

Microbeads from Crystalline Nanocellulose: Regulating Physical Properties by Chemical Modification and Hybridization

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DEDICATION

This document is dedicated to my family

Acknowledgments

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Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Junqi Wu

April 2020

Author Contribution and Contribution to Original Knowledge

1. Diffusion and Kinetics of Dye Uptake by Carboxylated Cellulose Nanocrystal Microbeads (Chapter 2)

Junqi Wu, and Mark Andrews

ACS Applied Nano Materials under revision

- J. Wu performed the experiments, collected, analysed and interpreted the data, and prepared the manuscript;
- M. Andrews conceived the ideas, supervised the project, provided valuable discussions and revised the manuscript.

Contributions to original knowledge:

1. We introduced a method to prepare microbeads from carboxylated crystalline nanocellulose (cCNC) *via* a one-step, water-based spray-drying method. We demonstrated that assembling a microbead from cCNC by spray drying is more advantageous when comparing the versatility and scope of the method to methods to make microbeads from cellulose. Both the method to make cCNC and the method to make the cCNC microbeads adhere to many of the principles of green chemistry and green engineering.
2. We explored methylene blue dye uptake kinetics and isotherms of cCNC microbeads and the results were compared with cellulose materials as dye absorbents.
3. We developed a film-pore diffusion-kinetic model to explore how methylene blue dye molecules diffuse and transport through cCNC microbeads. Diffusion coefficients were extracted from the experiments. There is little information in the published literature at

all on the transport properties of cellulose microbeads. This is despite the fact that conventional specialty cellulose polymers are widely used for time-release of pharmaceuticals, where well-known kinetic models dominate. Our study provided information about trafficking of MB as a charged probe molecule paradigm for drug transport in this new type of cCNC microbead.

4. With our film-pore diffusion model, we investigated how the variables like pH, ionic strength and temperature, affect methylene blue mass transport properties. The outcome of the study suggested procedures to enhance dye absorption.
5. From temperature dependent studies, we extracted activation energy, changes in Gibbs free energy, enthalpy and entropy pertaining to cCNC microbead dye uptake.

2. **pH responsive Carboxylated Cellulose Nanocrystal – Poly(acrylic acid) Hybrid Microbeads (Chapter 3)**

Junqi Wu, Ksenia Kolosova, Paul Wiseman and Mark Andrews

Patent “Hybrid Cellulose Microbeads”, in preparation;

Patent “Cellulose Nanocrystal-based Microbeads for Drug Fast Release”, in preparation;

Manuscript “Crystalline Nanocellulose - Poly(acrylic acid) hybrid microbeads” ready to submit

- J. Wu preformed the experiments, collected, analysed, and interpreted the data, and prepared the manuscript;
- M. Andrews conceived the ideas, supervised the project, provided valuable discussions and revised the manuscript;

- K. Kolosova performed the multi-photon spectroscopy third harmonic generation experiment.

Contributions to original knowledge:

1. We invented a one-step, water-based spray-drying method to make pH-responsive microbeads by hybridizing cCNC and the pH responsive polymer poly(acrylic acid) PAA.
2. We demonstrated spray-drying-induced crosslinking *via* esterification between cCNC and the carboxylic acid group on PAA.
3. We demonstrated how pH as a stimulus, triggers microbead expansion or contraction, and how pH regulates uptake of the methylene blue molecules.
4. We developed an XPS depth profiling to probe distribution of PAA and cCNC in the microbeads.
5. We established that third harmonic generation (THG) microscopy can give insight into the spatial distribution of cCNC throughout the neat cCNC microbead and how THG, in conjunction with other techniques, might be used to explore PAA distribution in cCNC-PAA microbeads.
6. We developed a super-resolution microscopy method to map the distribution of probe fluorescent molecules in 3-D within the cCNC microbeads.

3. Photo-gating Physical Response of Carboxylated Cellulose Nanocrystal Films and Microbeads (Chapter 3)

Junqi Wu, and Mark Andrews

- J. Wu preformed the experiments, collected, and analysed and interpreted the data, and prepared the manuscript;
- M. Andrews conceived the ideas and supervised the study.

Contributions to original knowledge:

1. We developed a method to graft 7-coumaryl-(6isocyanatohexyl) carbamate to the surface of cCNC to yield a coumarin bound cCNC.
2. We fabricated bulk films and microbeads of coumarin-cCNC and demonstrated how two-color photons trigger the reversible dimerization of the coumarin functionalities, hence, crosslinking and uncrosslinking of the bulk films. We termed this “photo-gating”, and showed how the gate event regulated methylene blue dye uptake.

4. Measuring Young’s Modulus of Carboxylated Cellulose Nanocrystal Microbeads via Atomic Force Microscopy

Junqi Wu, and Mark Andrews

Manuscript ready to submit

- J. Wu conceived the ideas, preformed the experiments, collected, analysed and interpreted the data, and prepared the manuscript;
- M. Andrews supervised the project, provided valuable discussions and revised the manuscript.

Contributions to original knowledge:

1. We developed a nanoindentation method to measure the Young's modulus of cCNC-based microbeads by atomic force microscopy (AFM).
 2. We critiqued methods that use conventional nano-sized probes to measure the Young's modulus of cCNC-based microbead. We contrasted findings from use of conventional probes with the findings when using a colloidal microbead tip.
 3. We demonstrated how esterification between small molecule citric acid and cCNC results in crosslinking that enhances the Young's modulus of hybrid cCNC microbeads.
 4. We established a correlation between the measured Young's modulus and the density of the cCNC microbeads, which were controlled by the concentration of the cCNC feed suspension during spray-drying: denser particles yielded larger Young's modulus.
5. **Tuning the Physical Properties of Carboxylated Cellulose Nanocrystal Microbeads by Hybridizing with Silk Fibroin**

Junqi Wu, Amelia Loesch and Mark Andrews

Patent "Proteinaceous Cellulose Microparticles", Mark Andrews, Timothy Morse, Monika Rak Junqi Wu, Zhen Hu and Mary Bateman, US provisional application No. 62/846, 281

Manuscript ready to submit

- J. Wu performed the experiments, interpreted the data, and prepared the manuscript.
- A. Loesch contributed to the small molecule uptake and release experiments, quantification of β -sheet in SF-cCNC microbeads through methanol treatment study.

- M. Andrews conceived the ideas, supervised the project, provided valuable discussions and revised the manuscript.

Contributions to original knowledge:

1. We demonstrated a one-step, water-based spray-drying method to incorporate silk fibroin (SF) protein molecules into cCNC microbeads.
2. We discovered that incorporation of silk fibroin conferred hydrophobic and lipophilic properties on the cCNC microbeads. We showed how the SF-cCNC microbeads were compatible with water-in-oil emulsions. The method and composition of matter were submitted as patent application.
3. We established how variations in SF-cCNC composition affect the morphology of the hybrid microbeads and provided an explanation based on the dimensionless empirical Péclet number to suggest how spray-drying parameters might give rise to the observed variations in morphology.
4. We established how SF β -sheet conformations, and their induction on exposure to methanol, can be used to regulate the Young's modulus in SF-cCNC microbeads.
5. We examined methylene blue uptake and release properties in the SF-cCNC microbeads and compared the properties with those of pristine cCNC microbeads. We showed that SF hinders the mass transport of methylene blue through combinations of hydrophobic barriers and physical barriers due β -sheet conformations .
6. We explored the spatial distribution of SF and cCNC within the microbeads *via* XPS depth profiling and found that SF is concentrated at the surface of the microbeads.

Abstract

This thesis describes how carboxylated cellulose nanocrystals (cCNC) can be co-opted for function at the micro-scale, establishing how judiciously regulated nanoscale assembly offers new avenues to cCNC-based “smart materials”.

Chapter 1 provides background to the thesis. In general, cellulose nanocrystals (CNC) are high aspect ratio, predominantly crystalline rod-like particles that have emerged as a leading sustainable resource “platform” material with potential for broad spectrum applications in technology. The incentive to deploy CNC in biomedicine, chemical sensing, film packaging, and polymer composites has been significant enough to stimulate full-scale manufacturing of CNC. In contrast to research on CNC particles themselves, there has been little activity on the study of aggregates of CNC, particularly those in which CNC particles are assembled into microscale objects, like spheres and capsules. Spheres and capsules with functionality and tunable mechanical properties have significant potential as self-healing and biomedical materials, as imaging media, in sensors, vehicles for drug delivery, for the replacement of microplastic beads, and as food ingredients. Rapid, facile, and green pathways to create capsules and microbeads with tunable mechanical and rheological properties remains largely elusive.

Chapter 2 is concerned with methods to assemble microbeads from carboxylated CNC sub-units. We describe the production of cCNC microbeads from carboxylated cellulose nanocrystals by the method of spray-drying. cCNC building blocks were obtained by dilute aqueous hydrogen peroxide hydrolysis of 100 % Forest Stewardship Certified wood pulp. We explore small molecule uptake and release properties of microbeads by using methylene blue as a probe molecule. A two-resistance model, based on film-pore diffusion, is used to provide insights into the mass transport of MB in the microbeads. The small molecule uptake is

investigated by exploring Langmuir, Temkin, and Freundlich isotherms. The linearized Langmuir isotherm is shown to best describe adsorption. With Langmuir type behaviour, the external-film mass transfer coefficients were found to be $\sim 10^{-6}$ m/s, and the effective internal diffusion coefficients were found to be $\sim 10^{-11}$ m²/s. The Biot number is calculated from the two mass transfer coefficients, and indicates that external film mass transfer dominates the dye uptake process. The influence of variables such as pH, ionic strength, and temperature on small molecule mass transfer is explored. MB dye uptake is both pH and ionic strength dependent. Thermodynamic parameters are derived from temperature dependent dye uptake studies. These show that uptake is spontaneous and endothermic. Arrhenius treatment of the kinetic data yielded an activation energy of 28 kJ/mol. This value is in line with those obtained from MB adsorption on inorganic mineral and other cellulosic materials. The study points to an opportunity for cCNC microbeads to replace environmentally damaging microplastic beads in areas like cosmetics and personal care, absorbents for water treatment, and as “smart gating” materials for drug delivery systems.

Chapter 3 consists of 2 case studies. Case study 1 is concerned generally with smart microbeads and membranes. These are of interest to the research and technology communities. With knowledge of small molecule mass transport in cCNC microbeads from Chapter 2, we introduce the notion of “gating” in the microbeads. We show how different types of microbeads express uptake and release functions that are controlled by molecular gates, switchable to open and closed states: a) by changes in proton concentration or b) by photons.

Gating by pH is studied in cCNC microbeads hybridized with poly(acrylic acid), PAA. These are produced by gas-phase esterification and crosslinking of aqueous cCNC-PAA suspensions. The PAA content can be varied at will. Optical imaging reveals that cCNC-PAA microbeads reversibly swell with changes in pH. In some cases, expansions by 100% are observed, an

increase that is unprecedented in the literature. We study the kinetics of diffusion and binding of methylene blue as functions of pH, showing that uptake and release are gated by pH under supervision of the PAA component. Basic pH impedes the release of MB dye molecules from the microbeads, while acidic pH promotes release.

XPS depth profiling is used to locate the spatial distribution of progargylated cCNC in the microbeads by monitoring the nitrogen element distributions at different etching depths. The experiment reveals that cCNC and PAA are intermixed in the hybrid microbeads. cCNC-PAA microbeads are imaged *via* multiphoton microscopy by monitoring the third harmonic response. This label-free method gives insight in the microbead particle morphology when swelled in water. Native cCNC microbeads show inhomogeneous interiors, *i.e.*, they were not dense, an observation that is confirmed by focused ion beam milling by scanning electron microscopy. Confocal microscopy is used to monitor the *in situ* release of a porphyrin fluor from cCNC-PAA microbeads. Structure illuminated super resolution microscopy (SIM) shows that the porphyrin probes are distributed in 3-dimensions throughout the microbeads, though non-uniformly.

Case study 2 describes how a photo-switchable swing-gate can be built from cCNC that is functionalized with a reversibly photodimerizable coumarin molecule. Quantities of coumarin-cCNC are mixed with coumarin-free cCNC that were elaborated from water suspensions into meso - and microbeads by spray-drying, or into films by evaporation. Methylene blue dye is used as a model compound to study gated reversible uptake and release. The gate, when closed, suppressed dye uptake/release.

Chapter 4 explores methods and outcomes of adjusting the mechanical properties of cCNC microbeads. We measured the Young's modulus of cCNC microbeads using Atomic Force Microscopy (AFM). The Young's modulus is extracted from the force-displacement curves

obtained by indenting each microbead with an AFM colloidal microsphere probe. The data are fitted to a Hertz model to obtain the modulus. We find that indentation with a *colloidal* microsphere probe yielded more meaningful results compared with the use of a conventional nanoprobe. The cCNC microbeads yielded a Young's modulus of 18.02 MPa. The elastic modulus was then systematically adjusted by incorporating citric acid as a small molecule crosslinking agent. With 10% citric acid, the Young's modulus is enhanced to 27.97 MPa. Increasing the concentration of cCNC suspension for spray-drying yields microbeads with denser internal structures comparing with dilute cCNC suspension generated microbeads. The higher density beads show a higher Young's modulus of 24.55 MPa, though not as high as the citric acid crosslinked beads. We concluded that mechanical properties of cCNC-based microbeads can also be adjusted by chemical crosslinking and through changes in spray-drying parameters.

Chapter 5 examines the outcome of incorporating the protein silk fibroin (SF) to cCNC microbeads. The empirical, dimensionless Péclet number is used to provide qualitative insight into the origins of the morphological changes in the microbeads that occur as the SF content is varied. We take advantage of the well-known β -pleated sheet conformational transition in SF to alter both the mechanical properties and hydrophobic/lipophilic character of the microbeads. We used self-deconvolution Fourier transform infrared spectroscopy to quantify random coil and β -pleated sheet conformations of SF in the microbeads. The technique is then used to follow how different aqueous methanol concentrations lead to controlled changes in the β -pleated sheet content of the microbeads. The hydrophobic domains in SF are used to confer hydrophobic and lipophilic character on the microbeads and it is established that the SF-cCNC microbeads are stable in water-in-oil emulsions. β -sheet conformational transitions induced by methanol and aqueous methanol solvent treatment are used to tune the mechanical properties of the beads and regulate small molecule uptake and release properties. Incorporation of 10%

silk fibroin enhanced the stiffness compared with neat cCNC microbeads. Methanol treatments of 10% and 20% SF-cCNC microbeads showed an even higher Young's modulus. The enhancement of the Young's modulus is found to be correlated with the percentage of SF β -sheet conformation in the microbead. Small molecule uptake and release kinetics are found to be impeded by methanol treatment, which is attributed to increases in both β -sheet crystallinity (physical barrier) and hydrophobicity.

Résumé

Cette thèse décrit comment les nanocristaux de cellulose carboxylée (NCCc) peuvent être employés pour fonctionner à l'échelle microscopique, établissant comment l'assemblage nanométrique contrôlé offre de nouvelles voies pour les « matériaux intelligents » basés sur les NCCc.

Le chapitre 1 fournit le contexte de la thèse. En général, les nanocristaux de cellulose (NCC) ont un large format, et consistent principalement en des particules cristallines en forme de bâtonnet qui se sont établis au fil du temps comme un matériau « plateforme » de ressource durable ayant un potentiel pour un large éventail d'applications technologiques. Le déploiement des NCC dans la biomédecine, la détection chimique, les films d'emballage et les composites de polymères a été suffisamment important pour stimuler la fabrication de NCC à grande échelle. Contrairement à l'importante recherche effectuée sur les particules de NCC elles-mêmes, les études sur les agrégats de NCC sont rares, et tout particulièrement sur les particules de NCC assemblées en forme d'objets à l'échelle microscopique, comme les sphères et les capsules. Les sphères et les capsules dotées de fonctionnalités et de propriétés mécaniques ajustables ont un potentiel important en tant que matériaux biomédicaux ou auto-cicatrisants, matériaux d'imagerie, dans des capteurs, des systèmes d'administration de médicaments, pour le remplacement de billes microplastiques et en tant qu'ingrédients alimentaires. À ce jour, des voies rapides, faciles et soutenables pour créer des capsules et des microbilles à propriétés mécaniques et rhéologiques ajustables restent introuvables.

Le chapitre 2 traite des méthodes d'assemblage des microbilles à partir de sous-unités de NCCc. Nous décrivons la production de microbilles de cCNC à partir de nanocristaux de cellulose carboxylée avec la méthode de séchage par atomisation. Les éléments constitutifs du NCCc ont été obtenus par hydrolyse au peroxyde d'hydrogène dilué en solution aqueuse de la pâte de

bois certifiée 100% Forest Stewardship. Nous explorons les propriétés d'absorption et de libération de petites molécules par les microbilles en utilisant du bleu de méthylène (BM) comme molécule modèle. Un modèle à deux résistances, basé sur la diffusion film-pore, est utilisé pour extraire des informations sur le transport de BM dans les microbilles. La capture de petites molécules est étudiée en explorant les isothermes de Langmuir, Temkin et Freundlich. Il est démontré que l'isotherme de Langmuir linéarisé décrit au mieux le processus d'adsorption. Avec un comportement de type Langmuir, les coefficients de transfert de masse du film externe sont de l'ordre de $\sim 10^{-6}$ m / s, et les coefficients de diffusion interne effectifs de l'ordre de $\sim 10^{-11}$ m² / s. Le nombre de Biot est calculé à partir des deux coefficients de transfert de masse et indique que le transfert de masse du film externe domine le processus d'absorption du colorant. L'influence de variables telles que le pH, la force ionique et la température sur le transfert de masse de petites molécules est également explorée. L'absorption de colorant BM dépend à la fois du pH et de la force ionique. Les paramètres thermodynamiques ont été dérivés depuis les études d'absorption de colorant dépendant de la température. Ceux-ci ont montré que l'absorption est spontanée et endothermique. Le traitement des données cinétiques à travers la méthode d'Arrhenius a révélé une énergie d'activation de 28 kJ / mol. Cette valeur est conforme à celles obtenues par adsorption du BM sur des minéraux inorganiques et sur d'autres matières cellulosiques. L'étude met en évidence une opportunité pour les microbilles de NCCc qui pourraient remplacer les perles microplastiques nuisibles à l'environnement dans des domaines tels que les cosmétiques et les soins personnels, les absorbants pour le traitement d'eau et en tant que matériaux à « déclenchement intelligent » pour les systèmes d'administration de médicaments.

Le chapitre 3 comprend deux études de cas. La première étude de cas concerne les microbilles et les membranes intelligentes. Celles-ci intéressent expressément les communautés de recherche et de technologie. Avec les connaissances acquises sur le transport de masse de

petites molécules dans les microbilles de NCCc au cours du chapitre 2, nous présentons ici la notion de « déclenchement » au sein des des microbilles. Nous montrons comment différents types de microbilles adoptent des fonctions d'absorption et de libération qui sont contrôlées par des portes moléculaires, et comment ces portes peuvent se trouver dans les états ouverts et fermés par : a) des changements de concentration de protons ou b) des photons.

Le déclenchement de ces portes par pH est étudié au sein de microbilles NCCc hybridées avec de l'acide polyacrylique (APA). Ces dernières sont produites par estérification et réticulation en phase gazeuse de suspensions aqueuses de NCCc-APA. La teneur en APA est ajustable. L'analyse par imagerie optique révèle que les microbilles de NCCc-APA gonflent selon le changement de pH. Dans certains cas, une expansion de 100% est observée, ce qui est sans-précédent au sein de la littérature. Nous étudions la cinétique de diffusion et de liaison au bleu de méthylène (BM) en fonction du pH, en montrant que l'absorption et le relargage sont contrôlés par le pH sous la supervision de l'APA. Un pH basique entrave le relargage des molécules de colorant de BM par les microbilles, tandis qu'un pH acide en favorise la libération. Des profils des profondeurs sont utilisés pour localiser la distribution spatiale des NCCc propargylés dans les microbilles en surveillant la distribution d'azote élémentaire sur différentes profondeurs d'attaque. L'expérience révèle une distribution homogène de NCCc et d'APA dans les microbilles hybrides. Les microbilles NCCc-APA sont analysées par microscopie multiphoton en observant la troisième réponse harmonique. Cette méthode sans marquage donne une idée de la morphologie des particules de microbilles gonflées dans l'eau. Les microbilles natives de NCCc montrent un intérieur non-homogène, *i.e.* non dense, observation qui fut confirmée par microscopie électronique à balayage avec fraisage par faisceau ionique focalisé. La microscopie confocale fut utilisée pour observer le relargage *in situ* de porphyrine fluorée depuis les microbilles de NCCc-APA. La microscopie

d'illumination structurée à super résolution (SIM) montre que les porphyrines sont distribuées en 3 dimensions dans les microbilles de manière non-uniforme.

L'étude de cas 2 illustre comment un interrupteur réversible photo-activable est construit à partir de NCCc fonctionnalisé avec des molécules de coumarine photo-dimérisable de manière réversible. Une quantité de NCCc-coumarine est mélangé avec des NCCc sans coumarine élaborés par suspension aqueuse dans des méso- et microbilles par séchage par atomisation, ou en tant que film produit par évaporation. Le bleu de méthylène est utilisé comme colorant modèle pour étudier les portes moléculaires et leur absorption et relargage. Lorsqu'elles sont fermées, les portes moléculaires suppriment l'absorption et relargage de colorant.

Le chapitre 4 explore les méthodes et les résultats de l'ajustement des propriétés mécaniques des microbilles de NCCc. Nous avons mesuré le module de Young des microbilles de NCCc par microscopie à force atomique (MFA). Le module de Young est extrait des diagrammes de force-déplacement obtenus par indentation de chaque microbille en utilisant une sonde colloïdale microsphérique par MFA. Les données sont ajustées pour obtenir le module en utilisant le modèle de Hertz. Nous avons trouvé qu'une indentation obtenue par sonde colloïdale microsphérique donne des résultats plus probants qu'en utilisant une nanosonde plus conventionnelle. Les microbilles de NCCc donnent un module de Young de 18,02 MPa. Le module élastique est alors systématiquement ajusté en incorporant de l'acide citrique en tant qu'agent de réticulation. Avec 10% d'acide citrique, le module de Young est amélioré en donnant une valeur de 27,97 MPa. En augmentant la concentration de suspension de NCCc, le séchage par atomisation génère des microbilles à structures intérieures plus denses que les microbilles obtenues en ayant utilisé des suspensions de NCCc plus diluées. Ces billes de hautes densités ont un module de Young de 24,55 MPa, une valeur élevée mais moindre que les billes obtenues par réticulation avec l'acide citrique. Nous concluons donc que les

propriétés mécaniques des microbilles basées sur les NCCc peuvent être ajustées par réticulation chimique et en changeant les paramètres de séchage par atomisation.

Le chapitre 5 examine les résultats obtenus lors de l'incorporation de protéine de fibroïne de soie (FS) dans les microbilles de NCCc. Le nombre de Péclet, valeur empirique et adimensionnelle, est utilisé pour fournir des informations qualitatives concernant l'origine des changements morphologiques obtenus lors de la variation de la teneur en FS. Nous tirons pleinement avantage de la transition conformationnelle des feuillets beta de FS qui est bien connue pour altérer les propriétés mécaniques ainsi que le caractère lipophile/hydrophobe des microbilles. Cette technique est alors utilisée pour observer comment différentes concentrations de méthanol engendrent des changements contrôlés dans la teneur en feuillet beta des microbilles. Les domaines hydrophobes de la FS sont aussi utilisés pour conférer aux microbilles des caractéristiques hydrophobes et lipophiles. Il est également établi que les microbilles de NCCc-FS sont stables dans des émulsions eau-dans-l'huile. Les transitions conformationnelles des feuillets beta induites par le méthanol et le traitement au méthanol aqueux sont utilisés pour affiner les propriétés mécaniques des billes et pour réguler les propriétés d'absorption de petites molécules ainsi que leur relargage. Une incorporation de 10% de fibroïne de soie améliore la rigidité de 67% en comparaison avec les microbilles de NCCc sans FS. Les traitements au méthanol à 10% et 20% des microbilles de NCCc-FS donnent des modules de Young encore plus élevés. L'amélioration des modules de Young est alors corrélée avec le pourcentage de feuillet beta des microbilles. Les cinétiques d'absorption et de relargage de petites molécules sont bloquées par les traitements au méthanol, ce qui est attribué à la cristallinité des feuillets beta (barrière physique) ainsi qu'à l'hydrophobicité.

Abbreviations

AFM	Atomic Force Microscopy
Crystalline Nanocellulose	CNC
Carboxylated Crystalline Nanocellulose	cCNC
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
XRD	X-ray Diffraction
XPS	X-ray Photoelectron Spectroscopy
UV-VIS	Ultraviolet–visible Spectroscopy
BET	Brunauer–Emmett–Teller Surface Area Analysis
TGA	Thermogravimetric Analysis
FT-IR	Fourier Transform Infrared Spectroscopy
BSE	Back-scattered Electron
SE	Secondary Electron
Pt	Platinum
DMF	Dimethylformamide

NMR	Nuclear Magnetic Resonance
DLS	Dynamic Light Scattering
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	N-Hydroxysuccinimide
PMMA	Poly(ethyleneglycol diacrylate)
PAA	Poly(acrylic acid)
PS	Polystyrene
PXRD	Powder X-Ray Diffraction
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
SF	Silk Fibroin
MT	Methanol Treatment
THG	Third Harmonic Generation
SIM	Structured Illumination Super Resolution Microscopy

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| 1 |

Introduction

Chapter 1 Introduction

This thesis explores the chemistry and physical properties of microbeads made from carboxylated cellulose nanocrystals (cCNC). Microbeads are defined as spherical particles that are in the size range 1 to 1000 μm in diameter. They are commonly made from petroleum sourced polymers, silica, metal oxides, and naturally occurring polymers like proteins and polysaccharides.^{1, 2} Microbeads are widely used in drug delivery,³ in cosmetics and skincare,⁴ in fluorescent immunoassay,⁵ as micro-carriers in biotechnology,² as viscosity modifiers,⁶ as stationary phases in chromatography,⁷ as abrasives,⁸ and as absorbents for water treatment.⁹

Among microbeads, microplastic beads are defined as plastics that are less than 1 mm in length with spherical shape and are purposefully manufactured.¹⁰ They are widely used in products for the cosmetic and personal care industries. This is because of their robust mechanical properties, sensorial benefits, straightforwardly tunable particle size, ease of manufacture, low production cost, and mature polymer production technologies.^{2, 11} Commonly used microplastic beads include poly(ethylene), poly(methyl methacrylate), poly(tetrafluoroethylene), poly(propylene), nylon, and poly(ethylene terephthalate).¹² Microplastic beads are often made by emulsion polymerization,¹³ suspension polymerization,¹⁴ or dispersion polymerization.¹⁵ With the tuning of the chemical compositions and the synthesis methods, the size, shape, and density of the microbeads can be tailored.

