow particles,^{57,58)} porous structures,⁵⁹⁾ and honeycomb structures.⁶⁰⁾

5. Objective and outline in this dissertation

The “material design in nature”, which enables to emerge functions from ordered assembly, has aspired as a platform for sustainable technology in the 21st century. This dissertation focuses on the potential of fluid interfaces as platforms for ordered assembly of polysaccharide-based nanofibrils, thereby attempting to induce structure-correlated functions similarly to the system of material design in nature.

The overview of this dissertation is shown in Fig. 4. Chapters 1 and 2 attempt to clarify how nanofibrils tend to locate at the fluid interface as a platform. Chapter 1 deals with the oil-water interface stabilized by polysaccharide-based nanofibrils as a Pickering emulsion, in terms of the adsorbability of nanofibrils and surface coverage ratio of the droplets. The results supposed to provide information of nanofiber immobilization efficiency to the oil-water interface as a platform. Chapter 2 deals with quantitative evaluation of surface free energy of various CNFs to allow prediction of affinity between the CNFs and fluid interfaces.

Chapters 3 and 4 attempt to utilize fluid interfaces to organize nanofibrils into ordered structures. Chapter 3 proposes a facile surface modification method of CNFs using oil-water interface of the Pickering emulsion as a reaction platform. The assembled structures of nanofibrils chemically modified at an oil-water interface were dependent on the heterogeneity of interfacial reaction platform, thereby exhibiting unique wetting behaviors. The facile surface modification approach mentioned above was used further for improvement of CNF adsorption onto polystyrene microspheres for resin-CNF compounding. The understanding of adsorption process was considered based on the surface free energy theory. In Chapter 4, non-uniaxial alignments of the C(h)NFs, which were observed as aligned 3D shell-like structures surrounding oil

droplets in the Pickering emulsions (Chapter 1), are extended to the 2D macroscopic membranes using an air-water interface. The anisotropic frictional properties depending on the non-uniaxial alignments of nanofibrils were investigated.

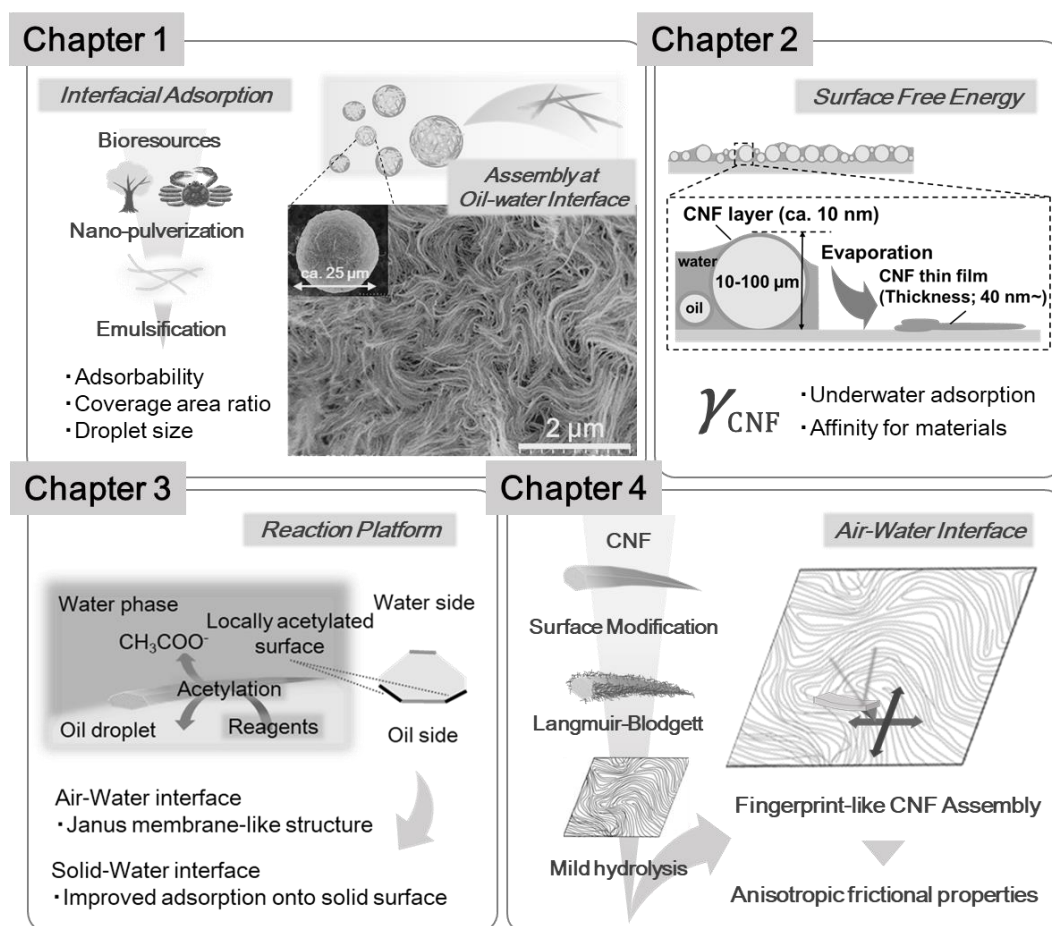


Fig. 4 Schematic illustration of entire outline in this dissertation.

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Chapter 1

Pickering Emulsion Stabilized by Polysaccharide-based Nanofibrils

Abstract

This chapter attempts to clarify how nanofibrils exist at the oil-water interface of the Pickering emulsion by characterizing interfacial adsorption behaviors of polysaccharide-based nanofibrils. The polysaccharide-based nanofibrils covered surface of droplets densely by only weak mixing, and the nanofibrils at the oil-water interface tend to align whilst flexing. In addition, the physicochemical characteristics of the Pickering emulsions were dependent on the raw materials of the nanofibrils. Minor charged groups on the chitin nanofibers affected significantly the physicochemical properties of edible canola oil emulsion due to ionic interactions between carboxy groups of fatty acids in canola oil and minor amino groups on the chitin surface.

These interfacial behaviors depending on the surface chemical structures are expected not only as development of additives to tune the physicochemical properties of emulsions, but also as the fabrication method of ordered nanofiber architectures.

1.1. Introduction

Emulsion is a system composed of liquid droplets (dispersed phase) dispersed in other immiscible liquid (continuous phase) and emulsifier. Emulsification of immiscible liquids occurs by stabilization of liquid interface via adsorption of stabilizer such as surfactants,¹⁾ amphiphilic polymers,²⁾ and solid particles.^{3,4)} In particular, the emulsions stabilized by solid particles such as clay, nanoparticles, bacteria, and fibers are termed Pickering emulsions,^{3,4)} which attract attentions as surfactant-free emulsion in the fields

of food, cosmetics, paintings, and pharmaceuticals. Recently, various studies on Pickering emulsion stabilized by cellulose nanofibrils (CNFs) and chitin nanofibers (ChNFs) as renewable non-toxic Pickering emulsifiers having high specific surface area^{5,6)} have been carried out. In chitin, amount of minor amino groups on C-2 position are related to its solubility and bioactivity.⁷⁾ Therefore, some reports on ChNFs-stabilized Pickering emulsions exhibiting antibacterial activities⁸⁾ and Pickering emulsion-templated scaffolds for cell culture⁹⁾ have appeared. In addition, Hartmann et al. reported on aqueous alkylation of amino groups on chitin nanocrystals (ChNCs) for bubble stabilization and following malachite flotation process.¹⁰⁾

Similarly to the CNFs, there are various ways proposed in the production of ChNFs, e.g., mechanical defibrillation,¹¹⁾ TEMPO-mediated oxidation,¹²⁾ sonication treatment in acidic media,¹³⁾ and the aqueous counter collision (ACC) method¹⁴⁻¹⁶⁾ that is focused on this chapter. According to the previous studies on ACC-produced cellulose nanofibrils (ACC-CNFs), the advantage of ACC method,^{16,17)} which is also capable to prepare chitin nanofibers^{18,19)} and collagen fibrils^{20,21)} solely by impinging a pair of water jets containing bio-based raw materials, is considered to provide characteristic nano-objects exhibiting unique amphiphilic properties in water^{16,22)} that would assist to stabilize oil droplets as a Pickering emulsion by surface-surrounding.^{5,23)} It is noted that Kose et al. reported that the ACC-ChNFs show unique physical properties different from that of ACC-CNFs.¹⁸⁾

The attempt in this chapter has been made to achieve emulsification using of ACC-ChNFs, and then some distinctive effects of ACC-ChNFs were found to work as an assistant for formation of their Pickering emulsification. Edible canola oil, mixture of fatty acids with carboxy group and long alkyl chain, were used as an oil phase to investigate the unique concentration-dependent interfacial stabilization behaviors of the

ACC-ChNFs via ionic interaction between the fatty acids and the minor amino groups on the surface of ACC-ChNFs. Further, adsorption ratio and surface-surrounding behaviors of nanofibrils were characterized using ACC-ChNFs and ACC-CNFs prepared from various cellulosic resources in terms of the adsorption ratio (R_{ad}) and surface coverage ratio (C), leading to understanding how nanofibrils exist at the fluid interface.

1.2. Materials and methods

1.2.1. Materials

α -Chitin powder, chitosan, hydrochloric acid (HCl) aqueous solution, monochloro acetic acid (CAA $\geq 99.0\%$), phenol ($> 99.0\%$), sulfuric acid ($> 95.0\%$), tetradecane ($> 99.0\%$), and toluidine blue O (TBO) were purchased from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, and the TBO was purified by recrystallization. Cyclohexane ($\geq 99.5\%$), dichloromethane (DCM: $\geq 99.0\%$), toluene ($\geq 99.5\%$), sodium hydroxide (NaOH) ($\geq 96.0\%$), and calcofluor white (CFW) were purchased from Sigma-Aldrich Japan, LLC., Tokyo, Japan. Vinyl acetate (VAc: $\geq 99.0\%$) was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Canola oil was purchased from The NISSIN Oillio Group, Ltd., Tokyo, Japan. Micro crystalline cellulose (MCC) powder was purchased from Funakoshi Co., Ltd, Tokyo, Japan, then purified with water and acetone according to the previous report.⁵⁾ Hardwood bleached sulfite pulp (HBSP) was provided from Dr. Orlando J. Rojas, a professor at University of British Columbia (Canada) and Aalto University (Finland). Ultrapure water obtained from a NANO pure Diamond (Thermo Fisher Scientific, Inc., Waltham, United States) was used for measurements of interfacial free energy. Deionized water was used in other experiments.

1.2.2. Preparation of polysaccharide-based nanofibrils

The aqueous dispersions of ACC-CNFs were prepared from bacterial cellulose (BC) pellicles, which does not contain other components such as hemicelluloses and lignin. Culture and purification of BC pellicles were carried out according to a previous report.¹⁷⁾ The purified pellicles were cut into ca. 1 cm³ cubes, then defibrillated using food mixer for 90 s.²⁴⁾ The aqueous dispersions of the pretreated BC, MCC powder, and HBSP were nano-pulverized using an ACC system¹⁴⁻¹⁶⁾ (CNNT Co., Ltd., Incheon, South Korea) under 200 MPa, 60 Pass (= cycles).^{17,24)}

Milled α -chitin powder was fractionated using stainless filter (180 μ m ϕ) to remove large particles, and then dispersed in deionized water. Chitosan powder was suspended in deionized water, then pre-pulverized using a Physcotron NS-51 (Microtec, Brixen, Italy) at 2×10^4 rpm for 10 min. The chitin and chitosan suspensions were nano-pulverized using ACC system under the same condition (200 MPa, 60 Pass) for the cellulosic materials. Concentration (w/w) of each suspension was determined from the dry weight of polysaccharides.

1.2.3. Preparation of surface carboxymethylated CNFs

Surface carboxymethylation of CNFs was carried out according to a previous report.²⁵⁾ An aqueous dispersion of BC-derived ACC-CNFs (ACC-BCNFs; 0.37 wt%, 200 mL) were poured into a 300 mL eggplant flask with 50 mL of NaOH aq. (25 wt%) while stirring, then the mixture was heated at 60°C for 1 h to activate OH groups on the CNFs. The NaOH treated sample (80.0 g) was poured in a 200 mL eggplant flask with CAA (0.08-6.02 g), then the mixture was heated at 80°C for 2 h. The obtained slurry was washed with pure water by 6 times of centrifugations at $9.0 \times 10^3 \times g$ and 25°C for 5 min.

1.2.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of each sample was recorded using an FT-IR-6700 spectrometer (JASCO Corp., Tokyo, Japan) equipped with a triglycine sulfate detector. The spectra were obtained from 16 scans at 2 cm^{-1} resolution and normalized using an absorption band at 1162 cm^{-1} attributable to C-O-C asymmetric stretching.²⁶⁾

1.2.5. Quantification of surface charged groups

Surface charged group content was measured according to the dye adsorption method.^{27,28)} Aqueous dispersions containing 0.4-0.5 wt% CNFs (0.5 mL) were mixed with 2.5 mL of water and 2.0 mL of $5.0\times 10^{-4}\text{ M}$ TBO aqueous solution in polypropylene tubes. After shaking at 120 rpm overnight for adsorption of TBO, the mixtures were centrifuged at $4.5\times 10^3\times g$ for 15 min, and the supernatant fractions were re-centrifuged at $2.0\times 10^4\times g$ for 15 min. The adsorption intensity at 628 nm of diluted supernatant fractions were measured using a UV/Vis spectrophotometer (Beckman coulter, Brea, California, United States) at least 3 times.

1.2.6. Atomic force microscopy (AFM)

The sizes of ACC-C(h)NFs were estimated from topographical images obtained by using an AFM (Asylum MFP-3D Origin; Oxford Instruments, Abingdon-Thames, United Kingdom) equipped with a silicon cantilever (OMCL-AC160TS-R-3; Olympus Corp., Tokyo, Japan). The AFM observation was conducted in tapping mode under ambient conditions. The AFM samples were prepared by dropping of aqueous dispersions on a glass slide substrate and subsequent drying at 50°C . Aspect ratios and specific surface areas (SSA) were calculated using the following equations:

$$\text{Aspect ratio} = \frac{2l}{h + w} \quad (1.1.).$$

$$\text{SSA} = \frac{2(lh + lw + hw)}{\rho lhw} \quad (1.2.).$$

where, l is the length of nanofibrils, h is the height of nanofibrils, w is the width of nanofibrils determined in previous works, and ρ is the density of cellulose I; 1.62 g cm^{-3} and α -chitin crystal; 1.46 g cm^{-3} , respectively.^{29,30)}

1.2.7. Oil-water interfacial free energy measurements

Interfacial free energy values between oil and water were determined by pendant drop tensiometry using a Drop Master DM300 (Kyowa Interface Science Co., Ltd., Saitama, Japan). The shape of droplet hanged from a needle is determined by the balance between the surface (interfacial) free energy and gravity. Hence, the interfacial free energy of the droplet in water can be calculated based on the following equation and curve fitting of droplet shape:

$$\gamma_{12} = -\frac{\Delta\rho g R_0^2}{\beta} \quad (1.3.).$$

where, γ_{12} is the interfacial free energy between phase 1 and phase 2, $\Delta\rho$ is the difference in density between two fluids, g is the gravitational constant, R_0 is the drop radius of curvature at the top, and β is the bond number depending on the droplet shape. The average values at least 10 times measurements were determined as the interfacial free energy values.

1.2.8. Preparation of Pickering emulsions stabilized by polysaccharide-based nanofibrils

Aqueous dispersions containing 0.02-0.30 wt% nanofibrils (5 mL) and organic solvents (5 mL) with different oil-water interfacial free energy values were poured into a 20 mL glass vessel. Then the mixtures were emulsified using a vortex mixer (Vortex-Genie 2; KENIS LIMITED, Osaka, Japan) for 2 min at room temperature.

1.2.9. Droplet diameter measurements

Droplet size distributions of the Pickering emulsions were measured using a laser diffraction particle size analyzer (Master Sizer 3000, Malvern Instruments Ltd., Worcestershire, United Kingdom or SALD-200V, Shimadzu Corporation, Kyoto, Japan).

Surface coverage ratio of the oil droplets were determined as follows:

$$C = \frac{A_{\text{eff}} R_{\text{ad}}}{A_{\text{ow}}} \quad (1.4.).$$

$$A_{\text{eff}} = \frac{m}{h\rho} \quad (1.5.), \quad A_{\text{ow}} = \frac{6V_0 R_{\text{eo}}}{D_{3,2}} \quad (1.6.).$$

$$\therefore C = \frac{m R_{\text{ad}} D_{3,2}}{6h\rho V_0 R_{\text{eo}}} \quad (1.7.).$$

where C is the surface coverage ratio, A_{eff} is the effective surface area of nanofibrils calculated by assuming the rectangular cross section, R_{ad} is the adsorption ratio of the nanofibrils, A_{ow} is the oil-water interfacial area, m is the dry weight of nanofibrils, V_0 is the total volume of the oil phase, R_{eo} is the emulsified ratio of the oil phase, $D_{3,2}$ is the Sauter's mean diameter of the oil droplets.

1.2.10. Adsorption ratio of polysaccharide-based nanofibrils

The ratio of the nanofibrils adsorbed onto the oil-water interface (R_{ad}) was determined by phenol-sulfuric acid method.^{6,31)} A nanofiber aqueous dispersion (0.10 wt%, 5 mL) and a 5 mL of tetradecane were poured into a 20 mL glass screw bottle, then the mixture was emulsified using a HG-200 rotation type homogenizer equipped with a K-12S generator (AS ONE Corporation, Osaka, Japan) at 2×10^4 rpm for 2 min. After 1 h from preparation, the aqueous phase containing non-adsorbed nanofibrils (0.5 mL) was poured into a glass vessel, then mixed with a 0.5 mL of the 5 wt% phenol aqueous solution. The mixture was reacted with a 2.5 mL of the sulfuric acid while cooling. The absorption intensity at 490 nm of the mixture was recorded using a UV/Vis spectrophotometer (Beckman coulter, Brea, California, United States) at least 3 times. The concentration of nanofibrils was determined using calibration curves made from nanofiber aqueous dispersions having known concentrations. Likewise, turbidity at 660 nm in wavelength was also used for determination of the nanofiber concentration in an aqueous phase.

1.2.11. Confocal laser scanning microscopy (CLSM)

Before observation, non-adsorbed nanofibrils were removed using a separation funnel. The Pickering emulsion (10 mL) was diluted with 50 mL of deionized water while stirring. After 10 min from stirring, oil droplets in the mixture were collected by creaming of emulsion. The dilution-collection steps were carried out 3 times. Then the washed droplets were mixed with a 15 mL of 1.3×10^{-3} wt% CFW aqueous solution, and the mixture was shaken at 0.3×10^3 rpm for 30 min to fix the fluorescent probes of cellulose and chitin. The CFW-stained droplets were purified with a 100 mL of deionized water 3 times according to the method mentioned above. Purified samples were placed on the

glass bottom dish for observation using a Leica SP8 (Leica microsystems, Wetzlar, Germany) equipped with a photomultiplier tube detector (Leica microsystems). The excitation and detection wavelength were set at 405 nm and 415-475 nm, respectively.

1.2.12. Zeta potential measurements

Interfacial properties of oil droplets were characterized in terms of pH- and concentration-dependency of zeta potentials. The formula of Smoluchowski³²⁾ was used for calculation of the zeta potential. Before measurements, 1 mL of emulsified phase was washed with deionized water 3 times for removal of non-adsorbed ACC-C(h)NFs in the continuous phase. The washed droplets were suspended in a 15 mL of deionized water. Then, the mixtures were hand-shaken before transferring into a measurement cell to avoid droplets aggregation. Zeta potential values of each sample were recorded using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd.) equipped with disposal cells (DTS1070, Malvern Instruments Ltd.) at 25°C. Averaged values from 5-15 times measurements were determined as the zeta potential values. The pH values of aqueous dispersion of 1.0×10^{-2} wt% nanofibrils or oil droplets dispersions were adjusted using diluted NaOH and/or HCl aqueous solution.

1.2.13. Field emission scanning electron microscopy (FE-SEM)

To investigate self-organization of polysaccharide-based nanofibrils at oil-water interface, morphological observation of Pickering emulsions was carried out. Pickering emulsion samples were prepared by mixing of 15 mL of 0.10 wt% polysaccharide-based nanofibrils (ACC-BCNFs, ACC-ChNFs, and ACC-CtNFs) with 15 mL of cyclohexane using a rotation type homogenizer at 2×10^4 rpm for 5 min. After removal of non-adsorbed

nanofibrils following the same manner for the zeta potential measurements, droplets were rapidly frozen and freeze-dried on a carbon tape using a FreeZone^{2.5} (Labconco, Kansas, United States). Subsequently, the dried samples were coated with osmium tetroxide (ca. 3 nm thickness) using an HPC-1SW (Vacuum Device Corporation, Ibaraki, Japan). Morphological imaging was conducted using an SU-8000 scanning electron microscope (Hitachi High-Tech Corp., Tokyo, Japan) at an acceleration voltage of 2.0 kV.

1.3. Results and discussion

1.3.1. Characterization of the polysaccharide-based nanofibrils

Fig. 1-1 shows the AFM height images of ACC-BCNFs and ACC-ChNFs. The size information of the polysaccharide-based nanofibrils were summarized in Table 1-1. The degree of deacetylation (DDA) values of chitin raw material was determined as 1.3% based on the elemental analysis. Fig. 1-2 draws that the zeta potential values of ACC-ChNFs and ACC-BCNFs depending on pH values of their aqueous dispersion media. ACC-ChNFs exhibited positive zeta potential values (30-40 mV) under acidic conditions (pH 2-5.5). With increase in pH values from 6 to 10, the zeta potential values were then significantly decreased. Eventually, ACC-ChNFs exhibited negative zeta potentials under alkali conditions over pH=10. The behavior is presumably due to a balance of association and dissociation of amino and/or OH groups on the surface of ACC-ChNFs, indicating a pH-responsive characteristic on their surface. In contrast, negative zeta potential values of ACC-BCNFs appeared all through from acidic till alkali pH conditions, although it was not a drastic changing, when compared with ACC-ChNFs. This implies that OH groups on the surface of ACC-BCNFs were predominant moieties that was not supposed to exhibit their pH-responsive surface potentials.

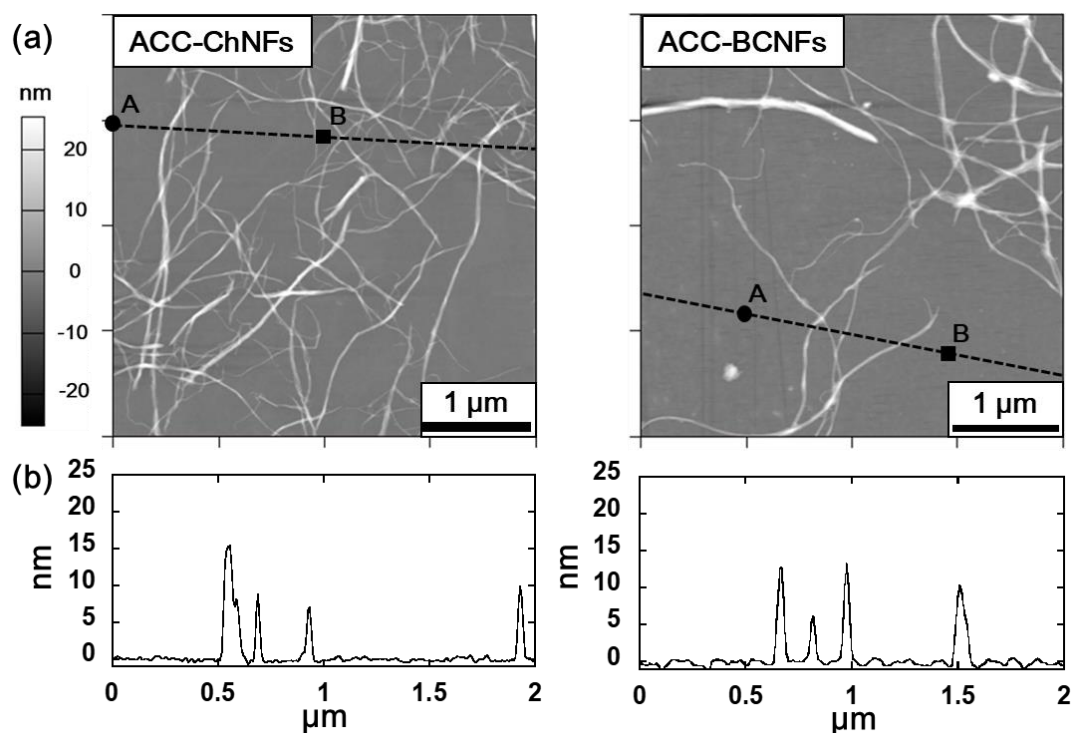


Fig. 1-1(a) AFM topographical images of ACC-ChNFs and ACC-BCNFs. (b) Cross section profiles along the between A and B dots on the line in each AFM image.

Table 1-1 Size information of polysaccharide-based nanofibrils prepared by ACC. Aspect ratio and specific surface area (SSA) values were calculated using equation (1.1.) and equation (1.2.), respectively.

	Length / μm	Height / nm	Width / nm	Aspect ratio	SSA / $\text{m}^2 \text{g}^{-1}$
ACC-ChNFs	2.3 ± 1.0	6.1 ± 3.1	$10 \pm 6^{18)}$	286	362
ACC-BCNFs	$4.4 \pm 2.3^{33)}$	$10.1 \pm 4.2^{24)}$	$22 \pm 13^{18)}$	274	179
ACC-MCCs	$0.9 \pm 0.6^{25)}$	$8.4 \pm 4.7^{25)}$	$16 \pm 3^{14)}$	74	226

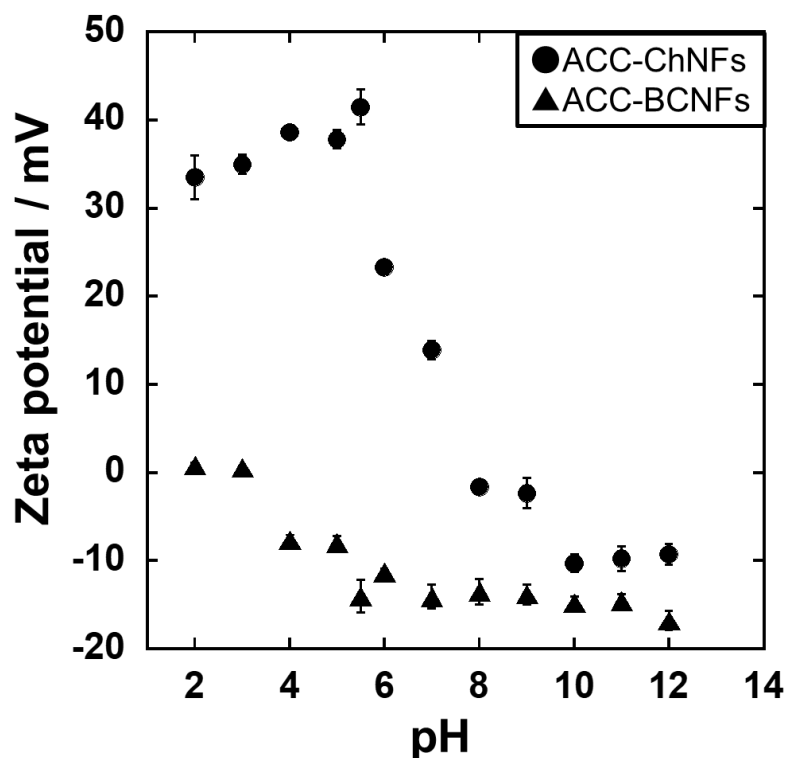


Fig. 1-2 pH-dependent changes of zeta potential values of each polysaccharide-based nanofibril.

1.3.2. Emulsifying properties of polysaccharide-based nanofibrils to typical organic solvents

To characterize emulsifying abilities of polysaccharide-based nanofibrils, agitated mixtures containing various oils and water dispersions of either ACC-ChNFs or ACC-BCNFs were investigated. Figure 1-3 displays the relative ratio of emulsified volumes after 5 min of mixing aqueous dispersions containing 0.02 wt% nanofibrils with oils having different oil-water interfacial energy (Fig. 1-3). Negligible emulsifications using VAc as an oil phase ($\times$ in Fig. 1-3) were observed in both nanofibril systems. When compared between the two systems, ACC-ChNFs and ACC-BCNFs cases, the ACC-ChNFs system (Fig. 1-3a) allowed the mixture to be emulsified more favorably possibly

due to a remarkable difference of the two in surface characteristics. The surface of ACC-ChNFs has positively charged amino groups, while the surface of ACC-BCNFs has negligible dissociable moieties. Jia et al. reported that the emulsifying properties of highly charged TEMPO-oxidized CNFs (TOCNFs) is lower than the TOCNFs having a lower carboxy content³⁴⁾ presumably due to high water dispersibility derived from polar groups. On the other hand, Wu et al. reported that Pickering emulsions were highly stabilized using weakly charged protein nanoparticles. This was due to the inhibition of the coalescence of oil droplets by the electrostatic repulsive force between the Pickering emulsifiers.³⁵⁾ The absolute zeta potential value of ACC-ChNFs surrounding non-polar hydrocarbon droplets (ca. +22 mV) was lower than those surrounded by TOCNFs³⁴⁾ (ca. -48 mV), therefore, the positively charged nanofibers presumably behaved as an electrostatic stabilizer for the Pickering emulsions. On the other hand, in the case of the ACC-BCNFs (Fig. 1-3b), the emulsified volume tends to be correlated to interfacial free energy of oils.

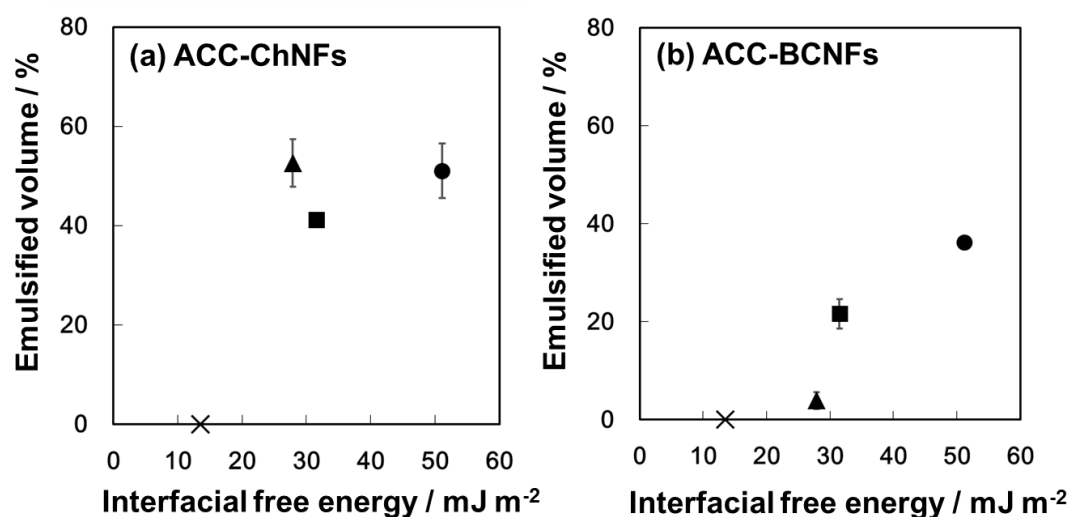


Fig. 1-3 Emulsified volumes of Pickering emulsions prepared from various oils and aqueous dispersions containing 0.02 wt% of (a) ACC-ChNFs and (b) ACC-BCNFs. Circle (●); cyclohexane, square (■); toluene, triangle (▲); DCM, and cross (×); VAc.

Goi et al. reported that the stabilization mechanisms of TOCNFs are due to both the roles as Pickering stabilizer and dispersion stabilizer.⁶⁾ Pickering emulsifier decreases oil-water interfacial free energy by adsorption onto droplet surface. Although the dispersion stabilizer doesn't adsorb onto the oil-water interface directly, the dispersion stabilizer increases viscosity of continuous phase, resulting in preventing coalescence of droplets. Hence, the dispersion stabilization effect decreases as decreasing in viscosity of CNF suspension. Tanaka et al. reported that the viscosity in TOCNF suspensions significantly increase until the TOCNF concentration reaches the critical concentration due to the increase in contact points of nanofibrils.³⁶⁾ Similarly, Ishikawa et al. reported that the viscosity of ACC-BCNFs significantly increase when the ACC-BCNF concentration reaches 0.04-0.05 wt%.³⁷⁾ Emulsification under the such low concentration condition (0.02 wt%) implies that ACC-C(h)NF mainly acts as Pickering emulsifier. The idea was supported by FE-SEM images of lyophilized oil droplets (Fig. 1-4). These images indicate that the polysaccharide-based nanofibrils densely covers oil-water interfaces. Moreover, although the ACC-HBSPs formed reticulated pattern, other nanofibrils tend to align whilst flexing like fingerprints.

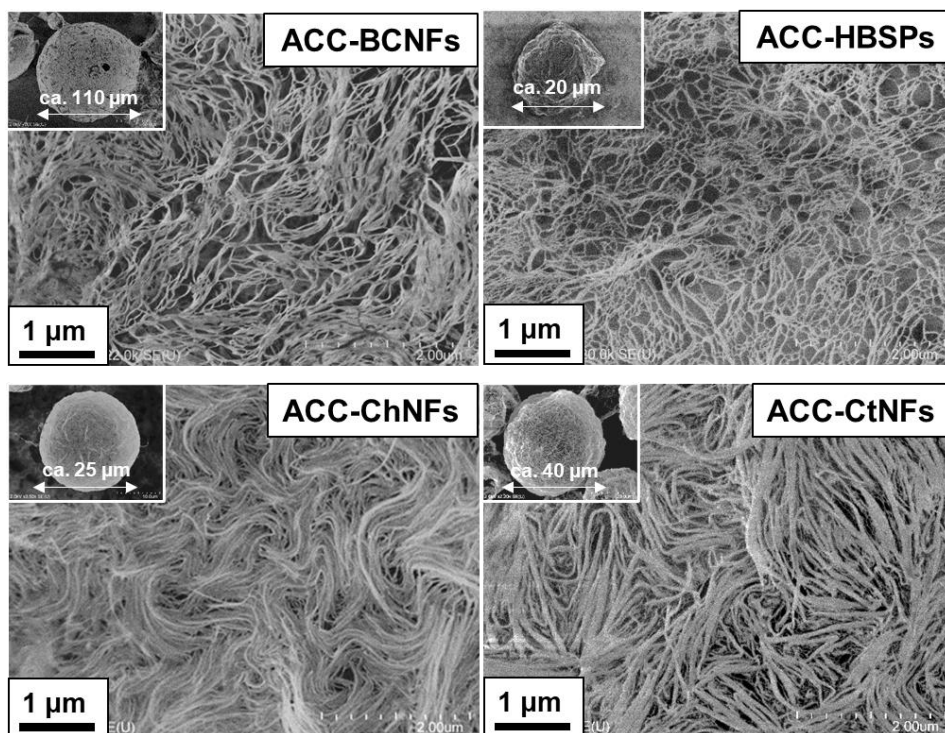


Fig. 1-4 FE-SEM images of the lyophilized shell-like nanofiber structures in Pickering emulsions. Inset: full images of the lyophilized cyclohexane droplets.

Figure 1-5 shows concentration dependency of emulsified volumes of the Pickering emulsion 5 min after standing still. The emulsified volume in the Pickering emulsions stabilized by ACC-ChNFs exhibited clearly higher than that in Pickering emulsions stabilized by ACC-BCNFs except the case of the canola oil (this will be discussed later). Namely, the ACC-ChNFs emulsified oil droplets favorably irrespective of the oil type, independent of their interfacial energy (Fig. 1-5ab). As for ACC-BCNFs system (Fig. 1-5b), Pickering emulsions of DCM were negligibly formed all through the entire concentration range measured, even if the concentration increased. In other words, ACC-ChNFs induced the emulsification of DCM, differently from ACC-BCNFs. In addition, the concentration-dependency in changes of emulsified volume in the ACC-ChNFs system is quite different from those of the ACC-BCNFs system.

In general, stability of nanofiber networks become stronger with an increase in the nanofiber concentration due to increase in their crosslinking points. In the current study, emulsified volumes of Pickering emulsions were increased with increasing in concentration of ACC-CNFs up to 0.20 wt%, which might be due to the increase in the total surface area of the individual Pickering emulsion oil droplets possibly for adsorption of the nanofibers. Then, the emulsified volume was rapidly decreased over 0.25 wt% in the cases of cyclohexane and toluene (Fig. 1-5b). This indicates that the higher concentration prevents the adsorption of ACC-BCNFs onto oil droplets because of generation of stable nanofiber networks.^{36,37)} Therefore, it was implied that the stirring energy of the vortex mixing could not reach the threshold which collapse the nanofiber networks in the dispersion of ACC-BCNFs. As a result, the adsorption of ACC-BCNFs to the oil droplet was possibly inhibited.

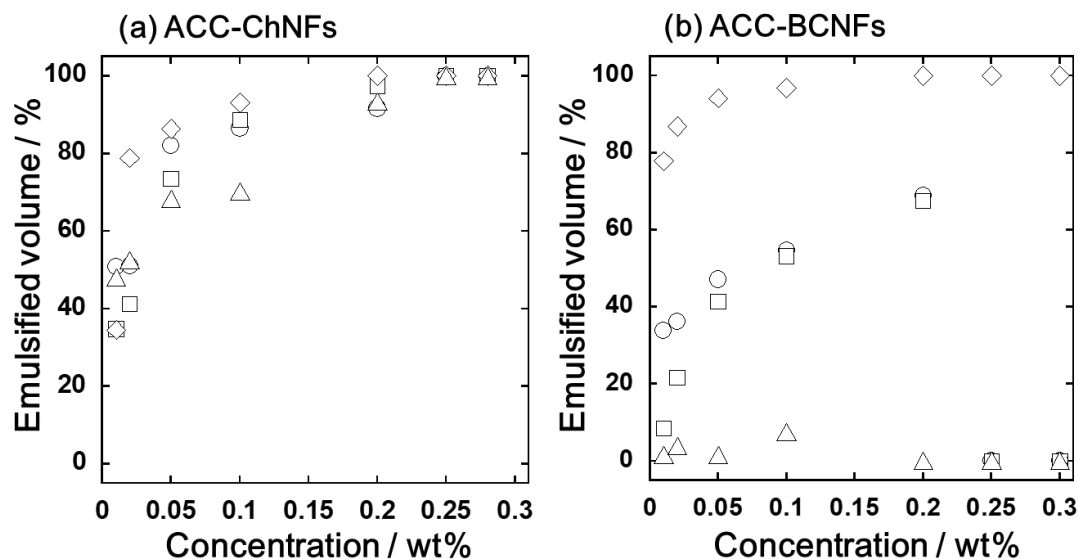


Fig. 1-5 Emulsified volumes of the Pickering emulsion stabilized by (a) ACC-ChNFs and (b) ACC-BCNFs. Circle (○); cyclohexane, square (□); toluene, diamond (◇); canola oil, and triangle (△); DCM.

In contrast, the ACC-ChNFs did not exhibit such a maximum point in emulsified volumes of Pickering emulsions. Although the aspect ratio between the two nanofibers was similar (Table 1-1), the length value of ACC-ChNFs was approximately half length of ACC-BCNFs, and ACC-ChNFs was thinner than ACC-BCNFs. In addition, the electrostatic repulsion force between ACC-ChNFs is supposed to be higher than that of ACC-BCNFs by taking account of the results of the zeta potential measurements (Fig. 1-2). Therefore, the mobility of the ACC-ChNFs in an aqueous medium is likely to be higher than that of ACC-BCNFs. This assumption further leads to the idea that ACC-ChNFs could adsorb more easily at the oil-water interface to exhibit higher emulsifying effects even under the higher nanofiber concentration. Investigations are still required to discuss quantitative relationships between emulsification properties and mixing energy, mobility and/or adsorbability of the nanofibrils.

1.3.3. Emulsifying properties of polysaccharide-based nanofibrils to canola oil

Investigation of the emulsion with edible natural oils and emulsifier/stabilizers is worth considering as a fundamental study for food, pharmaceutic and medical applications. Therefore, Pickering emulsions of a canola oil with ACC-ChNFs were also investigated in comparison of adding ACC-BCNFs.

In the case of canola oil-in-water emulsion, the volume of emulsified phase was larger than other organic solvents, as shown in Fig. 1-5ab. In particular, although the oil-water interfacial energy of the canola oil ($28.0 \pm 0.4 \text{ mJ m}^{-2}$) was similar to that of DCM ($27.9 \pm 0.6 \text{ mJ m}^{-2}$), ACC-ChNFs induced the emulsification of DCM, whereas ACC-BCNFs exhibited negligible emulsifying effects.

To observe the adsorption of the two nanofibrils on the surface of canola oil droplets in the Pickering emulsions, CLSM in situ was employed because it was hard to perform freeze-drying of the canola oil droplets. Figure 1-6 shows the fluorescent images of diluted emulsion phases stained with CFW as a probe for the localization of polysaccharides. This indicated that the interfacial stabilization appeared to be achieved by surface coverage of ACC-ChNFs at the interface on the canola oil droplets, where the fluorescent emission of the CFW attaching to the nanofibrils were clearly observed (Fig. 1-6ab). On the other hand, it appeared that the small droplets were trapped and stabilized by network of ACC-BCNFs (Fig. 1-6cd).

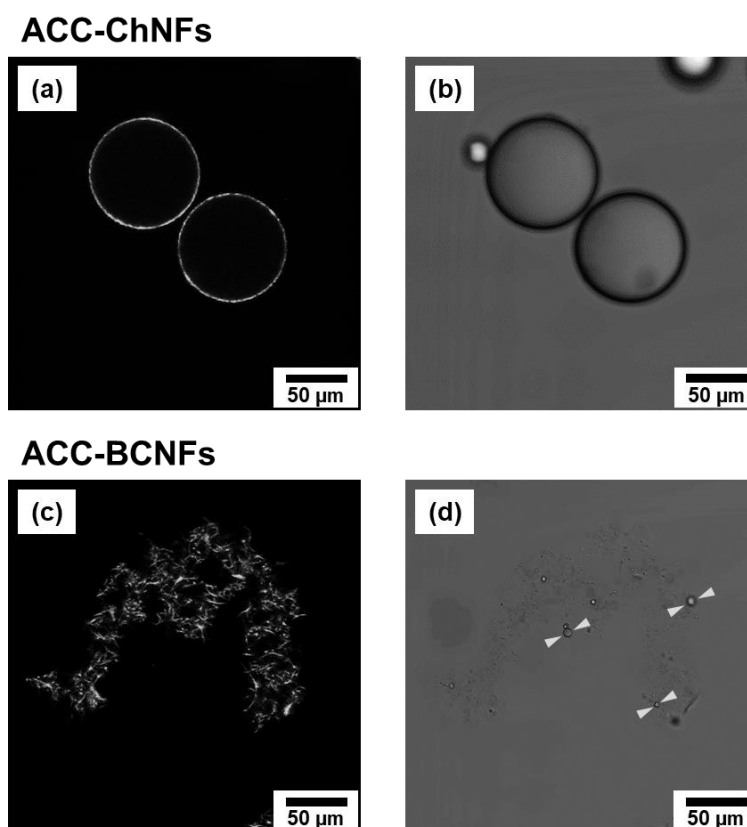


Fig. 1-6 CLSM sectioning images of droplets prepared from canola oil and aqueous dispersions at 0.25 wt% of the two nanofibers. (a, c) Fluorescent images and (b, d) bright field images. Arrows in Fig. 1-6d indicate canola oil droplets.

A canola oil is a complex oil that contains several fatty acids such as oleic acid, linoleic acid, and α -linoleic acid. Previously, the emulsification phenomenon in the system of water and fatty acids without any other additives was reported,³⁸⁾ which might be caused by surfactant-like chemical structures of fatty acids having hydrophobic long alkyl chains and hydrophilic carboxylic acids. In fact, the canola oil provided the emulsion in the nanofiber-free system (dotted lines in Fig. 1-7a-c), and it formed relatively smaller droplets having ca. 8 μm in volume average droplet diameter (D_V) than those of ca. 60-121 μm in D_V obtained in the ACC-ChNFs system. This indicates that the emulsification mechanism for the canola oil is different in the systems between ACC-ChNFs and ACC-BCNFs. According to Fig. 1-6ac, the predominant effect of ACC-BCNFs may be a dispersion stabilization of canola oil droplets, whereas ACC-ChNFs were allowed to cover the surface oil droplets, as well as the self-stabilization of fatty acids in the canola oil. It should be added that the emulsified phase of the canola oil emulsion prepared in the nanofiber free system was collapsed within 8 days, and the emulsified phase of canola oil/0.02-0.30 wt% polysaccharide-based nanofibril systems stayed at least for 4 months.

Figure 1-7 shows the D_V and number average droplet diameter (D_N) of nanofibril-stabilized canola oil droplets for both cases of ACC-ChNFs and ACC-CNFs. It is noted that the canola oil droplets stabilized by 0.20 wt% of ACC-ChNFs suspension exhibited relatively narrow D_V distribution. This indicates that the canola oil droplets stabilized by ACC-ChNFs were established, soon after formation of the canola oil/water emulsion.

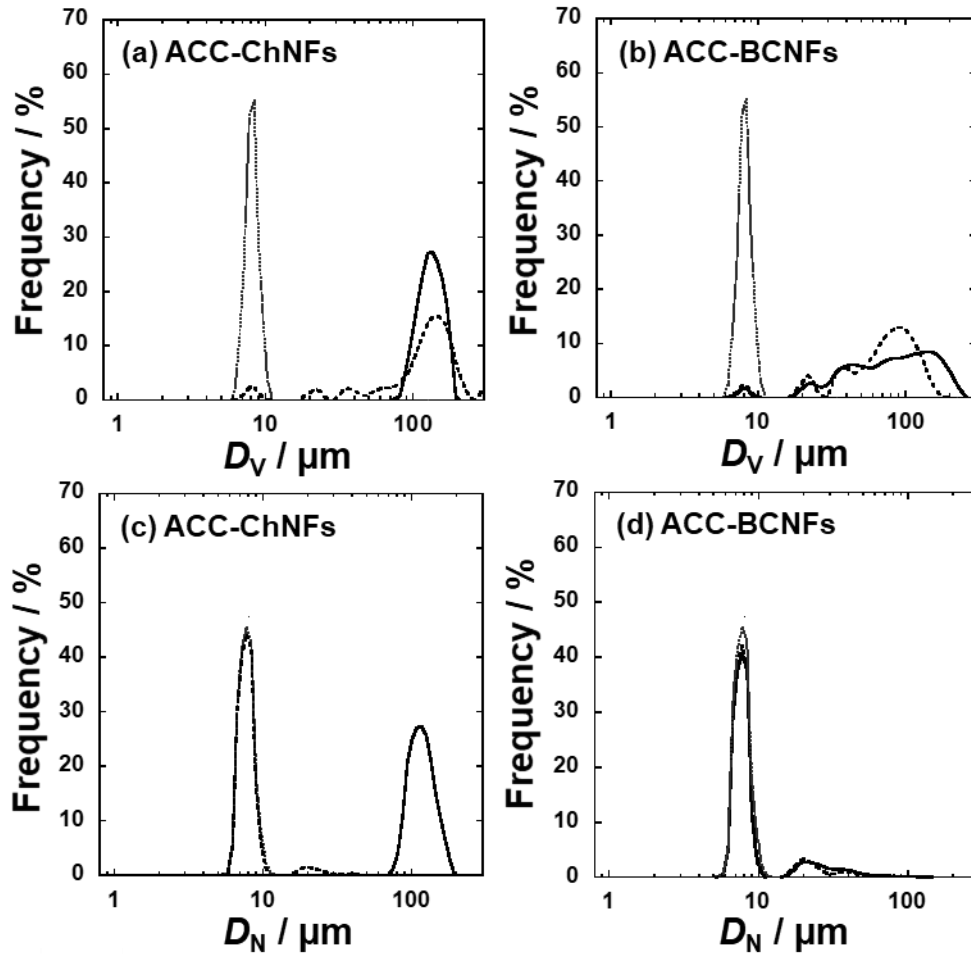


Fig. 1-7 Droplet diameter distributions of canola oil droplet assisted by ACC-ChNFs, ACC-BCNFs, and canola oil itself (nanofiber free condition). (a, b) volume average diameter, (c, d) number average diameter. Concentration of nanofibers were 0.20 wt% (solid line), 0.10 wt% (dashed line), and 0 wt% (dotted line), respectively.

The adsorption of anionic surfactants onto the chitin surface with cationic amino groups due to the electrostatic attraction was previously reported.^{39,40)} Therefore, the zeta potential values of the canola oil/ACC-ChNFs emulsion system in the current study were measured as shown in Fig. 1-8. There is no significant change in zeta potential values (ca. -21 mV) when the ACC-ChNF concentration is in the range of 0.20-0.28 wt%. The pH values of aqueous dispersion containing droplets at 0.20 wt% of ACC-ChNFs was kept

as 6.8, until the negative zeta potential turned to positive value of $(+ 9.5 \pm 3.5 \text{ mV})$ at pH of 4.0. Therefore, the negative zeta potential values might be due to the slight dissociation of positive amino groups of the Pickering emulsion shells constructed from ACC-ChNFs under a neutralized condition by negative carboxylic acids of the canola oil. The absolute values of negative zeta potential of canola oil droplets increased with decreasing in the concentration of ACC-ChNFs, and eventually the zeta potential value in the nanofiber-free condition reached ca. -51 mV. Notably, the concentration-dependent changes of zeta potential values corresponded to concentration-dependent droplet size distribution as shown in Fig. 1-7, indicating that presence of the critical concentration for Pickering emulsions stabilized only by ACC-ChNFs.

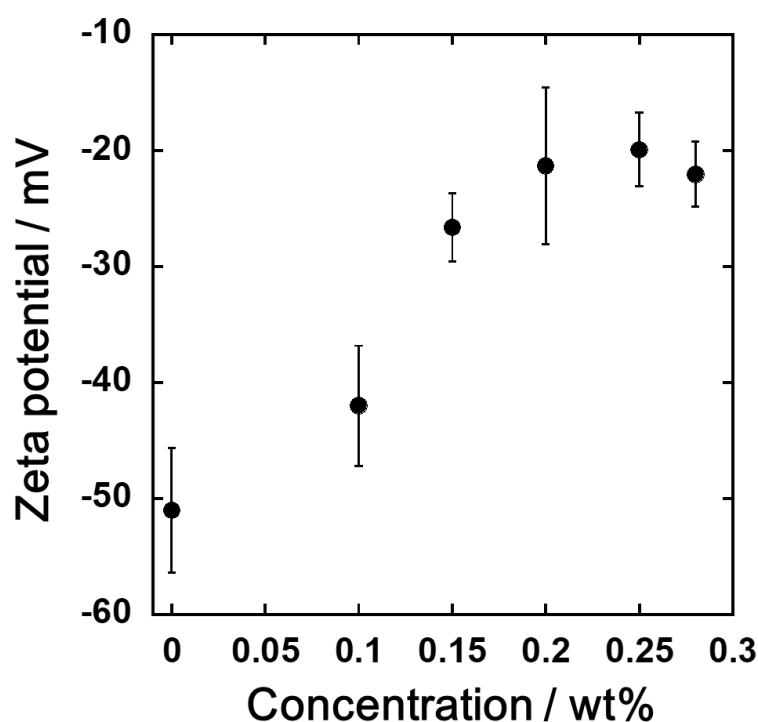


Fig. 1-8 Concentration-dependent changing in zeta potential values of canola oil droplets stabilized by ACC-ChNFs.

1.3.4. Adsorption and coverage ratios of various polysaccharide-based nanofibrils

In the later section of Chapter 1, nanofiber adsorption and surface surrounding of oil droplets were considered to clarify how nanofibrils exist at the fluid interface. Firstly, the adsorption ratio (R_{ad}) values of various CNFs were determined by phenol-sulfuric acid method. On the other hand, those values of the ACC-ChNFs were determined by turbidimetry because the ACC-ChNFs were not colored by the reaction with phenol and sulfuric acid. The concentration-dependent changing of the R_{ad} values of each polysaccharide-based nanofibril onto the tetradecane-water interface was presented in Fig. 1-9. The R_{ad} values of the ACC-ChNFs and wood-derived ACC-CNFs (MCCs and HBSPs) were significantly decreased as increasing in nanofiber concentration, which indicates that those excess nanofibrils were dispersed in continuous phase of Pickering emulsions. However, almost all of the ACC-BCNFs adsorbed onto the oil surface, and the R_{ad} values of the ACC-BCNFs were independent on the nanofiber concentration.

The difference of R_{ad} , which means absorption efficiency of nanofibrils onto the interface, might be due to the difference in affinity for oil-water interface. Excepting bacterial cellulose comprising pure cellulose, other polysaccharide-based nanofibrils have minor functional groups such as carboxy groups derived from hemicelluloses in HBSPs, amino groups on ChNFs, and sulfate half ester groups on MCCs introduced during sulfuric acid hydrolysis. Similar to the carboxylate groups on TOCNFs,³⁴⁾ these hydrophilic functional groups are presumed to decrease affinity for hydrophobic oils. However, the charged group contents of these nanofibrils were quite low (ACC-MCC; 0.02 mmol g⁻¹, ACC-HBSP; 0.06 mmol g⁻¹), although the reported decreasing in emulsifying properties of TOCNFs is in the range of high charged group content region (0.58-1.15 mmol g⁻¹).³⁴⁾

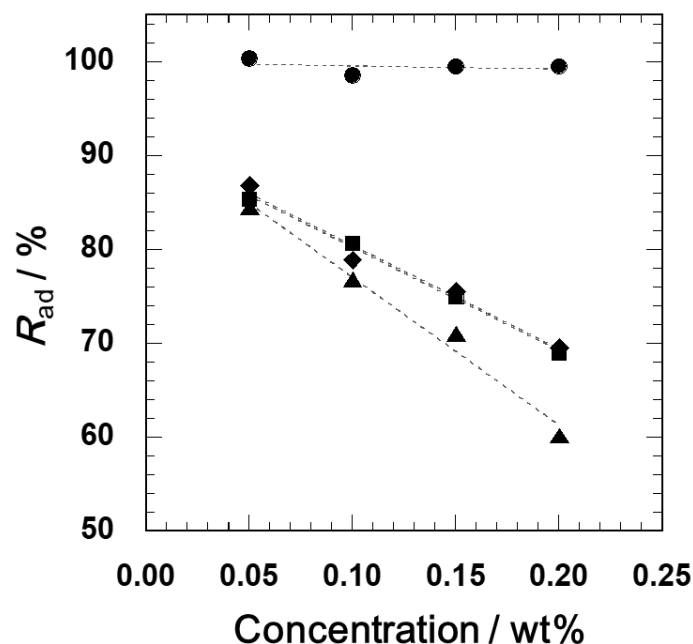


Fig. 1-9 Concentration-dependent changing in R_{ad} values of the polysaccharide-based nanofibrils in the tetradecane Pickering emulsions. Circle; ACC-BCNFs, triangle; ACC-MCCs, square; ACC-HBSPs, and diamond; ACC-ChNFs.

Therefore, the changing in R_{ad} values was investigated using slightly charged CNFs which are chemically prepared by carboxymethylation of uncharged ACC-BCNFs.²⁵⁾ The absorption bands due to carboxy and carboxylate groups were detected as shown in Fig. 1-10a. Carboxylate content increased as increase in added reagents (Fig. 1-10b), and the amount of these groups calculated by dye adsorption method^{27,28)} were in the range of 0.00-0.07 mmol g⁻¹. Figure 1-11 shows that the R_{ad} values decreased as slight increasing in charged groups (Fig. 1-10), which supports that minor charged groups on nanofibrils are efficient to regulate adsorbability of nanofibrils.

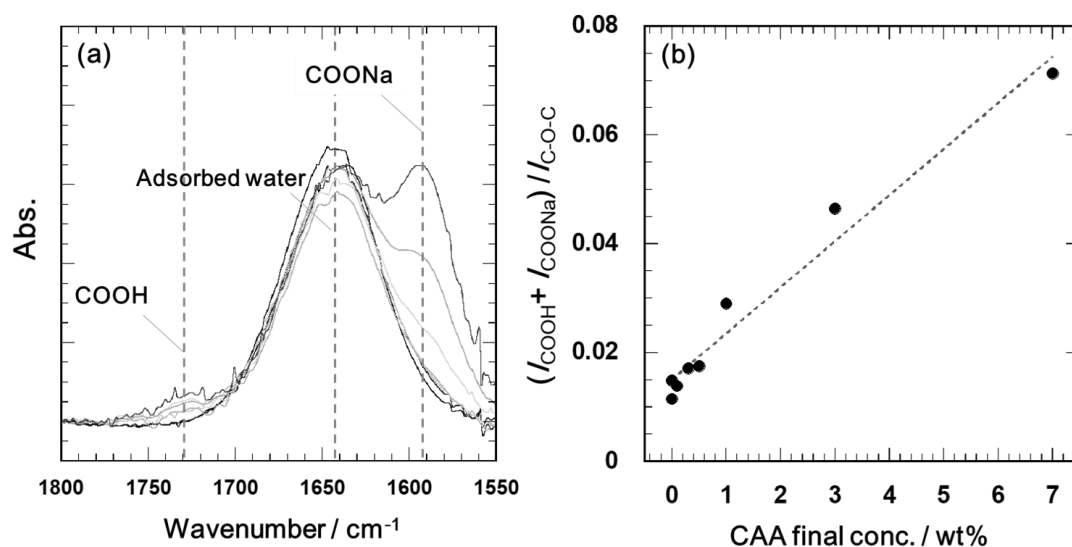


Fig. 1-10(a) FT-IR spectra of the carboxymethylated ACC-BCNFs. (b) Intensity ratio between the bands attributable to the C=O stretching and C-O-C stretching of the carboxymethylated ACC-BCNFs.

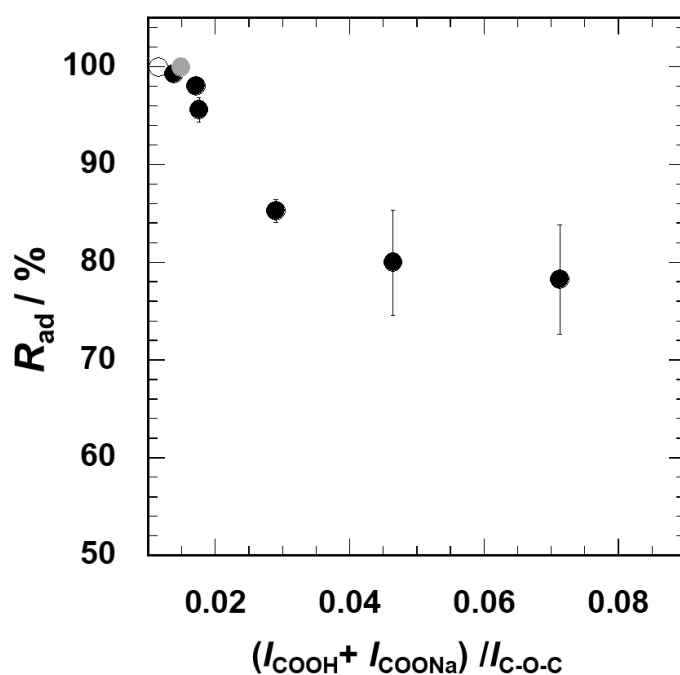


Fig. 1-11 R_{ad} values of the surface carboxymethylated ACC-BCNFs in the tetradecane Pickering emulsions. Open circle: ACC-BCNFs, closed gray circle: NaOH treated ACC-BCNFs, and closed black circle: carboxymethylated ACC-BCNFs.