There is compelling evidence that microbeads made from plastics cause harm to both the aquatic and terrestrial environment, including damage along the food chain.^{16, 17} Purification to eliminate unreacted monomer and other residues from polymerization is challenging. These toxic residues are eventually released into the aquatic environment. Also, the micro-sized particles may pass through water filtration systems and thereby enter the aquatic environment,

where they may be passed along the food chain, causing harm to the marine life.¹⁸ Indeed, with their high surface area, microplastic beads behave as absorbents in water, accumulating metal ions and organic molecules on the surface and within the beads. Pollutants contained in microbeads migrate up the food chain, eventually threatening human health. Machado *et al.* have explored the terrestrial fate of microplastics. They conclude that microplastics may present a similar or worse damaging effect on land and soil as they do in oceans.¹⁷

In this introduction, we begin by discussing how microplastic beads might be replaced by biodegradable materials from natural resources. While replacements for microplastics are incentives for our studies of microbeads from cellulose nanocrystals (CNC), the overarching purpose of this thesis is to establish the versatility and properties of CNC-based microbeads. We first explore conventional methods to make cellulose microbeads from well-known cellulose and chemical processes, distinguishing them from the use of CNC to produce them, and with greater diversity in properties (section 1.1). We then discuss the method of spray-drying, which can be used to make microbeads, and this method is discussed with a view to how different parameters influence properties of microbeads like morphology, polydispersity, sphericity, and surface properties. We describe how spray-drying can be used to make hybrid microbeads by adapting procedures from the pharmaceutical industry. Hybrid cCNC microbeads show new properties that are not easily obtained by heterogeneous covalent bond surface chemistry on the nanocrystals themselves (section 1.2). A list of physical properties, including surface tension, mechanical properties, and small molecule uptake and release, is introduced along with detailed measurement methods (section 1.3). We then explore the current applications of cellulose-based materials with an emphasis on the potential applications of CNC microbeads including absorbents, drug delivery systems and smart materials (section 1.4). Lastly, characterization techniques to identify chemical modification, and quantify particle physical properties are briefly discussed (section 1.5).

1.1. Biodegradable Microbeads

Increased concern regarding personal and environmental health has stimulated growth in research in microspheres made from renewable resources. Societal lifestyle changes and the urge to use organic/natural ingredients to replace traditional products have provided strong incentive for corresponding changes in personal care products, inks, pigments, coatings, composites, and thickeners for paints. “Sustainability” has become a buzzword as corporations and governments lobby for the evolution of the so-called “circular economy”. The principles of “green chemistry” and “green engineering” underpin what is now almost a mantra when it comes to sustainable and cradle-to-cradle practices. The implications for the microbead industry are obvious.

Besides the push for environmentally friendly chemistry and manufacturing, there is also push to develop biodegradable microbeads in the pharmaceutical industry for drug delivery applications, especially for delivering bioactive compounds.¹⁹ A limitation of non-degradable drug carriers is that they have to be removed from the body after drug release. Examples include silicone capsules and polyacrylic acids of various kinds.²⁰ In contrast, well-designed biodegradable carriers can degrade into nontoxic products in the body through processes like hydrolysis, or *in vivo* biological reactions (enzymic degradation).²¹

Biodegradable microbeads can be made from natural biodegradable polymers or synthetic ones.²² Figure 1-1 classifies different polymers that can be used to make biodegradable microbeads. These polymers have been widely applied in the pharmaceutical industry, in the textile industry, in biomedical engineering applications, in food, cosmetics, and plastic industries.²²

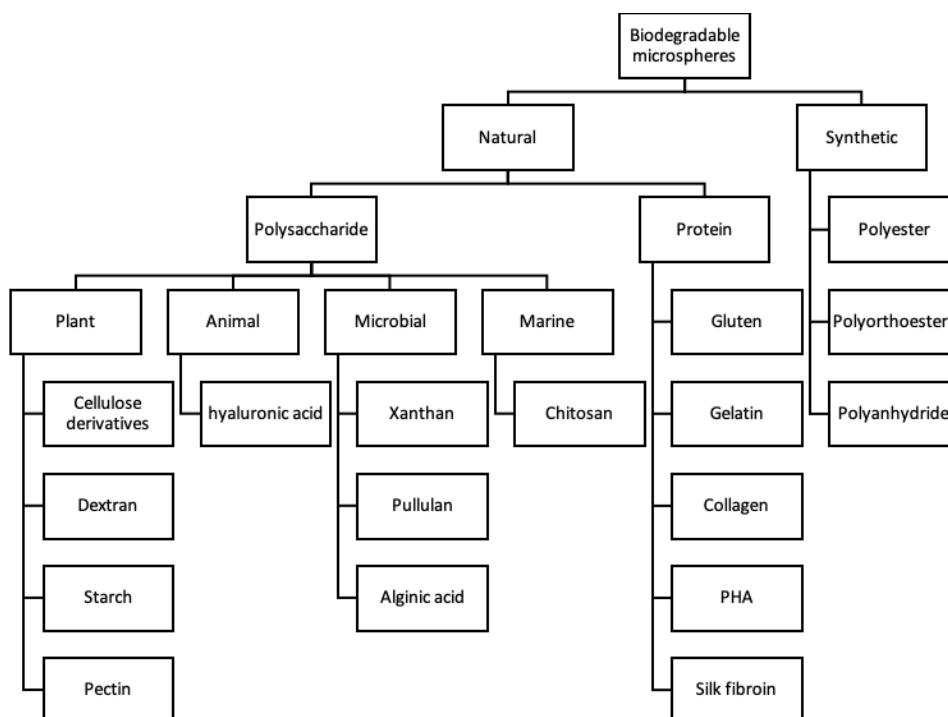


Figure 1-1 Classification of biodegradable polymers used for microbead synthesis.(Reprint with permission from ref²², copyrights managed by Talyor & Fancis)

Table 1-1 reviews biodegradable microbeads that can be made from natural or synthetic polymers. These microbeads are used primarily for drug release control, as cell scaffolds, and to encapsulate proteins. Emulsion-polymerization is a common method to make these microbeads, followed by small molecule crosslinking to enhance their stability. Emulsion polymerization usually involves organic solvents. This makes industrial scale-up challenging since the solvent residues may be hard to eliminate during mass production. Small molecule/ion crosslinkers include glutaraldehyde, Ca^{2+} , and epichlorohydrin. The toxicity of small molecule crosslinking agents remains a concern, especially for human health related applications.

Measurements of the mechanical properties of biodegradable microbeads have been performed by various researchers. Some of the methods are listed in Table 1-1. Biodegradable microbeads are often mechanically soft, exhibiting low values of the elastic modulus. This makes them challenging to compete with current synthetic non-biodegradable plastic microbeads that

outperform bio-sourced materials. Nevertheless, some materials have very interesting adhesive, viscoelastic, or thermoplastic properties that make them suitable materials for specific applications. Some of these are indicated in Table 1-1.

Table 1-1 Listing of biodegradable polymers for microbead fabrication.

	Methods	Applications	Comments	References
Dextran	Two-phase system Polymerization and emulsification	Controlled drug release	Elastic modulus: 4.5MPa with 50% H ₂ O content (Micromanipulation technique) Use of PEG solution for polymerization	23-25
Starch	Emulsification Emulsion crosslinking	Encapsulation of protein molecules	Use of PEG solution Cyclohexane and chloroform mixture used Toxicity of the crosslinker epichlorohydrin	26-28
Pectin	Spray-drying + crosslinking with CaCl ₂ w/o emulsion solvent Evaporation	Drug delivery	Elastic modulus: < 6MPa	29, 30
Hyaluronic acid esters	Solvent evaporation Water in oil emulsion method crosslinked by BDDE	Controlled drug release	Covalently bound drug molecules lead to zero-order release controlled by hydrolysis Elastic modulus: 2000Pa Well-known for its numerous biological functions and biocompatibility, mucoadhesion etc. involved in wound healing, and anti-inflammatory process.	31-33

Xanthan	Emulsion cross-linking with glutaraldehyde	Drug release	pH-responsive release Used in oral topical formulations as a suspending and stabilizing agent and a release sustaining agent in hydrophilic matrix tablet and pellets	34-36
Pullulan	Suspension crosslinking with epichlorohydrin dissolution using NaOH solution Double-emulsion-solvent evaporation	Drug release Protein delivery	pH and thermal responsive	37, 38
Alginate	Water-in-oil emulsion with Ca^{2+} crosslinking	Drug release Embolization	Low immunogenicity and mucoadhesive properties Elastic modulus: 490 kPa	39-42
Chitosan	Super critical drying Spray-drying Emulsion crosslinking Coacervation	Drug delivery, cosmetic plastic microbead replacements, enzyme immobilization		43-45
Gluten	Nanoprecipitation Emulsification/Polymerization	Agricultural slow release fertilizers encapsulation	Viscoelastic and adhesive properties	46, 47

Gelatin	Emulsion-congealing-chemical crosslinking method Emulsion-coacervation method in water-in-oil emulsion Crosslinked by glutaraldehyde	Controlled release		48, 49
Collagen	Water in oil emulsion	As cell carrier Controlled release	Elastic modulus: 132 Pa (microplate compression method)	50-52
PHA	Double emulsion evaporation	Drug controlled delivery	Thermoplastic	53
Silk fibroin	Spray-drying High pressure electrostatic generator	Auto-gelling device, cell culture carrier	Mechanical enhancement/crosslink	54-57

Synthetic biodegradable polymers (PLA, PGA, PCL, PPE, PLGA, etc)	Emulsion	Drug release	Enable copolymerization to alter their physical, chemical, and mechanical properties	22, 58

1.1.1. Cellulose Microbeads

Microbeads made from cellulose or cellulose derivatives have drawn much attention. Cellulose is the most abundant natural polymer in the world.⁵⁹ It is non-toxic, renewable, biodegradable, and biocompatible.⁶⁰ Cellulose also provides excellent mechanical and chemical properties that are attractive for microbeads applications. Furthermore, the abundant amount of hydroxyl group on cellulose simplifies chemical modification, enabling the tuning of the surface properties of the microbeads and introducing new functionalities. These properties make cellulose an excellent candidate as microbeads for drug delivery,⁶¹ chromatography,⁶² water treatment,⁶³ catalytic and other applications.⁶⁴ Detailed structural information for cellulose is given in section 1.1.2.

In general, cellulose beads are made in three steps: (1) cellulose dissolution, (2) shaping to spheres, (3) sol-gel transition, and solidification. Due to strong hydrogen bonding among polysaccharides, native cellulose is insoluble in water and in most organic solvents.⁶⁵ As shown in Figure 1-2, the research community generally adopts one of three pathways to dissolve cellulose. These involve direct dissolution using non-derivatizing solvents,⁶⁶ formation of the stable cellulose derivatives, and formation of the water-soluble cellulose derivatives.⁶⁷

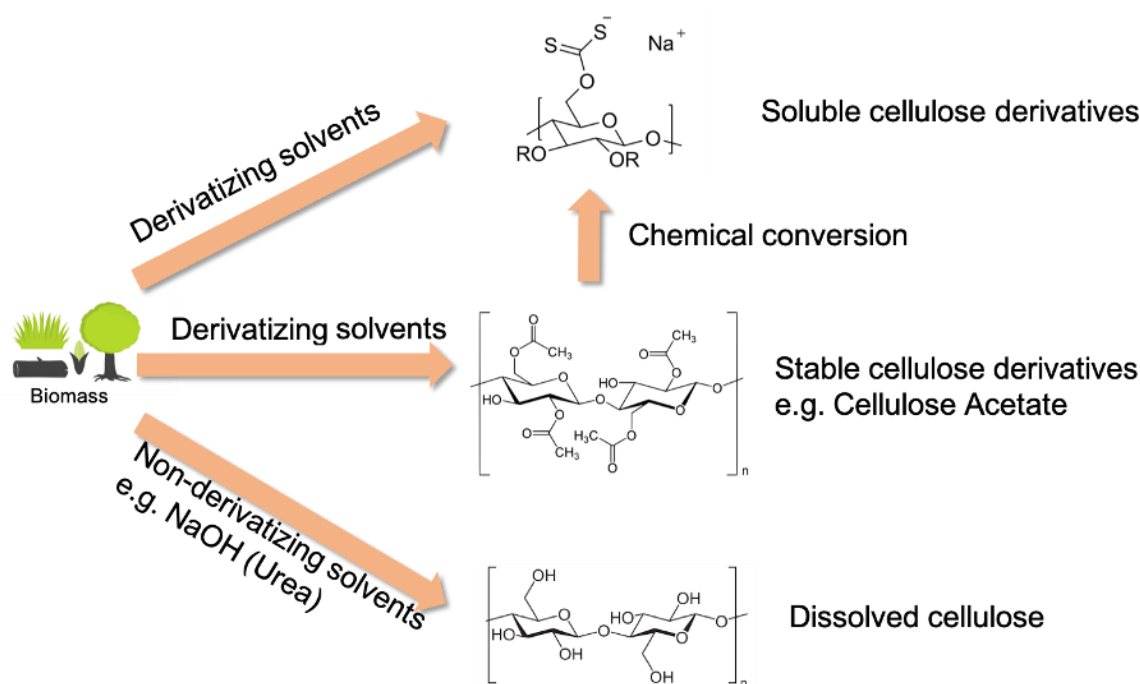


Figure 1-2 Cellulose dissolution methods. (Reprint with permission from ref.⁶⁸ Copyright © 2013, American Chemical Society)

Examples of non-derivatizing cellulose solvents are strong base and ionic liquids, particularly those that are imidazolium-based. Aqueous NaOH solutions with additives such as urea, thiourea, or ZnO are widely used to dissolve cellulose. This technique dates back to 1850 and its invention by John Mercer. Mercerization uses alkali treatment (dissolution) of cellulose to give many beneficial properties to textiles.⁶⁹ In these treatments, hydroxide ion disrupts the native cellulose type I crystallinity and usually forms the more thermodynamically stable cellulose type II crystalline structure if the system is allowed to dry. The polymorphism of cellulose I parallel chains and cellulose II antiparallel chains is shown in Figure 1-3. Ionic liquids are another type of cellulose solvent that show promising potential.⁷⁰ The drawbacks of ionic liquids are the high viscosity of cellulose/ionic liquid solutions, the recyclability of the ionic liquid, and difficulty to remove traces of the liquid.

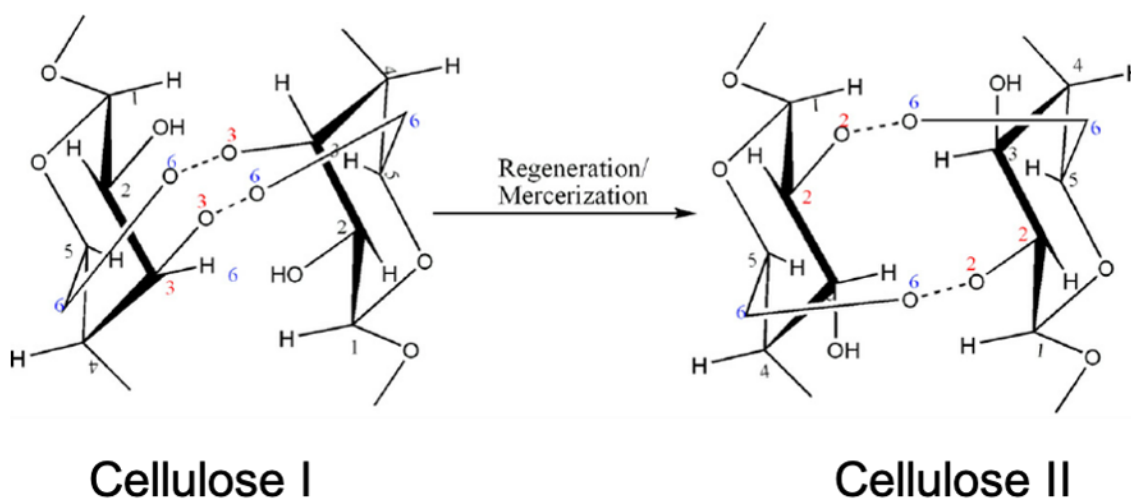


Figure 1-3 Conversion of the polymorphs from cellulose I parallel chains to cellulose II antiparallel chains. (Reprint with permission from ref. ⁷¹ Copyright © 2012, Elsevier B.V. All rights reserved)

Common cellulose derivatives used for making cellulosic microbeads include cellulose acetate,^{72, 73} cellulose triacetate, cellulose phthalate,^{74, 75} cellulose acetate butyrate,⁷⁶ nitrocellulose, cellulose sulfate, methylcellulose, ethylcellulose,⁷⁷⁻⁸² hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl methylcellulose, hydroxypropyl methylcellulose,⁸³ and carboxymethyl cellulose.^{84, 85} We use cellulose acetate (CA) to illustrate the processing of a cellulose derivative (technically, a cellulosic) to make cellulose microbeads. CA is produced by esterification of cellulose with acetic acid anhydride under either homogenous or heterogeneous conditions.⁸⁶ CA is much less crystalline compared with cellulose, exhibiting better solubility in common organic solvents.⁸⁷ The formation of derivatives usually relies on chemical modification of cellulose, which disrupts the native properties of this naturally derived material, making it a rather energy-consuming process. Because some cellulose derivatives are not water-soluble, a chemical conversion step is needed to dissolve them in water.

A shaping method is then used to convert aqueous cellulose to spherical particles. Shaping methods include droplet formation, jet cutting, spinning drop atomization, spinning disc atomization, and dispersion. This step is followed by solidification *via* coagulation, coacervation, freeze-drying, or solvent evaporation. These methods produce particles with sizes ranging from 10 μm to 300 μm . It is rather rare that cellulose microbeads prepared by these procedures generate particles less than 10 μm in size.

Evidently then, cellulose microbeads are made first by dissolving native cellulose, followed by a shaping method. Despite its cost effectiveness, dissolution disrupts the native cellulose crystalline structure. The use of organic solvents to shape the microbead yields a contaminated particle that must be freed of solvent. Purification is challenging for industrial mass production, hence, there is pressure to use green chemistry and green engineering principles to find alternative methods to make cellulose microbeads. In Section 1.2. and 1.3 below we describe naturally sourced cellulose materials, and how we can utilize CNC derived from cellulose fibers to make new types of cellulose microbeads.

1.1.2. Cellulose

Cellulose consists of β -(1 $\rightarrow$ 4)-glycosidically linked glucose units (Figure 1-4). The anhydroglucose (AGU) unit is the repeating unit of cellulose that contains three reactive hydroxyl groups, a primary one at position 6, and two secondary ones at positions 2 and 3.

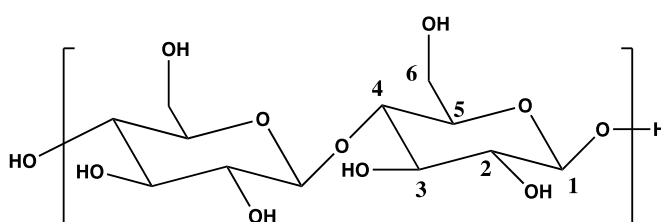


Figure 1-4 Chemical structure of Cellulose.

Cellulose is a linear chain polymer. Its multiple inter-molecular and intra-molecular hydrogen bonds provide enough force to hold the chains together side-by-side, forming microfibrils with high tensile strength.

Cellulose can adopt different crystalline structures. Their difference originates from the orientation of hydrogen bonds between and within the cellulose chains. Native cellulose exhibits the cellulose I structure with two different polymorphs: structure I_α and structure I_β . Dissolution with strong base or ionic liquids interrupts the native cellulose type I structure, yielding regenerated cellulose fibers typically with type II crystallinity. The conversion of cellulose I to cellulose II is often irreversible. Cellulose II is a more thermally stable phase. The structures are shown in Figure 1-3.

Wood is the most important source of cellulose but cellulose could also be derived from agriculture residues, water plants, grasses, algae, bacteria, fungi, tunicates and so on. In nature, cellulose plays important biological role. For example, cellulose is an essential structural cell wall component in plants. The coexisting amorphous and crystalline features of cellulose fibres make the cell wall rigid and tough. Cellulose also serves as an energy source for certain animals like ruminants and termites and serves as a dietary fiber for humans. Certain types of bacteria produce cellulose to form protective envelopes around the cells. Tunicates are the only known animal source for cellulose with high crystallinity, which acts as mechanical support for the body. Nature has evolved a supramolecular motor called the cellulose synthase complex (CSC) to manufacture its own “specialty cellulose”. The CSC does so with great fidelity, producing uniform nanocrystalline domains of cellulose interlocked by amorphous β -1,4-linked glucan chains. Figure 1-5 shows the amorphous and crystalline regions in cellulose microfibrils. Nanocrystalline cellulose building blocks are crucial for the cell to support the sizeable internal osmotic pressure necessary to withstand the pull of gravity.

By chemical, mechanical, or biological treatment of cellulose samples, the amorphous part can be removed, leaving largely rod-like cellulose nanocrystals. In this thesis, instead of putting energy into breaking up the crystalline structure of cellulose to manufacture specialty cellulose, we intend to co-opt the output of nature's supramolecular machinery and investigate how its cellulose nanocrystal building blocks can be recruited to build cellulose microspheres. This approach has the potential to make microbeads with wide-ranging functionalities.

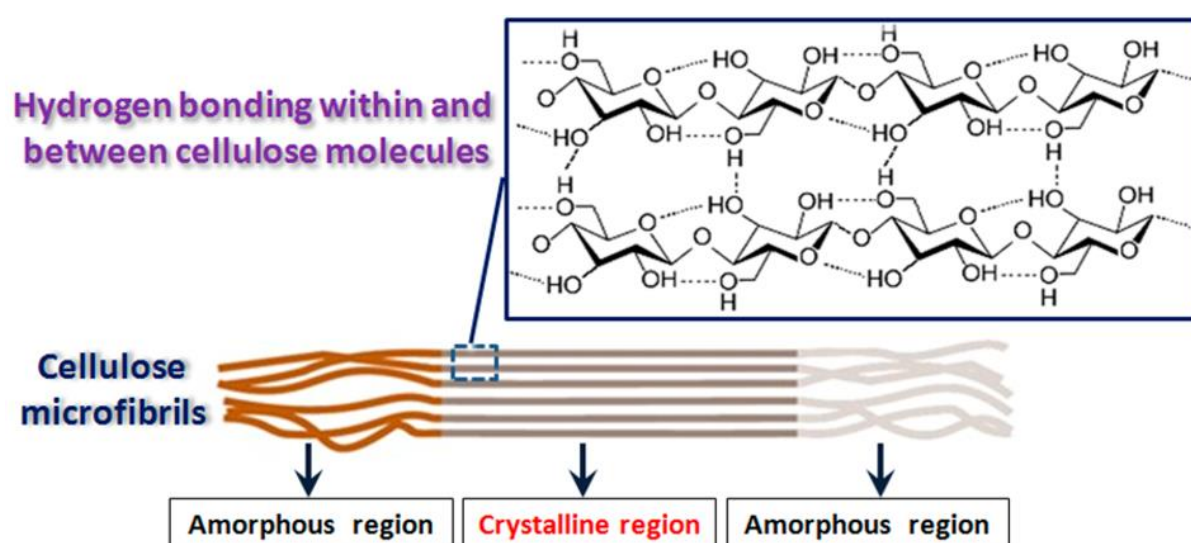


Figure 1-5 Schematic of amorphous and crystalline regions of cellulose microfibrils. (Reprint with permission from Zhou *et al.* ©2012 The Author(s). Licensee IntechOpen)

1.1.3. Crystalline Nanocellulose

Preparation

Synthesis of CNC requires preferential hydrolysis of the amorphous regions of cellulose, liberating the nanocrystals. Hydrolysis is usually achieved with concentrated sulfuric acid.⁸⁸ When hydrolysing and oxidizing the amorphous part of cellulose to generate CNC, the sulfuric acid also esterifies the surface of CNC to produce sulfate groups in the C6 position. Other acids

will hydrolyse cellulose to yield CNC. Examples include hydrochloric acid,⁸⁹ hydrobromic acid,⁹⁰ phosphoric acid,⁹¹ or a mixture of nitric acid and acetic acid.⁹² The sulfuric acid method has several disadvantages despite the fact it has been scaled to a mass production. Corrosive sulfuric acid increases the cost for equipment and operations. Handling of the acid also enhances the number and type of safety precautions, including waste management. The generated CNC contains sulfate surface groups, which are easily chemically transformed. The sulfate moiety is unstable at elevated temperatures. In recent years, non-acid hydrolysis methods have been explored by scientists, including the use of TEMPO,⁹³ ammonium persulfate,⁹⁴ ionic liquids,⁹⁵ enzyme,⁹⁶ aqueous hydrogen peroxide,⁹⁷ citric acid,⁹⁸ and deep eutectic solvents (DES)⁹⁹. In particular, TEMPO oxidation and aqueous hydrogen peroxide aqueous solution hydrolysis generate CNC with surface carboxylic acid functional groups. The carboxylic acid functional group is more versatile for chemical modification than the sulfate group. Aqueous hydrogen peroxide method is regarded as a more sustainable medium, with hydrogen peroxide being fully consumed in the process and the by-products being only water and largely hydrolysed sugar units.⁹⁷

Properties

CNC particles typically range in size from 3 - 20 nm (width) and 100 - 300 nm (length). The size depends on the origin of the cellulose, as well as the preparation conditions. As a high aspect ratio, rod-like material, CNC possesses unique mechanical and optical properties. These properties have been widely documented.¹⁰⁰⁻¹⁰⁶

Mechanically, since CNC originates from the crystalline part of cellulose, its elastic modulus and tensile strength depend on the crystallinity of the samples, defects in the nanocrystals, dimensions of the rods, and the chain directions of the CNC.¹⁰⁰ The crystallinity of cellulose is usually determined *via* X-ray diffraction (XRD), where it is calculated as the ratio of the areas

of the crystalline and amorphous regions in the diffractograms.¹⁰⁷ Currently, the quantification of CNC mechanical properties relies on AFM indentation studies, vibrational spectroscopy, X-ray diffraction, and computational modelling.

Tanaka and Iwata used molecular mechanics simulation to estimate the elastic modulus, yielding results on the order of 124 GPa and 155 GPa.¹⁰⁸ Their calculation assumes a linear relation between the changes of energy density of deformed crystals and the half of the square of strains under compressive conditions. A coarse-grain molecular dynamic simulation model was adopted by Majid and Bhnoush to calculate the tensile strength of CNC to be ~ 7.5 GPa under the influence of twist and interface energy.¹⁰⁹

Crystal deformation of cellulose I was first studied by Sakurada *et al.* via XRD. They measured elastic moduli of around 70 - 90 GPa, significantly smaller than the theoretical estimates of Tanaka and Iwata.¹¹⁰ Differences in the elastic modulus for different polymorphs of cellulose crystals were examined by Nishino *et al.*¹¹¹ They found that the elastic modulus decreased significantly from 138 GPa to 88 GPa between cellulose I and cellulose II.