Surface coverage ratio (C) of droplets is significant parameter providing packing density of nanofibrils on the oil-water interface. The C values calculated from eq. 1-7. are shown in Fig. 1-12. The changing in C values of the ACC-ChNFs was negligible, and those values of the ACC-MCCs also increased gently. In contrast, the C values of the ACC-BCNFs were remarkably increased as increasing in nanofiber concentration because of the constant high R_{ad} values of the ACC-BCNF. Ishikawa et al., reported that the ACC-BCNFs³⁷⁾ aqueous dispersions tend to form crosslinking points when the CNF concentration is over 0.05 wt%. Hence, these results imply that the ACC-BCNFs surrounded oil droplets densely, but slightly agglomerated due to their less-hydrophilic surface moiety⁴¹⁾ when the concentration is high. Alternatively, when the high concentration, slightly charged nanofibrils like ChNFs form non- or partially-adsorbed nanofiber layer around the oil-water interface, which act as dispersion stabilizer.

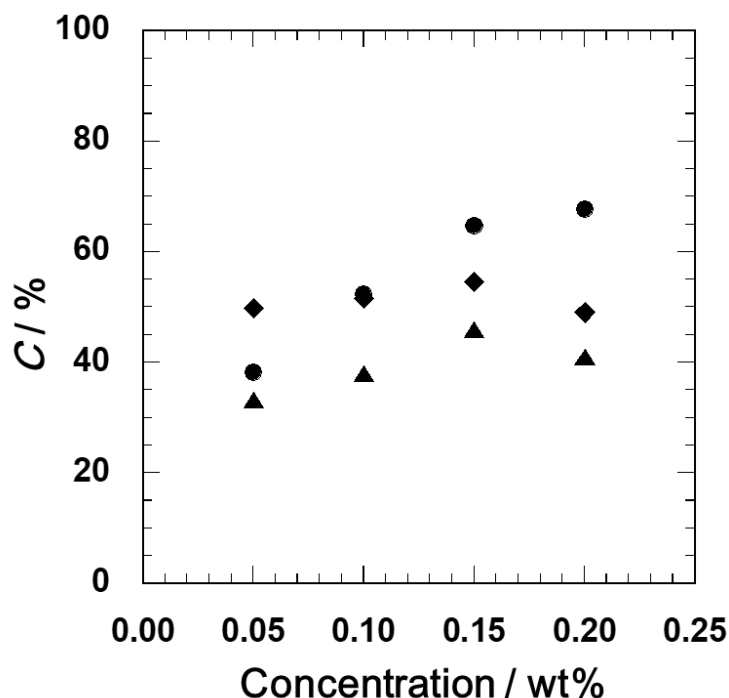


Fig. 1-12 C values of the tetradecane droplets stabilized by polysaccharide-based nanofibrils. Circle; ACC-BCNFs, triangle; ACC-MCCs, and diamond; ACC-ChNFs.

1.4. Conclusion

Polysaccharide-based nanofibrils, ACC-ChNF was found to stabilize oil-water interface as Pickering emulsifiers as well as ACC-CNFs. Interfacial adsorption of ACC-BCNFs was likely to correlate to the specific oil-water interfacial energy values, whereas the ACC-ChNFs exhibited more efficient emulsifier performance in the wide range of oil-water interfacial energy value.

Irrespective of the aqueous suspension concentration, ACC-ChNFs were allowed to stabilize Pickering emulsion in the aqueous dispersion phase, which may be owing to their higher electrostatic repulsion force and mobility. Among the combination of oil/aqueous suspension of nanofibrils in Pickering emulsion studied, the stability of emulsions for canola oil were the highest, and it was remarkably improved by adding the polysaccharide-based nanofibrils to the canola oil/water alone.

Concerning canola oil emulsions stabilized by the two polysaccharide-based nanofibrils, more complicated behaviors were monitored, which may be attributed to the emulsification mechanism due to not only characteristics of nanofibrils, but canola oil itself. It is noted that the emulsified volumes and surface charge of oil droplets were sensitive to the concentration of ACC-ChNFs, differently from the case for ACC-BCNFs. When their concentration was set to be higher than 0.20 wt%, interfacial adsorption of ACC-ChNFs was most likely to allow the oil droplets to be stabilized, rather than self-stabilization effects of the canola oil.

Accordingly, this chapter has highlighted the characteristics in the emulsification behaviors using ACC-ChNFs as an emulsifier for high versatility and thereby for the functional design of Pickering emulsions in the field of food emulsion, cosmetics, and drug delivery using bioactive chitin objects. In addition, from the concentration-

dependent physicochemical properties of Pickering emulsions, it is presumed that the low nanofiber concentration condition is suitable for fabrications of ordered structures on the fluid interfaces described in the following chapters.

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Chapter 2

Surface Free Energy of Janus-type Amphiphilic Cellulose Nanofibrils as an Indicator for the Surface Properties

Abstract

Chapter 2 attempts to clarify the adsorbability of ACC-CNFs based on the surface free energy theory. Work of adhesion, the energy required to separate two attached surfaces, was calculated using surface free energy values obtained by wettability measurements of the thin CNF films based on Wenzel's law and van Oss-Chaudhury-Good (VCG) theory. The work of adhesion in adsorption process indicated that the CNFs in water act like protective colloids, which surround unstable water-hydrophobic substance interface. In addition, the work of adhesion values of the CNFs with various oils or resins closely correlated with the actual CNF adsorption results in water media. Hence, the obtained surface free energy values are expected not only to predict ACC-CNF adsorption in water, but also become an indicator for surface physicochemical design of the CNFs to tune the interfacial interactions between CNFs and other materials.

2.1. Introduction

In nanomaterials with high specific surface areas, the interfacial interactions between materials are critical for adsorption, desorption, and interfacial stability. Examples include the responses of cells to scaffold materials,^{1,2)} and the interfacial fracture of nanocomposites.^{3,4)} In addition to the studies mentioned above, adsorption onto the surfaces of CNFs has been an important feature of numerous other studies, including on the binding of enzymes,^{5,6)} and on the interfacial concentration of reagents

at the surfaces of CNFs acting as catalysts.⁷⁾ However, the adhesion between CNFs and other substances is poorly understood owing to the nano-scale widths of the fibers.

The work of adhesion, which is defined as the energy required to separate two attached surfaces, is expressed as the surface free energy. There are various methods for estimating the surface free energy values of solids, including: inverse gas chromatography (iGC),^{8,9)} contact angle methods,¹⁰⁻¹³⁾ computational simulation,¹⁴⁾ atomic force microscopy (AFM),¹⁵⁾ chemical vapor deposition,¹⁶⁾ and Hansen solubility parameter¹⁷⁾ methods. Although iGC is suitable for powders and fibrous samples, Peng et al. reported that the surface free energy values of CNFs depend on the drying process.⁸⁾ Relini et al. reported that force–distance curves obtained by AFM using streptavidin crystals and a modified AFM tip could be used to quantitatively determine interfacial interactions.¹⁵⁾ Recently, Bruel et al. estimated the individual surface free energy values of a polar hydroxy group-rich plane and a non-polar glucan sheet face on rod-like cellulose nanocrystals (CNCs) using Hansen solubility parameters.¹⁷⁾ The contact angle method¹⁰⁻¹³⁾ is the most traditional and simple way of estimating the surface free energy values of solids using probe liquids with known surface free energy parameters. It should be noted that many cases of high correlation^{18,19)} between experimental adhesion strength and work of adhesion have been reported. This verifies the legitimacy of individual estimation using the contact angle method, although effective use requires an appropriate surface morphology, especially for nano- or micro-particle samples.²⁰⁾ There are some reports of contact angle measurements using molded colloidal particles²¹⁾ including CNCs^{22,23)} as the sample surface instead of a single particle, but they indicated that the penetration of liquid droplets influenced the contact angle values.^{20,23)} The correlation between errors derived from swelling and film thickness was also noted.^{22,24)}

Flat CNF monolayer films without gaps are ideal for precise contact angle measurements. However, the preparation of such thin CNF films is technically difficult owing to the aggregation induced by strong attractive inter-fibrillar interactions²⁵⁾ and entanglements among the fibers. The monolayer-like shell structures of CNCs and CNFs at the oil–water interfaces of Pickering emulsions have also been reported based on small-angle neutron scattering²⁶⁾ and electron microscope observations.^{27,28)}

In the current study, thickness-controlled CNF thin films were prepared from Pickering emulsions using a partly modified emulsion casting method,²⁹⁾ and the wetting behaviors of the films were used to estimate CNF adhesion.

2.2. Materials and methods

2.2.1. Materials

α -Bromonaphthalene (BN: $\geq 97.0\%$), bromoform ($\geq 99.0\%$), chloroform ($\geq 99.0\%$), cyclohexane ($\geq 99.5\%$), dichloromethane ($\geq 99.0\%$), ethyl acetate ($\geq 99.0\%$), pyrrole ($\geq 98.0\%$), and toluene ($\geq 99.5\%$) were purchased from Sigma-Aldrich Japan, Co. LLC., Tokyo, Japan. Acetone ($\geq 99.5\%$), ethylene glycol (EG: $\geq 99.5\%$), diethyl ether ($\geq 99.5\%$), and nitrobenzene ($\geq 99.5\%$) were purchased from FUJIFILM Wako Pure Chemical Corp., Osaka, Japan. The EG was dehydrated using molecular sieve 3A. Diiodomethane (DIM: $\geq 98.0\%$) was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. TEMPO-oxidized cellulose nanofibrils (TOCNFs, 1-2SX) were provided from DKS Co., Ltd., Kyoto, Japan. Other reagents were used without purification. Ultrapure water ($\sim 18 \text{ M}\Omega \text{ cm}$) was obtained using a NANOpure™ Diamond purification system (Thermo Fisher Scientific, Inc., Waltham, United States), and used in the measurement of water contact angles (WCA), and deionized water was used for all other experiments.

2.2.2. Preparation of CNFs

The ACC-CNFs derived from bacterial cellulose³⁰⁾ (ACC-BCNFs) and microcrystalline cellulose^{31,32)} (ACC-MCCs) suspension were prepared according to the method written in Chapter 1. Nanofibrillated cellulose (NFCs) suspension was prepared from hardwood bleached sulfite pulp (HBSP) according to the previous report.²⁸⁾ The HBSP were defibrillated using a microfluidizer (M110 P, Microfluidics Corp., Newton, MA, USA) equipped with 200 and 100 μm chambers under 2000 psi for 6 passes.²⁸⁾

2.2.3. Preparation of CNF films from Pickering emulsion

CNF-stabilized Pickering emulsions were used to obtain the CNF thin films. An aqueous dispersion containing the CNFs (15 mL) and cyclohexane (15 mL) was poured into a 50 mL glass vessel. Emulsification was conducted at 2×10^4 rpm and room temperature (23–25°C) for 3 min using an HG-200 rotation type homogenizer equipped with a K-12S generator (AS ONE Corporation, Osaka, Japan).

Glass slide substrates (76 $\times$ 25 $\times$ 1 mm) were thoroughly washed with acetone and dried at 50°C before being coated with the Pickering emulsion. The washed glass slide was perpendicularly placed on the bottom of a glass column. Then, 45 mL of deionized water was poured into the column. Subsequently, 5 mL of the Pickering emulsion was gently poured onto the water surface. The poured Pickering emulsion was favorably coated on the glass substrate by the flow-down of the subphase with the Pickering emulsion. The Pickering emulsion-coated glass slide was removed and dried in air for 1 day. The substrate was then dried at 105°C for 2 h to completely remove the cyclohexane.

2.2.4. Preparation of CNF cast films

The ACC-BCNF films were prepared by casting aqueous dispersions of ACC-BCNFs of various concentrations. Two washed glass substrates were placed on a 9 cm×9 cm polystyrene dish. Subsequently, 28.6 mL of an ACC-BCNF aqueous dispersion of the prescribed CNF concentration was poured into the polystyrene dish and air-dried for approximately 2 weeks.

2.2.5. Preparation of CNF pellets

To prepare CNF pellets, aqueous dispersions containing ACC-BCNFs were rapidly freeze-dried using a FreeZone™ system^{2,5} (Labconco, Kansas, USA). The dried samples were then pelletized at 50 MPa for 1 min.

2.2.6. Atomic force microscopy (AFM)

The surface roughness, actual surface area, and thickness of each CNF film were determined from the topographical images obtained by AFM. The AFM observations were made using an Asylum MFP-3D Origin system (Oxford Instruments, Abingdon-on-Thames, United Kingdom) with a silicon cantilever (OMCL-AC240TS-R3; Olympus Corp., Tokyo, Japan) in the tapping mode at 25°C. Except for in the case of the CNF pellets, which had surfaces that were too rough for evaluation, the root mean square (R_q) surface roughness, arithmetic average roughness (R_a), and actual surface area values of each film were estimated based on 10 μm ×10 μm images, and were the averaged values obtained from at least eight measurements. Prior to obtaining the film thickness (d) measurements, a portion of each CNF thin film was peeled from the glass substrate using a pair of stainless-steel tweezers. The gap between the CNF thin film and the glass

substrate was measured at least five times from the 10 μm ×10 μm images, and the average value was taken as the film thickness. The thickness of each cast film was measured using a micrometer.

2.2.7. Contact angle (CA) measurements

The apparent CA values of the liquid droplets (1 μL) were measured at $25 \pm 1^\circ\text{C}$ using a Drop Master DM300 contact angle meter (Kyowa Interface Science Co., Ltd, Saitama, Japan) with a polytetrafluoroethylene-coated needle. The values were recorded at least 1 s after dropping based on 10 measurements. The obtained apparent CA values were corrected using the Wenzel equation³³⁾ as follows:

$$\cos\varphi = r\cos\theta \quad (2.1.).$$

where θ is the contact angle, r is the roughness factor (the ratio of the actual surface area determined by AFM to the apparent surface area), and φ is the apparent contact angle.

2.2.8. CNF adsorption tests

Preparation of Pickering emulsions comprising CNFs

The adsorption of the CNFs at the oil–water interface was investigated to evaluate the accuracy of the obtained adhesion parameters of CNFs. An aqueous dispersion containing 0.10 wt% of the ACC-BCNFs (5 mL) and various organic solvents (5 mL) were poured into a 20 mL glass screw tube. The mixture was then emulsified using a vortex mixer (Vortex-Genie 2; KENIS LIMITED, Osaka, Japan) at 0.3×10^4 rpm for 60 s.

Assessment of the transparency of the continuous phase

The continuous (aqueous) phase containing the non-adsorbed CNFs was gently transferred to a quartz cell 1 h after emulsification. The transparency of the continuous phase was then determined at a wavelength of 660 nm using a UV/Vis spectrophotometer (Beckman Coulter, Brea, California, USA).

2.3. Theoretical model for the estimation of surface free energy

Expression of polar interactions

The theory pertaining to the interfacial interaction between non-polar surfaces¹⁰⁾ was extended to polar surfaces by Owens and Wendt, and the interactions were expressed as the geometric means of the dispersive and polar components.¹¹⁾ Wu also reported that the interactions of polar solids can be expressed as the harmonic means of each component.¹²⁾ Although the adhesion forces based on the Owens–Wendt¹¹⁾ and Wu¹²⁾ models correspond to the experimentally measured adhesion strength,^{18,19,34)} Fowkes et al. pointed out that the polar interactions should be described by two parameters: electron acceptor/electron donor components.³⁵⁾

van Oss–Chaudhury–Good (VCG) model

In the VCG model,¹³⁾ surface free energy (γ) is divided into the Lifshitz–van der Waals interaction component (γ^{LW}) and the acid–base interaction component (γ^{AB}), which is expressed by geometric means as the electron donor and acceptor components as follows:

$$\gamma = \gamma^{LW} + \gamma^{AB} \quad (2.2.).$$

$$\gamma^{AB} = 2\sqrt{\gamma^+ \gamma^-} \quad (2.3.).$$

$$\gamma_{12} = \left(\sqrt{\gamma_1^{\text{LW}}} - \sqrt{\gamma_2^{\text{LW}}} \right)^2 + 2 \left(\sqrt{\gamma_1^+ \gamma_1^-} + \sqrt{\gamma_2^+ \gamma_2^-} - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_1^- \gamma_2^+} \right) \quad (2.4.).$$

where γ^{LW} is the Lifshitz–van der Waals component of surface free energy, γ^{AB} is the acid–base interaction component of surface free energy, γ^+ is the electron acceptor component, and γ^- is the electron donor component.

The work of adhesion between the solid and the liquid (W_{SL}) is expressed by the Young–Dupré equation³⁶⁾ as follows:

$$(1 + \cos\theta)\gamma_{\text{L}} = W_{\text{SL}} = \gamma_{\text{S}} + \gamma_{\text{L}} - \gamma_{\text{SL}} \quad (2.5.).$$

where γ_{L} is the surface free energy of the liquid and W_{SL} is the work of adhesion between the solid and the liquid.

Equation 2.5. can also be expressed using γ^{LW} and acid–base parameters as follows:

$$(1 + \cos\theta)\gamma_{\text{L}} = 2 \left(\sqrt{\gamma_{\text{S}}^{\text{LW}} \gamma_{\text{L}}^{\text{LW}}} + \sqrt{\gamma_{\text{S}}^+ \gamma_{\text{L}}^-} + \sqrt{\gamma_{\text{S}}^- \gamma_{\text{L}}^+} \right) \quad (2.6.).$$

The adhesion strength calculated by VCG theory correlated well with the actual adhesion strength.^{34,37)} Therefore, we used the values estimated using the VCG model as the surface free energy of the CNFs for analysis of the adsorption characteristics.

2.4. Results and discussion

2.4.1. Structural characterization of CNF thin films

The cyclohexane droplets were stabilized by the adsorption of the ACC-CNFs. Prior to the subsequent experiments, all the obtained Pickering emulsions were confirmed as oil-in-water type emulsions.²⁸⁾

The glass slide substrates were successfully coated with the ACC-BCNF-stabilized Pickering emulsions. The Pickering emulsions on the substrates changed to medium internal phase emulsions, and subsequently to high internal phase emulsions as the continuous phase was evaporated, as described in our previous report.²⁹⁾ The coated substrates whitened slightly as they dried. The coating conditions used for the ACC-MCC- and NFC-stabilized Pickering emulsions are summarized in Table 2-1. The CNFs exhibited low adsorption with regard to the Pickering emulsions. This was presumably because they had high affinity for the water phase, highly negative zeta potential values (Table 2-1). Therefore, the ACC-MCC or NFC coatings were applied to nanofibrils of relatively high density.

Table 2-1 Preparation conditions of CNF thin films used in CA measurements and surface characteristics of CNFs.

	ACC-BCNFs	ACC-MCCs	NFCs	TOCNFs
Preparation method	Pickering emulsion	Pickering emulsion	Pickering emulsion	Casting
Flow rate / mm min ⁻¹	3-9	20~	20~	-
Coating cycle /	1	1	1-2	-
CNF conc. in water phase / wt%	0.10	0.40	0.20	-
Thickness range / nm	39-100	39-77	32-72	< 1 μ m
Roughness factor /	1.072	1.064	1.051	1.005
Zeta potential / mV	-19.0 $\pm$ 0.8	-37.9 $\pm$ 1.3	-48.8 $\pm$ 1.5	-75.6 $\pm$ 4.1
Charged group content / mmol g ⁻¹	Not detected	0.02 $\pm$ 0.01	0.06 $\pm$ 0.01	1.67 $\pm$ 0.07

Fig. 2-1 shows typical AFM topographical images of a thin film prepared from an ACC-BCNF-stabilized Pickering emulsion. The AFM images reveal that the substrates were successfully covered with the ACC-BCNFs, even in the narrow spaces between the dried droplets (Fig. 2-1b). Therefore, in the subsequent investigations of the Pickering emulsion-coated substrates we assumed that the surfaces of the glass slides had been completely covered with CNFs. Fig. 2-2 shows a partially peeled thin film on a glass substrate. The difference in height of the film in the 4 μm region from the unpeeled side of the film in the AFM images was taken as the film thickness (d), because fluffy structures were present on the peeled side (Fig. 2-2).

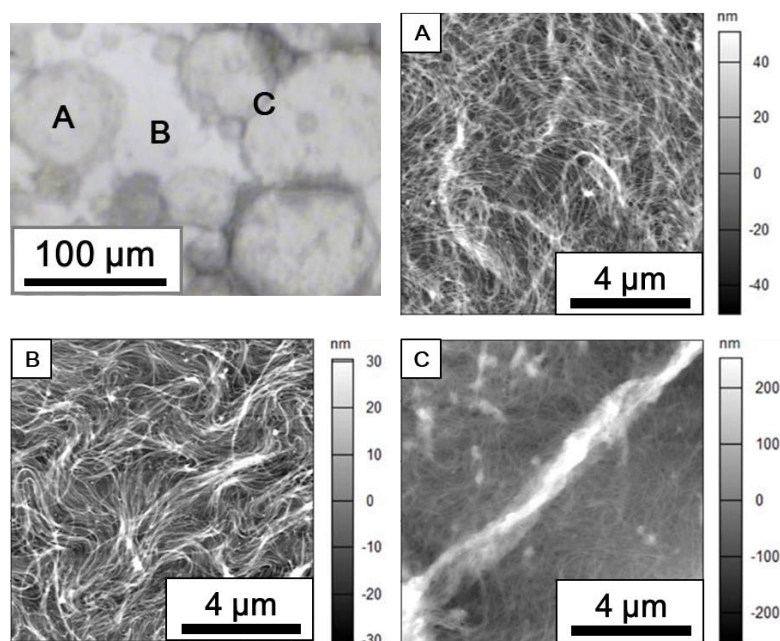


Fig. 2-1 Image of the Pickering emulsion film captured by CCD camera in an AFM system (top left), AFM topographical images of (A) a dried droplet, (B) the narrow space between dried droplets, and (C) the boarder of the dried droplets.

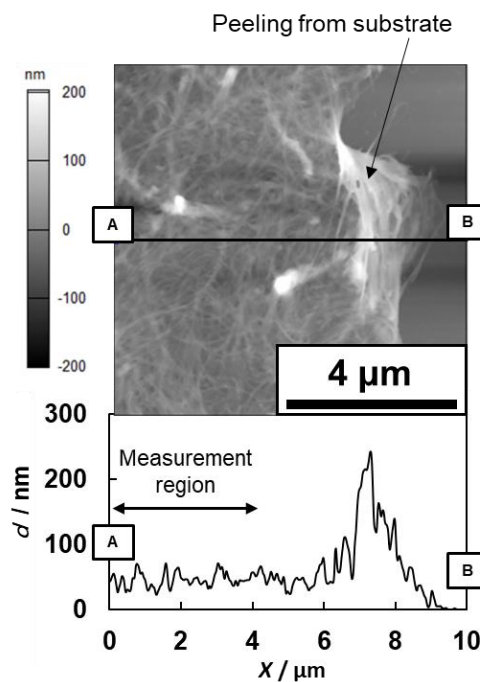


Fig. 2-2 Typical cross section profile along the A-B line in AFM topographical image of partially peeled ACC-BCNF thin film prepared from Pickering emulsion. Two direction arrow indicates measurement region in film thickness estimation.

Fig. 2-3 shows the correlation between the flow rate in the Pickering emulsion coating and the CNF film thickness. When the flow rate was less than 2 mm min^{-1} , the thickness distribution was wide, presumably owing to the overlap of the Pickering emulsion droplets. However, the film thickness decreased significantly when the flow rate increased to over 2 mm min^{-1} . From the reported width³⁰⁾ and height²⁷⁾ values of the ACC-BCNFs, it was estimated that there were 2–5 CNF layers.

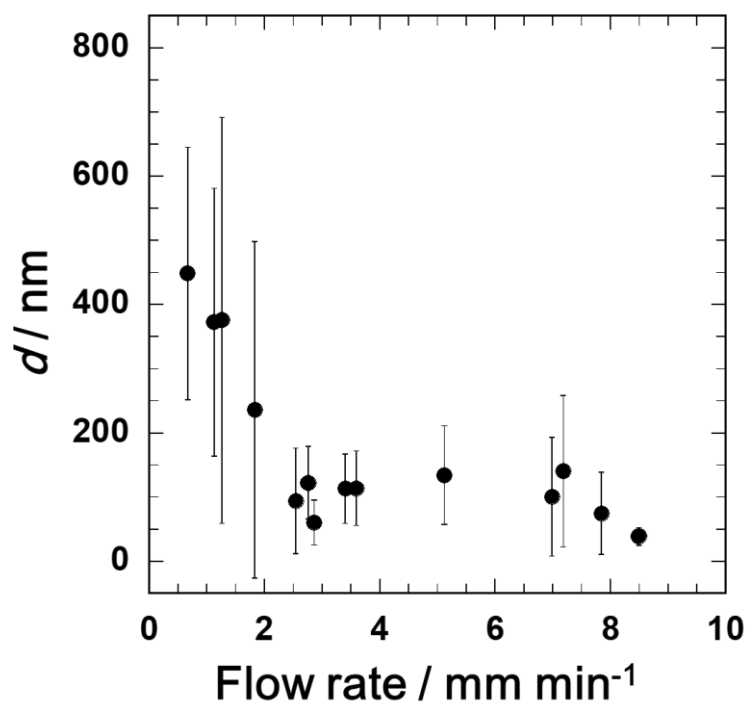


Fig. 2-3 Film thickness versus coating flow rate.

The correlation between surface roughness and wettability is known as Wenzel's law³³⁾. ACC-BCNF pellets with rough surfaces have significantly lower apparent water contact angle (AWCA) values ($19.3 \pm 1.6^\circ$) than cast films ($29.9 \pm 1.5^\circ$). However, there are reports whether the equation is applicable or not, especially in the low surface roughness region ($< 100 \text{ nm}$),³⁸⁻⁴⁰⁾ to which our samples belonged (Fig. 2-4). In the following study, it should be noted that the surface free energy parameters were calculated by assuming that the roughness was influential, according to a report on nanoscale roughness in cellulosic materials.⁴⁰⁾

The water droplets contacted on the CNF pellets were rapidly absorbed into the samples. The results implies that the pelletized CNF samples have a lot of small pores, thereby absorbing water into the film thickness direction. On the other hand, the AWCA values of cast films and Pickering emulsion films were decreased slowly, which might be due to the forming of strong inter-fibrillar interactions during drying process.²³⁾

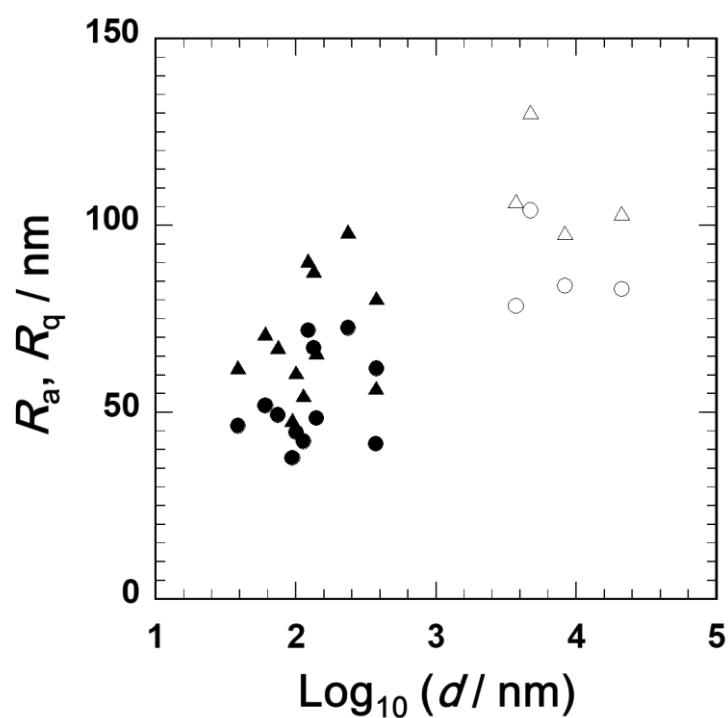


Fig. 2-4 Surface roughness values of ACC-BCNF films. Closed circles: Arithmetic average roughness (R_a) of Pickering emulsion films; closed triangles: root mean square roughness (R_q) of Pickering emulsion film; opened circles: R_a of cast films; and opened triangles: R_q of cast films.

2.4.2. Water contact angle (WCA) values of ACC-CNF films of various thickness

To evaluate the influence of liquid penetration into the CNF films, water with high affinity for CNFs¹⁷⁾ was selected as the first test liquid. Fig. 2-5 shows the WCA values of CNF films of various thickness. The low WCA values of the ACC-MCCs presumably resulted from the influence of the minor hydrophilic charged groups; this explanation is supported by the charged group content and zeta potential values (Table 2-1). The difference in the WCA values of the films prepared by the two different methods above, was negligible, and the WCA values of the ACC-BCNF films were similar to the equilibrium WCA values of a (110) cellulose I_β crystal plane (42.8°) calculated by computational simulation.⁴¹⁾ That is to say, the small pores between the fibrils in the CNF films, which are supposed to affect the WCA, had less effect on the current samples. Taking this result into account, it was considered that the prepared film samples were suitable for the evaluation of the actual wetting of a single CNF.

According to Bruel's report on CNC films of micro-scale thickness, film thickness does not necessarily affect the CA values.¹⁷⁾ The results were also confirmed in this case, CNF films with nano-scale thickness. Therefore, the relatively flat surfaces of the 40–100 nm-thick CNF films prepared from the Pickering emulsions were chosen for the subsequent experiments because they fell within the applicable range of the Wenzel equation (Fig. 2-4).

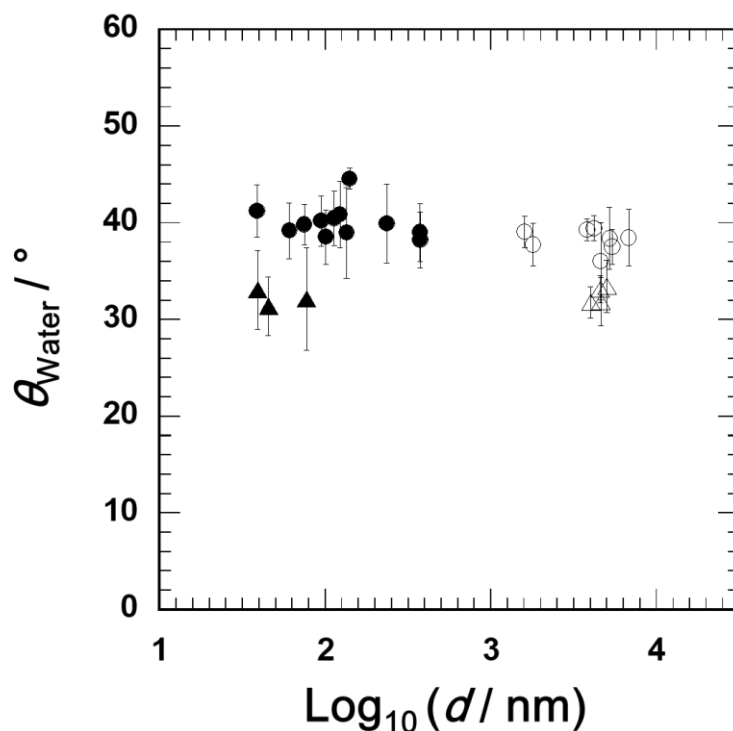


Fig. 2-5 WCA values of CNF films of various film thickness (d). Closed circles: ACC-BCNF Pickering emulsion films; closed triangles: ACC-MCC Pickering emulsion films; opened circles: ACC-BCNF cast films; and opened triangles: ACC-MCC cast films. The WCA values were obtained from AWCA values and roughness factor using Wenzel equation.

2.4.3. Estimation of surface free energy of CNFs

There are many probe liquids for estimation of surface free energy of solids such as DIM (non-polar), hexadecane (non-polar), and BN (non-polar), water (acidic), glycerol (acidic), dimethyl sulfoxide (basic), formamide (basic), and EG (basic). In the current study, hexadecane with low surface tension was excluded from the candidate of probe liquids,⁴²⁾ and other two non-polar solvents were used for estimation of Lifshitz-van der Waals interaction component of CNFs. Glycerol, very viscous acidic liquid, was excluded because the high viscosity makes it difficult to determine the equilibrium state of dropped

liquids on solid surface.⁴³⁾ Alternatively, water was selected as acidic probe solvent. EG was used as basic probe solvent because other two basic solvents are known as good solvents that swells cellulosic material.¹⁷⁾

The CA value of each sample is included in Table 2-2. With the polar solvents, the ACC-BCNFs and NFCs exhibited relatively high apparent contact angle values, θ_{Water} and θ_{EG} for water and EG, respectively, indicating less hydrophilic surface characteristics.

Table 2-2 Contact angle values of the probe liquids.

	$\theta_{\text{Water}} / ^\circ$	$\theta_{\text{DIM}} / ^\circ$	$\theta_{\text{EG}} / ^\circ$	$\theta_{\text{BN}} / ^\circ$
ACC-BCNFs	40.1 ± 1.4	45.1 ± 5.7	33.9 ± 2.3	30.2 ± 2.3
ACC-MCCs	30.9 ± 5.1	33.8 ± 2.1	26.8 ± 1.5	27.4 ± 3.6
NFCs	38.1 ± 2.4	40.4 ± 2.3	30.6 ± 1.9	27.9 ± 4.7
TOCNFs (cast film)	22.0 ± 1.7	44.8 ± 3.8	20.9 ± 1.7	31.3 ± 3.4

In the current study, the Lifshitz–van der Waals interaction component γ^{LW} was initially estimated from the CA values using two different non-polar probe liquids. Subsequently, the two unknown acid–base interaction parameters were estimated based on the CA values of polar probe liquids with known acid–base parameters. Although several acid–base parameters have been reported for probe liquids,^{13,44,45)} we chose the most reliable values reported by Della Volpe and Siboni,⁴⁴⁾ as listed in Table 2-3. Table 2-4 shows the estimated surface free energy values (γ) of each sample based on VCG theory. The γ value of the ACC-BCNFs was lower than that of the other CNFs. Furthermore, the ACC-MCCs and TOCNFs had high electron donor component (γ) values, indicating hydrophilic characters. Table 2-1 shows the zeta potential values and surface charged group contents of each CNF sample. The negligible surface charged group content of the ACC-BCNFs suggests that their surfaces comprised pure cellulose.

In contrast, the NFCs were negatively charged, presumably owing to the presence of groups derived from the carboxy groups of hemicellulose. It is well known that sulfate half ester groups with very low pKa values are introduced onto MCC and CNC surfaces during sulfuric acid hydrolysis.^{46,47)} That is, the relatively high γ^- values of the ACC-MCCs and TOCNFs were derived from minor sulfate half ester groups and abundant C6 carboxyl groups, respectively.

Table 2-3 Surface free energy and acid-base interaction parameters of probe liquids.

/ mJ m ⁻²	γ	γ^{LW}	γ^+	γ^-
Water ⁴⁴⁾	72.8	26.2	48.5	11.2
DIM ⁴⁴⁾	50.8	50.8	0.00	0.00
EG ⁴⁴⁾	48.0	33.9	0.966	51.6
BN ⁴⁴⁾	44.4	44.4	0.00	0.00

Table 2-4 Surface free energy (γ) with individual parameters including acid–base interaction parameters estimated using van Oss–Chaudhury–Good (VCG) theory.

/ mJ m ⁻²	γ	γ^{LW}	γ^{AB}	γ^+	γ^-
ACC-BCNFs	42.5	37.8	4.68	0.275	19.9
ACC-MCCs	45.7	41.1	4.57	0.229	22.8
NFCs	44.1	39.4	4.66	0.270	20.1
TOCNFs (cast film)	45.8	37.7	8.11	0.610	26.9

2.4.4. CNF adhesion in aqueous media

To evaluate the accuracy of the estimated surface free energy, the work of adhesion (W_{ad}) values were calculated from the surface free energy, then compared with the actual CNF adsorption.

• *Nanocomposites*

To date, CNFs, which have high mechanical strength, have mainly been used as fillers for nanocomposites. Kondo et al. recently proposed an ecofriendly compounding process (i.e., gentle shaking) that involved the underwater adsorption of ACC-CNFs onto hydrophobic resin microparticles.^{48,49)} Herein, the work of adhesion (W_{ad}) between the CNFs and the resin microparticles in the water is expressed as follows:

$$W_{ad \text{ CNF/Water/Resin}} = \gamma_{\text{CNF/Water}} + \gamma_{\text{Resin/Water}} - \gamma_{\text{CNF/Resin}} \quad (2.7.).$$

where W_{ad} is the work of adhesion and $\gamma_{1/2}$ is the interfacial free energy between 1 and 2 calculated from the surface free energy parameters using equation (2.4.). The reference values of the various resins used in the calculation of W_{ad} are summarized in Table 2-5.

Table 2-5 Surface free energy and acid-base interaction parameters of resin. The units of the electron acceptor and donor component are square root of mJ m^{-2}

/ mJ m^{-2}	γ	γ^{LW}	$\sqrt{\gamma^+}$	$\sqrt{\gamma^-}$
PP ⁴⁴⁾	28.3	28.3	0.00	0.00
PE ⁴⁴⁾	34.2	34.2	0.00	0.00
PMMA ⁴⁴⁾	37.7	37.4	0.104	1.49
PET ⁴⁴⁾	38.4	38.5	-0.0545	1.61
PS ⁴⁴⁾	38.2	38.4	-0.162	0.468
Nylon66 ⁴⁴⁾	42.8	37.4	1.73	1.57

Fig. 2-6 shows the differences in W_{ad} values between the ACC-BCNFs and various resins in water. These differences indicate that the acid–base interaction is the predominant component in adhesion interactions in water. In contrast, the W_{ad} values *in vacuo* in Fig. 2-7 indicate that Lifshitz–van der Waals interaction is the main component in ACC-BCNF–resin interfacial interaction. That is, ACC-BCNFs tend to interact with low-polarity resins through Lifshitz–van der Waals interactions.⁴⁹⁾

The stabilization of whole systems, including aqueous media, mainly depends on the replacement of an unstable resin–water interface with a stable CNF–water interface, enabling participation in acid–base and Lifshitz–van der Waals interactions. Ishikawa et al. reported that the readiness with which ACC-BCNFs were adsorbed onto resin microparticles in water followed the order: isotactic PP > PE >> PET.⁴⁹⁾ These results agree well to the current estimated W_{ad} values pertaining to ACC-BCNFs and various resin microparticles in water: polyethylene (PE) $\geq$ polypropylene (PP) > polystyrene (PS) > polyhexamethylene adipamide (Nylon66) > polymethyl methacrylate (PMMA) > polyethylene terephthalate (PET) (see Fig. 2-6). In particular, the W_{ad} values with regard to various CNFs and polypropylene (PP) in water followed the order: ACC-BCNFs $\approx$ NFCs > ACC-MCCs > TOCNFs (Fig. 2-8). These results also corroborated the previously reported confocal laser scanning microscopy observations: ACC-BCNFs > ACC-MCCs >> TOCNFs.⁴⁹⁾

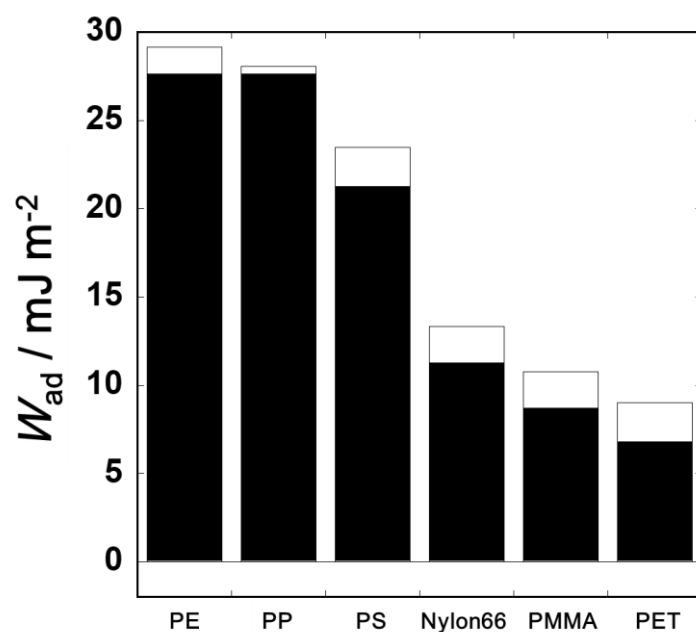


Fig. 2-6 Work of adhesion values pertaining to ACC-BCNFs and various resins in water. Black: acid–base interaction component, and white: Lifshitz–van der Waals interaction component.

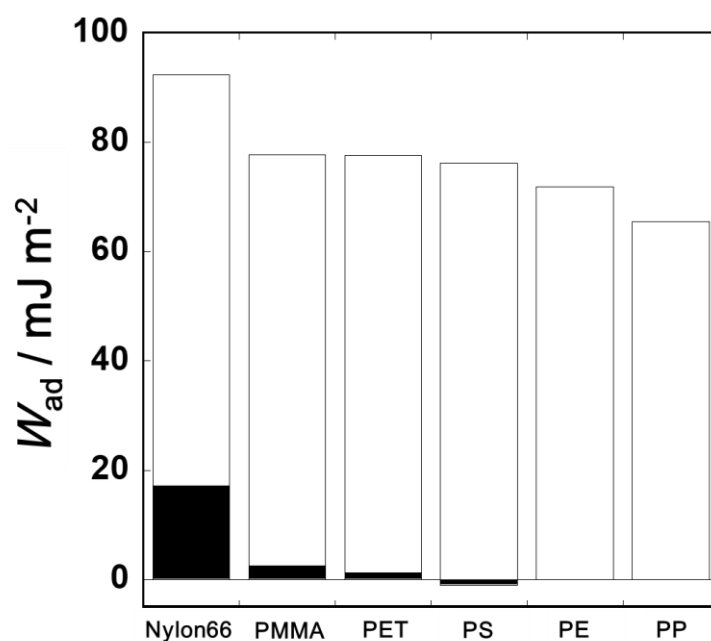


Fig. 2-7 Work of adhesion values pertaining to the ACC-BCNFs and various resins *in vacuo*. Black: acid–base interaction component, and white: Lifshitz–van der Waals interaction component.

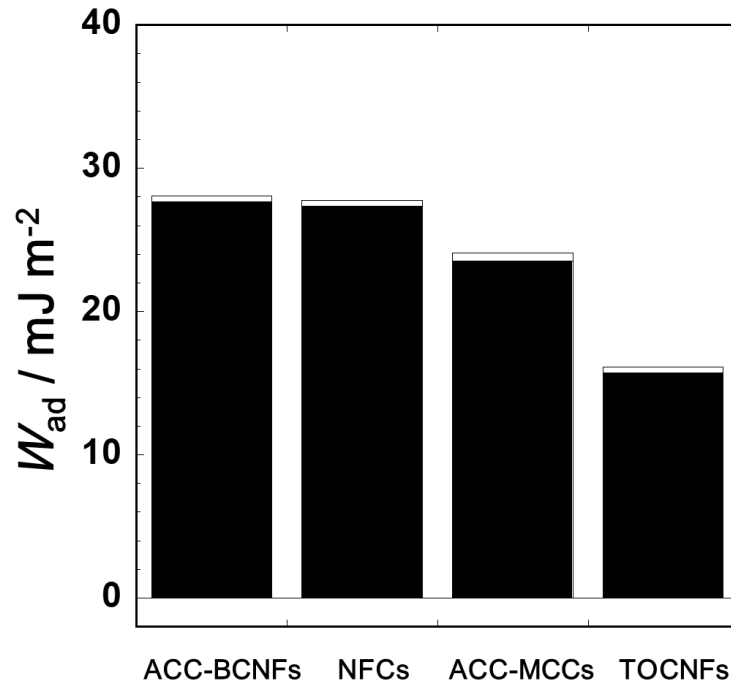


Fig. 2-8 W_{ad} between polypropylene and various CNFs in water. Black: acid-base interaction component, and white: Lifshitz-van der Waals interaction component.

• *Pickering emulsions*

Biocompatible CNFs with high SSAs significantly expand the potential applicability of Pickering emulsions.^{28,50)} To evaluate the accuracy of the estimated surface free energy, the work of adhesion values were calculated from the surface free energy, then compared with actual Pickering emulsion formation via CNF adsorption. Goi et al. reported that TOCNFs stabilize oil droplets by two mechanisms: they act as a Pickering emulsifier by decreasing the oil–water interfacial energy, and act as a dispersion stabilizer to prevent the coalescence of droplets.⁵⁰⁾ Ishida et al. reported that ACC-BCNFs are readily adsorbed at the oil–water interface.²⁷⁾ Therefore, the ACC-BCNF is poor dispersion stabilizer. In other words, effects as dispersion stabilizer can be easy for ACC-BCNFs to be separated on emulsifying. Therefore, ACC-BCNFs were chosen as contrasting model samples between experiments and work of adhesion for pursuing

surface free energy values.

The reference values of the various organic solvents used in the calculation of W_{ad} are summarized in Table 2-6. Table 2-7 presents the W_{ad} values pertaining to the underwater adsorption of the ACC-MCCs. It can be assumed that the oil–water interfaces of the Pickering emulsions were stabilized by the adsorption of the CNFs when $W_{ad} \geq 0$. However, negative W_{ad} values indicate that the Pickering emulsifier had detached from the oil–water interface. With the exception of several solvents with unknown acid–base interaction parameters, the estimations closely matched previously reported emulsification data for 10 solvents.²⁸⁾ The W_{ad} values of both the ACC-BCNFs correlated well with their actual adsorption behaviors. Except for diethyl ether, ethyl acetate, and nitrobenzene, which had negative W_{ad} values (see Table 2-7), the W_{ad} values with regard to the ACC-BCNFs and various organic solvents in water suggested adsorption.

Table 2-6 Surface free energy and acid-base interaction parameters of various organic solvents.

/ mJ m ⁻²	γ	γ^{LW}	γ^+	γ^-
Diiodomethane ⁴⁴⁾	50.8	50.8	0.00	0.00
α -Bromonaphthalene ⁴⁴⁾	44.4	44.4	0.00	0.00
Bromoform ⁴⁴⁾	41.5	28.1	19.9	2.24
Pyrrole ⁴⁴⁾	36.6	25.9	10.8	2.66
Hexadecane ⁴⁴⁾	27.6	27.6	0.00	0.00
Toluene ⁵¹⁾	28.3	28.3	0	2.7
Nitrobenzene ⁵²⁾	43.9	41.3	0.26	6.6
Cyclohexane ⁵²⁾	25.2	25.2	0	0
Dichloromethane ⁵³⁾	24.5	24.5	5.2	0
Chloroform ⁵⁴⁾	27.2	27.2	3.8	0
Ethyl acetate ⁵⁴⁾	23.9	23.9	0	19.4
Diethyl ether ⁵⁴⁾	16.7	16.7	0	16.4

Table 2-7 W_{ad} between ACC-BCNFs and various oils in water, and transparency of continuous phases.

	W_{ad} / mJ m^{-2}	W^{LW} / mJ m^{-2}	W^{AB} / mJ m^{-2}	Transparency / %
Diiodomethane	31.8	4.1	27.7	99.5
α -Bromonaphthalene	30.9	3.2	27.7	97.9
Bromoform	18.7	0.4	18.3	98.4
Pyrrole	13.8	-0.1	13.9	98.2
Hexadecane	30.0	0.3	27.7	96.4
Toluene	6.9	0.4	6.5	98.2
Nitrobenzene	-1.6	2.7	-4.3	95.8
Cyclohexane	27.5	-0.2	27.7	98.6
Dichloromethane	32.4	-0.3	32.7	98.7
Chloroform	32.2	0.2	32.0	99.4
Ethyl acetate	-29.6	-0.5	-29.1	34.4
Diethyl ether	-26.6	-2.1	-24.5	31.6
0.10 wt% ACC-BCNFs	-	-	-	29.7

Fig. 2-9 shows a Pickering emulsion stabilized by an aqueous dispersion containing 0.10 wt% ACC-BCNFs. The appearance (Fig. 2-9) and transparency (Table 2-7) of the continuous (aqueous) phase of each Pickering emulsion indicated the favorable adsorption of the ACC-BCNFs onto the oil–water interface, except when ethyl acetate or diethyl ether was the solvent. The experimental data closely matched the estimations based on VCG theory. Only the nitrobenzene result having W_{ad} value close to 0 differed from the predicted behavior. This mismatch was presumably owing to the influence of the Janus-type amphiphilic structures of the ACC-BCNFs, the fibers of which have both hydrophilic OH planes and hydrophobic glucan sheet planes.^{14,55,56)} The W_{ad} values based on wetting reflect the presence of minor CH groups and major OH groups, but the values

also suggest that the ACC-BCNFs have isotropic surfaces. This indicated the influence appeared from the averaged surface area fraction of each plane in the Janus-type amphiphilic fibers. However, it is considered that the hydrophobic CH planes in nanocelluloses effectively stabilize the oil–water interface when they face the hydrophobic oil side.^{17,26,28)} This implies a difference in interfacial stabilization efficiency between the averaged isotropic ACC-BCNF surface model and the actual anisotropic ACC-BCNF surfaces.

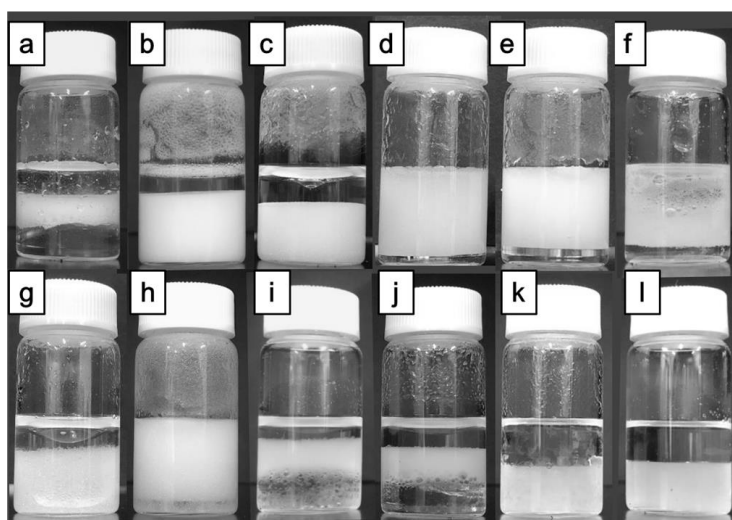


Fig. 2-9 Appearance of ACC-BCNF-stabilized Pickering emulsions of (a) diiodomethane, (b) α -bromonaphthalene, (c) bromoform, (d) pyrrole, (e) hexadecane, (f) toluene, (g) nitrobenzene, (h) cyclohexane, (i) dichloromethane, (j) chloroform, (k) ethyl acetate, and (l) diethyl ether.

Overall, results in this chapter demonstrated accurate wettability measurements enable reasonable estimations of surface free energy parameters, which can be used to predict CNF adhesion behavior. On the other hand, besides the anisotropic surface of CNFs, there are some problems e.g. incompleteness in theory, actual contacting area in solid-solid adhesion, and dynamic aspects in more complicated CNF adhesion systems.

2.5. Conclusion

An understanding of CNF adhesion is essential for their basic design and application. Reasonable estimations of the surface free energy parameters of CNFs can be obtained from VCG theory and the wetting behaviors of ultrathin films. Moreover, the work of adhesion values estimated from the wettability data closely matched the actual underwater adhesion behaviors of the CNFs at the oil–water interfaces. Besides the Pickering emulsion dealt with this chapter, the facile method for the quantitative analysis of CNF adhesion described herein may be extended to interpretation of other fields, including protein/enzyme adsorption, dispersibility of nanofibrils. Moreover, the current work may inform the surface modification of CNFs for the reinforcement of plastics.

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Chapter 3

Surface Modification of Cellulose Nanofibrils at an Oil-water Interfacial Reaction Platform for Induction of Structure-correlated Functions

Abstract

The self-organization of nano-sized fibrous building blocks is essential for the hierarchically constructed bio-based materials. The localization of hydrophobic moieties on the surface of such nanofibrils is key to hierarchical assembly in aqueous media.

In this chapter, fluid interface of the Pickering emulsion was used as a platform for facile surface modification of cellulose nanofibrils. Namely, assembled CNF structure surrounding oil droplets as a Pickering emulsion was used, thereby attempting to improve self-aggregation feature for inducing structure-correlated functions.

CNFs were successfully acetylated at the oil-water interface while maintaining its high crystallinity and fiber shape. Self-aggregation properties of the nanofibrils acetylated in Pickering emulsion system were quite different from those acetylated in the typical homogeneous dispersion system, and thereby, the films emerged unique wetting behaviors correlated with the assembled structure of nanofibrils.

Moreover, the acetylation of CNFs drastically improved nanofiber adsorption onto hydrophobic polymer surface, and the compounding via adsorption effectively improved mechanical strength of polystyrene films while keeping its original transparency.

3.1. Introduction

Natural organisms with complex 3D hierarchical structures-such as tree trunks, animal bones, and crustacean shells-are constructed from self-organized nano- or micro-

scaled molecular building blocks.¹⁾ Cellulose, building blocks of woods, self-assembles to form highly ordered hierarchical structures with excellent material properties. At the molecular level, cellulose chains have regular equatorially arranged hydrophilic hydroxy (OH) groups and axially arranged hydrophobic CH groups. Therefore, cellulose can be considered an “amphiphilic” molecule.

Nematic ordered cellulose (NOC) comprises a unique supramolecular structure of highly aligned molecular “tracks” or scaffolds with OH and CH groups.²⁾ NOC has relatively hydrophobic surface. The NOC surface can act as a template for unique processes such as 3D cell culture,³⁾ the patterned deposition of inorganics,⁴⁾ and the controlled movement of bacteria.^{5,6)}

Nanocelluloses⁷⁻¹¹⁾ are listed as high-order building blocks. They are categorized as cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs). In general, CNCs are produced via top-down chemical treatment,^{7,8)} and CNFs are produced via mechanical treatment,⁹⁾ or chemical treatment^{10,11)} followed by mechanical treatment. Both CNCs and CNFs are hydrophilic because they have abundant hydrophilic functional groups on their surfaces.

The aqueous counter collision (ACC) method¹²⁻¹⁴⁾ endows CNFs with unique amphiphilic characteristics. For example, hydrophilicity and hydrophobicity of substrate surfaces were inversed using ACC-CNFs as a coating agent.¹⁵⁾ ACC-CNFs could adsorb densely at the oil-water interface, resulting in formation and stabilization of Pickering emulsion at low energy.^{16,17)} Tsuji et al. recently reported that the CNFs prepared aqueous counter collision method are amphiphilic-like Janus-type fiber having both OH planes and CH planes on its single fiber.¹⁸⁾

Janus nano particles¹⁹⁻²³⁾ (JNPs) have been attracted attention as solid surfactants,²⁰⁾

active matters,²¹⁾ and nano building blocks for self-assembly^{22,23)} due to their chemical anisotropy. In general, synthesis of JNPs is achieved by using interfaces as reaction platforms. For example, fabrication of JNPs has been reported at the liquid/liquid (oil-water) interface²⁴⁾ of Pickering emulsion and the water/solidified oil interfaces of Pickering emulsion.²⁵⁾ Thus, using Pickering emulsion as a heterogeneous reaction platform, different two faces such as amphiphilicity (hydrophilicity and hydrophobicity) are provided to JNPs.

The amphiphilicity of JNPs is influenced by the size of hydrophilic and hydrophobic area, and Jiang et al. have explained the amphiphilicity of JNPs using an indicator reflecting the influence of their shape.²⁶⁾ Kang et al. reported that self-assembled suprastructures of JNPs are sensitive to their shape.²⁷⁾ Additionally, emergences of unique adhesion properties were proposed by two-dimensional anisotropic arrangements of JNPs.^{28,29)} To date, formations of higher dimensional structures materials have been limited to the objects having low aspect ratio. On the other hand, bio-based structural materials such as cellulose fibers are constructed from fibrous building blocks, and the understanding of assembling behaviors of fiber shaped Janus nano-objects is a significant issue for the functional material design.

Here, the surface acetylation of ACC-CNFs was carried out on the oil-water interfaces of Pickering emulsion composed of aqueous phase and oil phase containing reagents. The novelty of the current study is that it attempts to extend the surface modification approach to control the hydrophilic/hydrophobic balance of Janus-type structures on the surfaces of individual ACC-CNFs. That is, we used a heterogeneous reaction platform comprising the oil-water interfaces in a Pickering emulsion system to change the polarity of ACC-CNFs by introducing acetyl groups onto their surfaces. The

resulting locally acetylated ACC-CNFs were then characterized in terms of their self-aggregation. In addition, the surface acetylation effect on the improvement of CNF adsorption was considered based on the surface free energy, which is for development of facile compounding process with polymer microspheres.^{14,30,31)}

3.2. Materials and methods

3.2.1. Materials

Acetone ($\geq 99.0\%$), α -bromonaphthalene (BN: $\geq 97.0\%$), and toluene ($\geq 99.0\%$) were purchased from Sigma-Aldrich Japan, Co. LLC., Tokyo, Japan. Acetic anhydride (Ac_2O : $\geq 97.0\%$), *N, N*-Dimethylacetamide (DMAc: $\geq 97.0\%$), ethylene glycol (EG: $\geq 99.5\%$), Nile Red (for pathology research), phenol ($> 99.0\%$), and sulfuric acid ($> 95.0\%$), and triethylamine (TEA: $\geq 99.0\%$) were purchased from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, then EG was dehydrated using molecular sieve 3A. Diiodomethane (DIM: $\geq 98.0\%$) and 4-dimethylaminopyridine (DMAP) were purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. PS microspheres (S-20, 300 μm ϕ) were purchased from Sekisui Chemical Industries, Ltd., Osaka, Japan. PS microspheres were washed with ethanol and deionized water. Ethanol (99%) was purchased from Japan Alcohol Trading Co., Ltd., Tokyo, Japan. Ultrapure water ($\sim 18 \text{ M}\Omega \text{ cm}$) obtained using a NANOpure™ Diamond purification system (Thermo Fisher Scientific, Inc., Waltham, United States) was used for the wettability measurements. Deionized water was used in all other experiments.

3.2.2. Preparation of the ACC-CNFs

Bacterial cellulose-derived ACC-CNF aqueous suspension was prepared according

to previous work.^{13,15-17,32)} The concentration of the obtained aqueous dispersion of ACC-CNFs was determined based on its dry weight, and subsequently adjusted to the prescribed concentration with deionized water for each experiment.

3.2.3. Acetylation of the ACC-CNFs

Heterogeneous dispersion system comprising a Pickering emulsion

Acetylation in the interfacial reaction system was carried out using Pickering emulsion. An aqueous phase (15 mL) containing 0.10 wt % ACC-CNFs and a toluene (14 mL) phase containing TEA (1 mL, 7.17 mmol), DMAP (50 mg, 0.41 mmol), and Ac₂O (10-1500 μ L, 0.11-15.87 mmol) were poured in a 50 mL glass vessel. Emulsification and acetylation were simultaneously conducted using an HG-200 homogenizer equipped with a K-12S generator (AS ONE Corporation, Osaka, Japan) at $0.3\text{-}2.0\times 10^4$ rpm for 5 min under ambient conditions. Then, the obtained samples were purified with ethanol, acetone, and deionized water using a PTFE membrane filter (0.45 μ m ϕ , JHWP04700, Merck Millipore Ltd., Burlington, USA).