Atomic Force Microscopy (AFM) can measure the elastic modulus of cellulose crystals. One method requires that CNC bridge a groove. The AFM tip is then pushed on the nanorods to generate force-displacement curves. Depending on cellulose source, the measured elastic modulus varied from 78 GPa for bacterial CNC¹¹² to 150.7 GPa for CNC from tunicates.¹¹³

Figure 1-6 shows that CNC crystals in water can self-assemble at certain concentrations to form phase-separated layers: an isotropic upper layer and anisotropic chiral nematic bottom layer. The chiral nematic phase has motivated liquid crystal applications,¹⁰² and their use as nano-moulds for novel aerogel materials with chiral periodicity.¹¹⁴ Slow evaporation of water from the CNC liquid crystal phase yields a solid film with helicoidal structure and iridescent colours. This photonic property can be further tuned by adjusting the helicoidal pitch. The

chiral nematic texture has been utilized to imprint chirality into silica, hydrogels, and resins for applications in photonics.^{101, 115}

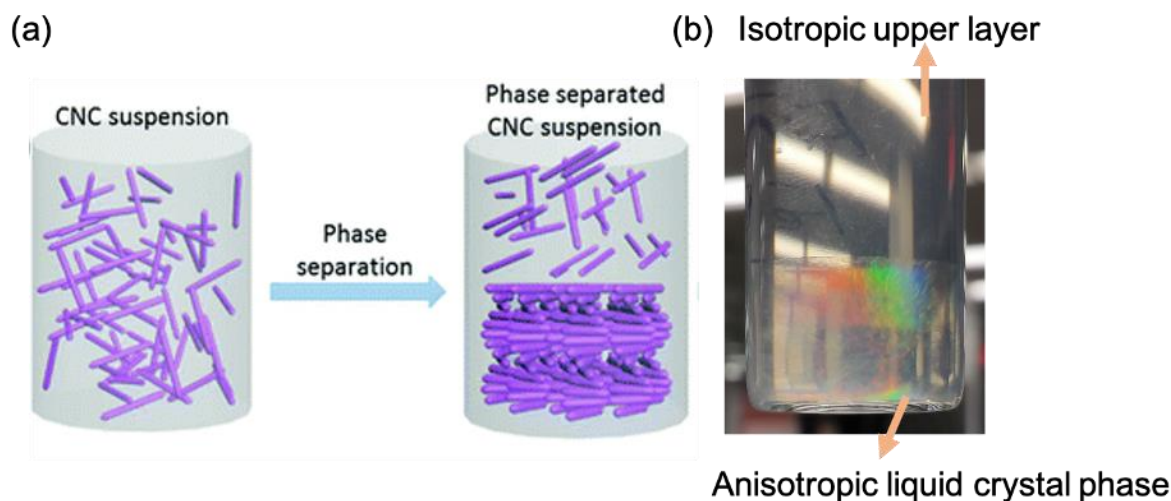


Figure 1-6 Liquid crystal formation of CNC. (a) Schematic illustration of CNC phase separation and formation of a helicoidal (chiral nematic) liquid crystal phase. (b) Iridescence from a chiral nematic suspension of 2.5 wt %CNC. (Reprint with permission from Xu *et al.*¹¹⁴, ©Royal Society of Chemistry 2018)

Chemical functionalization

The properties of CNC can be elaborated *via* chemical functionalization. Surface hydroxyl groups on the CNC can be converted to ethers and esters.^{116, 117} Silylation and urethanization can also be achieved using hydroxyl groups on the surface. Much of the chemical transformations are heterogeneous because of the insolubility of CNC, meaning that highly polar solvents like DMF, or aqueous alkali solutions must be used.¹¹⁷ Most CNC comes equipped with either sulfate or carboxylate moieties resulting from the chosen hydrolysis method.¹⁰⁰ The presence of the carboxylic acid group opens up new opportunities for surface functionalization in water. With sulfated CNC, carboxylation can also be achieved *via* TEMPO oxidation.¹¹⁸ Figure 1-7 illustrates the potential for chemical modification of CNC. Polymer

grafting is indicated in the scheme by the conventional “grafting to” or “grafting from” techniques. Click chemistry is a common transformation.^{119, 120} Click reactions make good use of Cu(I) catalysed 1,3-dipolar cycloadditions of an azide and an alkyne. Polymers and small molecules can be grafted by this means to give products with enhanced thermal stability and hydrophobicity.¹²¹

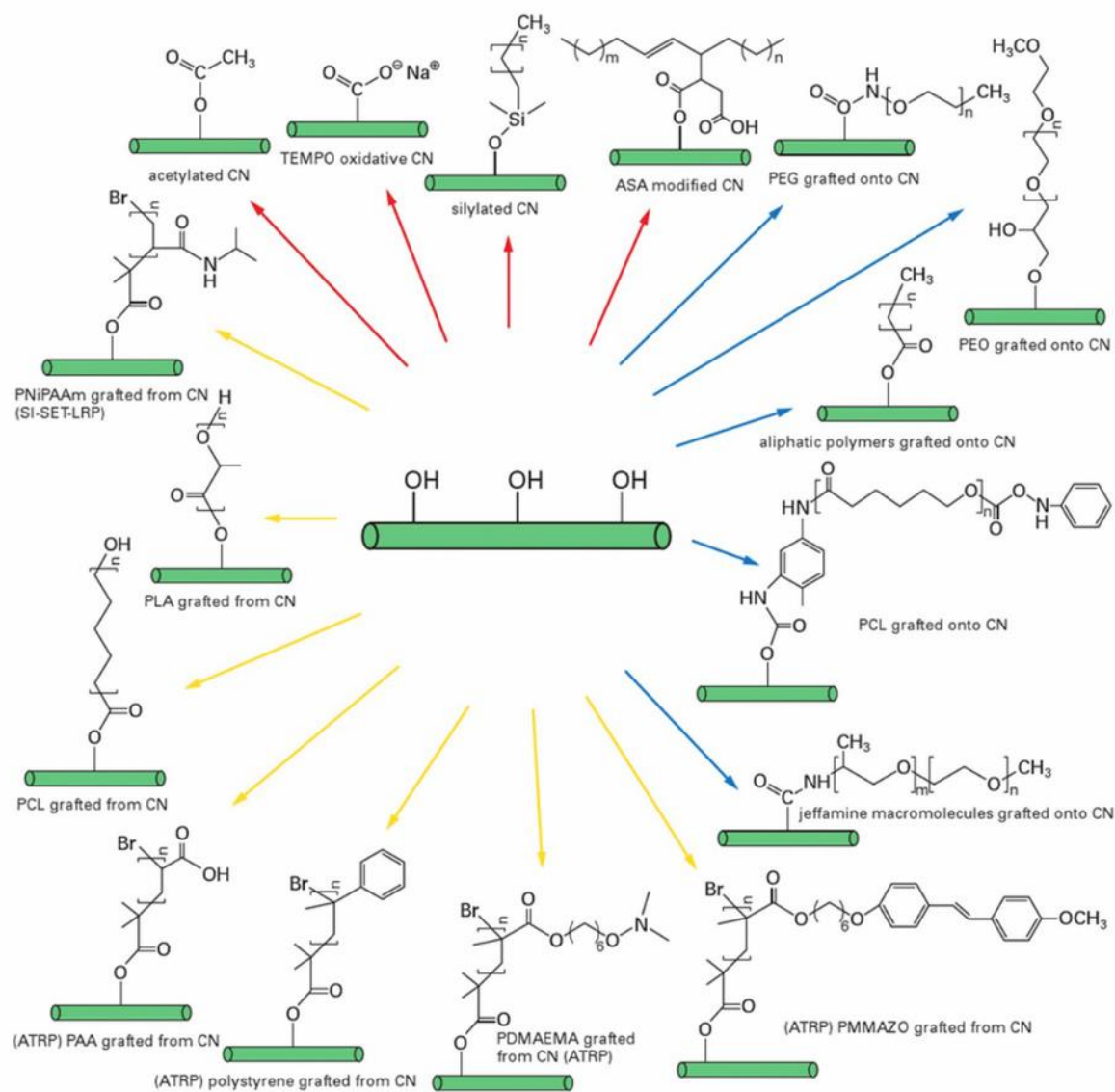


Figure 1-7 CNC surface chemical modification pathways. (Reprint with permission by ref,¹²²

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1.1.4. Nanocellulose microbeads

Nanocellulose and nanocellulose composite microbeads can be made by self-assembly,^{123, 124} spray-freeze-drying, and ultrasonic atomization-freeze drying. Microbeads so produced have been studied for applications in membrane separation systems,¹²⁵ as cell culture scaffolds,¹²⁶ and as absorbents for water treatment.¹²⁷ Self-assembly to make CNC microbeads from micro-sized water droplets by a microfluidics suffers from its lack of efficiency; the method is expensive and difficult to scale up.^{123 124}

Spray-freeze-drying and atomization-freeze-drying yield porous microbeads with high surface area that may be used as absorbents for water treatment. Crosslinking is usually necessary to prevent particle break-up in solvent media.^{126, 128 129}

Spray-drying has been used to make CNC microbeads. Lucia *et al.* demonstrated how the technique yields CNC-lignin composites. The resulting “donut” shaped microparticles showed a superhydrophobic effect.¹³⁰ Spray-drying to make CNC microbeads offers a flexible entry into complex compositions of CNC microbeads. There are several advantages to the technique. It offers a one-step, water-based approach that can be implemented in ways that adhere to several of the principles of green chemistry. Spray-drying is a well-known scalable industrial method that lends itself to high volume production, and the method can accommodate functional polymers to generate new and desirable physical properties in microparticles.

1.2. Spray-drying

1.2.1. Definition and Mechanism

Spray-drying is a particle production process that originated around 1860 and was subsequently patented by Samuel Percy in the United States in 1872. This technique converts a fluid that

contains suspended materials into powders. Figure 1-8 below shows a schematic of the spray-drying process.^{131, 132}

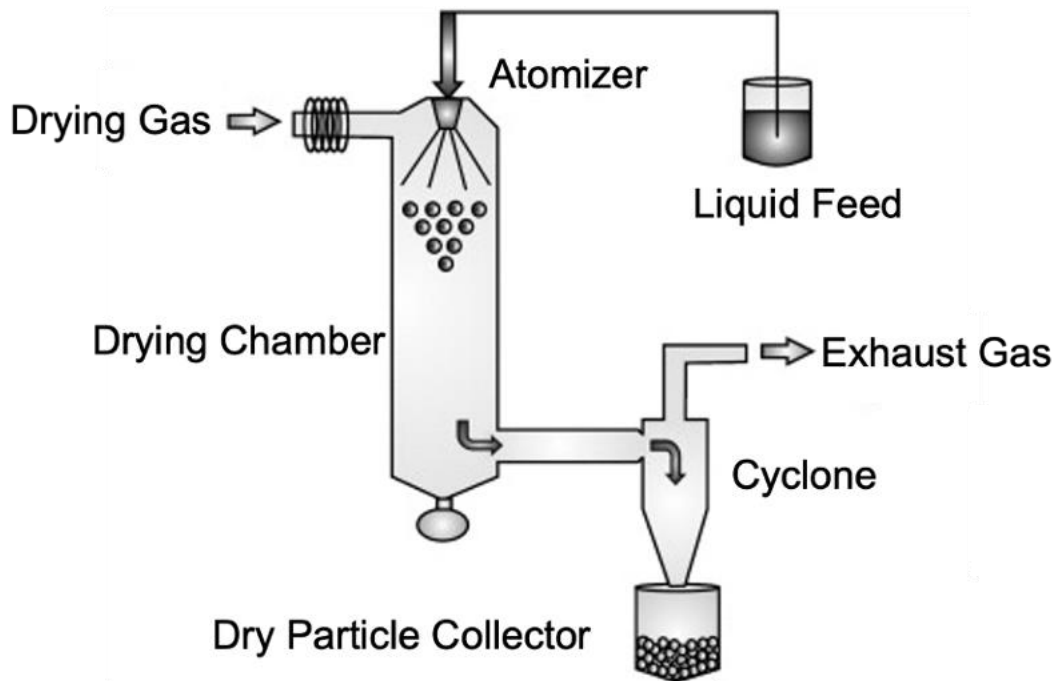


Figure 1-8 Schematic representation of a spray-drying apparatus. (Reprint with permission from ref,¹³³ Copyright © 2015 Elsevier B.V. All rights reserved.)

Materials to be dried must be well-dispersed or dissolved in a liquid feed vessel, with careful attention to viscosity. The liquid is then pressurized as it enters an atomizer, where water droplets are formed and sprayed into the drying chamber. Different types of atomizers give different sizes of water droplets. The drying chamber may pre-set to a temperature, often above 100 °C depending on the thermal stability of the product. The inner temperature setting depends primarily on the thermal stability of the materials. Researchers have found that the drying temperature correlates with particle morphology,¹³⁴ moisture content, and density.¹³⁵ In the drying chamber, water rapidly evaporates when encountering the high temperature, leaving the material to assemble into micro-particles. The dried particles are collected in the dry particle collector.

Several research groups have simulated the spray-drying process to understand its complexity. Spatially resolved single droplet drying models are one of the most common simulation models.¹³⁶ The development of such models has provided insight into factors that control particle morphology.

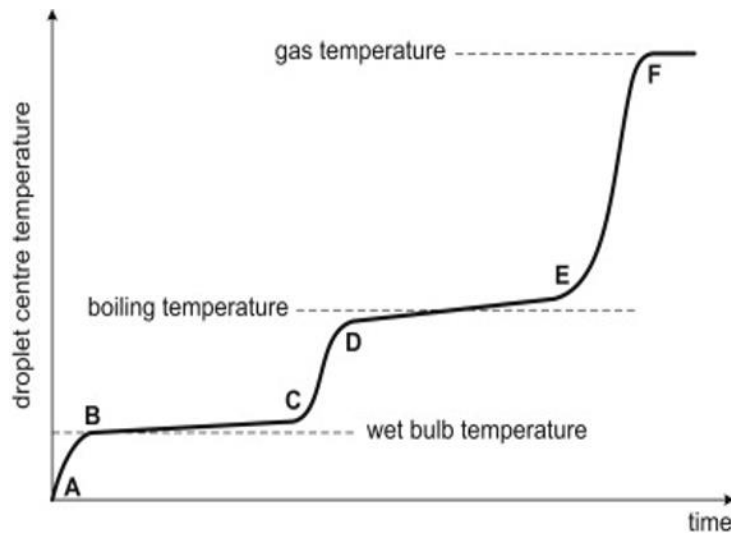


Figure 1-9 Temperature evolution during drying kinetics of a sprayed liquid droplet. (Reprint with permission from ref,¹³⁷ Copyright © 2008 Elsevier Ltd. All rights reserved.)

Figure 1-9 shows an idealized temperature profile at the center of a water droplet within the drying time in the spray chamber.^{137, 138} Period A-B occurs corresponding to the period over which droplet heats rapidly to the so-called wet-bulb temperature. This is the lowest temperature when water only evaporates. B to C, denotes a constant rate period, where the temperature in the center of the water droplet is assumed to remain unchanged, and the drying process is at a near-constant rate. During this period, the surface remains saturated with moisture. When the amount of moisture cannot saturate the surface, the temperature of the droplet center increases much faster. This period (C to D) is called the falling rate or second drying period. C-D occurs at a critical moisture content and is associated with the start of skin formation at the outer surface of the drying particle.¹³⁸ During the time D to E, vaporisation

starts when the droplet temperature reaches the solvent boiling point. The drying rate is then controlled by external heat transfer to the particle. When the free moisture has been removed, the temperature rises again until it equals the temperature of the surrounding environment.

The final properties of the particles are affected by parameters such as drying air flow rate, air humidity, inlet temperature, atomization air flow, feed rate, solid concentration in the feed, additional organic solvent, nozzle type, etc.¹³² Figure 1-10 illustrates the complex interplay of these parameters and the effect of outlet temperature on particle size, final product moisture content, and drying efficiency. In particular, particle size depends strongly on atomizing air flow and solids concentration in the feed liquid and the forming droplet. Additional solvent and feed rate have more indirect influence. Section 2.4. describes the factors that affect the particle size and morphology in more detail.

		OUTLET TEMPERATURE	PARTICLE SIZE	FINAL PRODUCT MOISTURE	EFFICIENCY		
INCREASING THESE VARIABLES	Drying air flow rate	Lower heat losses of the inlet energy	-----	Lower partial pressure of evaporated water	Better separation in cyclone		
	Air humidity	More energy contained in moisture	-----	Higher partial pressure of drying air	More moisture may lead to adherence of the product to chamber walls		
	Inlet Temperature	Direct proportion	-----	Lower relative humidity of air	Eventually dryer product prevent adhering		
	Atomizing air flow	Higher amount of cold air to be heated	Higher amount of available energy for atomization	-----	-----		
	Feed rate	More solvent to be evaporated	More liquid to disperse	Higher amount of water leading to its higher partial pressure	Depends on application		
	Solid concentration in feed	Less water to be evaporated	More solid available for particle formation	Less water evaporation, lower partial pressure	Bigger particles are easier to separate in cyclone		
.....	Organic Solvent (instead of water)	Less energy required for evaporation	Lower surface tension. More available energy to spend on particle fission	Lack of water in feed leading to very dry product	Lack of hygroscopicity results in easier drying		
		Minor increase	Moderate increase	High increase	Minor decrease	Moderate decrease	High decrease

Figure 1-10 Ways in which spray-drying parameters influence particle size, final product moisture, and drying efficiency. (Reprint with permission from ref, ¹³⁹ © 2017 The Author(s). Licensee, IntechOpen)

1.2.2. Particle Morphology

Particle morphology is determined by numerous factors. Besides the material itself, drying temperature and liquid feed concentration have significant impact on morphology. Figure 1-11 illustrates how particle morphology changes through the interplay of different factors.¹³⁷ From the figure, we conclude that the drying temperature directly influence the final particle morphology. Secondly, the formation of a dry or wet shell determines if internal bubble nucleation will occur, which directly impacts morphology. Referring to this figure, we can select variables to tailor the particle morphology to fit a specific application by adjusting the spray-drying parameters.

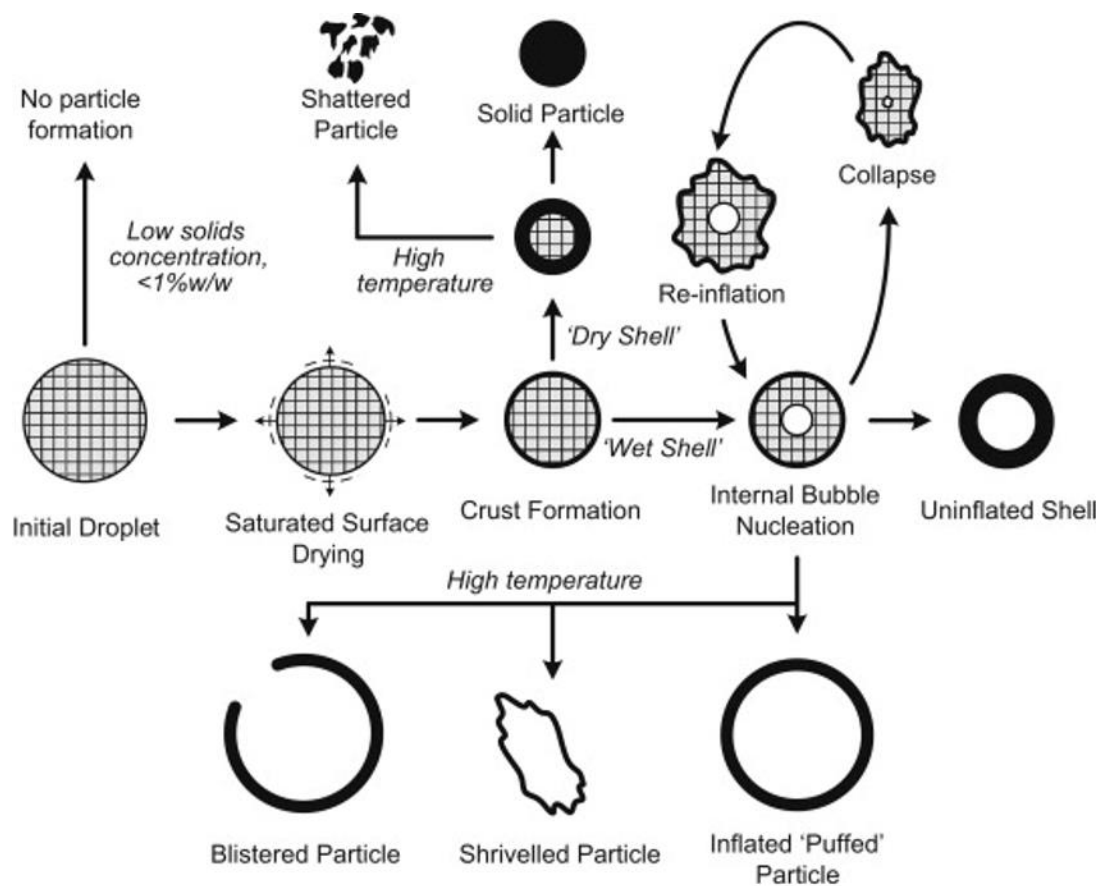


Figure 1-11 Schematic illustrating of how spray-drying parameters influence particle morphology. (Reprint with permission from reference¹³⁷. Copyright © 2008 Elsevier Ltd. All rights reserved.)

Competition between the particle diffusion and convection has significant impact on morphology. The competition can be quantified through the dimensionless Péclet number (P_e), defined in Equation (1).¹⁴⁰⁻¹⁴²

$$P_e = \frac{R^2}{Dt_{dry}} \quad (1)$$

In this equation, R is the radius of droplet, t_{dry} is the drying time, and D is the diffusion coefficient of the suspended particles.

For a spherical nanoparticle, D can be calculated from the Stokes-Einstein equation (2).¹⁴³

$$D = \frac{k_B T}{6\pi\eta r} \quad (2)$$

where, k_B is the Boltzmann constant, η is the viscosity of the solvent, T is the absolute temperature, and r is the effective hydrodynamic radius of a diffusing particle. The Péclet number can also be described in another way as shown in Equation (3).

$$P_e = \frac{\kappa}{8D} \quad (3)$$

In this equation, κ is evaporation rate, and D is the diffusion coefficient.

From Figure 1-12, when the Péclet number is low, diffusion dominates, leading to an evenly distributed particle concentration through the water droplet. During water evaporation, the droplet shrinks, to yield a dense particle, perhaps with irregular surfaces. In contrast, a high Péclet number results in convective transport. In this case, solute accumulates at the air-water interface. For CNC, based on Equation (2), the diffusion coefficient is calculated as $0.542 \times 10^{-11} \text{ m}^2/\text{s}$. The Péclet number of CNC nanoparticles is then calculated as $\sim 10^{-3}$ (using a drying time of $\sim 6 \text{ s}$, $r = 180 \text{ nm}$). This locates CNC in the range of low Péclet numbers. This value

was calculated when assuming spherical nanoparticles of CNC. For CNC as nanorods *in a dilute regime*, the translational diffusion coefficient of $D \sim 0.2 \text{ to } 7 \times 10^{-12} \text{ m}^2/\text{s}$ again yields a small Péclet number, $P_e \sim 7.7 \times 10^{-4}$. These Péclet numbers are misleading. With rapid water evaporation, the CNC particles will interact and probably transition from a sol to a gel phase. Accordingly, the diffusion coefficient will increase rapidly, shifting the Péclet number from low to higher values. Hence, spray-dried CNC microbeads are more likely to form a spherical shell with irregular surfaces. In Chapter 2-5, the morphologies of cCNC-based microbeads are revealed *via* SEM. We describe the cCNC microbead morphology in Chapter 2 (Figure 3) and Chapter 4 (Figure 5). Figure 5 in Chapter 4 reveals how the concentration of cCNC in water suspension affects spray-dried particle morphology both internally and at the surface. According to Equation (1), the drying time t_{dry} for pristine CNC microbeads plays a major role in determining morphology and porosity.

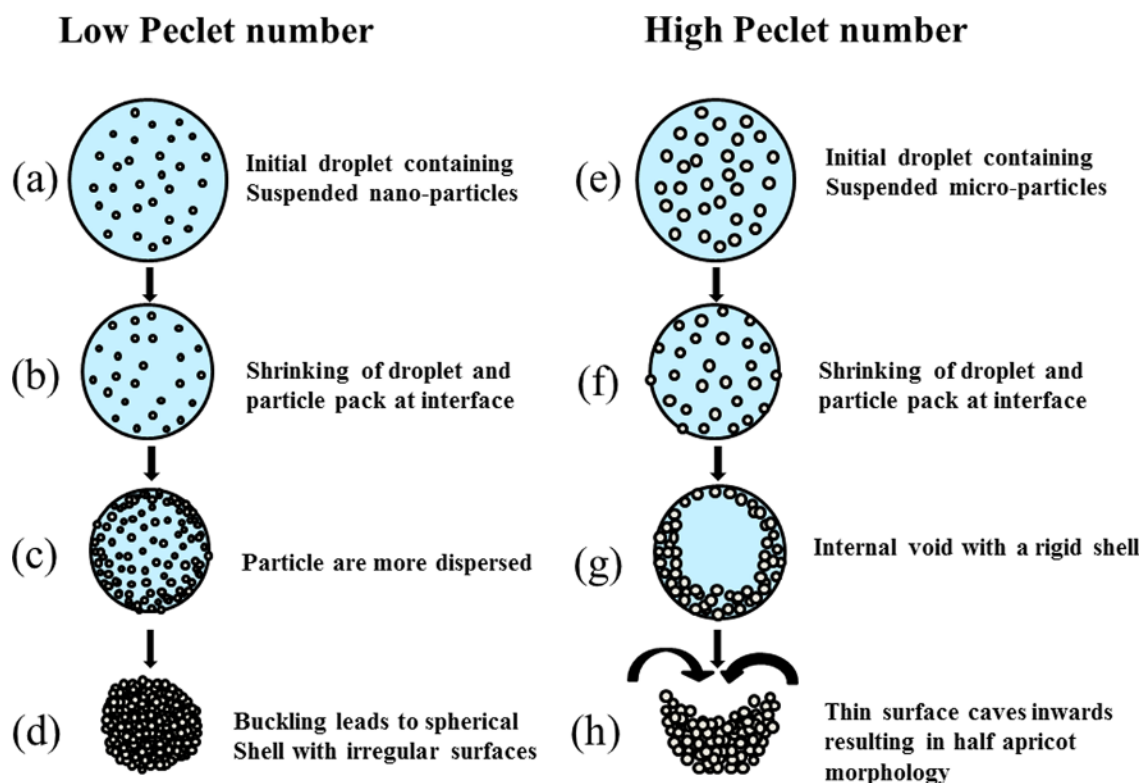


Figure 1-12 Demonstration of the influence of Péclet number on particle formation. (Reprint with permission from ref, ¹⁴² Copyright © 2017 American Chemical Society)

1.2.3. Composite Microbeads

Composite particles are defined as particles that are composed of several materials, giving unique or enhanced physical properties. Spray-drying enables the production of microbead composites. Okuyama *et al.*¹⁴⁴ have summarized the outcome of mixing two components *via* spray-drying. They categorize three different outcomes as shown in Figure 1-13: particles with homogeneously distributed components, microencapsulated particles, and “hairy” particles. The spatial distribution of the two components after spray-drying is related to their movement rate relative to the air-water interface during the spray-drying process, as well as the fractions of each component.¹⁴⁵ When the amount of two components in the mixture is equal, spray-dried particles with evenly distributed components can be obtained. But when the fractions are different, microencapsulation can occur, with one component coating the other. The rate of

material movement inside of a water droplet can be controlled by the diffusion coefficients and the relative sizes of the components. When the two components have quite different diffusion coefficients, it is more likely that the two components will be distributed heterogeneously in the final particles. In Chapter 3, hybrid microbeads made from poly(acrylic acid) (PAA) and CNC are discussed with respect to their spatial distributions after spray-drying. In Chapter 5, we describe how silk fibroin (SF) and CNC are mixed to make SF-CNC composite microbeads. With XPS depth profiling, the spatial distributions of the two components are revealed. PAA-CNC microbeads are intimately mixed, though not necessarily homogeneously. SF protein concentrates more on the surface than in the interior of SF-CNC microbeads.