Homogeneous dispersion system

Acetylated ACC-CNFs were prepared in traditional homogeneous dispersion system as control samples according to previous paper.³³⁾ Aqueous dispersions (30 mL) containing 0.33 wt% ACC-CNFs was centrifuged at $2.00\times 10^4\times g$ and 25°C for 3 min using an MX-300 high-speed refrigerated micro centrifuge (TOMY SEIKO Co., Ltd., Tokyo, Japan). The precipitation fraction was re-dispersed in DMAc, and then centrifuged twice under the same conditions. Eventually, the precipitated fraction was dispersed in 40 mL of DMAc. The mixture was stirred with TEA (1 mL, 7.17 mmol) and DMAP (15 mg, 0.12

mmol), and acetylated by the addition of Ac_2O (10-1500 μL , 0.11-15.87 mmol) while stirring at 22°C . After 30 min, the mixture was purified with deionized water by 6 times centrifugation under the same conditions.

3.2.4. Optical microscopy

After acetylation in Pickering emulsion system, a portion of the Pickering emulsion was diluted with 10 mL of deionized water. The microscopic images of droplets were immediately captured using a CKX-41 optical microscope (Olympus Corp., Tokyo, Japan), and the diameter of droplets were measured and averaged using Image J software. The oil-water interfacial areas (A_{ow}) of the Pickering emulsion systems were calculated using the following equations.

$$A_{ow} = 6V_0R_{eo}/d \quad (3.1.).$$

where, V_0 is the total volume of the oil phase, R_{eo} is the ratio of the emulsified oil phase, d is the average diameter of the droplets.

3.2.5. Confocal laser scanning microscopy (CLSM)

Oil droplets were visualized using a Leica SP8 (Leica microsystems, Wetzlar, Germany). Pickering emulsion containing 1.5 μmol of Nile Red was prepared according to the procedure described in section 3.2.3. (Ac_2O : 500 μL , 2.0×10^4 rpm). Prior to the observation, a portion of Pickering emulsions was diluted with deionized water. Nile Red in Pickering emulsion system was excited at 405 nm wavelength, and its fluorescent emission was detected at 540-590 nm.

3.2.6. Adsorption ratio of ACC-CNFs at the fluid interface of Pickering emulsion

The adsorption ratio of ACC-CNFs at the fluid interface as reaction platform was calculated from the adsorbed weight and the degree of substitution (DS) values. Pickering emulsion was prepared according to the method described in 3.2.3. (Ac_2O : 500 μL). The Pickering emulsion was diluted with excess deionized water and then stirred. After 15 min, non-adsorbed nanofibrils in the mixture were collected with aqueous phase. The dilution-separation steps were carried out twice, and then the collected whole aqueous dispersion of non-adsorbed nanofibrils was concentrated by evaporation. Water soluble impurity was removed by dialysis against deionized water, and then the dry weight of non-adsorbed nanofibrils was measured.

3.2.7. Field emission scanning electron microscopy (FE-SEM)

Observation of CNF-stabilized oil droplets was carried out according to Chapter 1. A portion of Pickering emulsion was diluted 3 times with excess deionized water. The washed droplets were placed on a carbon tape and freeze-dried using a FreeZone2.5 (Labconco, Kansas, United States). Then, osmium tetroxide coating (approx. 3 nm thickness) was carried out using an HPC-1SW (Vacuum Device Corporation, Ibaraki, Japan). Morphological imaging of lyophilized droplets was conducted using an SU8000 field emission scanning electron microscope (Hitachi High-Tech Corp., Tokyo, Japan) at an acceleration voltage of 2.0 kV.

3.2.8. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of each sample were recorded using an FT-IR-6700 spectrometer (JASCO Corp., Tokyo, Japan) equipped with a triglycine sulfate detector. The spectra

were obtained from 16 scans at 2 cm^{-1} resolution and normalized using an absorption band at 1162 cm^{-1} attributable to C-O-C asymmetric stretching.³⁴⁾

3.2.9. X-ray photoelectron spectroscopy (XPS)

Aqueous dispersions of acetylated ACC-CNFs were hand-shaken, and then rapidly frozen using liquid nitrogen. The frozen samples were freeze-dried using a FreeZone2.5 (Labconco, Kansas, United States), and then immobilized on a carbon tape. XPS measurements were performed using a KRATOS AXIS-ULTRA (SHIMADZU Corporation, Kyoto, Japan) equipped with a monochromatic $\text{AlK}\alpha$ X-ray source (1486.6 eV) at 15 kV and 5 mA . The pass energy and scan step in C1s narrow scan were set at 20 eV and 0.01 eV , respectively. The obtained spectra were peak fitted using GRAMS/AI spectra processing software (Thermo Fisher Scientific, Inc., Waltham, USA) with mixed Gaussian and Lorentzian function, then the peak attributable to the C–C (H) bond was adjusted to 285.0 eV .³⁵⁾ DS values of each sample were determined from peak fitted XPS spectra according to the previous report.³³⁾

3.2.10. Wide-angle X-ray diffraction (WAXD) measurements

WAXD profiles of lyophilized nanofibrils were measured using a MiniFlex600-C system (Rigaku Corporation, Tokyo, Japan) using $\text{CuK}\alpha$ radiation ($\lambda = 0.154\text{ nm}$) at 40 kV and 30 mA in the 2θ angle range of $5\text{--}40^\circ$. The diffraction patterns were separated using GRAMS/AI spectra processing software (Thermo Fisher Scientific, Inc., Waltham, USA) with mixed Gaussian and Lorentzian function. The crystallinity index (CI) of each sample was calculated from the area ration attributable to the cellulose I crystal region and the non-crystalline region.

3.2.11. Sedimentation behavior of acetylated ACC-CNFs

To investigate self-aggregation behaviors in aqueous media, the settling speed and the sedimentation volume (V_{sed}) were monitored. In the current study, V_{sed} is defined as the ratio between the clouding region volume due to the sedimentation and the total volume of dispersion. Aqueous dispersions containing 1.0×10^{-2} wt% acetylated ACC-CNFs were gently poured into glass tubes and sonicated at 43 kHz for 10 s using an ultrasonic bath (ASU-2D, AS ONE Corp., Osaka, Japan). Prior to the measurements, the dispersions were kept at prescribed temperature for a day.

3.2.12. Preparation of cast films

Glass substrates (18×18 mm) were pre-rinsed twice with ethanol by sonication at 43 kHz for 1 min. Then containers for casting were constructed from aluminum foil, and the washed cover glass was placed at the bottom. Aqueous dispersions containing nanofibrils (0.10 wt%, 1.5 mL) were casted into the container, then dried in a desiccator at room temperature for a week.

3.2.13. XPS analysis of both sides of cast films

The cast films were peeled from the cover glass slips, then the each side was immobilized on a carbon tape. The XPS spectra of both sides of cast films were measured using an ESCA5800 X-ray photoelectron spectrometer (ULVAC-PHI, Inc., Kanagawa, Japan) equipped with a monochromatic $\text{AlK}\alpha$ X-ray source (1486.6 eV) at 0.1 kW. For the C1 s narrow scan, the pass energy and scan step were set at 11.75 eV and 0.05 eV, respectively.

3.2.14. Compounding with PS microspheres

PS microspheres (10 g) and a 50 mL of aqueous dispersion containing 0.01-0.05 wt% acetylated ACC-CNFs with DS 0.28 (Ac_2O : 10-500 μL , DMAP: 15 mg, 2.0×10^4 rpm) were poured in a stainless vessel. The mixture was shaken at 150 rpm and 25°C for 30 min, then the microspheres were washed with water to remove non-adsorbed nanofibrils using a stainless mesh (100 μm ϕ). The washed microspheres were oven dried at 50°C overnight.

3.2.15. Evaluation of work of adhesion in water

To investigate adsorbability of the ACC-CNFs before and after surface acetylation, work of adhesion between the PS and nanofibrils was estimated from contact angle (CA) values based on the method written in Chapter 2. Acetylated ACC-CNF cast films for wetting analysis were prepared according to the 3.2.11. PS films were prepared from 0.30 wt% PS toluene solution by spin-coating at 1000 rpm for 15 s on a cover glass slip. CA values of water, ethylene glycol (EG), diiodomethane (DIM), and α -bromo naphthalene (BN) were recorded after 1 s from dropping using a Drop Master DM300 contact angle meter (Kyowa Interface Science Co., Ltd.) with a PTFE coated needle (Kyowa Interface Science Co., Ltd.). CA values were corrected by surface roughness parameters using Wenzel's law,³⁶⁾ according to the Chapter 2.

3.2.16. Preparation of composite films

The acetylated ACC-CNF/PS particles were molded into composite films by hot pressing at 130°C and 30 MPa for 25 min, then the films were cooled at room temperature.

3.2.17. Optical transparency of composite films

Composite films were cut into rectangle shaped test pieces (10 mm×40 mm). Transparency of the test pieces in 400-700 nm wavelength region was measured using a UV/Vis spectrophotometer (Beckman coulter, Brea, California, United States) under ambient conditions.

3.2.18. Thermal stability of composite films

Thermal degradation behaviors of nanofibrils and PS composites were recorded using a TG/DTA7300 (Hitachi High-Tech, Tokyo, Japan). Approx. 4 mg of samples filled in an aluminum pan were heated at 10 °C min⁻¹ from 30°C to 480°C under N₂ atmosphere.

3.2.19. Elongation tests of composite films

Elongation tests of the composite films were carried out using an STA-1225 (A&D Company Ltd.) equipped with a 2.5 kN load cell. 10 mm from both edges of test piece were clamped, then the measurements were carried out at 0.5 mm min⁻¹ of elongation speed under ambient conditions.

3.3. Results and discussion

3.3.1 Characterization of Pickering emulsion as reaction platforms

Pickering emulsions were stabilized by ACC-CNFs derived from BC pellicles (Fig. 3-1a), and the Pickering emulsion system containing fluorescent dye emits fluorescence only inside of the oil droplets (Fig. 3-1b). The diffusion behavior¹⁶⁾ of the emulsion phase in excess water indicated that all the Pickering emulsions were oil-in-water type emulsions, which agreed with the results for wood-based ACC-CNFs reported by Yokota

et al.¹⁶⁾ The average diameters of the oil droplets in the Pickering emulsion phases obtained under each stirring condition are listed in Table 3-1. The A_{ow} value increased as the rotation speed increased because smaller oil droplets are generated and dissipated when the mechanical energy is higher.

Table 3-1 Average diameters of oil droplets and A_{ow} values in Pickering emulsions prepared under various conditions.

Rotation speed / $\times 10^4$ rpm	0.3	0.6	1.0	2.0
Droplet diameter / μm ($N=100$)	140.4 ± 81.7	49.3 ± 17.7	33.9 ± 11.3	29.4 ± 10.2
A_{ow} / m^2	0.3	1.9	2.7	3.2

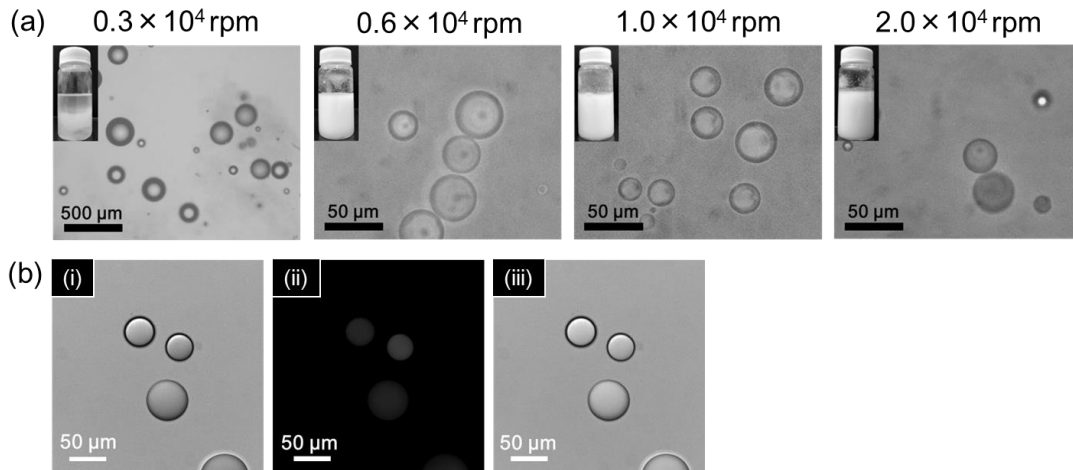


Fig. 3-1 (a) Optical microscope images of the Pickering emulsions under each stirring condition. Inset: photographs of Pickering emulsions (b) CLSM images of Pickering emulsion system (Ac_2O : 500 μL). (i) Bright field image, (ii) fluorescent image, and (iii) their merged image.

Fig. 3-2 shows FE-SEM images of the oil droplets in an ACC-CNF-stabilized Pickering emulsion after freeze-drying. The images reveal that the oil-water interfaces of the Pickering emulsions were densely covered with ACC-CNFs. The surface coverage

ratio of the Pickering emulsion (Ac_2O : 500 μL , 2.0×10^4 rpm) estimated from the FE-SEM images (osmium tetroxide coating thickness: 3 nm) was 33.2%. To estimate the adsorption ratio of the ACC-CNFs at the oil-water interfaces during emulsification, the weight of the adsorbed ACC-CNFs on the surfaces of the oil droplets was determined. Fig. 3-3 shows the adsorption ratio (R_{ad}) at each rotation speed. At a rotation speed of 0.3×10^4 rpm, the R_{ad} of the ACC-CNFs was 9.4%. The R_{ad} value increased dramatically when the rotation speed exceeded 0.6×10^4 rpm. This synergetic phenomenon indicates that the small oil droplets generated by high mechanical energy were stabilized by the adsorption of the ACC-CNFs. The oil droplets in the Pickering emulsions prepared at 0.6 - 2.0×10^4 rpm were stable for at least 1 month under ambient conditions. In the current study, the rotation speed was set to 1.0 - 2.0×10^4 rpm, which corresponded with the maximum R_{ad} value. The acetylation of the ACC-CNFs at the oil-water interfaces was most effective when the CNFs were densely coated on the droplets without overlapping.

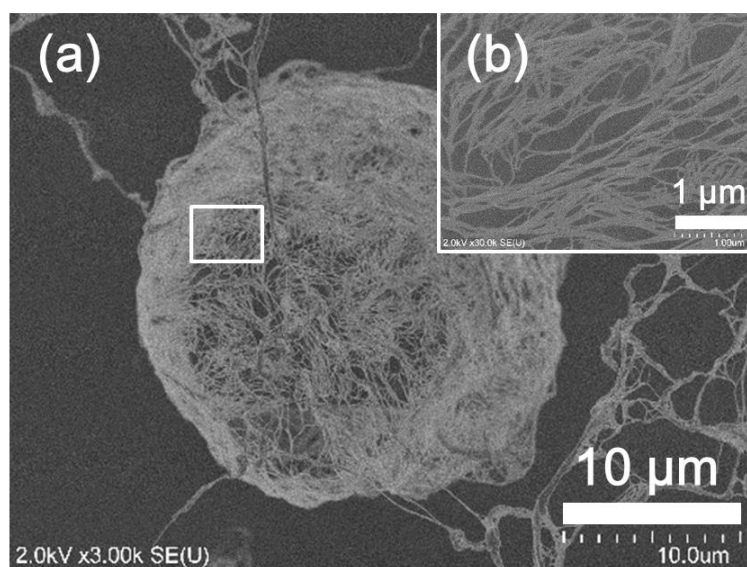


Fig. 3-2 FE-SEM images of (a) Pickering emulsion shells constructed with nanofibrils (b) magnified image of enclosed area.

3.3.2. Acetylation in Pickering emulsion system

The ACC-CNFs were monitored by FT-IR spectroscopy as their surfaces were acetylated. Fig. 3-4 (i) shows typical spectra produced by ACC-CNFs acetylated in a homogeneous dispersion system (HA-ACC-CNFs), ACC-CNFs acetylated in a PE system (PA-ACC-CNFs), and pre-treated ACC-CNFs. In the spectra produced by the HA-ACC-CNFs and PA-ACC-CNFs, absorption bands attributable to the acetyl group^{37,38)} (C=O stretching: 1742 cm^{-1} , C-O stretching: 1229 cm^{-1} , and CH_3 asymmetrical bending: 1369 cm^{-1}) were detected (Fig. 3-4 (i) b, c). The DS values of the HA-ACC-CNFs increased as the amount of Ac_2O increased (Fig. 3-4 (ii)). Conversely, the DS values of the PA-ACC-CNFs reached a maximum at approximately $0.25 (1.0 \times 10^4\text{ rpm})$, even when the amount of Ac_2O was increased. These results suggest that the reaction sites of the ACC-CNFs were limited to the portions immersed in the oil phase.

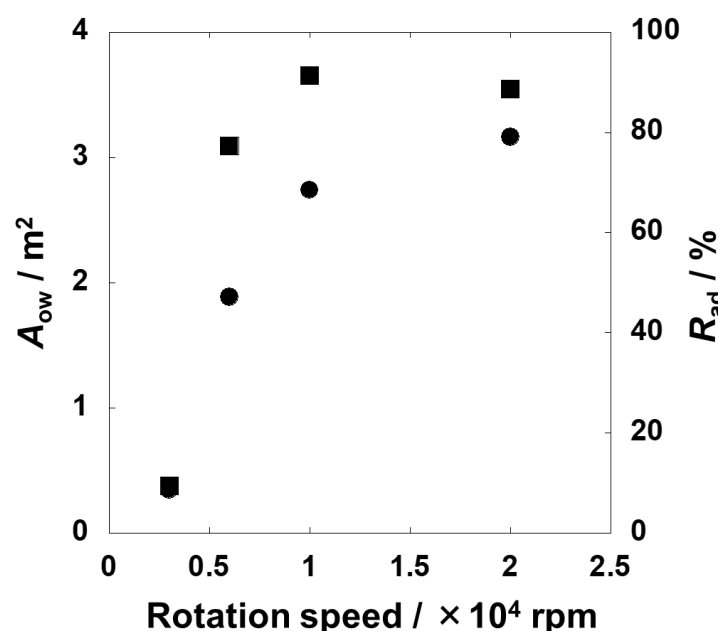


Fig. 3-3 Adsorption ratios (R_{ad} : squares) of the ACC-CNFs, and oil/water (O/W) interfacial areas (A_{ow} : circles) at various rotation speeds (Ac_2O : $500\text{ }\mu\text{L}$).

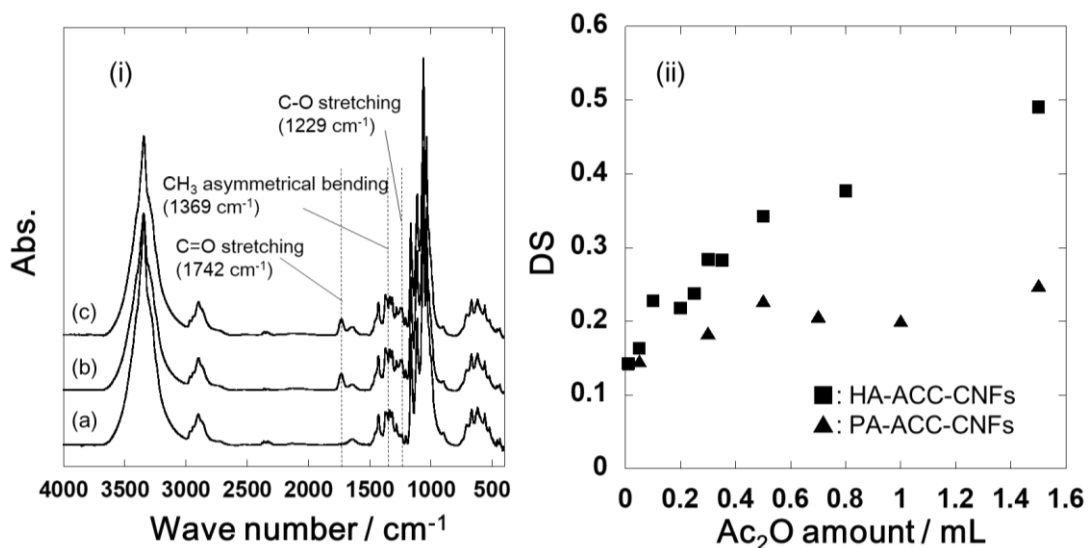


Fig. 3-4 (i) FT-IR spectra of (a) unmodified ACC-CNFs, (b) HA-ACC-CNFs with DS = 0.28, and (c) PA-ACC-CNFs with DS = 0.27. (ii) Influence of the amount of Ac₂O on the DS values calculated from XPS spectra. The PA-ACC-CNFs were prepared at 1.0×10^4 rpm.

Fig. 3-5 shows the correlation between the A_{ow} values and the DS calculated from the peak area ratios of the XPS spectra obtained for the Pickering emulsion reaction system. The DS values increased as the A_{ow} values increased. The A_{ow} values corresponded to the ACC-CNF-adsorbable areas. This indicates that the reaction sites of the ACC-CNFs that were at the oil-water interface and were partly immersed in the oil phase were acetylated in the Pickering emulsion.

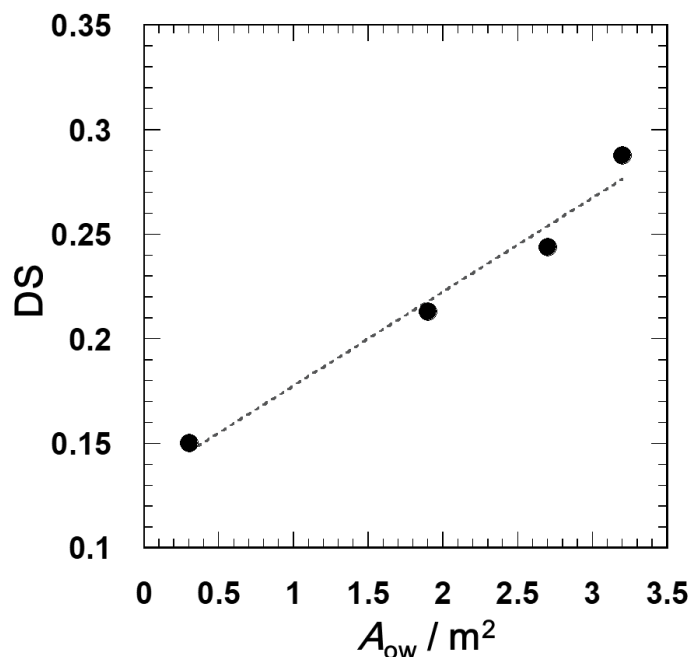


Fig. 3-5 Dependence of the DS values on the A_{ow} in the Pickering emulsion systems (Ac_2O : 500 μL).

Fig. 3-6 shows AFM topographical images of the unmodified ACC-CNFs and the acetylated ACC-CNFs with similar DS values—that is, the HA-ACC-CNF and PA-ACC-CNF samples depicted in Fig. 3-4 (i) b, c. The fiber morphologies of the acetylated ACC-CNFs hardly changed relative to the fiber morphologies of the original ACC-CNFs. There was no significant difference between the height values of the CNFs before and after reaction. The lengths of the PA-ACC-CNFs were slightly reduced by homogenization during the preparation of the Pickering emulsion (Table 3-2). However, acetylation had a negligible effect on the lengths of the HA-ACC-CNFs and PA-ACC-CNFs (Table 3-3).

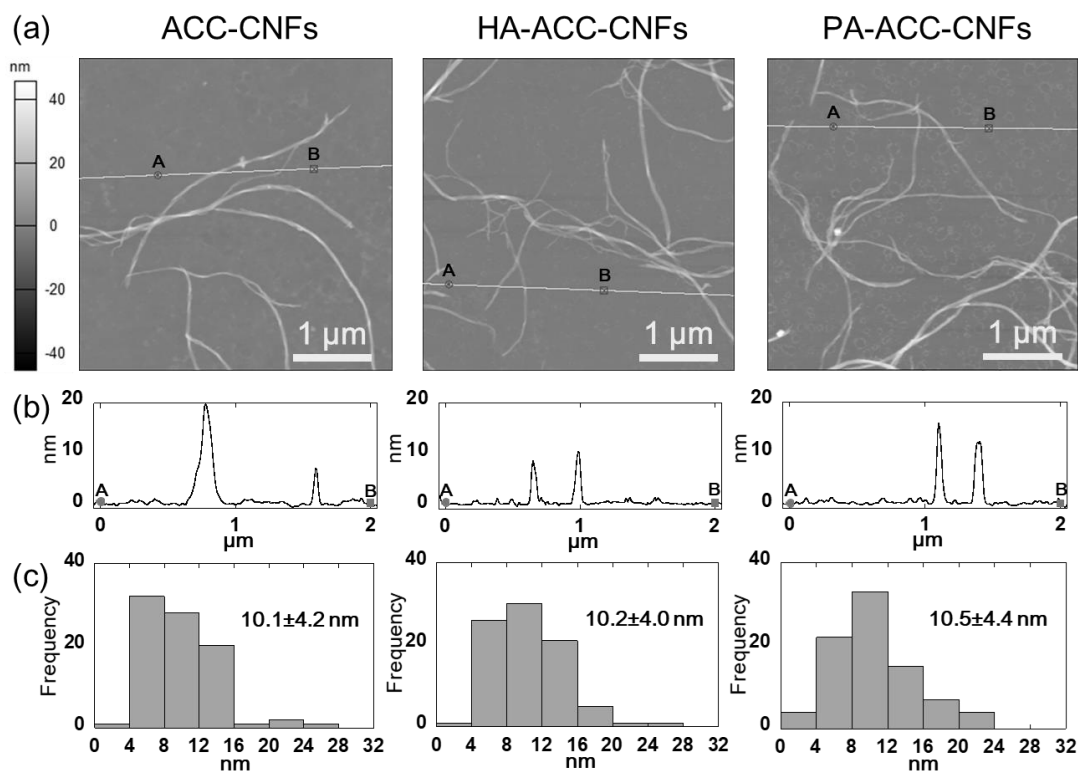


Fig. 3-6 (a) Atomic force microscopy (AFM) topographical images of unmodified and acetylated ACC-CNFs corresponding to Fig. 3-4 (i). (b) Cross section profiles along the perpendicular A–B line in each AFM image. (c) Histograms of fiber height values ($N = 85$) measured from each of the obtained AFM images: unmodified ACC-CNFs (left), HA-ACC-CNFs with DS = 0.28 (center), and PA-ACC-CNFs with DS = 0.27 (right).

Table 3-2 Influence of homogenization on length and height of ACC-CNFs.

Homogenization rate / $\times 10^4$ rpm	0 (control)	0.6	2.0
Length / μm ($N > 70$)	3.82 ± 1.91	3.91 ± 2.31	3.31 ± 1.68
Height / nm ($N > 75$)	10.1 ± 4.2	10.4 ± 3.2	10.5 ± 3.1

Table 3-3 Length of ACC-CNFs, HA-ACC-CNFs and PA-ACC-CNFs.

	ACC-CNFs	HA-ACC-CNFs DS = 0.28	PA-ACC-CNFs DS = 0.27
Length / μm ($N > 70$)	3.82 ± 1.91	3.71 ± 1.85	3.22 ± 1.66

The WAXD patterns of the unmodified ACC-CNFs and both of the acetylated ACC-CNFs indicated the typical profile of cellulose I crystal structure, as shown in Fig. 3-7. The CI value of the HA-ACC-CNFs was reduced by acetylation. The crystalline structure of the HA-ACC-CNFs may have decomposed to some extent when they were swollen in DMAc. The decrease in the CI value of the PA-ACC-CNFs was less than that of the HA-ACC-CNFs following acetylation. Highly crystalline CNFs are believed to be resistant to swelling in non-polar solvents such as toluene. Therefore, the CI values of CNFs are thought to be unaffected during reactions in such solvents^{38,39}). Nevertheless, non-polar solvents are less popular as media for use in the surface modification of cellulose nano-materials because they tend to promote aggregation.

However, in the Pickering emulsion system the ACC-CNFs underwent successful acetylation without modifications to their original crystallinity or crystal structures. Another advantage of this system is that the unintentional aggregation of ACC-CNFs during the reaction is suppressed by the selective adsorption of the ACC-CNFs onto the oil-water interfaces.

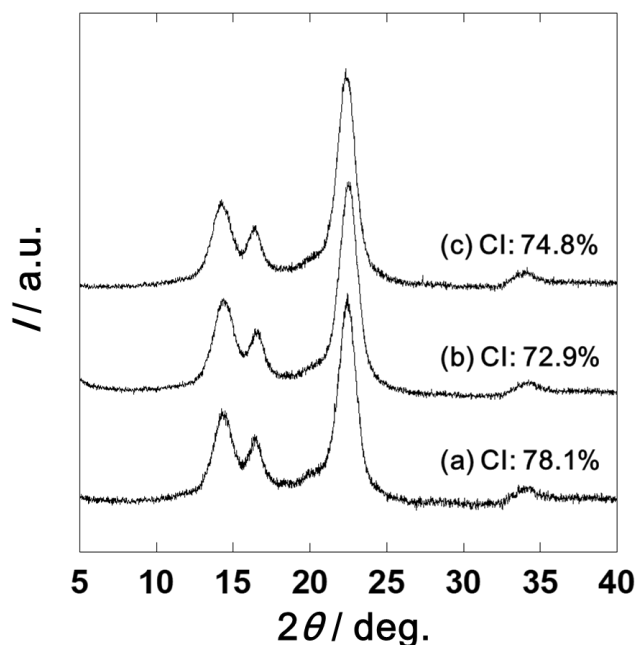


Fig. 3-7 WAXD patterns of (a) unmodified ACC-CNFs, (b) HA-ACC-CNFs with DS = 0.28, and (c) PA-ACC-CNFs with DS = 0.27. Samples (a)–(c) correspond to those in Figs. 3-4 (i) and 3-6.

Recently, a single ACC-CNF was reported to have a Janus-type structure comprising both hydrophilic and hydrophobic faces. Therefore, ACC-CNFs have greater amphiphilicity than other CNFs. In the present study, the negative surface charge due to unreacted OH groups in the PA-ACC-CNFs was exposed in the aqueous phase. Conversely, the more hydrophobic surfaces comprising the CH groups were attracted to the oil phase of the Pickering emulsion, which contained the reaction reagents. Therefore, in a Pickering emulsion system the reactive sites of the individual ACC-CNFs are presumably limited when they were immersed in the oil phase. Yokota et al. assumed that the more hydrophobic (200) planes of wood-based ACC-CNFs were adsorbed to the surfaces of the oil phase,¹⁶⁾ which stabilized the Pickering emulsion. Presumably the surface hydroxy groups neighboring the (200) planes of the ACC-CNFs are selectively

substituted in the oil phase (Fig. 3-8a).

On the other hand, the OH groups on the surfaces of the ACC-CNFs in the HA system were supposed to be homogeneously acetylated (Fig. 3-8b). Accordingly, the density of the hydrophobic acetyl groups differed between the PA-ACC-CNFs and HA-ACC-CNFs, even when they had the same DS values.

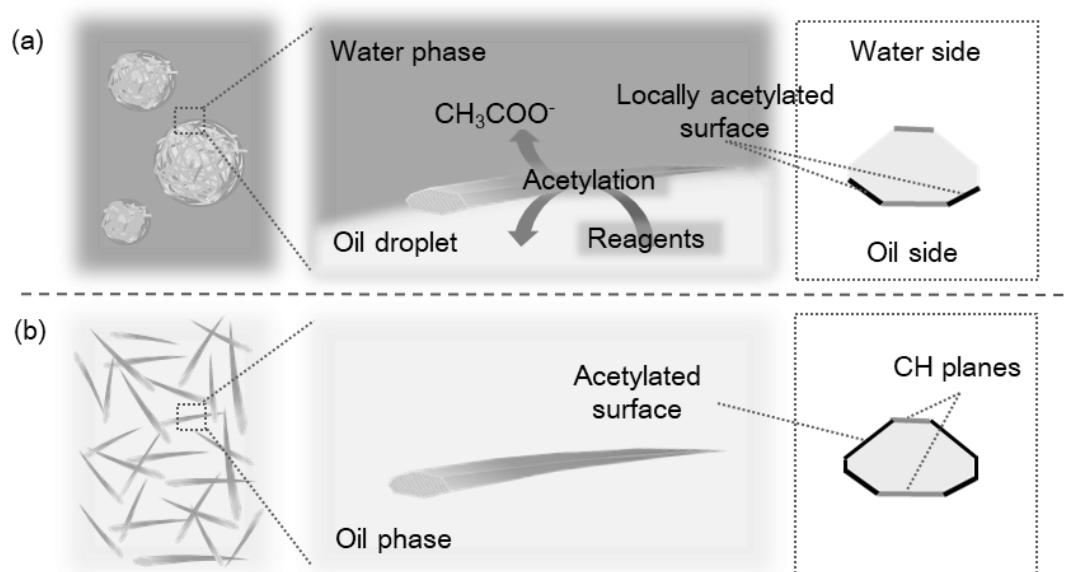


Fig. 3-8 (a) Schematic illustrations of surface acetylation in the Pickering emulsion system and the (b) homogeneous dispersion system.

ACC-CNFs in water settled as time passes, as shown in Fig. 3-9. Fig. 3-10 shows the time-dependent changes of the V_{sed} values of unmodified and acetylated ACC-CNFs. In cases of unmodified ACC-CNFs and PA-ACC-CNFs, the V_{sed} values reached an equilibrium state within 24 h. On the other hand, HA-ACC-CNFs needed approximately 2 weeks to reach an equilibrium state of the sedimentation. It is noted that the decreasing curve of V_{sed} of PA-ACC-CNFs had an inflection point at the initial stage of the sedimentation, while the unmodified and HA-ACC-CNFs had negligible inflection point

(Inset of Fig. 3-10). Chen et al. reported that the sedimentation speed of fiber shaped objects is sensitive to their mass and hydrodynamic constant.⁴⁰⁾ Hence, the presence of inflection points indicated that the hydrodynamic constant of the settling objects was changed during sedimentation, while the fiber mass was supposed to remain. Namely, the PA-ACC-CNFs might be precipitated while self-aggregating in an aqueous media. The results suggested that the hydrophilic/hydrophobic balance of unmodified ACC-CNFs was changed while keeping Janus-type fiber surface structures of ACC-CNFs.

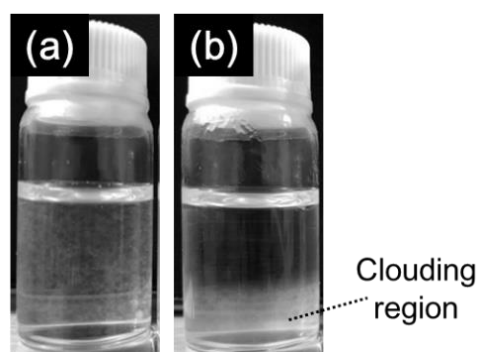


Fig. 3-9 Images of aqueous dispersion containing 1.0×10^{-2} wt% unmodified ACC-CNFs. (a) After 0 min from settling (b) equilibrium state of the sedimentation.

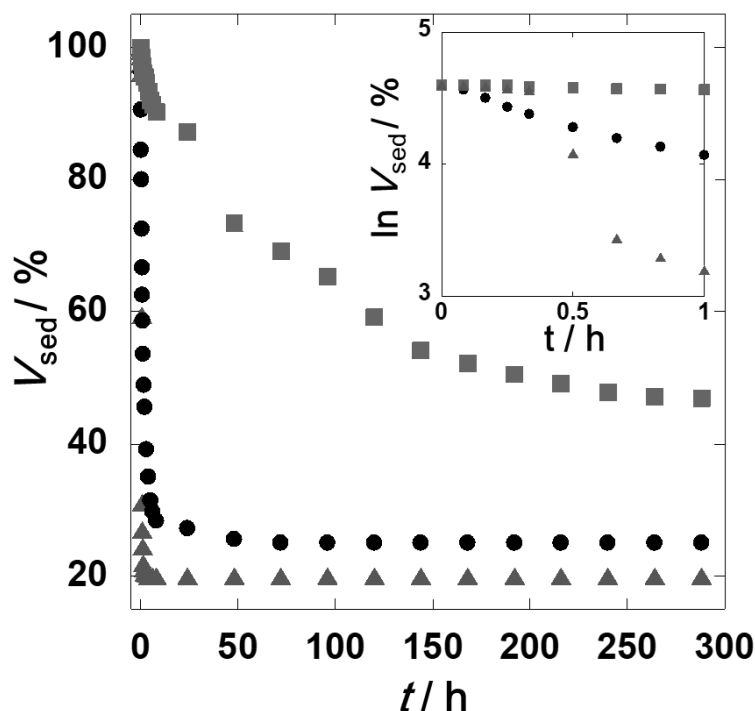


Fig. 3-10 Time-dependent change of sedimentation volume ratio (V_{sed}) of unmodified and acetylated ACC-CNFs. Inset: semi-logarithm plots of V_{sed} change at initial stage. Circle: unmodified ACC-CNFs, square: HA-ACC-CNFs with DS = 0.28, and triangle: PA-ACC-CNFs with DS = 0.27.

3.3.3. Wettability of films cast from acetylated ACC-CNFs on a glass substrate

The AWCA values of the cast films of both the acetylated ACC-CNFs increased with increasing DS values (Fig. 3-11). Therefore, comparisons of the AWCA values of the cast films prepared from the acetylated ACC-CNFs were used to indicate the localization of hydrophobic acetyl groups on the PA-ACC-CNFs. In fact, the AWCA values of the PA-ACC-CNF films were higher than those of the HA-ACC-CNF films with similar DS values. Surface roughness is an influential factor in WCA measurements.³⁶⁾ There were no significant differences in the root mean square roughness (R_q) values estimated by AFM between the HA-ACC-CNF films with DS values of 0.28 (R_q : 68.0 ± 19.1 nm) and the PA-ACC-CNF films with DS values of 0.27 (R_q : 66.9 ± 17.9 nm).

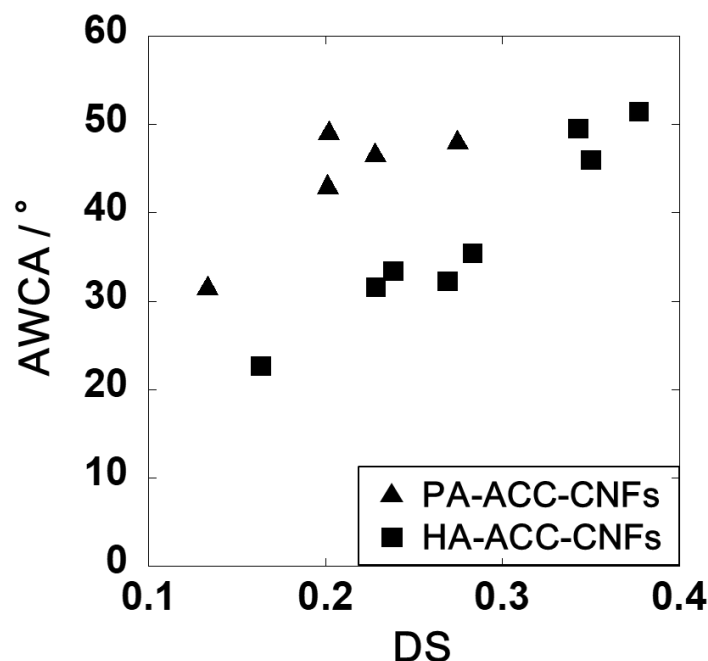


Fig. 3-11 Apparent water contact angle (AWCA) values of cast films prepared from HA-ACC-CNFs and PA-ACC-CNFs after 1 s from the dropping.

XPS analysis was performed to compare the chemical compositions of the air and glass sides of each cast film (Fig. 3-12). The XPS C1s spectra produced by the air and glass sides of the cast film prepared from HA-ACC-CNFs were similar (Fig. 3-12c). Conversely, the XPS C1s spectra produced by the air and glass sides of the cast film prepared from PA-ACC-CNFs were different, as shown in Fig. 3-12ab.

The peak intensity of C-C (H) (285.0 eV) attributable to the acetyl groups was higher in the spectrum produced by the air side than the spectrum produced by the glass side. These results corroborate the differences in the AWCA values between the PA-ACC-CNF and HA-ACC-CNF films described in Fig. 3-11. Wohlrab et al. reported that the wettability of a cast film prepared from amphiphilic molecules is dependent on the chemical characteristics of the substrate and the external environment in which the film is formed.⁴¹⁾ Therefore, in the present study the localized hydrophobic regions of the PA-

ACC-CNFs appeared at the hydrophobic air side of the cast films, whereas the less-modified regions were adsorbed onto the hydrophilic glass substrate.

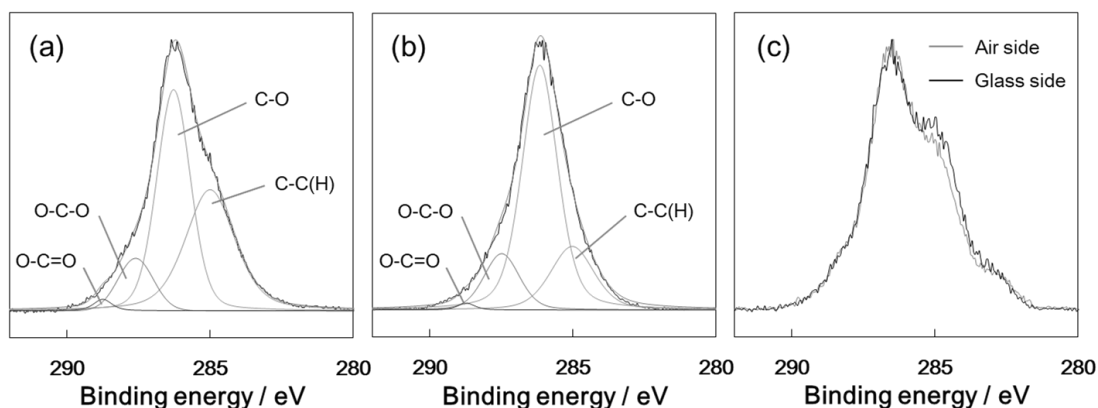


Fig. 3-12 XPS C1 s spectra of (a) the air side and (b) the glass side of cast films prepared from PA-ACC-CNFs with DS values of 0.27. (c) Both sides of cast films prepared from HA-ACC-CNFs with DS values of 0.28.

Fig. 3-13a-c shows the time-dependent AWCA values of cast films prepared from HA- and PA-ACC-CNFs. AWCA of cast films prepared from acetylated ACC-CNFs were decreased with the lapse of time due to the water soaking into the inside of cast films. In any case, AWCA values of cast films prepared from PA-ACC-CNFs were higher than the values of cast films prepared from HA-ACC-CNFs. In case of the HA-ACC-CNFs with DS = 0.28, the decrease of the AWCA value was relatively slow compared with the other samples (Fig. 3-13c). It is implied that the HA-ACC-CNFs with relatively high DS (= 0.28) prevent on penetrating of water into the internal area of the cast film. On the other hand, decreasing ratio of the AWCA on the cast film from PA-ACC-CNFs with DS = 0.27 was more remarkable. This indicates the different characteristics between the outer layer and the internal region of the cast films, which is due to the localized acetyl and OH groups on the nanofibrils surface supported by XPS spectra (Fig. 3-12c).

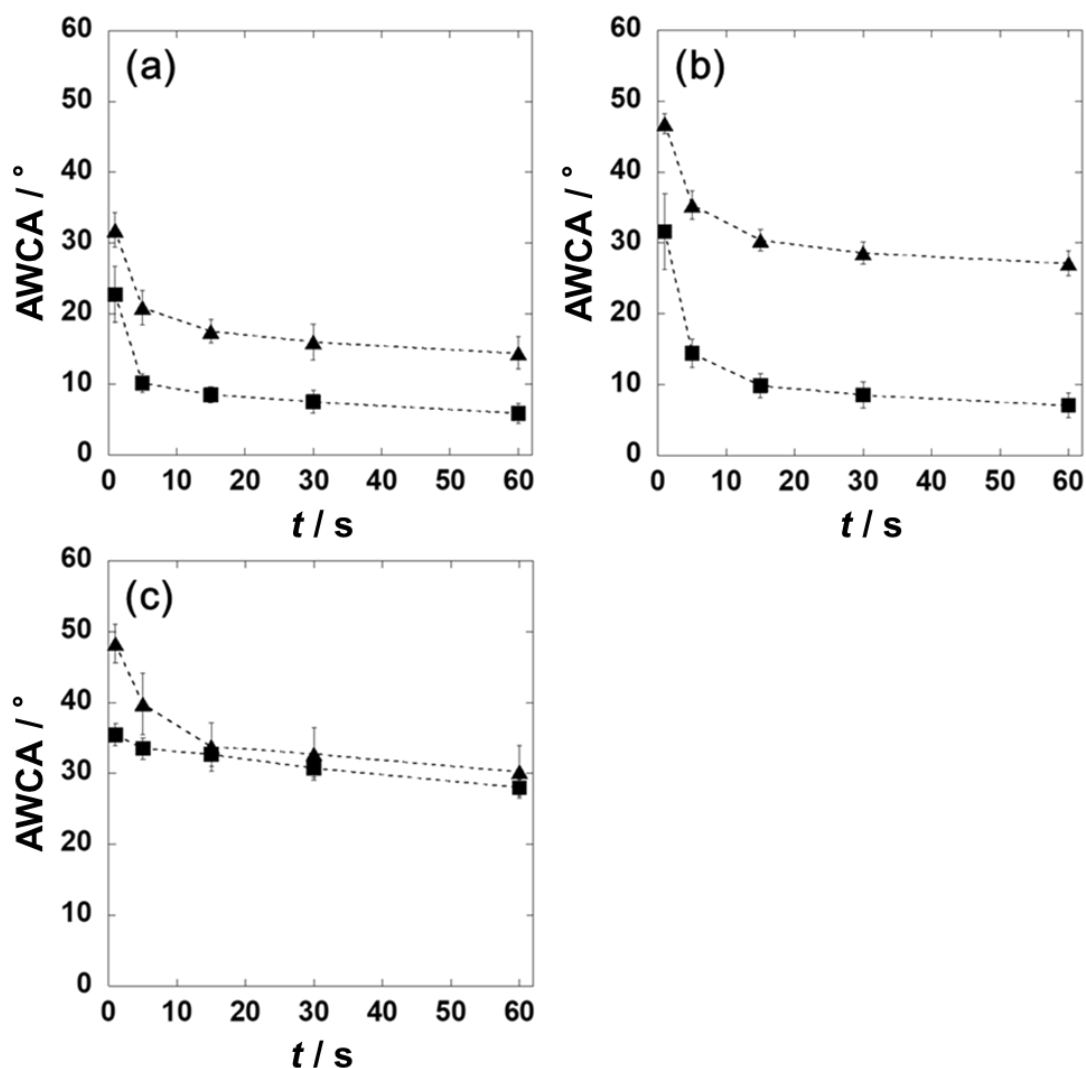


Fig. 3-13 Time-dependent change of AWCA of cast films prepared from HA-ACC-CNFs and PA-ACC-CNFs. (a) HA-ACC-CNFs with DS = 0.16 (square) and PA-ACC-CNFs with DS = 0.13 (triangle). (b) HA-ACC-CNFs with DS = 0.23 (square) and PA-ACC-CNFs with DS = 0.23 (triangle). c) HA-ACC-CNFs with DS = 0.28 (square) and PA-ACC-CNFs with DS = 0.27 (triangle).

These results suggest that the ratio between the localized acetyl groups and the OH groups has a significant influence on the hydrophilic/hydrophobic balance. Therefore, the amphiphilicity of Janus-type ACC-CNFs can be controlled by surface modification using fluid interface of Pickering emulsion as a reaction platform.

3.3.4. Improvement of nanofiber adsorption onto the polystyrene microspheres for ecofriendly compounding process

In the later section, the author attempted to improve adsorbability of CNFs onto the hydrophobic resin microspheres by the facile chemical modification mentioned above. Additionally, it is expected that the densely introduced hydrophobic groups on the CNF surface increase effectively affinity for the resin comparing with the nanofibrils hydrophobized in homogeneous dispersion system. The polymer compounding method via underwater adsorption of CNFs is simple and ecofriendly method.^{14,30,31)} However, the adsorbability depends on the polymers.³¹⁾ Tatsumi et al. also reported negligible adsorption of wood-derived ACC-CNFs onto the polystyrene (PS) microspheres.⁴²⁾

Likewise, it was confirmed that the adsorption of bacterial cellulose-derived ACC-CNFs onto the PS surface is negligible (C-PS; Fig. 3-14c). On the other hand, the acetylated ACC-CNFs were favorably adsorbed onto the PS microspheres as shown in (Fig. 3-14d,e) despite of the low (0.01 wt%) nanofiber concentration condition (A-PS 0.01), indicating that the surface acetylation improves underwater adsorption of nanofibrils onto the PS surface. Adsorption amount of the PA-ACC-CNFs was increased as increasing in suspension concentration (Fig. 3-15), but the PA-ACC-CNFs were tend to form aggregates on the PS surface when the suspension concentration is 0.05 wt% (A-PS 0.05; Fig. 3-15b,d).

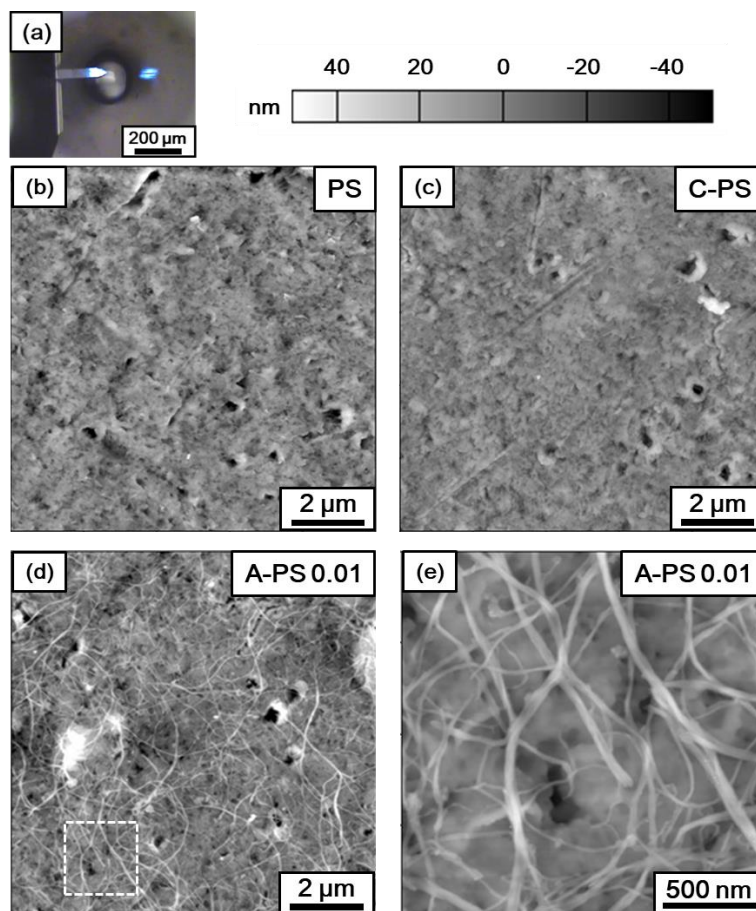


Fig. 3-14(a) CCD camera image of microsphere sample in AFM observation system. AFM topographical images of (b) PS microsphere surface (c) after adsorption treatment with the 0.01 wt% unmodified ACC-CNFs, and (d) 0.01 wt% PA-ACC-CNFs (DS 0.28). (e) Magnified image of enclosed area in (d).

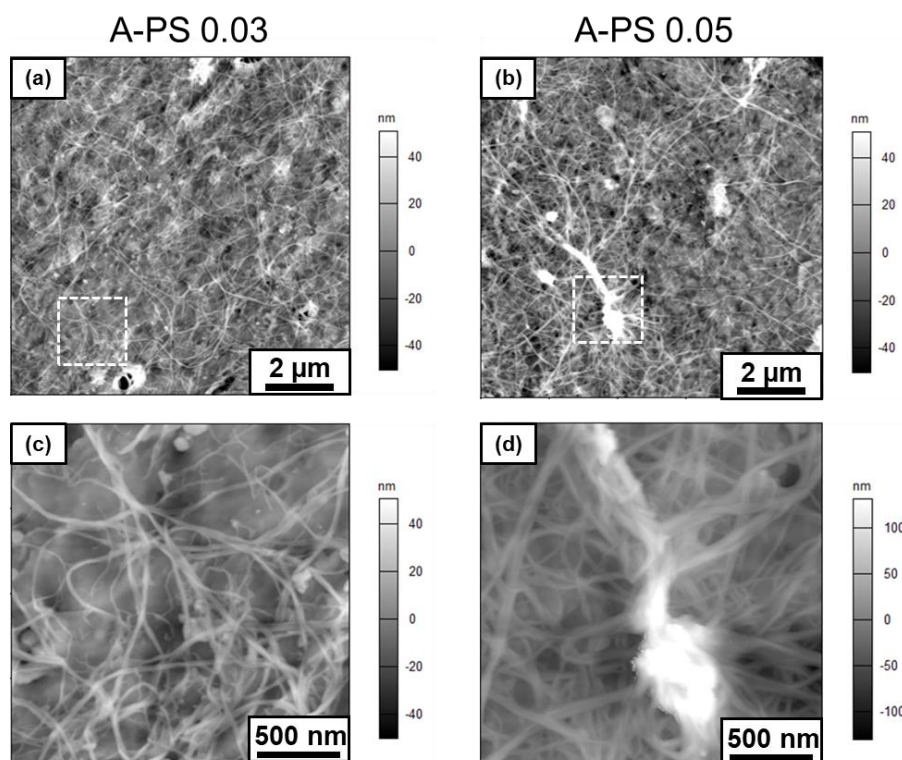


Fig. 3-15 AFM topographical images of the PS microsphere surface after adsorption treatment with 0.03 wt% PA-ACC-CNFs aqueous dispersion (a, c) and 0.05 wt% PA-ACC-CNFs aqueous dispersion (b, d). (c) and (d) are corresponded with the enclosed areas in (a) and (b), respectively.

The adsorption ratios of nanofibrils (R_{ad}) with various DS values are shown in Fig. 3-16, and the quite low R_{ad} value of the ACC-CNFs ($< 3.0\%$) corresponded with the negligible adsorption indicated by AFM observation. Similarly, most of the PA-ACC-CNFs with low DS values (0.14-0.20) did not adsorb onto the PS surface. On the other hand, R_{ad} value of the PA-ACC-CNFs with DS 0.22 increased drastically, then those values of nanofibrils with DS 0.25-0.26 reached to approx. 80%. The significant increase in R_{ad} values implies that there is a threshold-like point in nanofibril adsorption onto PS/water interface. It was noted that the adsorbability of the PA-ACC-CNFs with DS 0.21 was remarkably higher than those of the HA-ACC-CNFs with DS 0.24. Additionally,

affinity between water and composite particles was greatly changed by nanofiber adsorption (Fig. 3-16 inset). Due to the hydrophobic PS particle surface, the neat PS (density: 1.1 g cm^{-3}) and those compounded with PA-ACC-CNFs ($\sim \text{DS } 0.20$) partially formed Pickering foam near the water surface. However, those densely covered with A-ACC-CNFs with high DS ($\text{DS } 0.22 \sim$) did not form Pickering foam, which implies that the adsorbed nanofibrils offered the PS microspheres relatively polar surface.

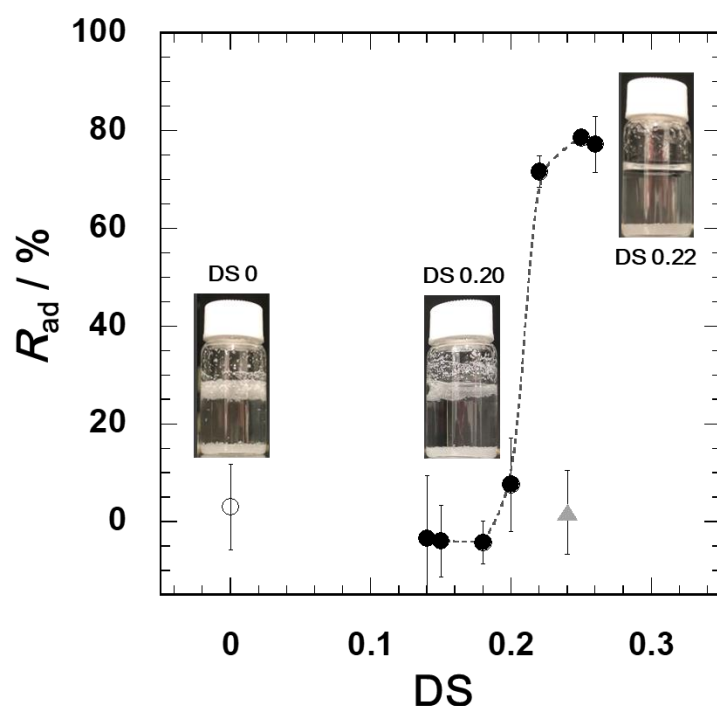


Fig. 3-16 Adsorption ratio (R_{ad}) values of each nanofibril with different DS values. Black circle; PA-ACC-CNFs with DS 0.14-0.26, gray triangle; HA-ACC-CNFs with DS 0.24, and white circle; unmodified ACC-CNFs. Three inset images; appearance of 500 mg PS composite particles in 5 mL of water.

Thermodynamic contributions for adhesion are expressed by surface free energy theory based on wetting dealt in Chapter 2. However, CA values of materials composed of multi components are influenced by the area fractions and wettability of each component known as Cassie equation.⁴³⁾ In the case of the PA-ACC-CNFs, it was

indicated that the interfacial reaction using Pickering emulsion locally modifies CNF surface while keeping its Janus-type fiber structure. Namely, the PA-ACC-CNFs are composed of several planes having different wettability (hydrophilic OH planes and less hydrophilic CH and acetylated planes), and the less hydrophilic planes are therefore presumed to adhere onto the hydrophobic PS surface.³³⁾ The locally acetylated planes on PA-ACC-CNFs were faced to the hydrophobic air side during drying process, resulting in forming hydrophobic cast film surface (3.3.3). Hence, we used air side of the cast films as suitable samples for wetting analysis to evaluate underwater adsorption process between PA-ACC-CNFs surface and PS microsphere surface.

Table 3-4 shows the CA values of each cast film. After acetylation, the CA value of water was increased, but the value of diiodomethane was significantly decreased. The results implies that the surface of nanofibrils became less hydrophilic. Nevertheless, changing in the CA value of ethylene glycol and α -bromonaphthalene were negligible.

Table 3-4 CA values of probe liquids on nanofiber samples. ※From Chapter 2

	$\theta_{\text{Water}} / ^\circ$	$\theta_{\text{DIM}} / ^\circ$	$\theta_{\text{EG}} / ^\circ$	$\theta_{\text{BN}} / ^\circ$
ACC-CNFs ※)	40.1 ± 1.4	45.1 ± 5.7	33.9 ± 2.3	30.2 ± 2.3
PA-ACC-CNFs (DS 0.28)	46.8 ± 3.7	33.6 ± 1.7	33.1 ± 1.8	30.3 ± 2.1

Tables 3-5 shows the estimated surface free energy values based on VCG theory.^{44,45)} After acetylation, the electron donor component was significantly decreased, although the Lifshitz-van der Waals component slightly increased. The low electron donor component implies low dispersibility in water, but the PA-ACC-CNFs were well dispersed in water as described in 3.3.2. presumably due to the decreasing in cohesion force of nanofibrils.⁴⁶⁾

Table 3-5 Surface free energy parameters of nanofiber samples. ※From Chapter 2

/ mJ m ⁻²	γ	γ^{LW}	γ^{AB}	γ^+	γ^-
ACC-CNFs※)	42.5	37.8	4.68	0.275	19.9
PA-ACC-CNFs (DS 0.28)	44.1	40.6	3.46	0.196	15.3

Table 3-6 shows the work of adhesion values between the PS and acetylated ACC-CNFs or unmodified one in water media. W^{LW} , and W^{AB} means the values of work adhesion derived from Lifshitz-van der Waals interaction and acid-base interaction component, respectively. Total work of adhesion (W_{ad}), the energy needed to separate two contacting materials, indicates that the nanofibrils stabilize water-PS interface via adsorption when those materials having positive work of adhesion values come into contact. In Chapter 2, the work of adhesion values based on VCG theory closely matched with the actual interfacial adsorption of ACC-CNFs onto the liquid-liquid interface of Pickering emulsions. However, despite of the negligible adsorption of the unmodified ACC-CNFs onto the PS surface, the work of adhesion value was positive value (Table 3-6). The results are presumably due to the contact area between the two rigid solids with surface roughness,⁴⁷⁻⁴⁹⁾ which is quite different from the CNF adsorption onto the flexible oil-water interface. The low contacting area decrease actual adhesion force, therefore we regarded the estimated work of adhesion values as relative values expressing nanofibril adsorption with PS surface in water.