Hybridization *via* spray-drying to create composite microbeads is a versatile method to introduce desired physical properties in microbeads. The term hybrid means to blend at a very small scale to make materials with enhanced or new properties. In chemistry, hybrid materials usually refer to the mixing of inorganic and organic components.¹⁴⁶ With spray-drying, inorganic clusters or nanoparticles with specific optical, electronic, or magnetic properties can be hybridized with organic polymer matrices.¹⁴⁷ For example, Costa Neta *et al.* discussed the role of silica when making a composite microbead of chitosan-silica *via* spray-drying.¹⁴⁸ The results showed that moisture affinity and acid stability of the microbeads depended on silica content. Choi *et al.*¹⁴⁹ made a composite microbead using NiO nanosheets and polyaniline (PANI) polymers for use as asymmetric superconductors. These hybrid PANI materials exhibited more polymer-like properties, making the hybrids easier to process and handle. In this thesis, we use hybridization *via* spray-drying to create cCNC-poly(acrylic acid) microbeads (Chapter 3) and cCNC-silk fibroin microbeads (Chapter 5).

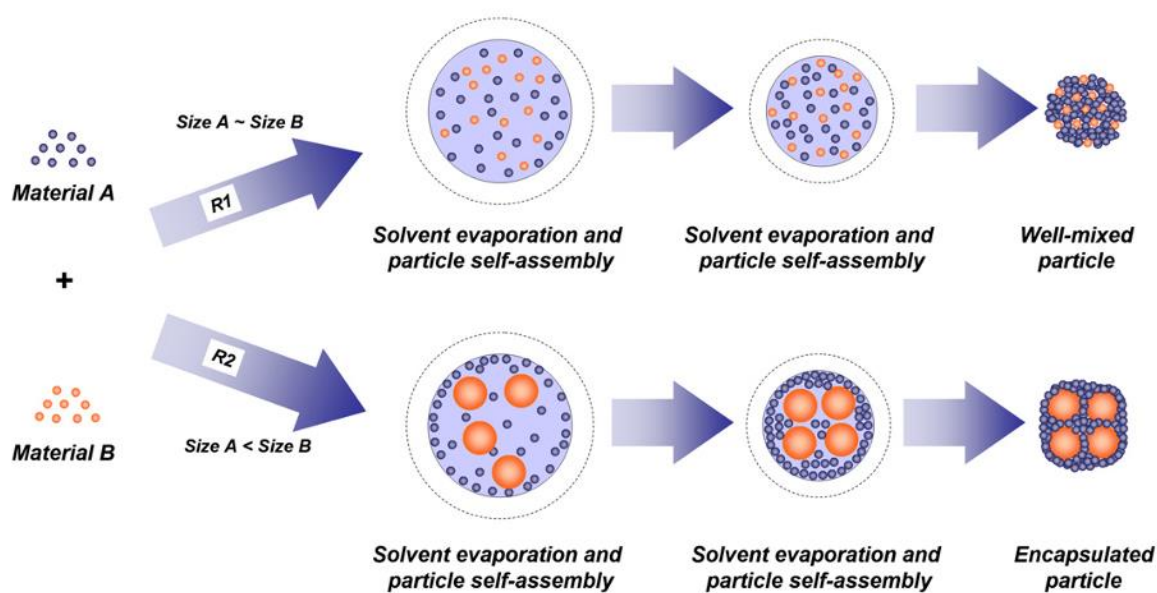


Figure 1-13 Composite particle formation *via* spray-drying with the outcome of the particle determined by a variety of factors. (Reprinted with permission from ref, ¹⁴⁴ Copyright © 2010 The Society of Powder Technology Japan. Published by Elsevier B.V. All rights reserved.)

1.2.4. Particle Size

Particle sizes vary depending on spray-drying conditions. The size of the particle influences mechanical properties in both dry states and fluid flows. It also affects whether the particles pack as powder or as tablets. In cosmetics, particle size determines dry powder softness and optical effects. In pharmaceutical applications, the efficiency of drug molecule encapsulation is highly influenced by the particle size.¹⁵⁰

Spray-drying produces polydisperse particles.¹⁴¹ Therefore, the size distribution is one of the critical characterization parameters for spray-dried particles. Since spray-drying usually gives distorted spherical particles, an average geometric diameter is adopted to represent the particle size.

Particle size can be measured by counting with an optical microscope by sizing with laser diffraction to measure geometrical dimensions.¹⁵¹

The geometric diameter of a spray-dried spherical particle is expressed as a function of formulation and processing variables as shown in Equation (4).¹⁵²

$$d_g = \sqrt[3]{\frac{c_F}{\rho_P}} d_D \quad (4)$$

In this equation, c_F is the concentration of the feed solution, ρ_P is the particle density, and d_D is the diameter of the liquid droplet. This equation shows that the particle diameter is directly affected by the concentration of the feed solution and the atomization process, which controls the diameter of the water droplet. Currently, four different types of atomizers are available: rotary atomizers, pressure nozzles, two-fluid nozzles, and ultrasonic atomizers. Different atomizers give different particle diameters.

1.2.5. Encapsulation via Spray-drying

Spray-drying has been used to encapsulate active ingredients, heat-sensitive substances, and small molecules in the applications in the food industry,¹⁴⁵ pharmaceuticals,¹⁴¹ and agriculture.¹⁵³ Encapsulation is used primarily to protect substances from the external environment, such as temperature, humidity, and UV radiation. Encapsulation creates a protecting layer for active ingredients. It helps to decrease the mass transfer rate of the core guest material to the outside environment. It can also mask undesired properties of the active component, such as odor, or chemical properties, like catalytic activity.

Spray-drying to encapsulate drug molecules for controlled release benefits from its one-step simplicity compared with other encapsulation methods, like coacervation, fluidized bed

coating, or extrusion. For encapsulation, drug molecules and organic polymer matrix are co-dissolved in a common solvent, preferably water, and spray-dried. Upon solvent evaporation, the polymer traps crystallites precipitated from solution or particles from suspensions. Aqueous solvents are preferred to avoid toxicity and improve sustainability and safety when spray-drying, but a small amount of organic solvent can be necessary to disperse hydrophobic drug molecules.¹⁵⁴

Cellulose derivatives like cellulose acetate,¹⁵⁵ oxidized cellulose,¹⁵⁶ bacterial cellulose,¹⁵⁷ sodium carboxymethyl cellulose,¹⁵⁸ and nanofibrillar cellulose¹⁵⁹ are commonly used as excipients for drug encapsulation *via* spray-drying. Evaluation of the success drug encapsulation includes drug encapsulation efficiency, drug release kinetics, and the percentage of burst release.^{154, 160}

Bioactive protein and peptide encapsulation for the food industry, agriculture, and pharmaceutical applications can also be realised using spray-drying. Protein-based drugs are becoming more and more important in the development of vaccines,¹⁶¹ and for gene therapy.¹⁶² Bioactive peptides and proteins are chemically unstable and may be highly hygroscopic. Encapsulation of bioactive ingredients improves stability, enhances shelf life, and maintains functionality like antioxidant activity.¹⁶³

In Chapter 3, we show that CNC-PAA hybrid microbeads with encapsulated fluorescent dye molecules can be imaged using super-resolution microscopy. This method reveals the 3-D spatial distribution of dye molecules in the microbeads. This method helps understanding how spray-drying encapsulates small molecules. The spatial distribution of small molecules is an important boundary condition to predict release kinetics and diffusion properties in microbeads.

1.3. Microbead Physical Properties

1.3.1. Surface Tension

In a liquid, surface tension is a physical force at the surface that arises due to the atoms of the liquid interacting through various forces with neighbouring atoms in all directions.¹⁶⁴ Surface tension has the dimension of force per unit length, or of energy per unit area. In materials science, surface tension is used to evaluate the interactions among material components and the air-solvent interface. Surface tension plays an essential role when evaluating materials for coatings, when dispersed in solvents, as precursors for dissolution, and in other practical applications.

It is difficult to measure the surface tension of solid materials directly. Indirect methods are usually adopted, with tensiometry and contact angle measurement among the most widely practised.¹⁶⁵ Tensiometry measures the dynamic surface tension.¹⁶⁶ Tensiometers include the force tensiometer, bubble pressure tensiometer, drop volume tensiometer and spinning drop tensiometer. Among them, the force tensiometer is best suited to measure the surface tension of microbead powders. In this technique, the relation between tensile force and the wetted length of a powder sample is recorded, from which the surface tension can be derived.

The contact angle can also be used to quantify surface tension. The contact angle is defined as the angle that is formed by the liquid of interest on a particular solid surface at the gas-liquid-solid interface.¹⁶⁷ It is one of the most common methods to quantify the wettability of a surface or a material. If the contact angle between a liquid and a solid is less than 90°, the liquid will wet the surface and spread. When the contact angle is larger than 90°, the affinity between the liquid and the surface is low and the droplet will stay at the surface as a bead. When the liquid is water, the contact angle can be used to differentiate hydrophilic from hydrophobic materials.

A common method to measure contact angle is the sessile drop technique. Here, a liquid drop is placed on a surface, and the contact angle is measured directly at the three-phase (liquid/solid/vapor) contact line of the formed drop.¹⁶⁸ This technique is straightforward for flat films, but it is not suitable for powder materials for several reasons. Firstly, the particles have to be packed into a tablet or a film. The liquid may penetrate through the loosely packed powders, making the contact angle difficult to measure. Secondly, the particle may swell when encountering a liquid droplet, leading to inconsistent measurement. Thirdly, the measured contact angle represents the surface energy of the film or the tablet from the particles, but it may not represent the real surface energy of a microbead. Thus, several indirect methods have been developed to measure the contact angle of powder materials. These include the Wilhelmy plate method, column wicking, thin-layer wicking, capillary pressure, drop penetration, dynamic contact angle, AFM, and Environmental Scanning Electron Microscopy (ESEM). Alghunaim *et al.* have reviewed and compared the available methods for wettability measurement.¹⁶⁹ The most challenging aspect is that the contact angle of a powder might deviate from the actual contact angle of the microbead. AFM and ESEM offer approaches that can yield contact angles of single particles, but repeatability remains a challenge.

Besides the contact angle, researchers also define the surface tension of microbeads using hydrophobicity, hydrophilicity, and lipophilicity. Hydrophobicity and hydrophilicity describe the affinity of the microbeads with water. Lipophilicity is defined as the ability to dissolve a chemical compound or a material in non-polar solvents (hexane, toluene, for example) and in fats, oils and lipids.¹⁷⁰ The lipophilicity of a microbead describes its affinity with these solvents.¹⁷¹ Cellulose microbeads are usually hydrophilic due to the abundance of hydroxyl groups on the surface that form hydrogen bonds with water and other polar solvents. Microplastic beads are usually hydrophobic, making them well dispersed in the oil phase when forming an emulsion. Several methods are developed to confer hydrophobic character on CNC.

Lin *et al.* have designed a two-step silanization method to graft dodecyltrimethoxysilane (DTMOS) onto CNC.¹⁷² A single step, organic solvent-free method was introduced by Baatti *et al.* to chemically bind methyltrimethoxysilane to CNC.¹⁷³ These methods require silanes. Covalent bond formation on CNC often limits its use in cosmetics and in health care application due to the toxicity, regulatory and compliance concerns. This makes it desirable to convert hydrophilic CNC to hydrophobic CNC without covalent bond formation. In Chapter 5, the surface tension of CNC-based microbeads is discussed emphasizing the tailoring the surface energy with silk fibroin molecules embedding hydrophobic protein segments without creating covalent bonds.

1.3.2. Mechanical Properties

The mechanical properties of microbeads are important characteristics of microbeads. There are many reasons why quantifying the mechanical properties of microbeads is essential. Mechanical properties are required for powder quality control.¹⁷⁴ They are needed to design microcapsules for controlled release.^{175, 176} In the pharmaceutical industry, knowledge of mechanical properties can be related to the stability of the microbead-based drug delivery system. Quantification of mechanical properties can also be used to understand interactions between drug molecules and the matrix.¹⁷⁷ Mechanical properties of microbeads are also crucial to predict their performance for needle injection processes for drug delivery.⁵² In cosmetics, mechanical properties are related to the sensory feeling of softness. For food applications, desired functionality and stability of the encapsulation system is essential. Mechanical properties of the microcapsules are highly related to the stability and the release patterns of the active ingredients.¹⁷⁸ Accordingly, mechanical properties are highly relevant when evaluating structure-function relationships in microbeads.⁵²

Mechanical properties are the physical response of a material to an applied force. They include the modulus of elasticity, viscoelasticity, elongation, hardness, and fatigue limit. These are briefly introduced next.

Young's Modulus

The Young's modulus, or tensile modulus, (also called modulus of elasticity), is a measure of the stiffness of an elastic material. The Young's modulus is defined as the ratio of stress along an axis to strain along the same axis. Stress is defined as the force per unit area, and strain is the ratio of deformation over initial length. Strain ε is the deformation of a solid due to stress, expressed in Equation (5).

$$\varepsilon = dL/L \quad (5)$$

In this equation, dL is the elongation or compression of the material, L is the length of the material. Stress is force per unit area and can be expressed by Equation (6)

$$\sigma = F/A \quad (6)$$

where, F is the applied force, A is the stress area of object.

The Young's modulus E is conventionally expressed by Equation (7)

$$E = \frac{\text{Stress}}{\text{Strain}} = \frac{\sigma}{\varepsilon} = \frac{(F/A)}{(\frac{dL}{L})} \quad (7)$$

Viscoelasticity

Viscoelasticity is the property of a material that characterizes its response when undergoing deformation. A material that has a viscous property resists shear flow and strain linearly with

time when a stress is applied. A material that has an elastic component returns its strain to its original state once the stress is removed. Viscoelastic materials have features of both.

Plasticity

Plasticity is defined as the response of a material that manifests irreversible changes in shape to an applied force. Figure 1-14 shows a stress-strain curve and the different regions of elastic deformation (Young's modulus) and plastic deformation.

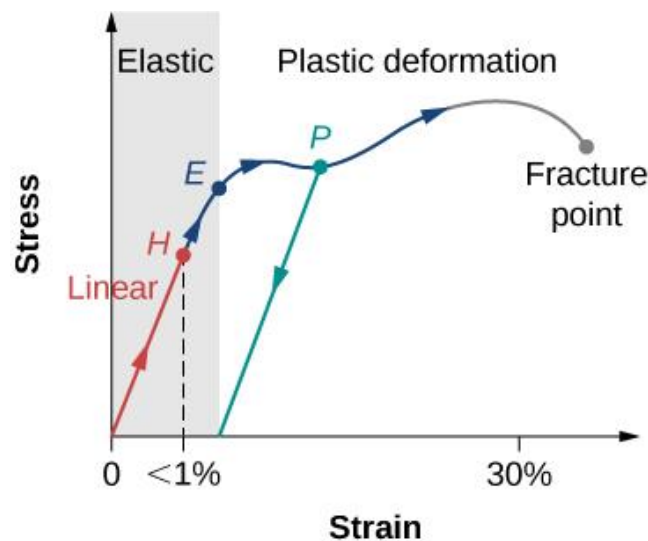


Figure 1-14 A stress-strain curve showing elastic and plastic deformation. H represents the elastic limit, E is the yield strength, and P is the start of the plastic region. (Reprint with permission from ref, ¹⁷⁹ Copyright © Jan 20, 2020 OpenStax)

Methods to Measure Mechanical Properties

Table 1-2 lists several test methods to determine the mechanical response of microbeads. Among them, nanoindentation¹⁸⁰ can measure mechanical properties of small spheres. Nanoindentation can be used to determine hardness, Young's modulus, fracture toughness,

interface toughness, and viscoelastic properties. Nanoindentation tests can be realised with a nanoindenter or by AFM. Figure 1-15 shows a force-distance curve generated by a single approach-retract cycle in AFM nanoindentation. Briefly, no force is detected at the beginning of the approach (red curve), where the tip is far away from the sample. When the tip moves towards the sample surface, long-range attractive and repulsive forces are detected *via* the response of the piezoelectric transducers used to control the approach or retraction of the tip. The dip in the red curve is a manifestation of adhesive forces that draw the tip and bend the cantilever downward. Further approach to the surface turns on repulsive forces, seen as a steep rise up the force curve. There is well-known hysteresis in the curves and this is exemplified in the blue curve in the Figure. Note the enhancement in adhesion as the cantilever attempts to withdraw from the surface.

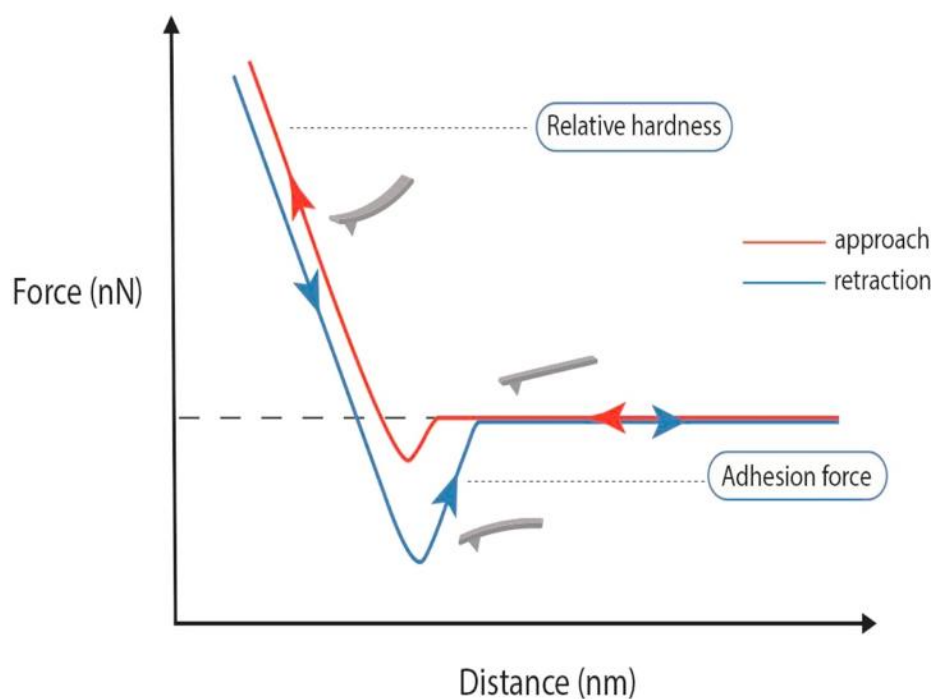
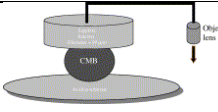


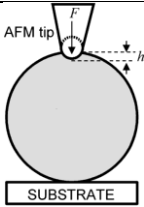
Figure 1-15 Schematic illustration of a force-distance curve from AFM nanoindentation.

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Nanoindentation is usually realised with an indenter tip. There are different shapes of indentation tips. For soft materials, spherical indentation tips are preferred.¹⁸² During the indentation process, a calibrated tip approaches the surface of the sample, where the force-displacement data are recorded, and the contact point is determined. After making contact with the sample, the force is increased linearly until the maximum force achieved over a short dwell time. This is the point where the sample is unloaded. AFM can also be used to generate a force-displacement curve to determine mechanical properties. In this case, AFM probes are used as nanoindentation tips.

Table 1-2 Listings of testing methods for the measurement of mechanical properties of microbeads.

Testing method		Mechanisms	Mechanical properties	Advantages	Disadvantages	References
Nanoindentation	Nanoindenter	Load-displacement curve	Hardness and Elastic modulus	Wide force range	Not suitable for soft materials due to surface adhesion, and tip dependency	183-185
	Atomic Force Microscope (AFM)	Force-distance curves on local material properties	Elastic modulus, adhesion force, and stiffness	No limitations on particle size Enable mechanical property measurement in fluid	Not suitable when there is particle plastic deformation Statistic based quantification Difficult to attach particles on the substrate Uncertainty from probe shape, and the point of contact	176, 186-189 190-193 194-198
Compression test	Nanoindenter		Failure load, fracture energy,	Enable time-dependent force curve study	Microplate is too big for microbead (< 5 μm) indenting	41, 52, 190, 199, 200

		microplate	and elastic modulus		particles less than 5 μm	
	AFM		Contact mechanistic, compressive modulus, surface modulus,	Tip size can be personalized	Localized mechanical properties	186, 201, 202
Optical tweezer, micromanipulation method		Via calibrated optical forces and its induced particle deformation	Elastic modulus	Procedure can be conducted under water, real time deformation can be measured by connecting with optical microscope	Force range is insufficient to induce any meaningful deformation in hydrogel-based microbeads (forces around 10^{-10} and 10^{-11})	25, 203, 204
Micropipette aspiration(MPA) & Microfluidic chamber		Trap a single sphere and precise control of the suction pressure to measure the deformability of the sphere	Elastic modulus	Non-invasive, simple and direct approach at the single sphere level	Usually used for single cell mechanical measurement, not suitable for dry state	205, 206
Microelectromechanical system (MEMS)		Differential displacement measurements in a two stage spring system, where one	Stiffness	Wide size range from sub-micrometers to hundreds of micrometers	In fluid measurement, usually used for cell force measurement	207, 208

	spring system consists of a MEMs reference spring, while the other one is the microparticle interested.		Displacement and force sensors possess high resolution down to sub-nanometer and sub-nanonewton levels		

Mathematical Models for Calculating the Young's Modulus

The Hertz model was developed by Heinrich Hertz back in 1882.²⁰⁹ In this model, the AFM probe and the sample are regarded as two spheres with radius R_1 and R_2 . The model considers only the elastic deformation, neglecting the adhesion force. But the Hertz model is not the only available model to interpret force curves. When the adhesion force is significant, several other models are available to calculate the Young's modulus. Table 1-3 summarizes three mathematical models for calculating the elastic modulus from force curves. In this table, R is the generalized radius, which can be derived from Equation (8), where R_1 and R_2 are the radii of the tip, and the sample, respectively.

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} = \frac{R_2 + R_1}{R_1 R_2} \quad (8)$$

Table 1-3 Mathematical models for calculating Young’s modulus from indentation force curves.

	Mathematical model	F_{adh}	References
Hertz Model	$F_{sphere} = \frac{4}{3} \frac{E}{1 - \nu^2} \sqrt{R} \delta^{\frac{3}{2}}$ $E = \frac{3}{4} \left(\frac{\Delta(F_{sphere})^{2/3}}{\Delta\delta} \right)^{3/2} \frac{1 - \nu^2}{\sqrt{R}}$	0	196, 197, 201
DMT (Derjagui, Muller and Toporov)	$F_{sphere} - F_{adh} = \frac{4}{3} \frac{E}{1 - \nu^2} \sqrt{R} \delta^{\frac{3}{2}}$ $E = \frac{3}{4} \left(\frac{\Delta(F_{sphere} - F_{adh})^{2/3}}{\Delta\delta} \right)^{3/2} \frac{1 - \nu^2}{\sqrt{R}}$	$2\pi RW$	²¹⁰
JFK (Johnson, Kendall and Roberts)	$F' = F_{sphere} + 3W\pi R$ $+ \sqrt{6WR F_{sphere} + (3W\pi R)^2}$ $E = \frac{3F'(1 - \nu^2)}{4\sqrt{R}\delta^{3/2}} \left[1 - \frac{2}{3} \left(\frac{6\pi RW}{F'} \right)^{1/2} \right]^{3/2}$	$\frac{3\pi RW}{2}$	196, 197, 211- 213

In this table, F_{sphere} is the force applied to the sample surface, R is the generalized radius, δ is indentation depth, E is Young’s modulus (elastic modulus), ν is the Poisson ratio of the sample, and W is the adhesion energy.

Colloidal Probe

Conventional AFM indentation uses nano-probes to measure mechanical properties. This method has the drawback that it focuses more on the localized elastic modulus in the vicinity of the nanoprobe and not the overall elasticity of a particle. A colloidal probe for AFM

indentation may provide a more accurate measure of the Young's modulus of soft microbeads. A colloidal probe consists of a sub-micron sphere (silicon, polystyrene, for example) attached to the end of a cantilever. This modification has several advantages.²¹⁴ Colloidal probes can be used to measure materials that have a rough or porous texture, which nano sized tips cannot do. A colloidal probe enables hydrodynamic force measurement. Colloidal microsphere probes show less tip wear than do nano-sized probes. Changes in the radii of nano-sized tips are often significant enough to affect the measured results. The use of microsphere probes significantly improves this situation.

Luo *et al.* have compared the elastic modulus of polystyrene nanospheres measured using a microsphere probe and a nanoprobe.¹⁹⁷ They showed that the moduli measured with a nanotip are larger than those measured with spherical micro-sized tips. Nano-sized tips were found to wear easily, usually increasing from 10 nm in the radius to 100 nm. The size of microsphere tips was shown to remain unchanged after measurement, leading to more predictable results.

1.3.3. Small Molecule Uptake and Release

Small molecule uptake has been widely studied for applications such as absorbents for dye molecules and metal ion removal for water treatment. Dye molecule or metal ion uptake are usually examined using absorption kinetics and isotherms. Absorption kinetics include the reaction-based kinetics and the diffusion-based kinetics. Pseudo-first order and pseudo-second order kinetics are commonly used when regarding the absorption process as a chemisorption event, as listed in Table 1-4. Diffusion-based kinetics considers the absorption process as a mass transport process, where diffusion related information can be explored. Relevant diffusion-based kinetics approaches are the film-pore diffusion model,^{215, 216} the intraparticle diffusion model,²¹⁷ the film-surface diffusion model, and the branched-pore-diffusion model.

²¹⁸ Absorption isotherm measurement is another type of small molecule uptake study, providing

insight into absorption capacity and thermodynamic properties. Some of the more common absorption isotherms are those due to Langmuir, Freundlich, and Temkin. These isotherm equations are listed in Table 1-5. Small molecule uptake is useful for applications other than water treatment because the uptake kinetics and isotherms can provide information on molecule transport mechanisms when using cellulose materials as the host matrix.

Table 1-4 Reaction-based kinetics model

Model	Equation
Pseudo-first order	$q_t = q_e(1 - e^{-k_1 t})$
Pseudo-second order	$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t}$

q_t : amount of molecules absorbed at time t , q_e : amount of molecules absorbed at equilibrium,

k_1, k_2 : rate constants.

Table 1-5 Three types of adsorption isotherms

Isotherm	Non-linear Form	Linear Form
Langmuir Isotherm	$q_e = q_m K_L \frac{C_e}{1 + K_L C_e}$	$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$
Freundlich Isotherm	$q_e = K_F C_e^{1/n}$	$\log q_e = \log K_F + \frac{1}{n} \log C_e$
Temkin Isotherm	$q_e = \frac{RT}{\beta} \ln (\alpha C_e)$	$q_e = \frac{RT}{\beta} \ln \alpha + \frac{RT}{\beta} \ln C_e$

q_e : amount of molecules absorbed per gram of adsorbent (mg/g) at equilibrium. q_m : the maximum absorption capacity (mg/L), K_L : the Langmuir isotherm constant, C_e : equilibrium concentration of molecule solutions. K_F : Freundlich isotherm constant, $1/n$: heterogeneity factor, R : gas constant, T : Temperature, β : Temkin isotherm constant, α : Temkin equilibrium binding constant.

Several mathematical models exist to describe small molecule release kinetics. These models provide information about mass transport mechanisms that are relevant to drug release profiles. Mathematical models are also useful to predict and design a drug release profile to meet a specific need. As listed in Table 1-6, commonly used kinetic models include zero-order kinetics, first-order kinetics, the Higuchi model, Hixson-Crowell model, Ritger-Peppas and Korsmeyer-Peppas model, Weibull model, and Peppas-Sahlin model.²¹⁹ These models describe the dependence of release as a function of time. The type of release profiles is relevant to the type and amount of the drug molecules, polymer matrix, size, and shape of the system.²²⁰

Table 1-6 Mathematical models for drug release kinetics, adapted from ²¹⁹.

Mathematical model	Equation
Zero-order	$Q_t = Q_0 + K_0 t$
First-order	$\ln Q_t = \ln Q_0 + K_1 t$
Higuchi	$Q_t = K_H t^{1/2}$
Hixson-Crowell	$Q_0^{1/3} - Q_t^{1/3} = K_s t$
Korsmeyer-Peppas	$\frac{Q_t}{Q_\infty} = K_K t^n$
Weibull	$\log[-\ln(1 - m)] = \beta \log(t - T_i) - \log \alpha$
Hopfenberg	$\frac{Q_t}{Q_\infty} = 1 - [1 - k_0 t / C_0 a_0]^*$

Q_t : Amount of drug released in time t , Q_0 : Initial amount of drug in the dosage form, Q_∞ : Total amount of drug dissolved when the dosage form is exhausted, K_0 , K_1 , K_H , K_s , K_K : release rate constant, n : release exponent (indicative of drug release mechanism), m : accumulated fraction of the drug, β : shaper parameter, α : scale parameter, T_i : location parameter, C_0 : Initial concentration of drug in the dosage form, a_0 : initial radius for a sphere or cylinder or the half-thickness for a slab. In the Hopfenberg equation, * is the exponent of 1, 2, and 3 for a slab, cylinder, and sphere, respectively.

1.4. Applications of Cellulose-based Materials

Collectively, nanocellulose refers to nanofibrillated cellulose, cellulose nanofibers and cellulose nanocrystals. Nanocellulose materials have been demonstrated as absorbents for

water treatment for dye and metal ion removal, as drug excipients for drug delivery and controlled release, for smart microbeads, and as replacements of microplastic beads in cosmetics. In this section, the above four applications are introduced with a focus on the microbeads.

1.4.1. Absorbents for Water Treatment

Cellulose-based materials are commonly used as absorbents for water treatment due to their lack of toxicity, good biodegradability, renewability, and low cost. Among the cellulose-based materials, cellulose nanomaterials, such as cellulose nanocrystals are alternative absorbents that show potential due to their high surface area-to-volume ratio, and their robust mechanical properties.²²¹ Besides, chemical modification of the nanocrystals can enhance binding affinities for pollutants.

For example, TEMPO oxidation is a well-known method to increase the content of the carboxylic acid groups on nanocellulosics. Increasing the concentration of carboxylic acid groups on nanocellulose substantially increases absorption capacity for anionic dye molecules, such as methylene blue.²²² A carboxylated “hairy” CNC has been described by van de Ven and coworkers.²²³ Mandana *et al.* examined the dye uptake capacity of hairy CNC. A composite hydrogel system was designed combining hairy CNC with alginate, a hybridization that shows relatively high dye removal capacity.²²⁴

The dye removal capacity of CNC materials is affected not only by the carboxylic acid content, but also by the choice of drying method. He *et al.* tested the dye removal capacity of lyophilized cCNC powders and compared the absorption properties with those of porous carbon monoliths.²²⁵ In this thesis, we describe the dye removal capacity of cCNC microbeads

produced from spray-drying (Chapter 2). The drying conditions affect the surface area and porosity of the generated materials. These can be used to adjust the dye removal capacity.

Cellulose-based microbeads are excellent candidates for water treatment applications.²²⁶ Setyono *et al.* showed how porous cellulose microcapsules absorb metal nanoparticles.²²⁷ Wang *et al.* used a cellulose-based microbeads to bind metal ions, Cu^{2+} and Mn^{2+} .²²⁸

To target hydrophobic organic contaminants, scientists use surface chemistry to graft organic functionalities onto nanocellulose to enhance its hydrophobicity. For example, Kohrhonen *et al.* used atomic layer deposition to deposit titanium dioxide onto CNC aerogels. The effect is to lower the surface energy of CNC, enabling oil and organic solvent absorption.²²⁹ Vapor deposition of silane onto CNC²³⁰ and trapping graphene oxide within a nanocellulose matrix²³¹ have been demonstrated to bind hydrophobic contaminants.

One of the challenges of using cellulose-based adsorbents is reusability. It is also one of the primary goals when designing sustainable absorbents. The number of reusable cycles is typically between 3 to 5.²²¹ Washing with acid or salt solutions will increase the number of reusable cycles.

1.4.2. Drug Delivery Systems

Conventionally, cellulose microbeads are used for controlled drug release. Table 1-7 gives a comprehensive literature review on using cellulose-based microbeads for drug encapsulation and controlled release. The synthesis method, encapsulated drug types, and drug release time are reviewed to provide a database for future design and comparison of drug release systems. From this table, the equilibrium time for drug release is influenced mainly by particle size, material composition, and the identity of the drug molecule.

Table 1-7 Cellulose-based microbeads for drug encapsulation and controlled release.

Material compositions	Synthesis method	Encapsulated drug	Time for controlled release	Refs
Eudragit S 100 Hydroxylpropyl Methylcellulose phthalate (HPMCP)	Solvent evaporation	Sodium pantoprazole	5 h	⁸³
Eudragit S100 and HPMC	Spray dry	Pantoprazole	500 min	²³²
Eudragit RS 100 and Ethyl cellulose	Solvent evaporation method	Verapamil HCL	12 h	²³³

Ethyl cellulose	Oil-in-oil solvent evaporation method	Fenoterol HBr	10 h	⁷⁷
Ethyl cellulose	Emulsion solvent evaporation	Caftazidime	10 d	⁷⁸
Ethyl cellulose	Emulsion solvent diffusion method	Terbinafine hydrochloride	5 h	⁷⁹
Ethyl cellulose	Emulsion method	Paliperidone pamitate		
Ethyl cellulose	Water-in-oil emulsion	Salbutamol sulphate	10 h	⁸⁰
Ethyl cellulose	Emulsion solvent evaporation	Theophylline	12 h	⁸¹
Ethyl cellulose	Water-in-oil type of emulsification solvent evaporation	Aspirin	18 h	⁸²
Ethyl cellulose/chitosan	w/o/w emulsion and solvent evaporation	Berberine hydrochloride	15 h	²³⁴
Ethyl cellulose HPMC	Diffusion evaporation technique	Carvedilol	10 h	²³⁵
Chitosan/nanohydroxyapatite /ethyl cellulose	Granulation	Gentamicin	50 d	²³⁶
Ethyl cellulose / chitosan	Solvent evaporation	Ciprofloxacin hydrochloride	90 d	²³⁷

Ethyl cellulose and Eudragit RS 100	Solvent evaporation method	Verapamil HCL	12 h	233
Ethylcellulose, HPMC, Carbopol 934P, chitason	Solvent evaporation method	Metformin	16 h	238
Ethyl cellulose, cellulose acetate phthalate, PLGA	Solvent evaporation method	Urea	160 h	239
EC and PVP	Solvent diffusion evaporation method	5-fluorouracil	15 h	240

Cellulose acetate butyrate and poly(epsilon-caprolactone)	Spray dry	Ketoprofen	5 h	155
Cellulose acetate-polystyrene	W/O/W emulsion method	Ketoprofen	+6 h	241
Cellulose acetate phthalate (CAP)	Oil/water solvent evaporation method	Plasmid DNA	4 h	74
Cellulose acetate butyrate	Oil-in-oil emulsion solvent evaporation method	Theophylline	12 h	76
Eudragit S100 Cellulose acetate butyrate	Emulsion-solvent evaporation method	Theophylline	12 h	242
Cellulose acetate phthalate	Solvent evaporation method	Tenoxicam	200 min	75
Cellulose acetate	Emulsion solvent evaporation	Theophylline	12 h	72
Cellulose acetate	o/w emulsification and solvent evaporation	Nifedipine, and verapamil hydrochloride	500 min	73
Chitosan/cellulose acetate	w/o/w emulsion and solvent evaporation	Ranitidine	30 h	243

Cellulose graft poly(L-lactide)	Phase separation method	Vitamin C	150 h	244

Carboxymethylcellulose	Oil-in-water emulsion method	2,7-dichlorofluorescein	30 h	⁸⁴
Gelatin and hydroxyethyl cellulose	Water-in-oil emulsion method	Theophylline	100 min	²⁴⁵
Carboxymethyl cellulose microbeads	Crosslinking	Aldicarb	10 h	⁸⁵
Collagen/gelatin/hydroxyethyl cellulose	Emulsification/crosslinking method	Calendula officinalis	3 days	²⁴⁶
Oxidized cellulose	Spray dry	Camptothecin Carboxylic content enhances dissolution	200 h	¹⁵⁶
PMMA/CNF composite	Pickering emulsion method	Methylene red	33 h	²⁴⁷
PMMA/SCNC composite	Pickering emulsion method	Curcumin	33 h	²⁴⁸
Cellulose Hydroxylpropyl cyclodextrin	Solvent evaporation method	Prednisolone	10 h	²⁴⁹
Nanocrystalline cellulose		Doxorubicin hydrochloride Tetracycline hydrochloride	Half day	²⁵⁰
Oxidised cellulose		Ranitidine HCl	100 min	²⁵¹
Chitosan and carboxymethyl cellulose	Inverse emulsion-crosslinking method	Doxorubicin	6 d	²⁵²
Carboxymethyl cellulose and carboxymethyl chitosan	Inverse emulsion method	Doxorubicin	200 min	
Chitosan, sodium cellulose sulfate and sodium tripolyphosphate	Crosslink	BSA	8 h	²⁵³

Nanocellulose provides a new opportunity to incorporate ingredients for drug delivery. Researchers have comprehensively reviewed the use of nanocellulose materials for this purpose. For example, Salimi *et al.* reviewed the production of nanocellulose and its use as a drug delivery system. The review focused on the toxicity of nanocellulose in drug delivery,

and the applicability of different types of drug delivery systems such as oral administration, transdermal drug delivery, and local drug delivery.²⁵⁴ Pachuaui reviewed the cytotoxicity and uptake mechanisms of cellulose nanoparticles. The biodegradability, drug binding, drug release mechanisms, and targeted delivery systems have been evaluated. The review also focused on internalization of nanocellulose by cell, as it provides fundamental studies on how the cell interacts and uptakes nanocellulose materials.²⁵⁵

Nanocellulose-based materials have also been used as building blocks to make microcapsules to deliver drug molecules. For example, Lin *et al.* studied a cellulose nanocrystal-alginate nanocomposite microbead as a drug delivery capsule for theophylline release.²⁵⁶ Hybrid CNC-chitosan microcapsules have been made using the layer-by-layer method and then used for controlled drug delivery.²⁵⁷

Several research groups have explored the potential of using spray-drying to encapsulate vaccines, drug molecules, gene therapy drugs, and active ingredients to understand their release profiles.¹⁵⁴ Spray-drying provides a one-step, industrially scalable, and water-based method for encapsulation. For example, Kolakovic *et al.* used spray-drying to encapsulate drug molecules like ibuprofen, atenolol, and nadolol in a nanofibrillar cellulose matrix to achieve controlled release over ~60 days.¹⁵⁹

1.4.3. Smart Materials

Smart materials are defined as materials that react to environmental stimuli or conditions.²⁵⁸ This environmental response can come from pH, light, temperature, electricity, magnetic fields, and mechanical forces.²⁵⁹ The abundance of hydroxyl groups makes surface functionalization of cellulose of interest in making so-called smart materials. Figure 1-16 describes three strategies that have been used to design smart materials from cellulose. They include

assemble/disassemble strategies, swell/deswell strategies, and swell/collapse strategies. Designing smart materials from cellulose means the materials can inherit unique properties such as strong mechanical response, biocompatibility, and biodegradability, while elaborating new functionalities.

The preparation of smart materials from cellulose usually proceeds by two methods: either chemical modification or hybridization. Chemical modification utilizes the surface functional group of cellulose, including hydroxyl and carboxylic acid groups, to graft “intelligent” small molecules or polymers into the system. For hybridization, spray-drying provides a flexible doping strategy to incorporate “intelligent” polymers into the cellulose host matrix to yield smart composite cellulose materials.

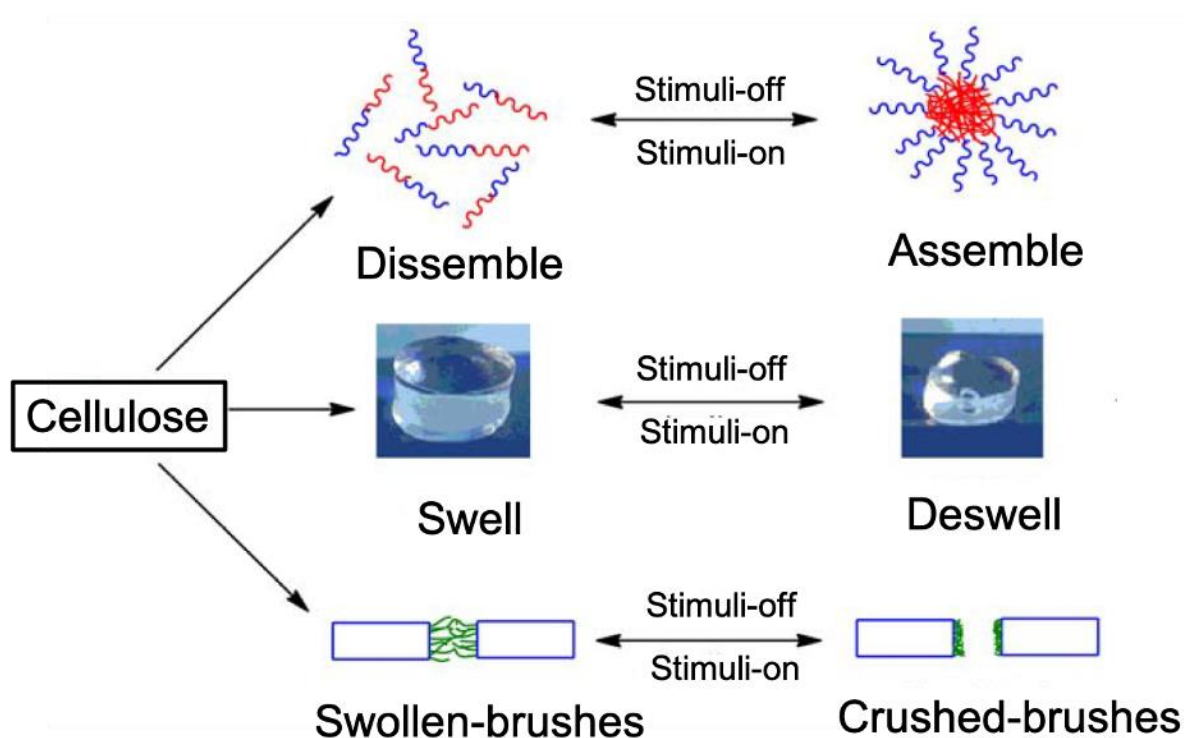


Figure 1-16 Examples of using cellulose materials to create smart responsive materials.

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MDPI, Basel, Switzerland.)

1.5. Characterization Methods

These are described as they emerge in Chapters 2-5. The workhorse tools include X-ray Photoelectron Spectroscopy (XPS), Fourier Transform-infrared (FT-IR), solid-state NMR, and powder X-ray Diffraction Spectroscopy (pXRD). Morphology was interrogated by Scanning Electron Microscopy (SEM), optical microscopy, and Focused Ion Beam Electron Microscopy (FIB). We give a terse explanation of the FT-IR self-deconvolution method, XPS depth profiling and confocal, super-resolution and multi-photon microscopy in the paragraphs below, since these techniques may be less familiar to the reader.

1.5.1. FT-IR Spectroscopy – Data Analysis

This thesis uses the method of Fourier self-deconvolution (FSD) to provide a quantitative analysis of the percentage of β sheet formation in silk fibroin protein.²⁶¹ FSD is a signal-processing method applied to infrared spectral analysis. It is a mathematical method for reducing the bandwidth to resolve overlapped bands from each other.²⁶² In Chapter 5, The deconvolution procedure follows the algorithm of Lorenz-Fonfria that applies a high pass filter to resolve the silk fibroin amide I band into narrowed peaks that quantitatively reflect the amorphous and silk I and II conformational contributions, and even some amino acid contributors.

1.5.2. X-ray Photoelectron Spectroscopy (XPS) and XPS Depth Profiling

X-ray photoelectron spectroscopy (XPS) is a well-known technique for surface chemical bond speciation and element identification. XPS is widely used to probe cellulosic materials, especially to validate chemical derivatives at the surface. For example, functionalization of cellulose by click reaction and EDC coupling can be traced through the evolution/identification of the nitrogen 1s peak and by analysing its chemical state through shifts in binding energy.²⁶³

In this thesis, we use XPS primarily to profile the spatial distribution of certain entities on the surface and within the microbeads. The method can yield a 3D map of elemental and percent composition. Taketa *et al.* studied the internal chemical composition of chitosan-based layer-by-layer films using depth profiling XPS analysis. The axial composition was assessed with this technique by tracing the spatial evolution of the nitrogen binding energy at 400 eV and 402 eV.²⁶⁴ XPS depth profiling was also used to probe interlayer diffusion of chitosan in multilayer films by Gilbert *et al.*²⁶⁵

1.5.3. Microscopy

Confocal Microscopy

Confocal microscopy can record three-dimensional (3-D) images with enhanced resolution and contrast compared with conventional light microscopy. In conventional microscopes, light is detected in multiple focal planes to create a single two-dimensional image. This technique is not ideal for thick samples. Confocal microscopy uses a spatial pinhole to focus light on a single point within a defined focal plane, enhancing the image resolution, suppressing noise, and image distortions.²⁶⁶ Confocal fluorescence microscopy requires dye labeling of the sample. Even with its enhanced resolution, the confocal method still suffers from optical diffraction limitations (200 nm). In Chapter 3, Case study 1, confocal microscopy is used to monitor encapsulated small molecule release from cCNC-based microbeads *in situ*, providing kinetic information through direct visual images.

Super-resolution Microscopy

Super-resolution microscopy has been developed to circumvent the diffraction limitations of optical microscopy. Super-resolution microscopy includes stimulated emission depletion microscopy (STED), Structured Illumination Microscopy (SIM), Photo-activated localization

microscopy (PALM), and Stochastic Optical Reconstruction Microscopy (STORM). Table 1-8 summarizes the benefits and limitations of different super-resolution microscopy techniques.

Table 1-8 Benefits and limitations of different super-resolution techniques. (Reprint with permission from ref. ²⁶⁷ Copyright © 2014 Elsevier Inc. All right reserved.)

Modality	Resolution (nm)	Illumination	Probes	Acquisition Time	Post-Acquisition Processing	Other Concerns
Standard fluorescence microscopy	250	Confocal/TI RF/Epi	Conventional fluorescent probes			
SIM	100	Wide-field	Conventional fluorescent probes	Short (s)	Yes (Fourier transforms)	Out of focus signals and sample thickness
STED	30-70	Laser scanning confocal	Specific fluorescent probes	Short (s)	No	Photobleaching
PALM	10-55	Wide-field	Photo-activatable fluorescent proteins	Long (min)	Yes (Centroid identification)	Over/under labelling
STORM	10-55	Wide-field	Photoswitchable Blinking fluorophores	Long (min)	Yes (Centroid identification)	Over/under labelling

Among them, SIM enhances spatial resolution by eliminating conventional Köhler illumination, replacing it with non-uniform excitation light patterns that have sinusoidal intensity variations due to diffraction from a grid that is translated and rotated to give a Moiré pattern that is deconvoluted.²⁶⁸ Coupled with powerful image reconstruction techniques, resolution can be enhanced by a factor of two.²⁶⁹ Besides enhanced resolution, an advantage of SIM is that it places no special requirements on the fluorophore except that it should resist photobleaching.²⁷⁰ In Chapter 3, Case Study 1, we utilize SIM to reveal the 3-D spatial distribution of a porphyrin

molecule encapsulated in cCNC microbeads. SIM provides a direct visual image of the locations of the porphyrin and helps to predict release profiles from the microbeads.

Multi-photon Microscopy and Third Harmonic Generation (THG)

Super-resolution microscopy requires covalent labeling with a fluorescent dye. A limitation of this technique is photobleaching and over/under labeling artifacts. Label-free light microscopy provides another route to microbead imaging. An example of a label-free method is multi-photon microscopy. Multi-photon microscopy utilizes two-photon excited fluorescence, second harmonic generation (SHG) and third harmonic generation. Among them, second harmonic generation is emerging as a powerful tool for imaging biomaterials like collagen, actomyosin, and tubulin. Second-harmonic generation is a non-linear optical effect. The contrast is obtained from the variations in a sample's ability to generate second-harmonic light from the incident light. Often, laser light passing through a material with a noncentrosymmetric molecular structure leads to the signal for SHG. Brown *et al.* have found that the macromolecular structure of cellulose can be revealed with second harmonic generation imaging microscopy, making it a useful tool to monitor the dynamics of cellulose structures in biological systems.²⁷¹

Third harmonic generation (THG) microscopy, as used in this thesis, is a label-free multiphoton technique also based on a non-linear scattering mechanism. It utilizes the $\chi^{(3)}$ process where three incident photons at frequency ω_F sum to one photon at $\omega_{THG} = 3\omega_F$. The wavelength of the third harmonic output is $\lambda_F/3$. The third-order induced polarizability is represented in Equation (9). Here, ϵ_o is the permittivity of free space. $E(\omega)$ is the incident electric field at frequency ω . The third-order susceptibility $\chi^{(3)}$ is a fourth rank tensor. The indices $ijkl$ are summed over the Cartesian linear polarization directions, x, y, and z.

$$P_i^{(3)}(3\omega) = \varepsilon_o \sum_{jkl} \chi_{ijkl}^{(3)}(\omega, \omega, \omega) E_j(\omega) E_k(\omega) E_l(\omega) \quad (9)$$

THG occurs at material interfaces like local transitions in the refractive index or the third-order nonlinear susceptibility $\chi^{(3)}$, both of which are intrinsic physical properties of materials. Thus, THG can be found at interfaces between water and physical bodies, like cCNC microbeads, contained in it.²⁷² THG requires coherent scattering at interfaces, a process that is subject phase matching between the excitation and scattered fields near focus. The phase-matching condition for THG is $\Delta k = 3k_1 - k_3 = 0$, where k_3 and k_1 are the wave vectors of the third harmonic field and the fundamental field.²⁸⁵ The intensity of the THG signal depends on the sample size and its structural features, the excitation and detection scheme, the power and polarization of the incident light. Generally, one tunes the focal volume to optimize THG intensity for the structure of interest. The advantages of $\chi^{(3)}$ microscopy are as follows: the technique provides structural information about the microbeads, both internally and externally; it offers 3D sectioning (depth profiling); it enables imaging thick samples; and it decreases photodamage.^{274, 275} Despite its advantages, it is important to note the potential for artefacts. THG intensity depends strongly on the excitation power density at the depth of focus. It also depends on excitation pulse length and laser repetition rate, excitation wavelength and the numerical aperture of the objective. When imaging, THG signal is strongly affected by light scattering and wavelength dispersion that is caused by out-of-focus structures and by increasing distortions of the focus in depth. In multi-photon microscopy, spatial resolution depends on the size of the volume of the focal spot and of background fluorescence when trying to image at depth. Changing the depth of focus can bring different structural features into play or make it appear that there is spatial inhomogeneity in samples where inhomogeneity does not exist. The application of this technique in imaging cCNC-based microbeads in water are discussed in Chapter 3, Case Study 1.

1.6. Conclusions

In conclusion, cellulose-based microbeads may be of value in many different application scenarios. Their appeal lies in their biodegradability, unique mechanical properties, reusability, low toxicity, and capacity to be chemically modified to suit a given application. In this review, we explored a range of different types of microbeads, focusing largely on those derived from native cellulose. We discussed cellulose microbead fabrication methods and critiqued shortcomings of conventional preparations. We then reviewed the potential of spray-drying to make nanocrystalline cellulose-based microbeads. A brief introduction was given to the complex parameter space of spray-drying and how different variables can affect morphology, surface energy, mechanical and transport properties. We provided a brief introduction to the most relevant methods used in this thesis to characterize cCNC microbeads.