Table 3-6 Work of adhesion values calculated from surface free energy parameters. The units of each parameter is mJ m⁻².

	PA-ACC-CNF/PS (DS 0.28, in water)	ACC-CNF/PS (in water)	PA-ACC-CNF/PS (DS 0.28, in water)	ACC-CNF/PS (<i>in vacuo</i>)	PA-ACC-CNF/water (DS 0.28, in water)	ACC-CNF/water (<i>in vacuo</i>)
W_{ad}	25.3	16.3	79.4	76.4	122.7	128.5
W^{LW}	3.2	2.6	81.5	78.7	65.2	62.9
W^{AB}	22.1	13.7	-2.1	-2.3	57.5	65.6

Both components of work of adhesion, especially acid-base interaction component, were increased after surface acetylation. The results imply that the improvement of interfacial adsorption of ACC-CNFs is mainly due to the decreasing in affinity between ACC-CNFs and water, similarly to the adsorption onto the oil-water interface.

3.3.5. Characterization of surface acetylated ACC-CNF/PS composite films

Changing in appearance of the PA-ACC-CNF/PS composite films (Fig. 3-17) and its transparency in visible wavelength region was negligible when the PA-ACC-CNFs concentration during adsorption treatment is 0.01-0.03 wt% (Fig. 3-17). On the other hand, the transparency in composite films of A-PS 0.05 was slightly decreased presumably due to the existence of nanofiber aggregates observed in AFM topographical image (Fig. 3-15b,d).

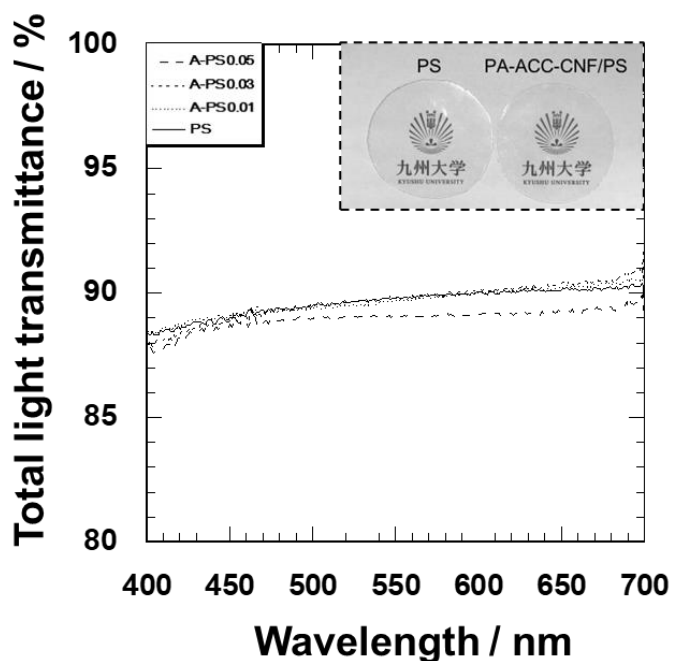


Fig. 3-17 Transparency of composite films in the visible wavelength region. Inset: appearance of the neat PS and A-PS 0.01 films (DS 0.28).

Fig. 3-18 shows the thermogravimetric changing of PS films and composite films. Because the low PA-ACC-CNFs in nanocomposites, there is no significant change in thermal degradation behavior of both films (Fig. 3-18a). On the other hand, in the case of CNFs before and after surface acetylation, starting point of thermal degradation was shifted from ca. 290°C to ca. 310°C after surface acetylation (Fig. 3-18b). Taking the results into account, it was implied that the slight thermal degradation of composite film was occurred from approx. 310°C in nanocomposite.

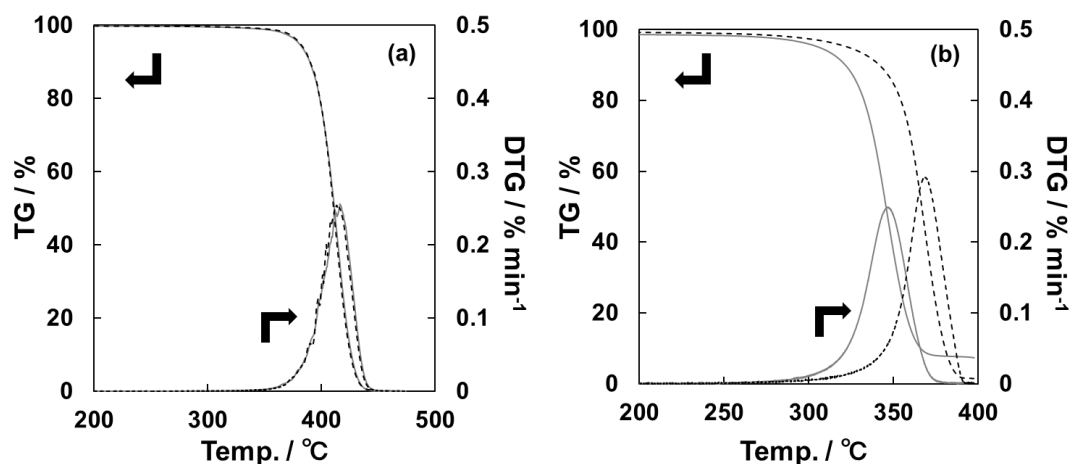


Fig. 3-18 (a) Thermal degradation behaviors of the PS composite. Black dashed line: PS/PA-ACC-CNF (DS 0.28) composite, and gray solid line: neat PS. (b) Thermal degradation behaviors of the ACC-CNFs. Black dashed line: PA-ACC-CNFs with DS 0.28, and gray solid line: unmodified ACC-CNFs.

Mechanical strength of the composite films prepared from A-PS 0.01 and 0.03 increased by compounding with PA-ACC-CNFs despite of the extremely low CNF addition, 0.05 wt% and 0.15 wt% to the PS weight, respectively (Figs. 3-19, 20). However, sufficient reinforcement effects were not obtained when we added 0.25 wt% of PA-ACC-CNFs to the PS weight (A-PS 0.05) presumably due to the stress concentration to the fiber

aggregates supported by AFM data (Fig. 3-15bd). Fujisawa et al. reported about approx. 27% increase in Young modulus of PS by addition of the 0.5 wt% TEMPO-oxidized cellulose nanofibrils.⁵⁰⁾ On the other hand, our results exhibit outstanding reinforcing efficiency, approx. 11% increase in Young modulus by addition of 0.05 wt% PA-ACC-CNFs to the PS weight ratio while keeping original high transparency.

Overall, we proposed simple surface modification approach for aqueous compounding with resin particles, and the method effectively improved mechanical strength of the PS materials in spite of the very low nanofiber loading, thereby leading to design of materials to be effective in minute quantities.

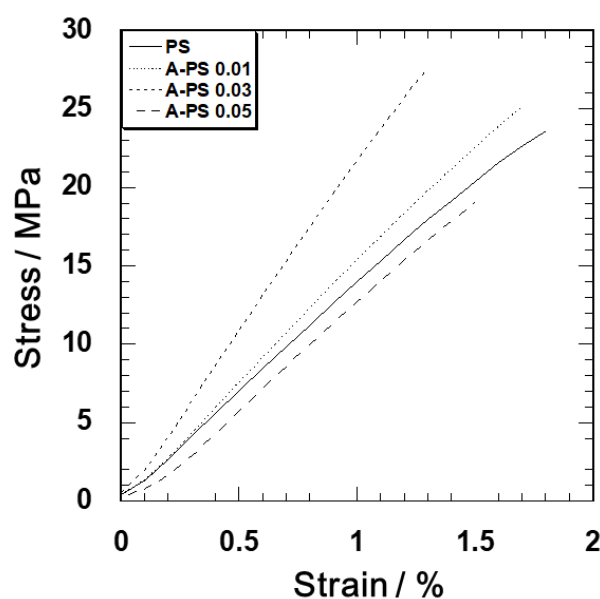


Fig. 3-19 Stress-Strain curve of the PS/PA-ACC-CNF (DS 0.28) composite films.

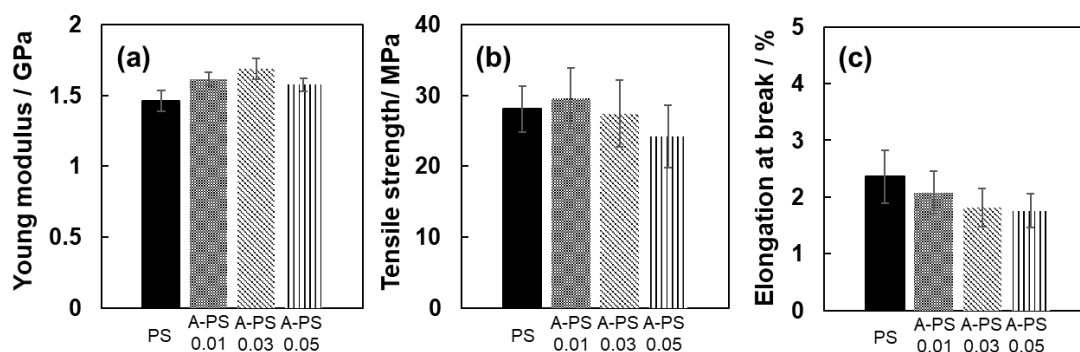


Fig. 3-20 Mechanical properties of the PS/PA-ACC-CNF (DS 0.28) composite films estimated from stress-strain curves.

3.4. Conclusion

In the current study, the surfaces the oil droplets in a Pickering emulsion, of which tend to adsorb ACC-CNFs, were used as a heterogeneous reaction platform. The quantity of ACC-CNFs adsorbed at the oil-water interfaces depended on the diameters of the droplets, which could be controlled by varying the mixing rate. The DS of the introduced acetyl groups increased as the surface area increased because the adsorption of ACC-CNFs at the oil-water interfaces stabilized the Pickering emulsion. The acetylated products retained the original fibrous morphology and CI, and the surface modification procedure successfully produced localized acetylated ACC-CNFs without the use of polar aprotic solvents. The products were readily re-dispersed in an aqueous medium, and the PA-ACC-CNFs exhibited unique self-association behavior in an aqueous media. It should be noted that the locally acetylated ACC-CNFs exhibited environment-responsive self-aggregation at both the air/water and glass/water interfaces during drying. When films were cast from the aqueous suspensions, membrane-like Janus structures with anisotropic

surfaces were produced and the assembled structure correlated surface properties was exhibited.

In addition, it was revealed that surface acetylation of CNFs drastically improve adsorbability of nanofibers onto PS surface above the certain DS value, and thereby, mechanical strength of composite films effectively increased while maintaining original high transparency of PS.

The surfaces of CNFs can be chemically modified using the oil-water interfaces in Pickering emulsions as reaction platforms. The method is simple and ecofriendly and eliminates the need for solvent exchange. Furthermore, localized surface modification enables the control of Janus-type amphiphilicity. The modified ACC-CNFs thus obtained have high aspect ratios and can be used as fibrous building blocks for higher-order hierarchical materials based on biomimetic architectonics.

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Chapter 4

Anisotropic Frictional Properties Induced from Fingerprint-like Assembly of Cellulose Nanofibrils at an Air-water Interface

Abstract

In this chapter, the author focused on an air-water interface as an interfacial platform for macroscopic 2D organization of nanofibrils. Namely, the objective in this chapter is enlargement of the concept in this dissertation “Architectonics on the Fluid Interface” from liquid-liquid interface of Pickering emulsions to the macroscopic 2D air-water interfacial platform using Langmuir-Blodgett (LB) technique.

Here, long alkyl chains as molecular floats were introduced on the surface of cellulose nanofibrils (CNFs) for suppression of sedimentation from the water surface. The nanofibrils were gradually compressed at the water surface of LB trough, thereby assembling into the fingerprint-like pattern, which is similar to the aligned structures observed in the Chapter 1. The unique alignments were kept even after the mild hydrolysis of the LB films to regenerate natural CNF surface. In addition, lateral force microscopy (LFM) revealed that the oriented microdomains in the CNF-LB films exhibit anisotropic frictional properties depending on the fiber orientation angles. This fabrication method for ordered architectures of polysaccharide-based nanofibril makes the architectonics using interfacial platforms into the more versatile concept.

4.1. Introduction

Alignments of nanofibrillar materials strongly correlated with functions e.g. mechanical strength,^{1,2)} electronic conductivity,³⁾ and cell responses.⁴⁻⁷⁾ Rao et al. reported

that the uniaxially oriented collagen nanofiber surface induced cell elongation along with the orientation direction.⁵⁾ Wang et al. reported that alignments of polycaprolactone/collagen nanofiber mediate single cell migration.⁶⁾ Therefore, various fabrication methods for alignment of nanofibrillar materials have been extensively studied.^{1,3,7-11)} However, these reports are mostly limited in relatively simple uniaxial orientation due to the restriction of fibrous geometry.^{12,13)}

Langmuir-Blodgett (LB) technique^{14,15)} is one of the fabrication methods capable to prepare ordered molecular films,¹⁴⁻¹⁷⁾ and the LB method has greatly contributed to investigation of molecular assembly.¹⁴⁻¹⁷⁾ In this method, membrane components are spread on the water surface individually, then the components are gradually compressed by moving wall, thereby forming membrane-like structures as increasing in surface density of those components. The compressed structures at each point are not only correlated with the surface pressure, changing in surface free energy of water surface, but also the compressed structures can be transferred on a solid substrate as a membrane.

The LB method is also applied to the colloidal building blocks.^{18,19)} Habibi et al. reported fabrication of densely packed rod-like cellulose nanocrystal (CNC) monolayers.^{20,21)} Ahola et al. reported enzymatic degradation behaviors of CNCs using CNC monolayers as model films.²²⁾ Furthermore, Chae et al. reported that the compression mediates uniaxial CNC alignments in the LB films, and the CNC monolayer thus prepared was found to induce anisotropic frictional and optical properties.²³⁾ In the studies, the higher density of cellulose I crystal (1.6 g cm^{-3}) than density of water forced immobilization of CNCs surfaces, which had minor negative sulfate ester groups, to the air-water interface by assistance of adsorption with cationic surfactant.²⁰⁻²³⁾ Similarly, LB films of the cellulose nanofibrils (CNFs) were prepared by surface hydrophobization²⁴⁾

or mixing with the amphiphilic polymer.²⁵⁾ Through the studies described above, the CNFs in the film forms had no structural order such as orientation,^{24,25)} and moreover, Bashar et al. reported that the CNFs structures at fluid interface was in random order.²⁵⁾

In this chapter, the aligned CNF LB films were fabricated by reversible surface hydrophobization of CNFs, LB deposition, and subsequent mild hydrolysis. Then the surface characteristics of the CNF-LB films were studied in terms of structure-function correlation.

4.2. Materials and methods

4.2.1. Materials

Triethylamine (TEA: $\geq 99.0\%$) was purchased from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan. Dichloromethane (DCM: $\geq 99.0\%$), toluene ($\geq 99.5\%$), and sodium hydroxide ($\geq 96.0\%$) were purchased from Sigma-Aldrich Japan, LLC., Tokyo, Japan. 4-Dimethylaminopyridine (DMAP) and n-octanoyl chloride ($\geq 99.0\%$) were purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. All reagents were used without purification. Ultrapure water obtained from a NANO pure Diamond (Thermo Fisher Scientific, Inc., Waltham, United States) was used for all measurements.

4.2.2. Synthesis of surface octanoylated ACC-CNFs (C8-CNFs)

Aqueous dispersions containing 100 mg of the bacteria cellulose derived-ACC-CNFs (prepared in Chapter 1, 200 MPa, 60 Pass) was concentrated by centrifugation using an MX-300 high-speed refrigerated micro centrifuge (TOMY SEIKO Co., Ltd., Tokyo, Japan) at $9.0 \times 10^3 \times g$ and 25°C for 5 min, then re-dispersed in acetone. The solvent-

exchange steps were repeatedly carried out 4 times with acetone, followed by 2 times with toluene under the same conditions. Eventually, the precipitation fraction was added in 50 mL glass vessel with 20 mL of toluene. TEA (185 μ L, 1.33 mmol, 10 equiv. to the surface OH groups of ACC-CNFs) and DMAP (16.2 mg, 0.133 mmol, 1 equiv.) were added in the toluene dispersion while stirring at 25°C. 5 mL of toluene solution containing octanoyl chloride (22.7 μ L, 0.133 mmol, 1 equiv.) was added dropwise into the reaction system while stirring, the mixture was heated at 70°C for 1 h. The obtained slurry was purified by 6 times centrifugation with toluene under the same conditions. The centrifuged sample was filtrated by suction filtration using a PTFE membrane filter (0.45 μ m ϕ , JHWP04700, Merck Millipore Ltd., Burlington, USA). To exchange dispersion medium to the DCM, 40 mL of DCM was quickly poured over the filtrated sample 4 times to avoid aggregation while filtrating. Finally, the products were dispersed in DCM. The concentration of nanofiber dispersion was determined by the dry weight of the dispersion.

4.2.3. Preparation of Langmuir-Blodgett/Schaefer (LB/LS) films

LB films were fabricated using an NL-BIO40-MWCT (Laser Techno Co., Ltd., Aichi, Japan) equipped with a Wilhelmy plate. 20 μ L of DCM containing 0.05 wt% hydrophobized ACC-CNFs was spread on the surface of water filled in Teflon-coated LB trough (40 mm $\times$ 200 mm) using a stainless syringe. After 20 min for evaporation of DCM, the spread nanofibrils were compressed at 5 mm min⁻¹ of barrier moving rate. The barrier was in parallel with the deposition substrate, and the moving direction was orthogonal to the deposition substrate. The compressed structures on the water surface were transferred onto a pre-washed cover glass slip (18 mm $\times$ 18 mm) under the constant surface pressures (*II*) by the vertical deposition method at 2 mm min⁻¹ of upward transfer speed.

Additionally, the structures were also transferred onto a cover glass slip by horizontal contacting for ca. 3 s at 5.0 mJ m⁻² of surface pressure.

4.2.4. Atomic force microscopy (AFM)

AFM observation was carried out using an Asylum MFP-3D Origin (Oxford Instruments, Abingdon-Thames, United Kingdom) equipped with a silicon cantilever (OMCL-AC240TS-R-3; Olympus Corp., Tokyo, Japan) of spring constant 1.7 N m⁻¹. AFM topographical images were obtained in tapping mode under ambient conditions. The area fraction of CNFs (F_{CNF}) was determined from binarized 6 $\mu\text{m} \times 6 \mu\text{m}$ topographical images. Surface roughness values, arithmetic average roughness (R_a) and root mean square (R_q) roughness, were determined from at least 12 topographical images. To determine the CNF orientation angle (ψ) to the scan direction of AFM cantilever, obtained topographical images of LB films were assessed using 2D-FFT analysis.

4.2.5. Estimation of the fiber density in C8LB films

To reveal the influence of compression directions on the fiber density in microdomains, the C8LB films were scanned laterally to the compression direction (from left to right) during LB procedures. Then, the C8LB films were set rotated 90° and scanned perpendicularly to the compression directions (from top to bottom). The density of C8-CNFs was estimated from the center-to-center distance of adjacent two nanofibrils in aligned microdomains. The average values of center-to-center distance in 6 AFM images (2 $\mu\text{m} \times 2 \mu\text{m}$, ca. 25 nanofibrils per single image) were determined as fiber-to-fiber distance.²⁶⁾

4.2.6. Hydrolysis of LB films

The obtained LB films were hydrolyzed to regenerate cellulosic surface. The LB films were immersed in 0.1 wt% NaOH aqueous solution at 20-22°C for 2-48 h. After the hydrolysis, the films were extensively washed with ultrapure water, then dried in a desiccator at 25°C.

4.2.7. Apparent water contact angle (AWCA) measurements

AWCA values of each sample were recorded using a Drop Master DM300 contact angle meter (Kyowa Interface Science Co., Ltd., Saitama, Japan) with a stainless needle. The AWCA values were recorded after 1 s from 1 µL dropping, and the average values of 10 times measurements are regarded as AWCA values. The obtained AWCA value was corrected using Cassie equation²⁷⁾ (4.1.) as follows:

$$\cos\theta_{\text{CNF}} = \frac{(\cos\varphi - F_S \cos\theta_S)}{F_{\text{CNF}}} \quad (4.1.).$$

where θ is contact angle, φ is apparent contact angle, F_S is the area fraction of substrate, and F_{CNF} is the area fraction of CNF.

4.2.8. Lateral force microscopy (LFM)

To characterize frictional properties on the LB films, cantilever deflection of lateral direction was recorded using an Asylum MFP-3D Origin (Oxford Instruments) equipped with an Al coated silicon cantilever (HQ: CSC17/Al BS; Nano And More Japan, Saitama, Japan) of spring constant 0.18 N m⁻¹. The lateral scans were conducted under constant load in contact mode at 25°C under constant humidity. The LFM images were obtained

by subtracting of lateral signal in the retrace images from those of trace images to suppress noises derived from sudden changing in height values during lateral scans. The lateral cantilever deflection signals of each oriented region in the LFM images were normalized by the lateral signal of the whole LFM images ($6\ \mu\text{m}\times 6\ \mu\text{m}$).

4.3. Results and discussion

4.3.1. Characterization of LB films

Fig. 4-1 shows the FT-IR spectra of ACC-CNFs before and after esterification. After esterification, the absorption band at $1750\ \text{cm}^{-1}$ attributable to the C=O stretching²⁸⁾ was detected. The shape of the absorption bands at $2800\text{--}3000\ \text{cm}^{-1}$ attributable to the C-H stretching were slightly changed, but the changing in those of O-H stretching ($3200\text{--}3600\ \text{cm}^{-1}$) was negligible. Degree of substitution value of the surface octanoylated ACC-CNFs (C8-CNFs) determined by peak area ratio in XPS spectra was 0.11.

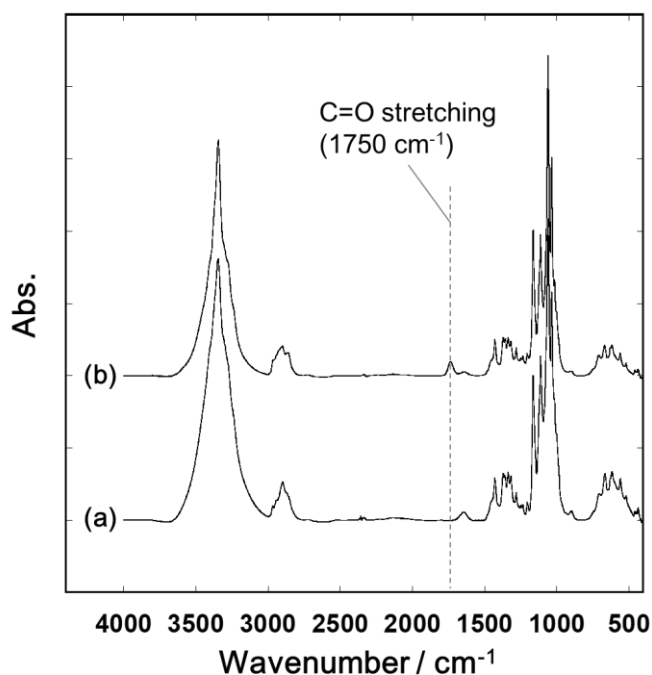


Fig. 4-1 FT-IR spectra of the (a) ACC-CNFs and (b) C8-CNFs.

The surface octanoylated ACC-CNFs (= C8-CNFs) were well dispersed in non-polar organic solvents, whereas the C8-CNFs have poor dispersibility in the water. To spread C8-CNFs on the water surface of the LB trough, dichloromethane (DCM) with high volatility and positive spreading coefficient to the water surface was selected as spreading solvents for the LB deposition. Fig. 4-2 shows the typical surface pressure-area (Π - A) curve of the C8-CNFs. The Π values were gradually increased as decreasing in A , and the Π - A curve of the C8-CNFs didn't have clear inflection points.

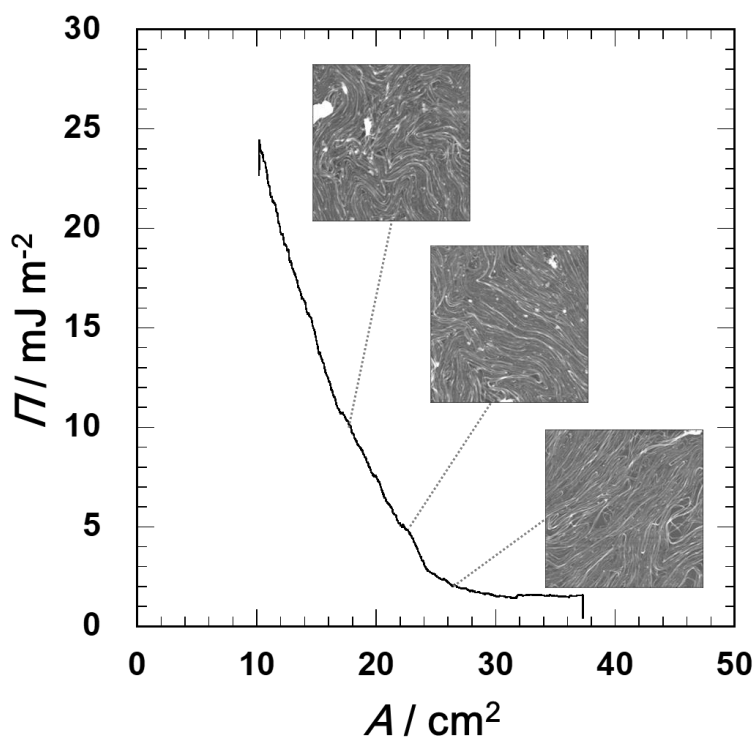


Fig. 4-2 Typical Π - A curve of the C8-CNFs. Inset images: 6 μm ×6 μm AFM topographical images transferred at each point (corresponding to Fig. 4-3).

Fig. 4-3 shows the AFM topographical images of the C8LB films transferred at each surface pressure value. In the cases of $\Pi \leq 1.0 \text{ mJ m}^{-2}$ (Fig. 4-3a), objects were not observed on the substrate, due possibility to the low stability of sparsely dispersed C8-

CNFs at the air-water interface. When the Γ values were over 2.0 mJ m^{-2} (Fig. 4-3b-d), nano-ordered fibrous objects were observed as monolayer-like membrane structures. It was noted that the LB films were composed of aligned microdomains of nanofibrils orienting to the various directions, which is similar to those observed as shells of Pickering emulsions (described in the Chapter 1). The fingerprint-like patterns of the nanofiber alignments were quite different from the random structures reported previously.^{24,25)}

Jorden et al. reported the alignment formation of semiflexible amyloid fibrils having ca. 10 nm in width on the air-water interface.²⁹⁾ The amyloid fibrils bound at water surface once straighten with increasing in surface fiber density, and the alignment undergoes in-plane bending as surface density increases further.²⁹⁾ Hence, it was considered that the well-dispersed C8-CNFs were spread on the water surface sparsely, then the nanofibrils aligned and bended due to the compression together with increasing in surface density of C8-CNFs.

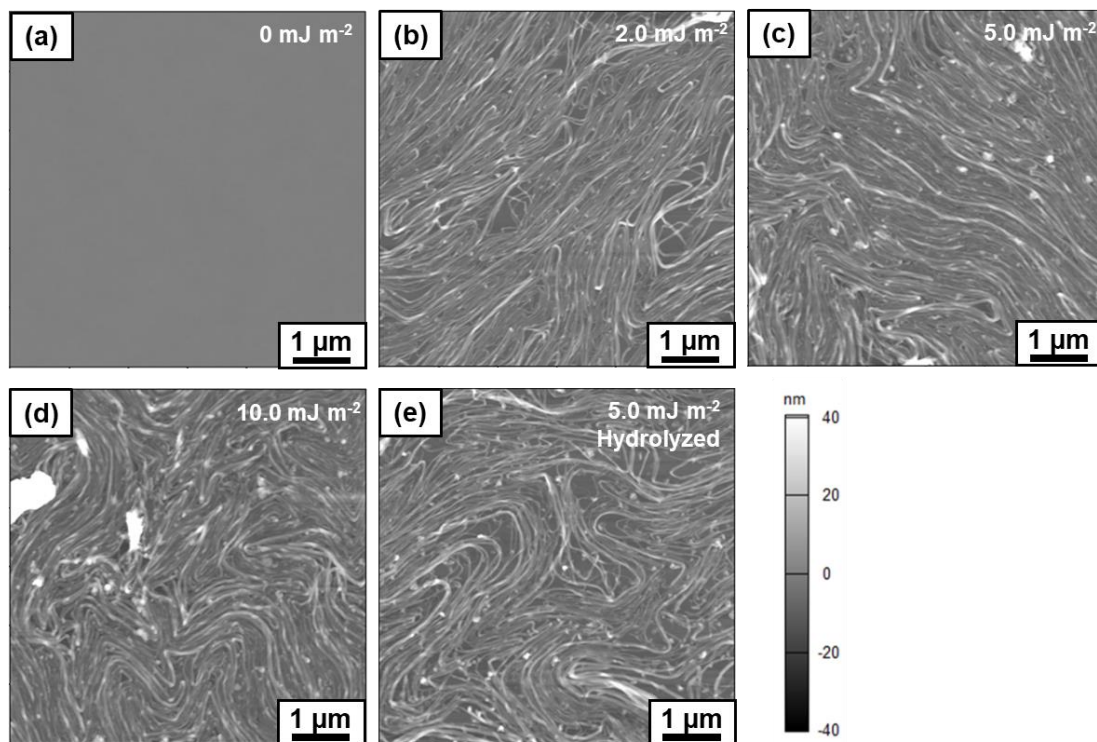


Fig. 4-3 AFM topographical images of the C8LB films transferred at (a) 0 mJ m⁻², (b) 2.0 mJ m⁻², (c) 5.0 mJ m⁻², (d) 10.0 mJ m⁻², and (e) C8LB films hydrolyzed for 24 h (transferred at 5.0 mJ m⁻²). Compression directions of those images are left to right.