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| 2 |

**Diffusion and Kinetics of Dye Uptake by Carboxylated
Cellulose Nanocrystal Microbeads**

Chapter 2 Diffusion and Kinetics of Dye Uptake by Carboxylated Cellulose Nanocrystal Microbeads

This chapter includes a submitted peer-reviewed paper. The bibliographic details of the paper are:

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My contribution to the paper involved:

1. Experimental design and implementation;
2. Data collection and analysis;
3. Preparation of the manuscript;

Preface

This study began with a search for a performing alternative to plastic microbeads whose use is now under scientific, legislative and consumer pressure to be banned. This pressure creates incentive to adopt *naturally sourced* materials that are biodegradable and manufactured according to sustainable practices. Naturally sourced microbeads are not a “drop-in” solution to the microplastics dilemma. There is a need to quantify the performance characteristics of the replacements.

Figure 2-1 shows that conventional cellulose microbeads may be made by dissolving cellulose, usually by the viscose process or mercerization. Often the purpose of dissolution is to permit chemical modification of the dissolved polysaccharide to confer desirable properties on the microbead. After dissolution of the cellulosic, emulsion or coacervation methods may be used to shape the polymer into microbeads. But why put energy into breaking up the crystalline domains, why not conserve entropy, if this important component of natural cellulose can be incorporated into a process that brings chemical and physical diversity to cellulose microbeads by some other method? Microbeads are under aggressive development because of their uptake and release capabilities. Our paper examines how microbeads from carboxylated crystalline nanocellulose cCNC can regulate trans-microbead trafficking of a charged dye molecule, offering the cCNC microbead as a model uptake and release system. Spray-drying is as the Aufbauprinzip (building-up principle) to make the microbeads from aqueous cCNC suspension. In effect, this is a “re-scaling” of nanoscale matter to the microscale.

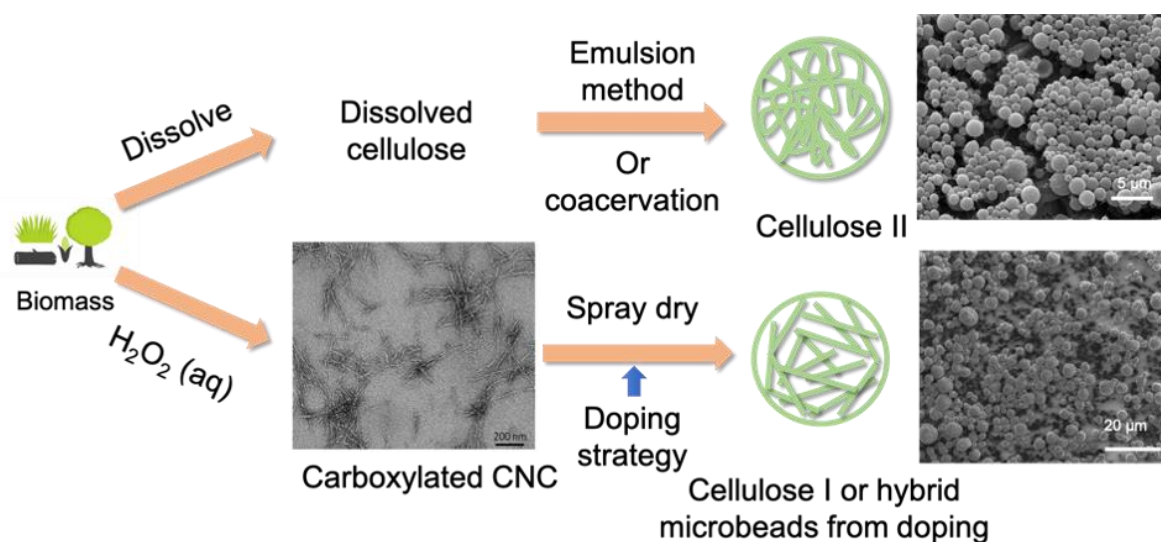


Figure 2-1 Pathways to make cCNC or conventional cellulose microbeads.

Surprisingly, there is little information at all in the published literature on the transport properties of cellulose microbeads. This is despite the fact that conventional specialty cellulose

polymers are widely used for time-release of pharmaceuticals, where well-known kinetic models dominate. Our interest is in providing information about trafficking of a charged probe molecule paradigm, methylene blue, in this new type of cCNC microbead.

Our study moves beyond standard uptake kinetics to explore the impact of pH and ionic strength on transport properties within a two-resistance framework that examines external and internal mass transfer. Our implementation of a film-pore diffusion model discriminates how protonation-deprotonation affects MB diffusion. We probe kinetics and diffusion in suspensions of the microbeads in water and how the transport characteristics are modified by changes in pH and ionic strength. We identify a “gating” mechanism where MB uptake is regulated by a combination of charge potential and reversible hydrogen bonding among the nanocrystals.

This gating mechanism puts our microbeads in the class of “smart materials” for ion uptake. Our research anticipates applications in drug delivery, separation science, and the creation of pigments from plant-based charged dye molecules.

Our study in this chapter is the first of several studies that deal with the physical properties of cCNC microbeads. These include pH responsive smart drug delivery systems (Chapter 3), atomic force microscopy studies of mechanical properties (Chapter 4), and lipophilic energetic interfaces created by hydrophobic silk fibroin protein/cCNC hybrids (Chapter 5).

Diffusion and Kinetics of Dye Uptake by Carboxylated Cellulose Nanocrystal Microbeads

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Key words:

Crystalline Nanocellulose, microbeads, diffusion kinetics, methylene blue, isotherms, plastic microbeads replacement

Abstract

Cellulose microbeads can be made by spray drying aqueous suspensions of carboxylated cellulose nanocrystals (cCNC). The rescaling of cCNC to micron-size particle agglomerates yields a new type of biodegradable cellulose microbead with potential to replace plastic microbeads, and to add to the repertoire of cellulosic microbeads that find application in chromatography, water treatment, protein immobilization, solid-phase synthetic chemistry and pharmaceuticals. The present study offers insight into small molecule transport properties that are important to design and assess the performance of cCNC microbeads. In our study, cationic methylene blue (MB) dye was used as a model compound to probe uptake kinetics and diffusion. The study goes beyond the framework of pseudo-first or second order kinetics in that it explicitly engages the problem of diffusion. We introduce a two-resistance model based on film-pore diffusion that is conditioned by an adsorption isotherm. The numerical study is combined with experiment to give more detailed insight into mass transport of MB in the

microbeads. Langmuir, Temkin and Freundlich isotherms were investigated. Film-pore diffusion based on Langmuir adsorption reveals how trafficking of MB molecules in the microbeads depends on pH and ionic strength. At neutral pH the effective diffusion coefficient is $7.5 \times 10^{-11} \text{ m}^2/\text{s}$. A “gating” mechanism is proposed to account for MB inhibition and uptake. Thermodynamic parameters were determined. The process is spontaneous, but slightly endothermic with activation energy on the order of 20 kJ/mol.

Introduction

In this paper, we quantify small molecule uptake and release kinetics and diffusion in cellulose microbeads assembled from carboxylated cellulose nanocrystals (cCNC). The study focuses on the model compound, methylene blue (MB). Binding and release studies show that adsorption and transport properties in the microbeads can be gated by controlling ionic strength and pH. Our studies are relevant to uses of nanoparticles and their rescaling to biodegradable microbeads for wastewater purification, cationic drug release and other microbead transport processes where knowledge of diffusion, adsorption and their coupling to changes in ambient pH and ionic strength is required.

Synthetic polymer microbeads^{1,2} find applications in personal care products,³ pigments,⁴ water treatment absorbents,⁵ catalyst supports,⁶ and as drug carriers.⁷ Performance, cost, availability and mature industrial scalability have driven widespread adoption of microplastics. But microplastics are now recognized as a significant environmental threat. Legal and moral imperatives are driving the microplastics industry to replace them with benign alternatives while maintaining competitive performance. The push towards sustainability provides an additional constraint on the introduction of suitable replacements. Cellulose is a major candidate to replace microplastics. Microbeads made from cellulose have a long history of

applications in chromatography, water treatment, protein immobilization, solid-phase synthetic chemistry and pharmaceuticals.⁸⁻¹⁶

Cellulose microbeads are typically prepared from dissolved cellulose derivatives like cellulose acetate,¹⁷ HPMC (Hydroxypropyl methyl cellulose),¹⁸ carboxymethyl cellulose,²⁰ or ethyl cellulose.²¹ Emulsion-solvent evaporation²³⁻²⁸ and coacervation methods are commonly used to prepare microbeads from these sources.²⁹⁻³¹ The emulsion-solvent evaporation method can be challenging to scale up due to the large quantities of solvent required.³² Moreover, residual solvent may be difficult to remove from the final product. The coacervation method cannot produce microbeads below 10 μm .³¹ Spray-drying from water suspensions has been used to produce microbeads from ethylcellulose³³ and hydroxypropylmethylcellulose.³⁴ This method is scalable to industrial quantities.³⁵

The starting materials for the cellulose microbeads referred above belong to the class of specialty cellulose derivatives. They are typically made by separating the desired starting material from lignin and hemicellulose and then dissolving the crystalline or semicrystalline cellulose in strong base. Chemical work-up followed by functional group transformations yield the different types of cellulose polymers. Life cycle analyses show that there are energy and environmental costs associated with the production of specialty cellulose.³⁶

Nature has evolved a supramolecular motor called the cellulose synthase complex (CSC) to manufacture its own “specialty cellulose”.³⁷ The CSC does so with great fidelity, producing uniform nanocrystalline domains of cellulose interlocked by amorphous β -1,4-linked glucan chains. Nanocrystalline cellulose building blocks are crucial for the cell to support the sizeable internal osmotic pressure necessary to withstand the pull of gravity. Instead of putting energy into breaking up the crystalline structure of cellulose to manufacture specialty cellulose derivatives, it makes sense to co-opt the output of Nature’s supramolecular machinery and investigate how

its cellulose nanocrystal building blocks can be recruited to build cellulose microbeads. This approach has the potential to reduce the energy requirements and processing steps associated with conventional methods to make cellulose microbeads from chemically modified cellulose. It also has the potential to make microbeads with wide-ranging functionality based on uptake and release of non-covalently bound molecules. In this paper, we show how carboxylated cellulose nanocrystals (cCNC) can be assembled into microscale objects, yielding cellulose microbeads that exhibit small molecule uptake and release. The resulting cCNC-based microbeads are shown to belong to the class of “responsive” matter where uptake (and release) can be gated by changing pH and/or ionic strength.

Depending on the cellulose source and processing conditions, CNC typically ranges in length from 100 – 200 nm and 5 – 10 nm in width, giving the crystal a high aspect ratio. CNC has emerged as a leading sustainable resource “platform” material with potential for broad spectrum applications in nanotechnology.³⁸ CNC is typically made by hydrolysis in concentrated sulfuric acid.³⁹ The CNC product contains a sulfate ester moiety in the C₆ position. Carboxylated CNC (cCNC) is a much sought-after modification that offers advantages of higher thermal stability and the versatile chemical handle of the carboxylic acid group. cCNC is typically made by TEMPO or periodate oxidation.^{40, 41} A carboxylated “hairy” CNC has been described by van de Ven and co-workers.⁴² Recently, a new catalyst-free dilute aqueous hydrogen peroxide method to make cCNC has been introduced.⁴³ The scalable procedure uses 100% Forest Stewardship Certified (100% FSC) pulp. Peroxide is fully consumed in the process that produces little waste in the course of yielding cCNC in one step. cCNC based microbeads are produced by spray-drying from water. Diffusion, uptake and release kinetics can be important to understand how cCNC-derived microbeads in general might perform in different applications. Kinetic and transport properties of cCNC microbeads are therefore the subject of this paper.

Below we examine features of the uptake of methylene blue (MB) dye cation by cCNC microbeads. MB is a widely used model compound in studies of the uptake of charged entities in heterogeneous media.⁴⁴ Apart from release kinetics related to pharmaceutical applications, little has been published in the open literature about the mass transport properties of cellulose microbeads in general. Our study contributes new knowledge to cellulose microbead uptake in the context of a film-pore diffusion model with spherical boundary conditions. A gating mechanism is identified where MB uptake is regulated by a combination of charge potential and reversible cross-link formation. We use MB to probe kinetics and diffusion in suspensions of the microbeads in water and show how the transport characteristics are modified by changes in pH and ionic strength.

Dye uptake may be examined in terms of reaction-based and/or diffusion-based kinetics. Pseudo-first order and pseudo-second order models based on adsorption capacity are commonly used to explore solid–liquid interactions. They have been invoked in studies of MB adsorption on clay,⁴⁵ walnut shell powders,⁴⁶ activated carbon,⁴⁷ activated silica gel⁴⁸ and polyacrylamide/CNC hydrogel nanocomposites.⁴⁹ Kinetic models, however, have the shortcoming that they do not consider diffusion of the dye molecule. Several diffusion-based models are available to address this. These include the intraparticle diffusion model,⁵⁰ the film-surface diffusion model,⁵¹ the film-pore diffusion model,^{52, 53} and the branched-pore-diffusion model.⁵⁴ In our study, we use a two-resistance mass transport model, namely external mass transfer and internal diffusional mass transport, where internal diffusion transport is assumed to occur by pore diffusion.⁵⁵ The model invokes a lumped “effective” diffusion coefficient D_{eff} to incorporate both pore and surface diffusion. Below we show how the film-pore diffusion model incorporating the Langmuir isotherm gives insight into the transport properties of MB in the cCNC microbeads. We clarify how diffusion depends on pH, ionic strength and

temperature. Thermodynamic parameters are extracted to illustrate the temperature dependence of dye transport.

Experimental Section and Theoretical Modelling

2.1. Materials

cCNC samples were provided by Anomera Inc. cCNC microbeads were supplied as a spray-dried free flowing white powder. 4.22 wt % cCNC suspensions were provided in distilled water. Methylene blue hydrate, hydrochloric acid, sodium hydroxide, and sodium chloride were purchased from Millipore Sigma and used without further purification. 10 mm poly(styrene) cuvettes for electronic absorption spectroscopy were purchased from Sigma Aldrich.

2.2. Preparation of cCNC Aqueous Particle Suspensions and cCNC Microbeads

cCNC was prepared by reaction of 100% Forest Stewardship Certified high purity dissolving pulp, Temalfa 93 originating from stands of black spruce, *Picea mariana*. The catalyst-free reaction uses only aqueous hydrogen peroxide, which can be fully consumed in the process. The “waste” consists of dilute sugars in water. Carboxylated cellulose nanocrystals are produced simultaneously in the reaction, ie, there is no post-modification of the CNC. Accordingly, the preparation of cCNC was carried out in a 12 L grease free glass reactor equipped with a motorized glass stirring rod with a Teflon paddle. 30 wt % aqueous H₂O₂ (Peroxal®, Arkema) (2.5 L) was heated to reflux (115 °C). 200 g of dry pulp was cut into 1 cm x 5 cm strips and added to the refluxing H₂O₂. The mixture was stirred vigorously for 8 hours under reflux. The viscosity of the white suspension gradually diminished over time. The reaction was quenched by adding ice to dilute the solution to 4 L. The c CNC was then purified by diafiltration⁵⁶ using a Spectrum Labs 10 kDa mPES (modified polyethersulfone) hollow

fiber filter until the conductivity of the permeate was brought below 100 $\mu\text{S}/\text{cm}$. The cCNC suspension was neutralized with 1 M NaOH and then dispersed by sonication for 5 - 20 min at 80% output using a Sonics Vibra-cell VCX130.). cCNC microbeads were obtained as a white powder by spray-drying of a 4.22 wt % suspension in a commercial spray dryer (American Custom Drying, Burlington, New Jersey).

2.3. Physical Properties of cCNC Particle Suspensions

The carboxylate content of cCNC in an aqueous suspension of was determined by potentiometric titration. Zeta potential was determined with a Brookhaven NanoBrook Omni. TEM (FEI Technai 12 BioTwin 120 kV TEM) was used to image and measure cCNC particle dimensions of cCNC labeled with uranyl acetate (Figure 1). Nanocrystals ranged in width from 5 to 10 nm with an average length of 170 nm (Image J software, standard deviation 62.8 nm, χ^2 0.00493).

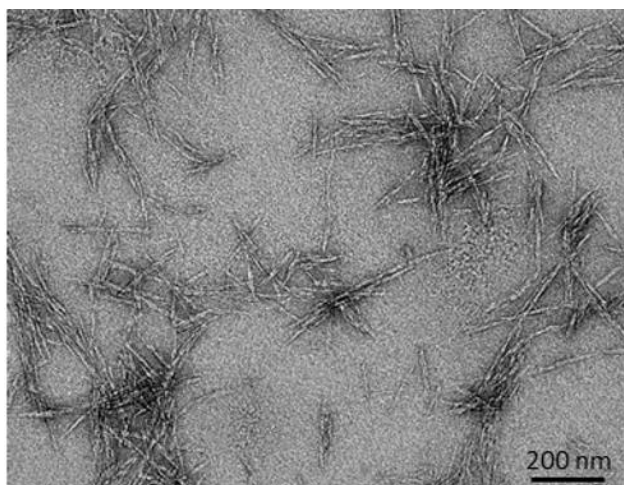


Figure 2-2 TEM image of cCNC labeled with uranyl acetate.

2.4. Characterization of cCNC Microbead Powder.

cCNC microbead morphology and size distribution were determined and measured using Scanning Electron Microscopy (FEI Inspect F50 Scanning Electron Microscope (FE-SEM)). An FEI Helios Nanolab 660 DualBeam (Focused Ion Beam-Extreme High-Resolution Scanning Electron Microscope) was used for cCNC cross section imaging. The samples were mounted on an aluminum SEM stub and coated with a 4-nm layer of Pt to enhance electrical conductivity. Milling of sample was carried out with the FEI Helios Nanolab 660 DualBeam instrument (Thermo Fisher Scientific, Hillsboro, OR USA). After selecting a specific particle, a 2 micron thick of protective platinum stripe was deposited on the surface of the region of interest to protect the surface from ion beam damage. Microbead cross-sections were then prepared by gallium ion milling. A primary trench to clean the sample was milled at 30 kV ion beam accelerating voltage and 21 nA beam current. Following cleaning cross-sections were obtained with beam current ranging between 9.3 nA to 0.79 nA. The smooth cross-section was imaged with electron beam at an accelerating voltage of 2 kV, beam current of 0.40 nA and working distance of 4 mm in the secondary electron mode.

Porosity and surface area were analyzed with a Micromeritics TriStar 3000 surface area unit. Nitrogen was used for adsorption analysis (BET method) and the samples were pre-treated by flowing nitrogen at 120 °C overnight. 0.5432 g cCNC powder was used for this study. Porosity was calculated from Equation 1. V_T is the total volume of sample. V_p is the total volume of pores. The density ρ of the cCNC microbeads was calculated according to Equation 2.

$$\varepsilon = \frac{V_T - V_p}{V_T} \quad (1)$$

$$\rho = \frac{m}{V_T - V_P} \quad (2)$$

2.4. Dye Adsorption Equilibrium Experiments

Freundlich, Langmuir and Temkin isotherms (Section 2.8.1) were determined from measurements of C_e and q_e . C_e is the concentration of dye in solution (mg/L) after equilibrium. This was obtained by measuring the absorbance of MB at 665 nm. q_e is the amount of dye adsorbed at equilibrium (mg/g). It was determined from Equation 3. For the isotherm studies, 50 mg samples of cCNC microbeads were added to dye samples over a range of MB concentrations spanning 5 - 100 mg/L. MB concentrations at C_e in water were determined by interpolation from a standard calibration curve.

$$q_e = \frac{(C_0 - C_e) \times V}{m_s} \quad (3)$$

2.5. Dye Adsorption Kinetic Experiments

A stock solution consisting of 100 mg/L MB was diluted to initial concentrations of MB of either 7 or 10 mg/L. Distilled water (conductivity: 0.0003 mS/cm measured by Metrohm 865 Conductivity Module) was used throughout. For each initial concentration, 5 mg of cCNC microbeads were added to 3 mL MB solution in a 10 mm pathlength polystyrene cuvette. The cuvette was equipped with a mini stir bar and thermally equilibrated in a temperature regulated (Peltier Control and Cooling Unit (PCCU1)) cuvette holder in the UV-Vis spectrometer chamber (Evolution 260 Bio UV-Visible spectrophotometer). Data acquisition began immediately and measurements were acquired at 1 s intervals at 665 nm until there were no

longer any changes in absorbance. The measurement was repeated several times showing almost identical dye uptake kinetics. For clarity in this paper, we report only one data set for kinetic modeling studies. The measurements were repeated at 5.00 °C, 25.0 °C and 45.0 °C.

2.6. Dye Adsorption Kinetic Experiments at Different pH

A stock MB solution at 100 mg/L was prepared and diluted with water adjusted to a predetermined pH. The pH was adjusted by diluting 1 M HCl or 1 M NaOH solutions, respectively. Dye adsorption experiments were performed as in 2.5 above. A pH meter (pHTestr 10) was used to determine the pH of the MB solutions.

2.7. Dye Adsorption Kinetic Experiments at Different Ionic Strength

A stock MB solution at 100 mg/L was prepared and diluted with distilled water adjusted to a predetermined ionic strength. Accordingly, 0.01 M, 0.001 M, and 0.0001 M salt concentrations were prepared by successive dilutions of 0.1 M NaCl stock solution. Dye adsorption experiments were performed as described in 2.5 above.

2.8. Numerical Treatment of the Data

2.8.1. Adsorption Isotherm

Adsorption isotherms provide insight into the properties of adsorption active sites and are also related to thermodynamic properties of the adsorption.^{8, 52} Langmuir, Freundlich and Temkin isotherms were tested. The Langmuir isotherm⁵⁷ assumes that adsorption takes place at homogeneously distributed sites at a solid surface and the surface of the adsorbent is uniform. Adsorbed molecules do not interact and all adsorption occurs through the same mechanism. The linearized Langmuir form is:

$$\frac{1}{q_e} = \frac{1}{K_a q_m} \frac{1}{C_e} + \frac{1}{q_m} \quad (4)$$

q_e is the amount of dye adsorbed per g of adsorbent (mg/g) at equilibrium. q_m is the maximum adsorption capacity (mg/L). K_a is the Langmuir isotherm constant related to the affinity of the dye for the binding sites. q_m and K_a were determined by plotting $1/q_e$ against $1/C_e$. The Langmuir equilibrium adsorption constant K_a is related to the Gibbs free energy (ΔG) of sorption *via* $\Delta G = -RT \ln K_a$.

The Freundlich model⁵⁸ is an empirical model that attempts to account for heterogeneous adsorption at a surface with non-uniformly distributed binding sites. Adsorption is assumed to be reversible and not limited to the formation of the monolayer. Accordingly, the Freundlich expression is:

$$\ln q_e = \ln K_f + \frac{1}{n} (\ln C_e) \quad (5)$$

K_f (L/mg) is a constant that reflects the adsorption capacity of the solid, and $1/n$ is a heterogeneity factor introduced to account for the type of isotherm: irreversible ($1/n = 0$), favorable ($0 < 1/n < 1$) and unfavorable ($1/n > 1$).

The Temkin isotherm⁵⁹ attempts to account for the effects of indirect adsorbate/adsorbate interactions on the adsorption process. The model assumes that the heat of adsorption of all molecules in the coverage layer decreases linearly, rather than logarithmically. The Temkin isotherm is expressed *via* Equation 6. Here, α represents the Temkin equilibrium binding constant (L/g), corresponding to the maximum binding energy. β is the Temkin isotherm constant related to the heat of adsorption (J/mol). Plots of the quantity of dye adsorbed at equilibrium, q_e , against $\ln C_e$, yield α and β from the slope-intercept form.

$$q_e = \frac{RT}{\beta} \ln \alpha + \frac{RT}{\beta} \ln C_e \quad (6)$$

2.8.2. Mathematical Modelling: Reaction-based Kinetic Models

Pseudo-first order kinetics associated with adsorption processes were first introduced by Lagergren,⁶⁰ and subsequently elaborated by Ho and McKay.⁶¹ The solution for pseudo-first order is given by:

$$q_t = q_e(1 - e^{-k_1 t}) \quad (7)$$

The rate constants k_1 can be determined from the slope in the plot of $\ln (q_e - q_t)$ versus t .

For pseudo-second order kinetics, one writes,⁶¹

$$\frac{dC}{dt} = -k_2 C^2 \quad (8)$$

with solution,

$$\frac{t}{q_t} = \frac{1}{q_e^2 k_2} + \frac{1}{q_e} t \quad (9)$$

A linear plot of t/q_t versus t yields both k_2 .

2.8.3. Mathematical Modelling: Diffusion-based Kinetic Model

This model incorporates diffusion-controlled kinetics of dye adsorption. We use the film-pore diffusion model, first developed by Spahn and Schlunder for adsorption on activated carbon.⁶² The model yields a governing equation by parsing the adsorption kinetics into two resistance steps: external mass transfer and internal mass transfer. Figure 2 clarifies these steps for our case of methylene blue dye uptake in the cCNC microbeads. The first external diffusion step

requires that the dye overcome a resistance diffusion barrier (dashed line) adjacent to the surface of a microbead. The second step is described by Fickian diffusion of MB inside the pores.⁵²

External Mass Transfer

At the initial adsorption stage, external-film diffusion⁶³ dominates and controls the adsorption rate. This process is represented in Equation 10.

$$\frac{dC}{dt} = -k_f \frac{A_s}{V} (C - C_s) \quad (10)$$

The meanings of the variables and constants are given in Table 1. The solution to Equation 10 yields:

$$\ln \frac{C_t}{C_0} = -k_f \frac{A_s}{V} t \quad (11)$$

From this expression, we extracted the external film mass transfer coefficient k_f . We used this value of k_f as the initial value for numerical modeling.

Film-Pore Diffusion Model Development and Methodology

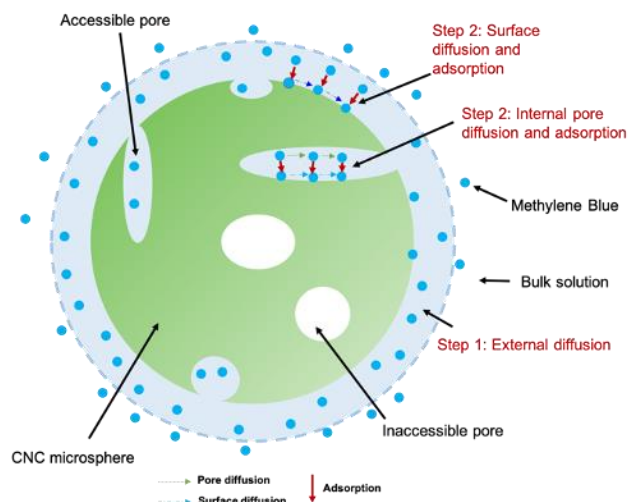


Figure 2-3 Illustration of external and internal diffusion for MB transport at the surface and within a cCNC microbead. The duplicate label, Step 2, indicates that the two processes are simultaneous in the model.

Table 2-1 Physicochemical parameters of cCNC microbead dye uptake used to solve the Film-pore diffusion model

A_s	Total surface area of the cCNC microbeads
V	Volume of solution
k_f	External liquid film mass transfer coefficient
C_t	Bulk MB concentration at time t
C_s	Surface concentration
C_e	Equilibrium dye concentration in liquid phase
C_{b0}	Initial bulk concentration
C_i	Solid state concentration at grid I at time t
D_{eff}	Internal effective diffusion coefficient
K_a	Langmuir adsorption constant
m_s	Mass of microbeads added
r	Radial position in the particle
R	Radius of the particle
q_{avg}	Average dye concentration in solid phase
q_e	Solid phase dye concentration at equilibrium
q_i	Solid phase dye concentration at grid I at time t
t	Time (s)
τ	Dimensionless time τ
ε	Particle porosity
ρ_p	Particle density
R^2	Coefficient of determination

Film-Pore Diffusion

Material balance yields Equation 12 for adsorption of dye with pore diffusion in a spherical particle:

$$\varepsilon \frac{\partial C_i}{\partial t} + (1 - \varepsilon) \rho_p \frac{\partial q_i}{\partial t} = \varepsilon D_{eff} \left[\frac{\partial^2 C_i}{\partial r^2} + \frac{1}{r} \frac{\partial C_i}{\partial r} \right] \quad (12)$$

The initial condition at $t = 0$ is $C_i = 0$ for $0 \leq r \leq R$. The boundary condition at the center of the particle ($r = 0$) is $\partial C_i / \partial r = 0$. At the solid surface where $r = R$, the rate of the MB transferred through the external film must be equal to the rate at which it diffuses into the solid.

In this way, we obtain:

$$k_f(C_t - C_s) = D_{eff} \frac{\partial C_i}{\partial r} \quad (13)$$

Assuming that MB equilibrates rapidly at the liquid-solid interface, the amount of MB adsorbed in the solid phase q_i at any radial location and time is related to the aqueous phase concentration C_i by any of the isotherm equations. The isotherms are incorporated in the variable q_i expressed in general form by Equation 14:

$$q_i = f(C_i) \quad (14)$$

Combining (12) and (14) gives of the general diffusion equation:

$$\varepsilon \frac{\partial C_i}{\partial t} + (1 - \varepsilon) \rho_p \frac{\partial f(C_i)}{\partial t} = \varepsilon D_{eff} \left[\frac{\partial^2 C_i}{\partial r^2} + \frac{1}{r} \frac{\partial C_i}{\partial r} \right] \quad (15)$$

For example, for Langmuir type dependence, we substitute the Langmuir isotherm expression into $f(C_i)$ in Equation 15, *i.e.*:

$$f(C_i) = \frac{q_e K_a C_i}{1 + K_a C_i} \quad (16)$$

The average dye concentration q_{avg} is obtained by integrating from the surface over the radius, R , of the particle, *i.e.*,

$$q_{avg} = \left(\frac{3}{R^2}\right) \int_0^R q_i r^2 dr \quad (17)$$

The bulk concentration of MB is obtained by considering solute balance for the adsorption process. This yields the overall balance:

$$V \frac{dC_t}{dt} = -m_s \frac{dq_{avg}}{dt} \quad (18)$$

The corresponding initial conditions are:

At $t = 0$, $C_t = C_0$ and $q_{avg} = 0$

We make the above equations dimensionless by defining the following variables:

$$Z = \frac{r}{R}; \quad \bar{C}_l = \frac{C_l}{C_0}; \quad \bar{C}_t = \frac{C_t}{C_0}; \quad B_i = \frac{k_f R}{D_{eff}}; \quad \tau = t \left(\frac{D}{R^2}\right)$$

These substitutions reduce Equation 15 to

$$\frac{\partial \bar{C}_l}{\partial \tau} = A(\bar{C}_l) \left[\frac{\partial^2 \bar{C}_l}{\partial Z^2} + \frac{1}{Z} \frac{\partial \bar{C}_l}{\partial Z} \right] \quad (19)$$

where,

$$A(\bar{C}_l) = 1 + \frac{(1 + \varepsilon) \times \rho_P \times q_m \times K_a}{\varepsilon \times (1 + K_a \times \bar{C}_l \times C_{b0})^2} \quad (20)$$

The substitutions lead to the following modified initial and boundary conditions:

When $0 \leq Z \leq 1$:

$$\bar{C}_l|_{\tau=0} = 0 \quad (21)$$

For $\tau \geq 0$:

$$\left. \frac{\partial \bar{C}_l}{\partial \tau} \right|_{Z=0} = 0 \quad (22)$$

$$B_i(\bar{C}_t - \bar{C}_s) = \left. \frac{\partial \bar{C}_l}{\partial Z} \right|_{Z=1} \quad (23)$$

The integral for the dimensionless average concentration in the solid phase is:

$$\bar{q}_{avg} = 3 \int_0^1 \bar{q}_l Z^2 dZ \quad (24)$$

The overall mass balance for the solute then becomes:

$$\frac{d\bar{C}_t}{dt} = - \frac{m_s q_h}{V C_{b0}} \frac{d\bar{q}_{avg}}{dt} \quad (25)$$

The corresponding initial conditions are:

$$\bar{C}_\tau|_{\tau=0} = \bar{C}_0; \quad \bar{q}_{avg}|_{\tau=0} = 0;$$

We solved this nonlinear adsorption model by the method of lines.⁵² The governing partial differential equations (PDEs) with their boundary conditions were converted to a set of first order ordinary differential equations (ODEs). The radial derivatives were discretized using the central finite difference method. The radius of the particles was divided into $N-1$ segments of

equal length. The grid points were indexed as $I = 1, 2, 3, 4 \dots N$. Here, $I = 1$ refers to the center of the particle and $I = N$ refers to the outer surface.

The resulting ODEs are given below:

- i. At the center ($Z = 0, j = 1$):

$$\frac{d\bar{C}_1}{d\tau} = A(\bar{C}_1) \times \frac{2 \times \bar{C}_1(2) - 2 \times \bar{C}_1(1)}{h^2} \quad (26)$$

- ii. At the interior points ($I = 2, 3, 4, \dots N-1$)

$$\frac{d\bar{C}_i}{d\tau} = A(\bar{C}_i) \times \left(\frac{\bar{C}_i(i+1) - 2 \times \bar{C}_i(i) + \bar{C}_i(i-1)}{h^2} + \frac{1}{Z} \times \frac{\bar{C}_i(i+1) - \bar{C}_i(i-1)}{2h} \right) \quad (27)$$

- iii. At the external surface ($Z = 1, I = N$)

$$\begin{aligned} \frac{d\bar{C}_N}{d\tau} = A(\bar{C}_i(N)) \times & \left(\frac{2h \times bi \times (\bar{C}_t - \bar{C}_i(N) + \bar{C}_i(N-1))}{h^2} - \frac{2 \times \bar{C}_i(N) + \bar{C}_i(N+1)}{h^2} \right) \\ & + \frac{1}{Z} \times \frac{2h \times bi \times (\bar{C}_t - \bar{C}_i(N))}{2h} \end{aligned} \quad (28)$$

MATLAB was used to solve the equations to yield the time evolution of the bulk liquid phase MB concentration. D_{eff} and k_f were determined by fitting the experimental data to the film-pore diffusion model using a genetic algorithm. Chi square values were determined according to Equation 29.⁶⁴ All parameters are reported in SI units, except as otherwise specified.

$$\chi^2 = \sum \frac{(C_{exp} - C_t)^2}{C_t} \quad (29)$$

Results and Discussion

3.1. Physical Characterization of the cCNC Suspension and Microbeads

The cCNC microbeads used in our study comprise agglomerates of cCNC constituent particles held together by van der Waals and hydrogen bond forces. The cCNC constituent particles were prepared by hydrolysis with < 15 wt % hydrogen peroxide, purified by diafiltration⁵⁶ and neutralized with NaOH. The reaction is effectively “one-step” since carboxylation occurs simultaneously with the production of the nanocrystals. Hydrogen peroxide is fully consumed in the reaction so that there is no need for peroxide capture and recycling. The reaction by-products are water and dilute, predominantly pentose and hexose sugars. This method avoids the harsh conditions that are commonly used to produce CNC, such as the use of sulfuric acids.⁶⁵ Potentiometric titration gave 0.15 mmol/g of carboxylic acid groups on the cCNC. TEM revealed that the cCNC particles have average dimensions of 170 nm (length) by 10 nm (width). cCNC microbeads were provided as a spray-dried white powder (see experimental section 2.2.). The morphology of the cCNC microbeads depends on spray-drying process parameters, which in our case were selected for industrial scale production.^{43,66-69} Higher particle concentrations lead to more irregular particles; the somewhat viscose suspension of cCNC causes deformation of the sprayed droplets, as well as pre-aggregation of cCNC in the source reservoir.

By SEM, the cCNC microbeads are textured distorted irregularly shaped particles (circularity ~ 0.7 (Sysmex FPIA 3000)) with diameters ranging from 2.1-8.7 μm (Figure 2-4 (a)). We selected several particles for focused ion beam (FIB) analysis to determine if the particles were hollow or densely packed with nanocrystals. Figure 2-4 (b) shows one of several particles selected for study. The dotted line shows where the particle was transected. From FIB-SEM images in 2-4 (c) and (d), the particle shows a dense internal structure, though the resolution of the SEM is not high enough to discern porosity.

BET measurements yielded surface area 1.49 m^2/g . This low value compared with other porous microbeads reflects the high packing density of the cCNC particles in the microbead. From the BET measurement and Equation 1, we calculate a porosity (void fraction) of 0.99. Density was determined to be 1000 kg/m^3 from Equation 2. Overall, the microparticles appear to be dense agglomerates of nanocrystals with low porosity.

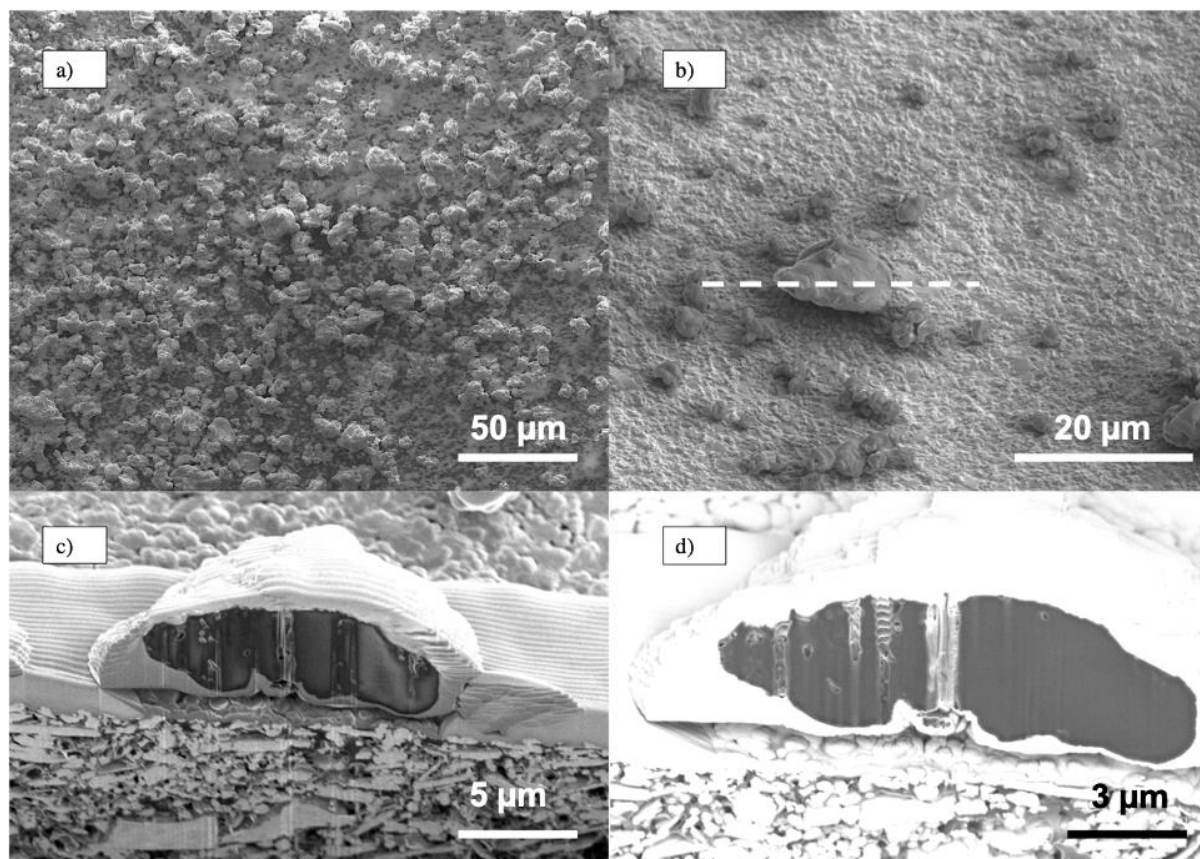


Figure 2-4 SEM images characterize the cCNC microbead surface and internal morphology. (a) and (b) SEM images of cCNC microparticles with different magnification. (c) and (d) Focused Ion Beam (FIB)-SEM cross-section images of the internal structure of the distorted cellulose microbead indicated in (b). The dashed line indicates the position of the cut through the particle.

3.2. Adsorption Isotherms

We investigated dye uptake according to Langmuir, Freundlich and Temkin isotherms. Figure 2-5 (a) shows that as the equilibrium concentration C_e increases, the amount of dye adsorbed, q_e , rapidly increases until $C_e \cong 3$ mg/L. After this point, dye adsorption begins to saturate. Beyond $C_e \cong 20$ mg/L, q_e continues to increase but with a diminished slope. We believe the large errors beyond $C_e = 25$ mg/L, are the result of competing processes of adsorption and MB self-aggregation.⁷⁰ Therefore, we fitted the different isotherms for C_e in the region below 20

mg/L. From Table 2-2, we cannot distinguish between the Langmuir and the Temkin isotherms based on the coefficient of determination alone. Fits to the Freundlich isotherm were unsatisfactory. In the following sections, we focus our discussion primarily on the Langmuir isotherm in order to compare our findings with extant literature data.^{13, 71, 72}

Equilibrium for MB adsorption was reached within 300 s. From the Langmuir isotherm, the maximum absorption capacity of the cCNC microbeads at room temperature is $q_m = 130.7$ mg/g. The isotherm constant is $K_a = 0.347$ L/mg for our carboxylate content of 0.15 mmol/g. We compare these results with those reported by He *et al.* for MB adsorption on lyophilized cCNC powders (carboxylate content ~ 0.006 mmol/g to 0.012 mmol/g). They find $K_a \sim 0.0194$ L/mg and $q_m \sim 101.16$ mg/g.⁷² This suggests that cCNC microbeads may offer advantages of faster adsorption and higher capacity over lyophilized CNC and perhaps other cellulose for water treatment.^{70, 72} It has been noted that the adsorption capacity of cCNC depends on the carboxylate content. TEMPO oxidation can produce large quantities of surface carboxylate. Tam *et al.* reported that TEMPO oxidized CNC (2.1 mmol/g) yielded values of 769 mg/g and 0.0091 L/mg for q_m and K_a , respectively.⁷¹ Room temperature adsorption of MB on sulfated CNC in aqueous suspension yielded values of $q_m = 118$ mg/g and $K_a = 0.0137$ L/mg.⁶⁷ These data for K_a indicate that our cCNC microbeads exhibit a greater propensity for adsorption than do either the lyophilized powders of cCNC or aqueous suspensions of sulfated CNC.

MB uptake is influenced by pH and ionic strength. The effects of these variables show up in the adsorption isotherms of Figure 2-6. The results of Langmuir fitting in Table 2-3 show that both K_a and q_m increase when increasing pH. Above pH 5.0, the carboxyl groups on the cCNC are deprotonated yielding an increase in negative surface charge density (pK_a of carboxylic acid ~ 4.74). Hence, the number of active sites for MB dye uptake increases. Under acidic conditions, protonation decreases negative charge and increases hydrogen bonding. We

observed that the latter effect reduces particle swelling in water, and together these decrease both K_a and q_m . Figure 2-6 (b) shows the influence of ionic strength on the Langmuir isotherm. Increasing NaCl concentration leads to decreasing K_a and q_m (Table 2-3). This is expected, since Na cation will compete with MB for charge at higher concentrations of Na^+ . Inspection of the microbead suspension by optical microscopy reveals that increases in ionic strength promote aggregation of the microbeads. This too can reduce the binding MB by occluding sites because of particle-particle condensation. Yang *et al.*¹³ point out that increasing the ionic strength may promote MB self-aggregation in solution, leading to decreased MB diffusivity in water, thus, decreasing the maximum adsorption capacity.

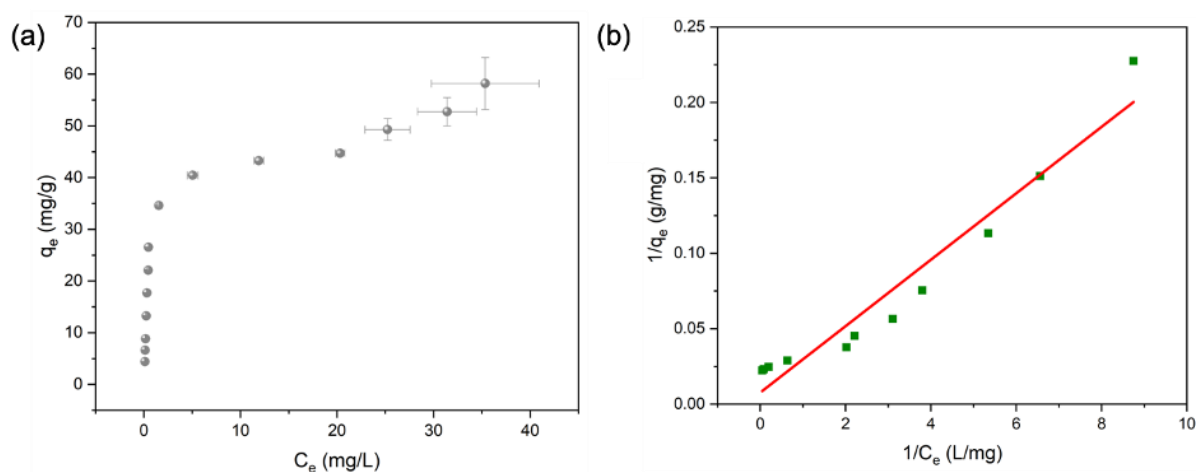


Figure 2-5 MB dye uptake isotherms and the Langmuir fit. (a) The relation between the amount of adsorbed methylene blue per unit mass of cCNC microbeads and methylene blue concentrations at equilibrium derived from experimental data for methylene blue adsorption by cCNC microbeads in water. (b) Linear fit to the Langmuir isotherm.

Table 2-2 Freundlich, Langmuir and Temkin isotherm parameters for MB adsorption by cCNC microbeads at room temperature

Freundlich Isotherm			Langmuir Isotherm			Temkin Isotherm		
k_f (L/mg)	n	R^2	K_a (L/mg)	q_m (mg/g)	R^2	α (L/g)	β (J/mol)	R^2
20.12	2.584	0.7631	0.3474	130.7	0.9436	3.176	309.7	0.9295

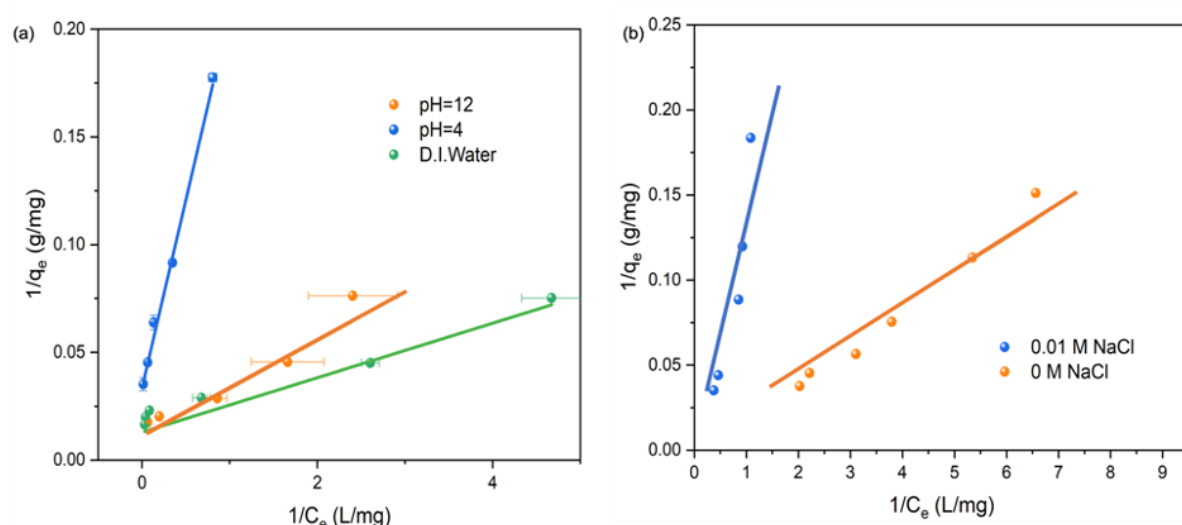


Figure 2-6 Langmuir isotherm dependence on pH and ionic strength. (a) MB dye uptake Langmuir isotherm dependency on pH for cCNC microbeads at room temperature. (b) Langmuir isotherm plots for MB uptake with 0 M NaCl and 0.01 M NaCl at room temperature.

Table 2-3 Langmuir isotherm parameters for MB dye uptake by cCNC microbeads at room temperature at different pH and salt concentrations

Langmuir Isotherm	K_a (L/mg)	q_m (mg/g)	R^2
pH = 4	0.1923	29.90	0.9968
pH = 10	0.5208	90.91	0.9948
pH = 12	0.2169	166.7	0.9249
0.01 M NaCl	0.04673	163.0	0.9499

3.3. Dye Uptake: Pseudo-First and Pseudo-Second Order Kinetics

We conducted dye uptake kinetics experiments at initial concentrations of 5 mg/L and 8 mg/L, and at temperatures of 278 K, 298 K and 318 K. The kinetics were analyzed according to pseudo-1^o and pseudo-2^o schemes. The results are shown in Table 2-4 and Figure 2-7 (a) and (b). Based on higher R^2 values, the pseudo-second order model is slightly favoured over first order kinetics. Table 2-4 also documents the influence of temperature on dye adsorption, where we observe that an increase in temperature leads to an increase in the adsorption rate constants, although the dependence on concentration is puzzling.

Reaction-based kinetics regard dye uptake as a chemisorption process, where the rate of sorption is described by k . From Table 2-4, the k values change with initial dye concentration C_0 . This indicates that the microbead dye uptake active sites are not saturated for a given C_0 .

But the MB dye uptake is not reducible to a chemisorption process alone. Diffusion plays an important role in the process, especially in the case of objects like cCNC microbeads. We examine the role of diffusion in the next section.

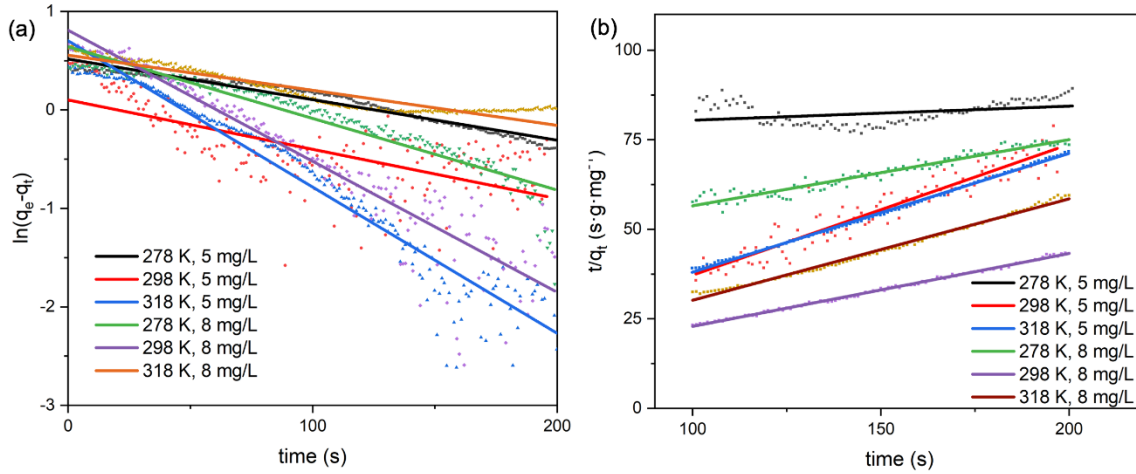


Figure 2-7 Pseudo-first order and second order kinetics. (a) Linear fit to pseudo-first order kinetics at different temperatures and initial concentrations. (b) Linear fit to pseudo-second order kinetics at different temperatures and initial concentrations.

Table 2-4 Pseudo-first and Pseudo-second order parameters for MB adsorption by cCNC microbeads at different temperatures and initial concentrations

Temperature	C_0 (mg/L)	Pseudo First order		Pseudo Second order	
		k_1 (s ⁻¹)	R^2	k_2 (mg·g ⁻¹ ·s ⁻¹)	R^2
278 (K)	5mg/L	1.5×10^{-3}	0.9539	1.7×10^{-3}	0.1264
	8mg/L	2.6×10^{-3}	0.9028	3.3×10^{-3}	0.9411
298 (K)	5mg/L	1.7×10^{-3}	0.4851	5.5×10^{-2}	0.9048
	8mg/L	2.8×10^{-3}	0.8983	1.9×10^{-2}	0.9969
318 (K)	5mg/L	5.3×10^{-3}	0.9421	2.7×10^{-2}	0.9979
	8mg/L	0.8×10^{-3}	0.8849	2.7×10^{-2}	0.9923

3.4. Temperature Dependent Dye Uptake Using the Film-Pore Diffusion Model

In this section, we explore diffusion-controlled kinetics in light of a film-pore diffusion model.