Chae et al. reported that the CNC alignments in LB deposition were influenced not only the compression, but also the water flow in subphase induced by vertical pulling of substrate for deposition.²³⁾ Hence, Langmuir-Schaefer (LS) method,³⁰⁾ a horizontal deposition technique that does not need vertical pulling, was attempted to clarify which factors are predominant for assembling. Fig. 4-4 shows the monolayer film of C8-CNFs transferred at 5.0 mJ m⁻² of constant surface pressure by the LS method. Similarly to the C8LB films, horizontally deposited C8LS film also has fingerprint-like arrangement of the C8-CNFs (Fig. 4-4). Hence, the bending of aligned microdomains is mostly due to the increasing in surface density during compression rather than the pulling effect, and

the negligible pulling effect in C8LB films might be due to the low mobility of fibrous C8-CNFs comparing with rod-like CNCs.

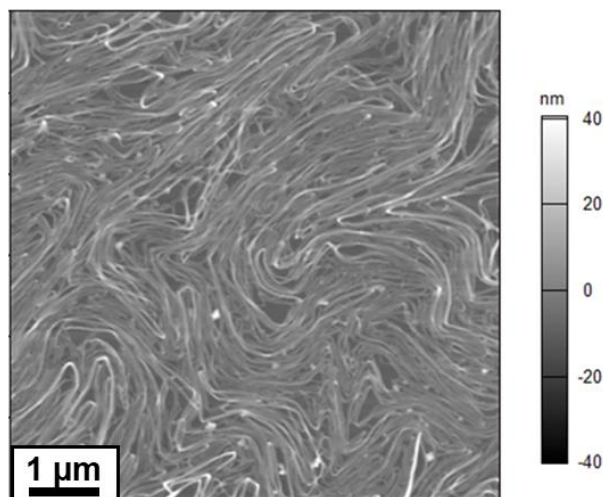


Fig. 4-4 AFM topographical image of the C8-CNF Langmuir-Schaefer film. Compression direction of the image is left to right.

Influence of the compression directions was also investigated in terms of fiber density in the aligned microdomains of nanofibrils. Instead of the nanofiber density, fiber to fiber distance of adjacent two nanofibrils was measured from $2\ \mu\text{m} \times 2\ \mu\text{m}$ AFM topographical images. However, there is no significant difference in the distance (lateral orientation to the compression direction is $55.6 \pm 4.9\ \text{nm}$, perpendicular orientation to the compression direction is $55.9 \pm 4.5\ \text{nm}$ (Fig. 4-5)).

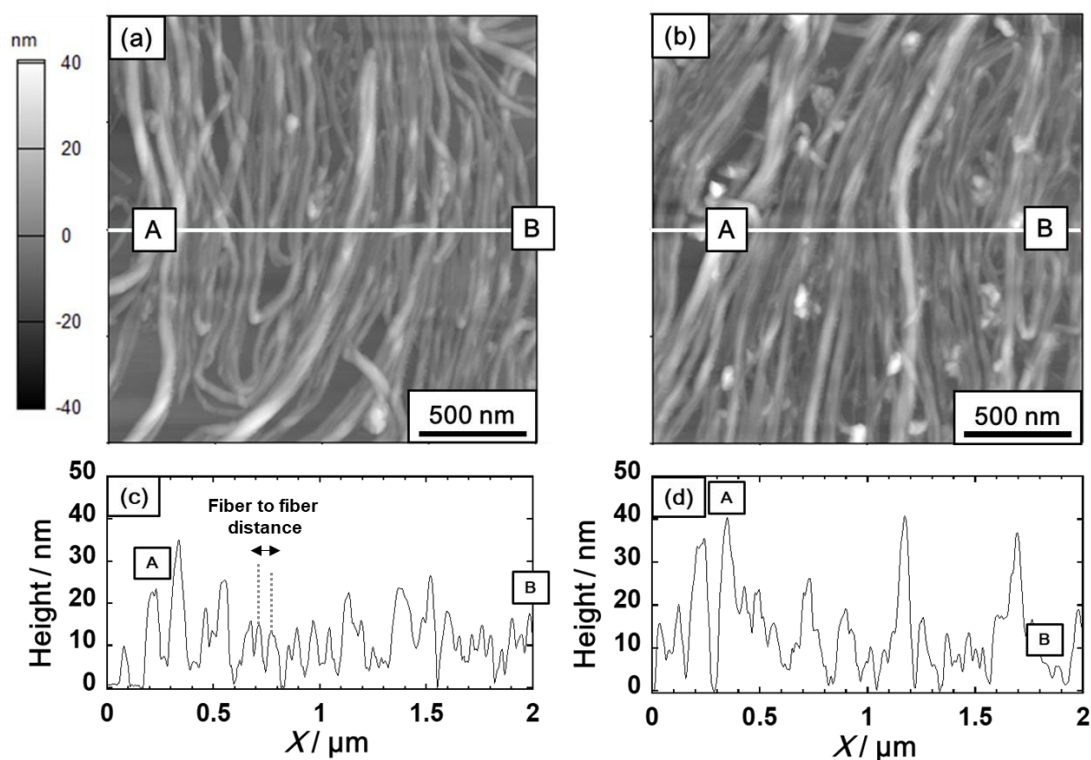


Fig. 4-5 Typical AFM topographical images of the aligned microdomains in the C8LB films. AFM scan directions in these images are the same to the A-B cross sectional lines. Compression directions during LB procedure are left to right (a) and top to bottom (b). Fig. 4-5c,d Cross section profiles of the Fig. 4-5a,b, respectively.

Although the surface roughness values of the C8LB films transferred at 5.0 mJ m^{-2} were higher than those transferred at 2.0 mJ m^{-2} , the difference between the C8LB films transferred at 5.0 mJ m^{-2} and those transferred at 10.0 mJ m^{-2} is negligible (Table 4-1). The area fraction of nanofibrils in the LB films was increased as increase in surface pressure values (Fig. 4-6). The difference between the area fraction values estimated by AFM and Π -A isotherm is presumably due to the size broadening derived from AFM chip effect.³¹⁾

Table 4-1 Surface roughness values of C8LB and h-CNF LB films ($N \geq 12$).

$\Pi / \text{mJ m}^{-2}$	2.0	5.0	10.0	5.0 (hydrolyzed)
R_a / nm	10.0 ± 3.4	17.4 ± 7.4	16.6 ± 5.9	15.9 ± 11.0
R_q / nm	20.0 ± 8.5	35.0 ± 13.3	34.9 ± 13.4	28.4 ± 18.4

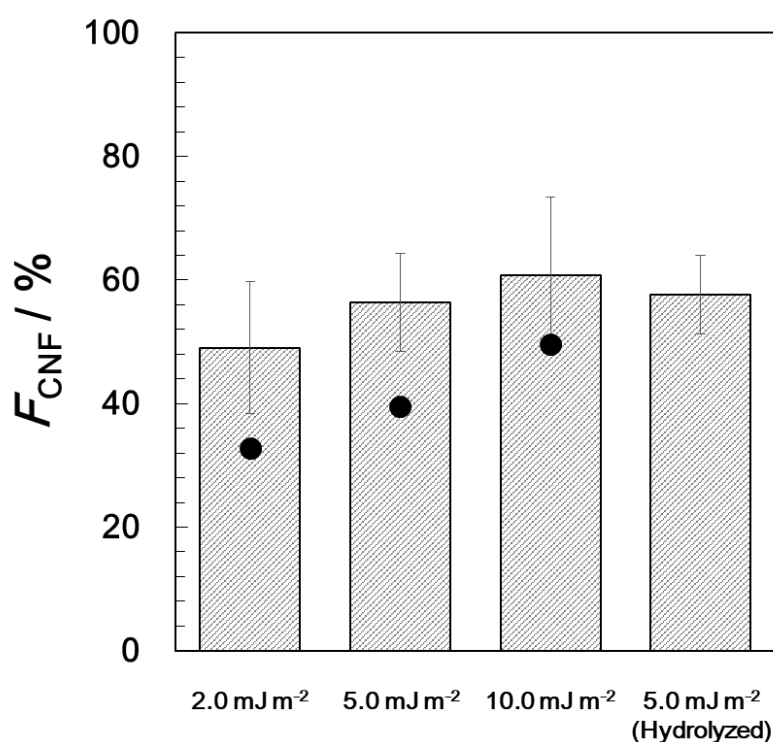


Fig. 4-6 Area fraction of C8-CNFs transferred at each surface pressure. Bars; area fraction estimated by AFM topographical images. Plots; area fraction calculated from the Π -A isotherms.

The fingerprint-like alignments of C8-CNFs were maintained (Fig. 4-3e), without significant changes in the area fraction of nanofibrils before and after hydrolysis, except slight decrease in the surface roughness values. This is presumably due to the detachment of fiber aggregates from substrate during hydrolysis and washing procedures (Table 4-1).

4.3.2. Regeneration of CNF surface

To investigate effects of the subsequent hydrolysis, changing in surface chemical structures and wettability during were monitored by XPS analysis and apparent water contact angle (AWCA) measurements. As shown in Fig. 4-7, the peak at 285.0 eV attributable to the C-C (H) bonding in XPS spectra indicated that the octanoyl groups on C8LB films were removed by the hydrolysis for 24 h (Fig. 4-7).

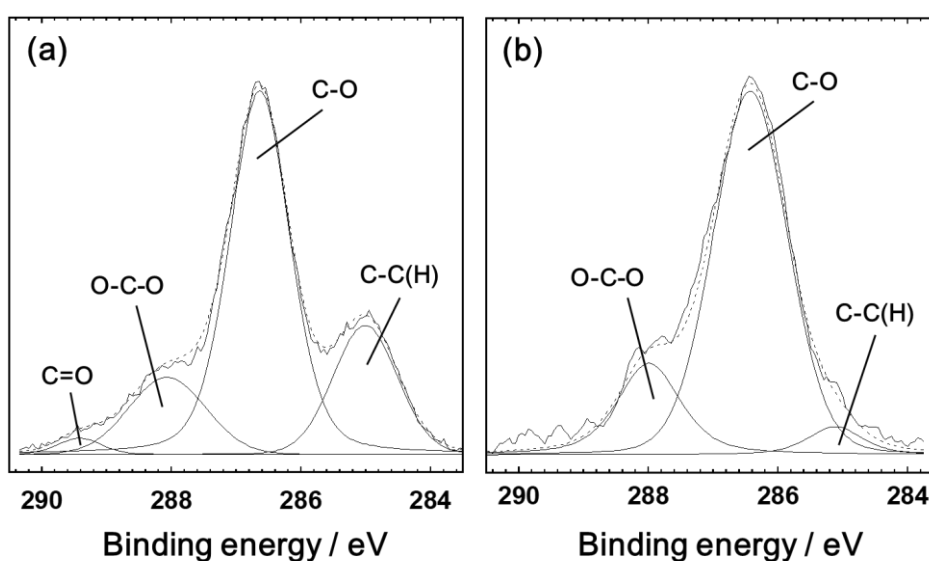


Fig. 4-7 XPS C1s spectra of (a) C8LB and (b) C8LB film hydrolyzed for 24 h.

Fig. 4-8 shows the apparent water contact angle (AWCA) values of the C8LB film transferred at 5.0 mJ m^{-2} (thickness; $27.0 \pm 15.0 \text{ nm}$) under the various hydrolysis times. While the AWCA values significantly decreased as increasing in hydrolysis time, the changing in AWCA values was negligible when the hydrolysis time is 20-48 h. Taking these results into account, it was suggested that the surface chemical structures of C8LB films are regenerated to the natural surface structures of ACC-CNFs when the hydrolysis time is more than 22 h. When the C8LB films were hydrolyzed for 22 h, the WCA value

corrected using Cassie equation²⁷⁾ was $42.7 \pm 2.6^\circ$. The value matched greatly with the WCA on the (110) plane of cellulose I_β crystal (43°) simulated by Mazeau et al.³²⁾ Hence, the hydrolyzed OH surface of the hydrolyzed-CNF LB (h-CNF LB) films was mostly exposed to the air side, which implies that the hydrophobized OH planes of the C8-CNFs were facing to the air side during compression. Taking the wettability data and fiber to fiber distance (Fig. 4-5) into account, the interfacial structures of C8-CNFs on the water surface was presumed as shown in Fig. 4-9.

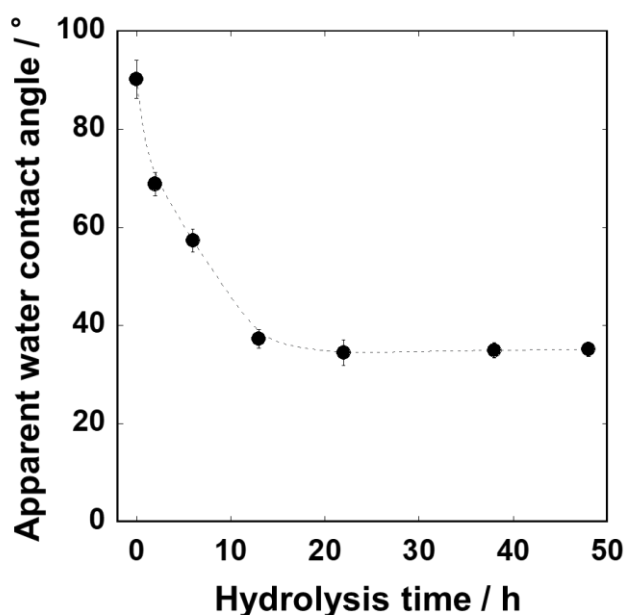


Fig. 4-8 Correlation between AWCA values and hydrolysis time of the C8LB films.

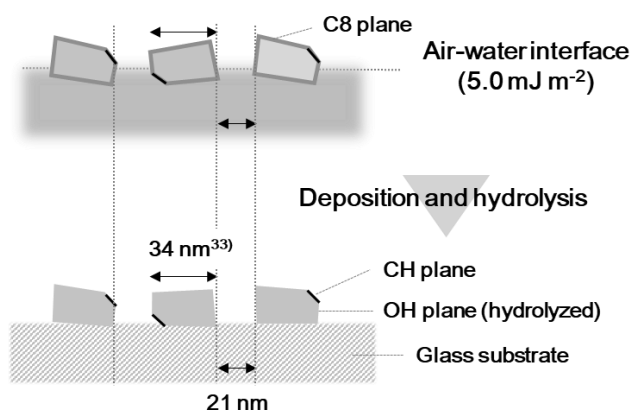


Fig. 4-9 Structural model of the C8-CNFs on the air-water interface at 5.0 mJ m^{-2} .

4.3.3. Frictional properties of the LB films

Fig. 4-10 shows AFM topographical images together with their LFM images for the C8LB films. The contrast in the LFM image reflects degree of cantilever lateral deflection due to the lateral force between LFM tip and samples. Enclosed areas in topographical image (Fig. 4-10(a)) indicate aligned microdomains of the C8-CNFs corresponding to enclosed area in Fig. 4-10(b). Here, the numbers in Fig. 4-10(a) are correlated with the numbers of the 2D-FFT images. The CNF microdomains with parallel alignment to the scan direction exhibited relatively low lateral deflections of cantilever, whereas those having orthogonal alignments exhibited high lateral deflections.

Fig. 4-11 depicts the correlation between the sine of orientation angles (ψ) to the AFM scan direction and relative lateral deflections at microdomains (ca. $1\text{-}3\ \mu\text{m}^2$) in C8LB and hydrolyzed-CNFLB (h-CNFLB) films normalized by lateral signal of whole ($36\ \mu\text{m}^2$) LFM image comprising many microdomains with various orientation directions. When the $\sin\psi$ is in the range of 0-0.51, it was negligible changing in relative lateral deflection values are (ca. 0.8). On the contrary, the corresponding values were significantly increased when the $\sin\psi$ became higher than 0.51. The lateral signals are also considered as influenced by sudden changing in height values e.g. fiber aggregation. Fig. 4-11(b) shows the root mean square surface roughness (R_q) values on the aligned microdomains of C8LB and h-CNFLB films, indicating that the orientation angle values were independent on the R_q values. Hence, the relative lateral deflection values and results in Figs. 4-10 and 4-11 can be reflected friction forces between aligned nanofibrils and LFM probe directly. These results indicated that the fingerprint-like nanofiber alignments comprising aligned microdomains exhibit anisotropic frictional properties depending on the fiber orientation angles.

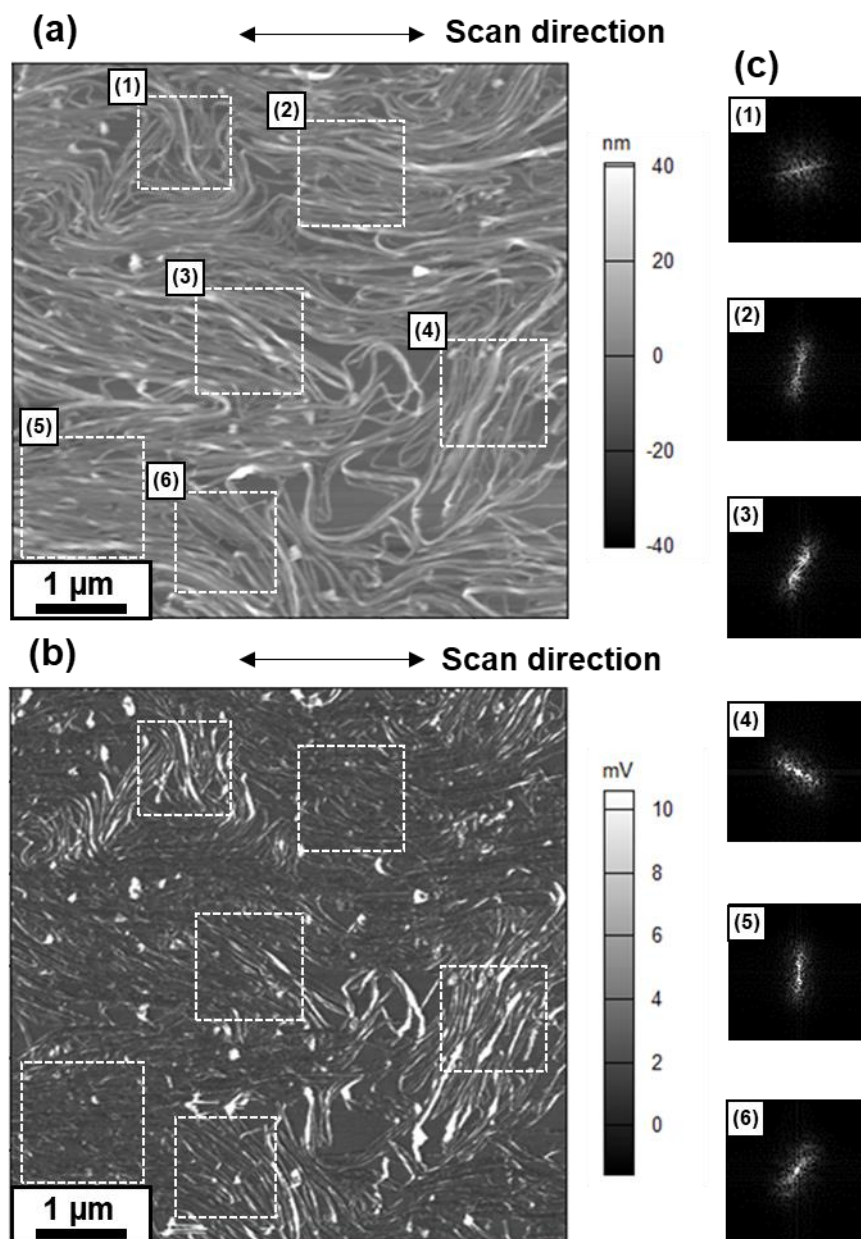


Fig. 4-10 Contact mode AFM images of the C8LB film transferred at 5.0 mJ m^{-2} . (a) Topographical image and (b) LFM image of the C8LB film. (c) The number of enclosed areas correlated with the 2D-FFT images.

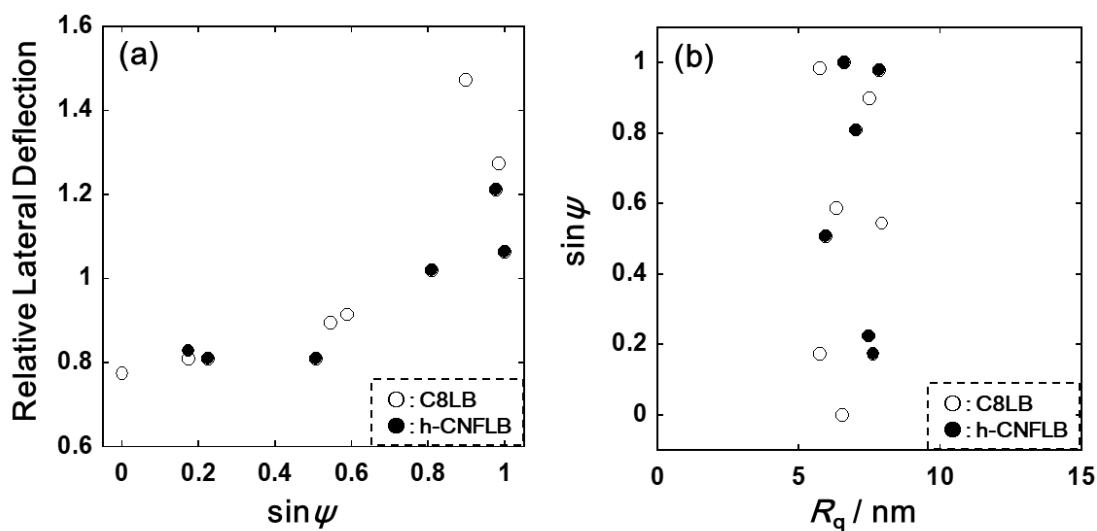


Fig. 4-11 (a) Nanofiber orientation angle dependency of the relative cantilever lateral deflections at the aligned microdomains. (b) Correlation between the root mean square surface roughness (R_q) of aligned microdomains and nanofiber orientation angle values.

4.4. Conclusion

The highlight in this study has focused on CNF assembled structures at the air-water interface, which has been regarded as a random structure. In the current study, non-uniaxial fingerprint-like alignments of the hydrophobized ACC-CNFs were fabricated by compression of nanofibrils at the air-water interface of the LB trough. The WCA values and XPS spectra of the LB films indicated that the cellulosic surface was regenerated by the mild hydrolysis with its aligned structures kept. Furthermore, the non-uniaxial orientation of nanofibrils induced unique anisotropic frictional properties. The LB fabrication of CNF alignments can lead to establishment not only for a nanofiber coating method offering anisotropic frictional properties, but also for scaffolds in cell culture, which possibly induce anisotropic cell migration and/or elongation by controlling in

aligned CNF microdomain size and degree of orientation. The insights obtained in this study for the nanofibrils at the air-water interface have referred to the exploitation of the CNF-stabilized Pickering emulsions and foams.

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Summary

In this dissertation, the author attempted to make material functions emerge from assembling of fibrous nano building blocks according to the concept of architectonics.

Chapter 1 described assistance of polysaccharide-based nanofibrils for the interfacial stabilization of the oil-water interface. Although the polysaccharide-based nanofibrils favorably formed Pickering emulsions, adsorbability and surface coverage behaviors of nanofibrils strongly depended on the raw materials. Regardless of the nanofiber concentration, the less hydrophilic ACC-BCNFs mostly adsorbed onto the oil droplets. On the other hand, adsorption ratio of nanofibrils with minor charged groups were remarkably decreased as increasing in concentration, and the excess nanofibrils were dispersed in continuous phase. In addition, it was noted that the polysaccharide-based nanofibrils surrounding oil droplets tend to form various assembled patterns depending on the raw materials.

The positively charged chitin-derived nanofibrils exhibit unique concentration-dependent interfacial behaviors for stabilization of canola oil emulsion system due to the Pickering stabilization effect and self-association of anionic fatty acids as main components of canola oil. The minor amino groups on the ACC-ChNFs also acted as electrostatic stabilizer of oil droplets, resulting in forming stable Pickering emulsions.

Chapter 2 described surface characteristics of CNFs evaluated in terms of surface free energy based on the VCG theory. For the accurate evaluation, thickness-controlled ultrathin films were prepared using CNFs with Pickering emulsion-templates, and the influence of penetration of the probe liquids was considered. The obtained surface free energy parameters were correlated with the minor surface functional groups. Moreover, the accuracy and the utility of the obtained parameters were demonstrated by close

matching with actual interfacial adsorption behaviors of the CNFs. On the other hand, to obtain more detailed information on CNF adhesion, the evaluation of surface free energy on the local domains of nanofiber surface (e.g. Hansen solubility parameter method¹⁾) and averaged values obtained by macroscopic wetting analysis (Chapter 2) should be considered in combination.

In Chapter 3, to modulate the “chemical anisotropy” in a single ACC-CNF, the author attempted to utilize the oil-water interface of oil droplets in a Pickering emulsion as a reaction platform for the ACC-CNFs. Most of the CNFs ($\approx 90\%$) were adsorbed onto the surface of oil droplets containing acetyl reagents for surface modification. As a result, the surface of CNFs was successfully acetylated without any solvent-exchange step with keeping the original fibrous morphology and crystallinity. The interfacial reaction system provided locally acetylated ACC-CNFs, exhibiting unique self-association behavior in an aqueous media. When the films were cast from the aqueous suspensions of the chemically modified ACC-CNFs, membrane-like Janus structures which show unique wetting behaviors were formed at the air-water and glass-water interfaces.

Furthermore, the adsorbability of nanofibrils onto the polystyrene microspheres was significantly improved also by the facile surface acetylation. VCG theory explained that the key issue in the underwater adsorption of acetylated CNFs was decrease in affinity between CNFs and water media rather than changing of affinity in CNF/resin interface. In addition, the mechanical strength of the nanocomposite films was effectively improved with keeping high transparency of polystyrene.

Chapter 4 dealt with the interfacial assembly of hydrophobized ACC-CNFs at the air-water interface for extending the concept of architectonics at fluid interfaces, from the 3D microscopic oil-water interface of Pickering emulsions to the 2D macroscopic air-

water interface. LB films of CNFs were successfully prepared by surface hydrophobization of CNFs, followed by compression at the air-water interface. AFM observation of the LB films composed of CNFs revealed that the fingerprint-like alignments can be constructed as 2D macroscopic membranes. This finding was in contrast to the previous and common concept for LB films that have been appeared as random network structures.^{2,3)} The fingerprint-like alignments comprising microdomains with various orientation directions were maintained even after hydrolysis of octanoyl groups on the CNFs. The surface of the LB films exhibited the unique anisotropic frictional property depending on the orientation directions in fingerprint-like CNF architectures reflecting “geometrical anisotropy” of nanofibrils.

Overall, fluid interfaces were used as platforms for nanofiber assembly. Due to the geometric anisotropy, polysaccharide-based nanofibrils assembled into fingerprint-like patterns at the oil-water/air-water interfaces. Additionally, the assembly-disassembly behaviors of nanofibrils were significantly changed by the modulation of chemical anisotropy in a single CNF via surface modification at an oil-water interface. In other words, the surface free energy/adhesion parameters on local domains of CNFs were tuned by the interfacial reaction of CNFs. Hence, by applying the interfacial reaction of nanofibrils, it is expected that the building blocks can be organized into more various structural patterns at oil-water/air-water interfaces, thereby designing functions from the assembled structures.

The author hopes that the concept of nanofiber assembly at fluid interfaces become a versatile strategy for bottom-up material design process mimicking architectonics in nature.

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List of publications

1) Emulsifying properties of α -chitin nanofibrils prepared by aqueous counter collision

Koichiro Ishida, Shingo Yokota and Tetsuo Kondo

Journal of Fiber Science and Technology, **77**(8), 203-212 (2021).

(Chapter 1)

2) Evaluation of surface free energy inducing interfacial adhesion of Janus-type amphiphilic cellulose nanofibrils

Koichiro Ishida and Tetsuo Kondo

Submitted to Biomacromolecules

(Chapter 2)

3) Localized surface acetylation of aqueous counter collision cellulose nanofibrils using a Pickering emulsion as an interfacial reaction platform

Koichiro Ishida, Shingo Yokota and Tetsuo Kondo

Carbohydrate Polymers, **261**, 117845 (2021).

(Chapter 3)

4) Modulation of adsorption of cellulose nanofibrils onto polymer microspheres for facile compounding process

Koichiro Ishida and Tetsuo Kondo

Submitted to Journal of Applied Polymer Science

(Chapter 3)

5) Anisotropic frictional properties induced by cellulose nanofibril assembly

Koichiro Ishida and Tetsuo Kondo

Submitted to Biomacromolecules

(Chapter 4)

6) Facile size evaluation of cellulose nanofibrils adsorbed on polypropylene substrates
using fluorescence microscopy

Satomi Tagawa, Koichiro Ishida, Tsubasa Tsuji and Tetsuo Kondo

Cellulose, **28**, 2917-2929 (2021).

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