Referring back to Figure 2-2, we first concentrate on the process of external film diffusion. The external resistance model is known to apply in the early stage of dye uptake.⁷³ From Equation 11, we determined the external film mass transfer coefficient k_f from the slope of the linear portion of the curves in Figure 2-8 (b) after plotting them in the form $\ln(C_t/C_0)$ versus t (compare Figure 2-8 (a)). We found that the coefficient of determination was sensitive not only to the choice of the contact time where Equation 11 is presumed to be valid, but also to fluctuations in extinction caused by optical scattering by small amounts of suspended microparticles that had remained unsettled. We believe optical scattering is largely responsible for the variation in R^2 values. Table 2-5 shows that k_f values increase by a factor of about 10 with increasing temperature. Moreover, the time required for external mass transfer of MB decreases from 50 s at 278 K to 15 s at 298 K. These values of k_f were used as initial guesses to solve the film-pore diffusion problem, which is discussed next.

Application of the film-pore diffusion model (*cf.* Equations 19 *et seq.*) gives k_f on the order of 10^{-6} , and D_{eff} on the order of 10^{-11} . Internal diffusion coefficients for Methylene Blue adsorption on different types of leaf powders have been found to be on the order of 10^{-12} .⁵² The higher effective diffusivity in our case could be due to the enlarged pore size caused by swelling of the cellulose microbeads in water. When modelling film pore diffusion kinetics, we have assumed that particle swelling is essentially instantaneous or at least very rapid compared with the rate of dye diffusion. This assumption is justified by optical microscopy where we observe rapid particle swelling in water (Figure S2-1).

The film-pore diffusion model was then used to examine the effect of temperature on dye uptake. The results are collected in Table 2-5. Temperature increases lead to increases in k_f . The increase in k_f may be due to the enhanced mobility of MB in water at the higher temperatures; and decreases in the solution viscosity combined with decreases in the external resistance, *i.e.* decreases in the thickness of the boundary layer.⁵² More insight into the effect of temperature can be gleaned from the Biot number (see below).

Table 2-5 indicates that increasing the temperature seems to have an anomalous effect on D_{eff} - the effective diffusion coefficient is observed to *decrease* with increasing temperature. We do not have an explanation for this, although we observe that Ponnusami *et al.* reported a similar decline in D_{eff} with increasing temperature in their studies of MB adsorption onto Guava leaf powder.⁵²

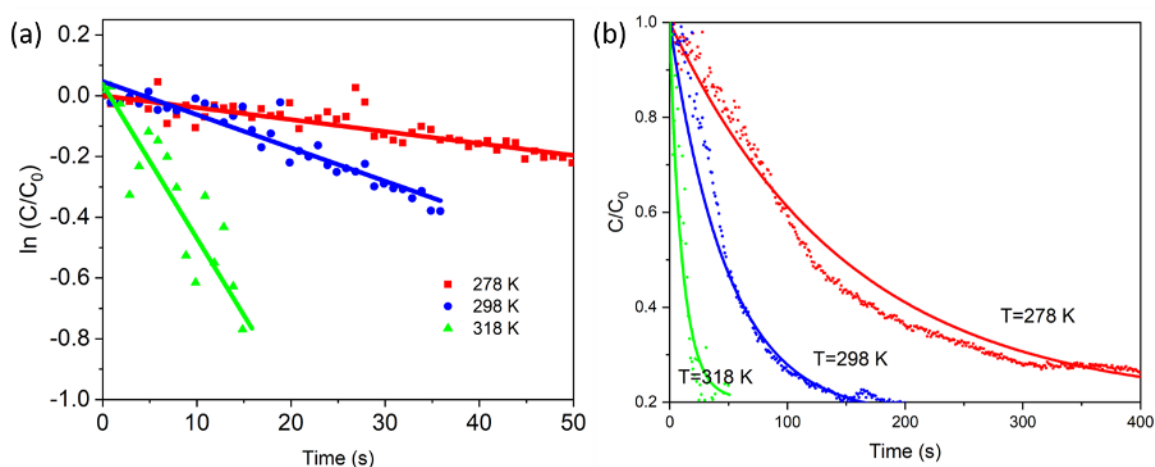


Figure 2-8 Film-pore diffusion model. (a) Linear fit to single external diffusion model for experiments at different temperatures for initial adsorption. (b) Film-pore diffusion model based on Langmuir isotherm fitted at different temperatures.

Table 2-5 Single external diffusion model and film pore diffusion model based on Langmuir isotherm and Freundlich isotherm parameters for cCNC microbead adsorption kinetics

T	Single external diffusion		Film-pore diffusion			
	k_f (m s ⁻¹)	R ²	k_f (m s ⁻¹)	D_{eff} (m ² s ⁻¹)	R ²	χ^2
278 K	1.6×10^{-6}	0.831	3.0×10^{-6}	9.9×10^{-11}	0.971	1.12
298 K	5.3×10^{-6}	0.910	7.3×10^{-6}	7.5×10^{-11}	0.956	0.776
318 K	20×10^{-6}	0.878	9.9×10^{-6}	2.0×10^{-11}	0.893	1.15

Table 2-6 Biot number calculated at temperature 278 K, 298 K, and 318 K for Film-pore diffusion model

Temperature	278 K	298 K	318 K
Biot number	0.060	0.19	0.99

The Biot number may be used to determine the rate-limiting step in film-pore diffusion.⁷⁴ As shown in Equation 30, it represents the ratio of the rate of transport across the liquid layer (external mass transfer) to the rate of intraparticle diffusion. If the Biot number is greater than 100, the adsorption process is controlled mainly by internal diffusion. A Biot number less than 100 indicates that dye uptake is controlled by external mass transfer. Table 2-6 shows Biot numbers for different temperatures, clearly indicating that dye uptake is controlled by external mass transfer. The fact that the Biot number increases with temperature suggests that external resistance becomes less dominant at higher temperatures.

$$B_i = \frac{k_f \times d}{D_{eff}} \quad (30)$$

3.5. pH and Ionic Strength Dependent Dye Uptake Kinetics in the Film-Pore

Diffusion Model

The state of aggregation of cCNC nanorods in water is well-known to depend on pH.⁷⁵⁻⁷⁷ At low pH, a gel may form due to enhanced hydrogen bonding between carboxylic acid groups and the abundant hydroxyl groups on cCNC surface. At higher pH, electrostatic repulsion among negatively charged cCNC particles stabilizes the nanorods, yielding a well-dispersed cCNC water suspension. MB dye uptake by suspensions of cCNC microbeads will be affected by changes in pH. Figure 2-9 (a) and (b) offer a model for what might occur in the microparticles with changing pH. The left-hand image in Figure 2-9 (a) shows 2 nanocrystals interacting *via* hydrogen bonds at low pH. The right-hand image shows the particles separated from one another due to electrostatic repulsions that ensue as the pH is increased. The latter case should lead to a more expanded microparticle, which is what we observe by optical microscopy. In effect, MB uptake is gated by the response of the microbead to changes in pH.

Figure 2-9 (c) shows the dye uptake kinetic data and the fit according to the film-pore diffusion model. Dye uptake is inhibited between pH = 2 to pH = 4. Close examination of the early part of the data for pH 4 reveals downward curvature from $t = 0$ to about $t = 25$ s. This shows that dye uptake does not depend exclusively on surface charge, but also implicates diffusion processes. There is a sudden change in the curves between pH 4 and pH 7 where MB uptake dramatically increases. MB uptake then decreases from pH 10 to 12. The pH experiments were not adjusted for changes in ionic strength. The influence of ionic strength is clarified in Figure 2-9 (d) at a fixed pH of 6.2 and temperature of 294 K. Increases in the medium ionic strength lead to decreases in MB uptake, showing how increasing the Na^+ concentration competes with

MB for binding sites. Table 2-7 and Table 2-8 collect the fitting results for the pH and ionic strength experiments in the film-pore diffusion model. We find that k_f values for *external mass transport* depend on both pH and ionic strength. Increasing the pH causes k_f to increase because there are more negatively charged binding sites (see Figure 2-9 (a) and (b)). Decreasing the ionic strength reduces competition between MB and Na^+ for binding sites, and this is reflected in the larger value of k_f at the lowest concentration of Na^+ . The increase in D_{eff} between pH 7 and 12 is real. Microparticles swell under basic conditions and swelling increases the available pore volume, resulting in an enhanced D_{eff} . In contrast, D_{eff} is insensitive to ionic strength in the range examined. Our findings locate the cCNC microbeads in the class of materials where the uptake (and release) of molecules is gated by pH and/or ionic strength.

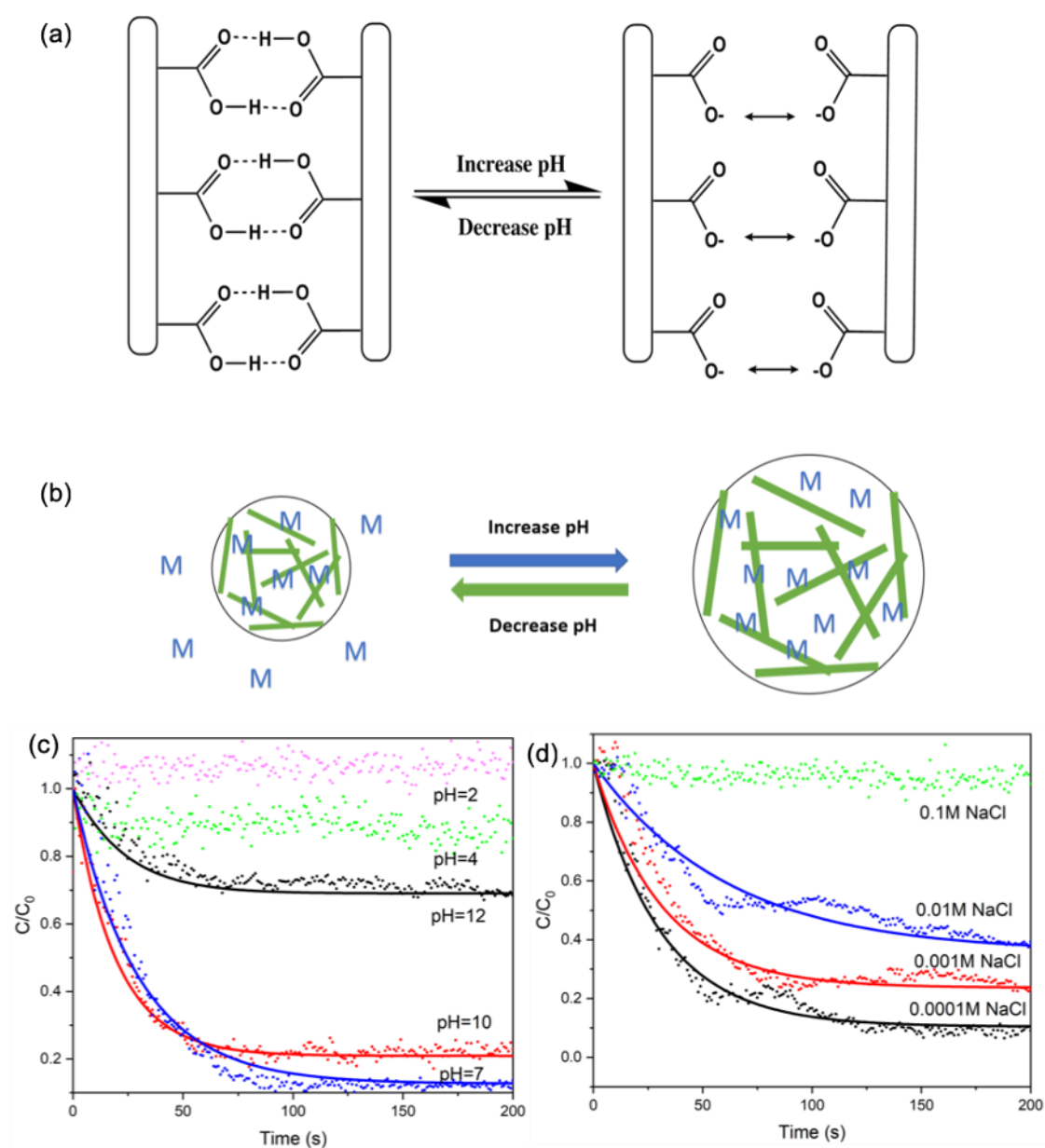


Figure 2-9 pH dependent MB dye uptake kinetics and film pore diffusion model fitting. (a) cCNC microbead pH response proposed at molecular level. (b) cCNC microbeads MB dye uptake pH response illustration at micro level. (c) MB dye uptake by cCNC microbeads at different pH fitted to film-pore diffusion model based on a Langmuir isotherm. (d) MB dye uptake on cCNC microbeads at different ionic strength fitted to the film-pore diffusion model based on the Langmuir isotherm.

Table 2-7 The Film-pore diffusion kinetic model based on Langmuir isotherm parameters for MB adsorption on cCNC microbeads at various pH at room temperature

	pH = 12	pH = 10	pH = 7	pH = 4	pH = 2
k_f (m s ⁻¹)	9.7×10^{-6}	6.3×10^{-6}	6.2×10^{-6}	-	-
D_{eff} (m ² s ⁻¹)	5.7×10^{-11}	1.2×10^{-11}	1.2×10^{-11}	-	-
R ²	0.9844	0.9771	0.9614	-	-
χ^2	0.337	0.5652	1.560	-	-

Table 2-8 The Film-pore diffusion kinetic model based on Langmuir isotherm parameters for MB Adsorption on cCNC microbeads at various ionic strength at room temperature

	0.1 M NaCl	0.01 M NaCl	0.001 M NaCl	0.0001 M NaCl
k_f (m s ⁻¹)	-	2.5×10^{-6}	3.3×10^{-6}	9.8×10^{-6}
D_{eff} (m ² s ⁻¹)	-	2.4×10^{-11}	1.1×10^{-11}	2.0×10^{-11}
R ²	-	0.9146	0.8648	0.9555
χ^2	-	0.827	1.55	1.28

3.6. Thermodynamics

We extracted thermodynamic parameters for MB adsorption shown in Table 2-9 from the temperature dependence of the Langmuir constant, K_a and the following equations:⁷⁸

$$\Delta G = -RT \ln K_a \quad (31)$$

$$\ln K_a = -\frac{\Delta H}{R} \frac{1}{T} + \frac{\Delta S}{R} \quad (32)$$

$$\Delta G = \Delta H - T\Delta S \quad (33)$$

For our cCNC microbead samples, ΔG varies from -0.61 kJ/mol to -1.0 kJ/mol, indicating spontaneous adsorption. The small positive value of the enthalpy ΔH shows that the MB dye adsorption on the cCNC microbeads is somewhat endothermic.⁷⁹ The positive values of ΔS suggest considerable reorganization at solid-liquid interface as a result of dye uptake.^{80, 81}

Table 2-9 Thermodynamic parameters for MB adsorption on cCNC microbeads at various temperatures

Temperature	K_a (L/mg)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/(mol·K))
278 K	0.26	-0.61	6.9	14
298 K	0.35	-0.86		
318 K	0.39	-1.0		

Activation energies for MB uptake were determined from the Arrhenius Equation 34.

$$k = k_0 e^{-Ea/RT} \quad (34)$$

The external mass transfer constant k_f from film pore diffusion yielded 22 kJ/mol. The activation energy for MB adsorption obtained from pseudo-second order kinetics on lyophilized cCNC yielded 18.52 kJ/mol.⁷² MB adsorption on cereal chaff, wheat shells, and Palygorskite gave energies of 22.40 kJ/mol,⁸² 16.63 kJ/mol,⁸³ and 13.97 kJ/mol,⁸⁴ respectively. Thus, the activation energies for MB adsorption on cCNC microbeads fall within range of these studies, despite differences in the substrates.

Conclusions

This study has provided new insights into the mass transport characteristics of cellulose microbeads, and in particular a new class of microbead comprising carboxylated cellulose nanocrystals (cCNC). The cCNC was obtained by a sustainable manufacturing method from 100% Forest Stewardship Certified (FSC) pulp and a benign oxidative hydrolysis with dilute aqueous hydrogen peroxide. Methylene blue dye uptake by spray-dried cCNC microbeads was found to obey pseudo-first order kinetics. More detailed knowledge of the transport properties of the CNC microbeads was obtained by exploring a film-pore diffusion model. The model was conditioned by incorporating one of several possible adsorption isotherms. The model considered transport of the dye across a resistance boundary (external diffusion layer) and intraparticle transport (internal diffusion). With Langmuir type behavior, the external-film mass transfer coefficients k_f were found to be $\sim 10^{-6}$ m/s. Effective internal diffusion coefficients D_{eff} , were on the order of 10^{-11} m²/s. cCNC microbead MB uptake was found to be both pH and ionic strength dependent. Biot numbers indicate that external film mass transfer dominates the dye uptake process. Thermodynamic parameters show that MB uptake is

spontaneous and slightly endothermic. Arrhenius treatment of the kinetic data from pseudo-first order analysis and from the film-pore diffusion model for cCNC microbeads yielded values in line with those obtained from MB adsorption on inorganic mineral and other cellulosic surfaces. In the future work, we suggest that the film-pore diffusion model be elaborated with more complex isotherm models to address the limitations of the simpler isotherms explored in this paper.

Supplementary

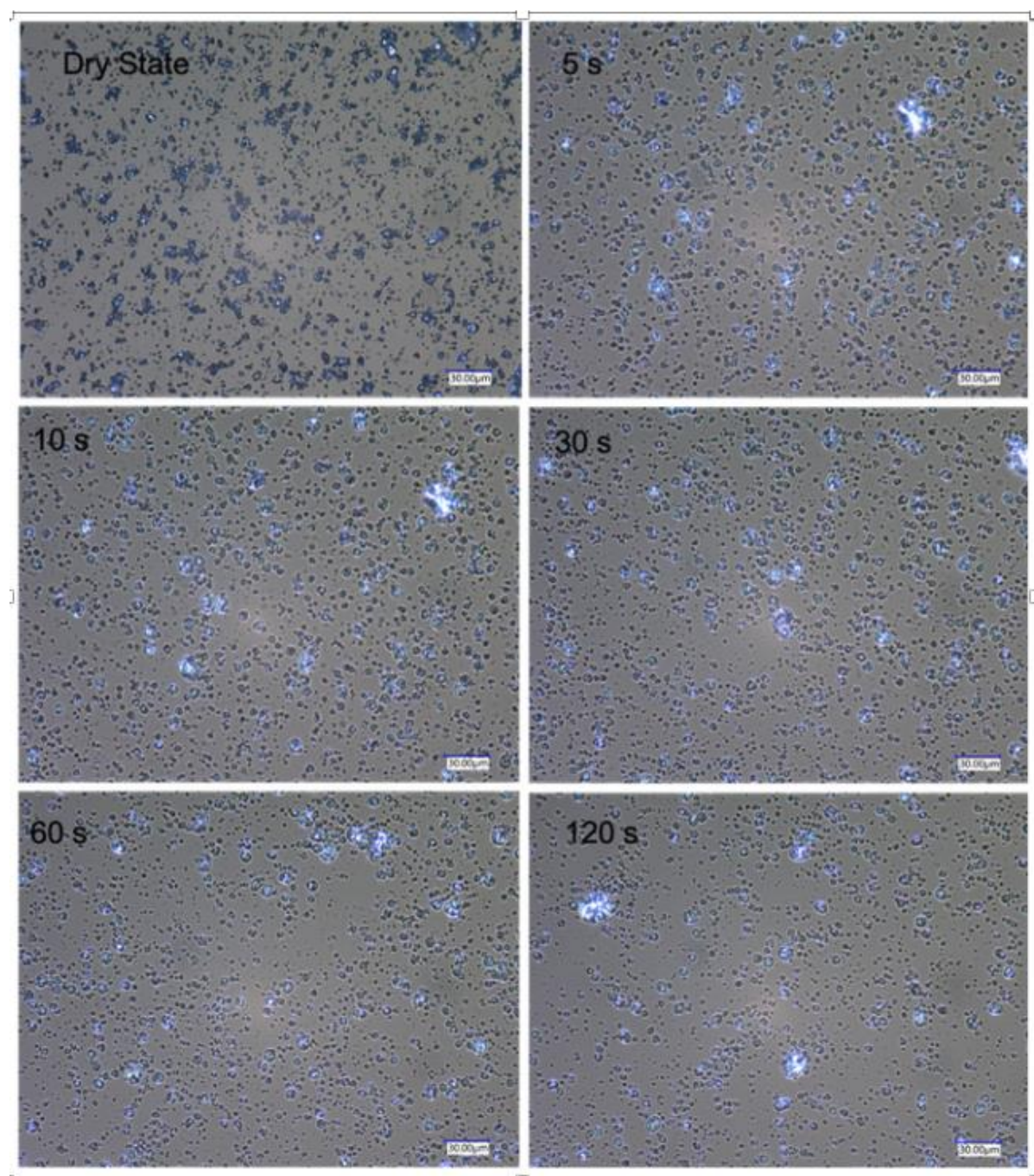


Figure S 2-1 Optical microscopic images of cCNC microbeads at dry state and when immersed in water after 5 s, 20 s, 30 s, 60 s and 120 s.

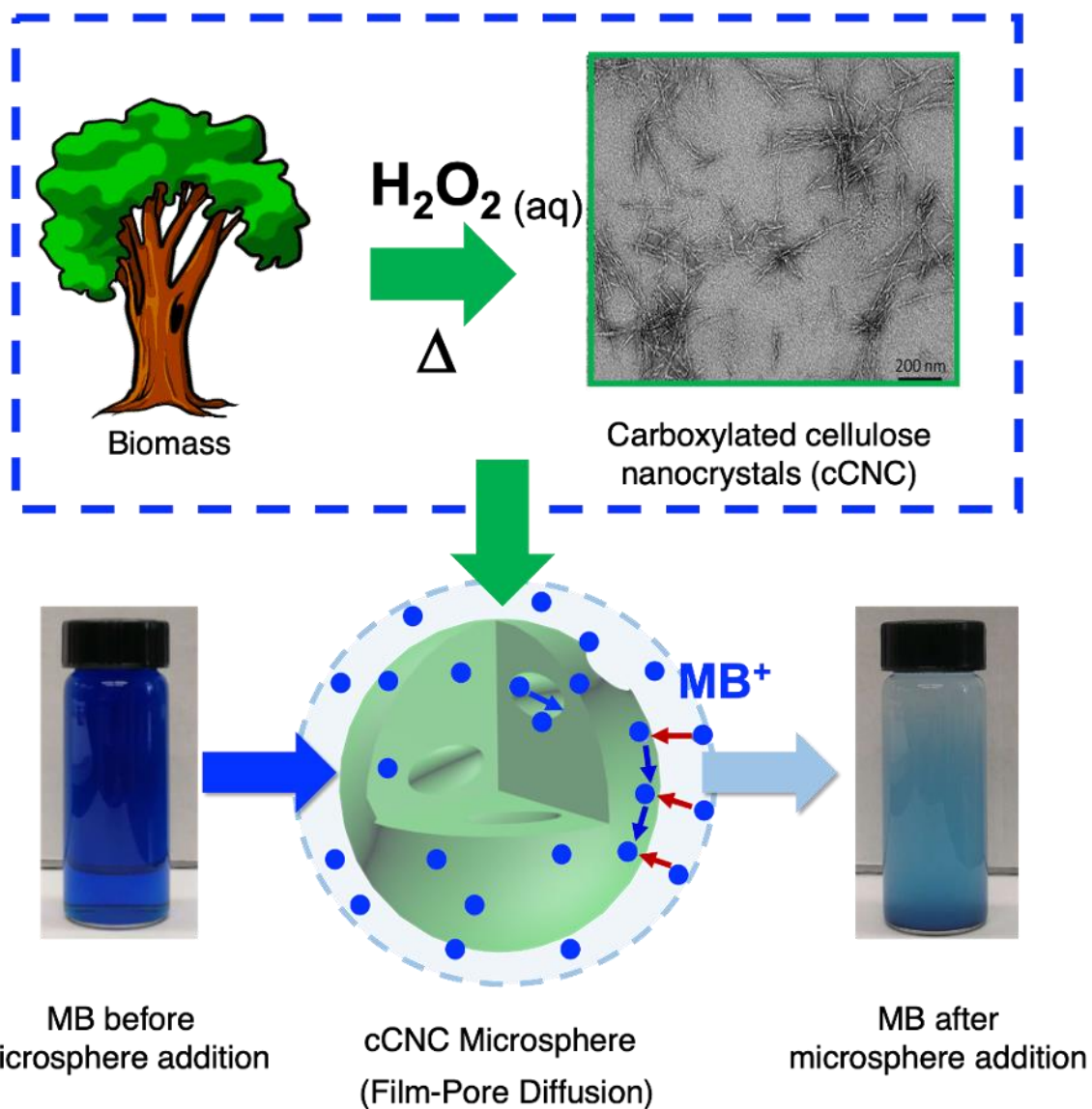


Figure S 2-2 Table of content

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| 3 |

**Stimuli-Responsive Carboxylated Cellulose Nanocrystal
Microbeads**

Chapter 3 Stimuli-Responsive Carboxylated Cellulose

Nanocrystal Microbeads

This chapter includes one manuscript that is ready to submit, one on-going project and one patent that is in preparation.

- Manuscript: “Stimuli-Responsive Carboxylated Cellulose Nanocrystal Microbeads ”
Ready to submit
- Patent: “Hybrid Cellulose Microbeads”, patent pending

The bibliographic details of the projects are:

Junqi Wu, and Mark P. Andrews

My contribution to the project involved:

1. Experimental design and implementation
2. Data collection and analysis
3. Preparation of the manuscript

Preface

In Chapters 1 and 2, we introduced concept that cellulose microbeads can be assembled from carboxylated cellulose nanocrystal (cCNC) building blocks. In Chapter 2 we demonstrated conditions under which cCNC microbeads mediate uptake and release of the small molecule model compound, methylene blue (MB). MB was used to probe kinetics and diffusion in the cCNC microbeads in water and to show how the transport properties can be gated (controlled) by changes in pH and ionic strength. In this chapter, we explore the response of cCNC

microbead composite hydrogels to triggers like pH and light. We alter the composition of the microbeads selectively. We consider two cases. In the first case, we combine poly(acrylic acid) with cCNC to make a bead that responds to changes in pH. In the second case, we attach a coumarin molecule that undergoes reversible photodimerization, locking and unlocking cCNC units to introduce a gate for molecular transport. Both cases underscore the potential of the modified cCNC microbeads to behave as “smart materials”. Smart materials are defined as materials that demonstrate changes in physical properties in response to environmental changes or external stimuli such as light, heat,¹ electric field,² or pH.³ These physical properties include particle size, mechanical properties, and small molecule mass transport properties. Smart materials can present in various forms such as those in solutions,⁴ in gels,⁵ in microbeads,⁶ in aerogels,⁷ in capsules,⁸ and in bulk solids and films forms.⁹ Researchers have paid much attention to smart materials in recent years due to their significant potential in pharmaceuticals,^{10,11-14} as membranes,¹⁵ for sensors and actuators,¹⁶ in smart coating materials,¹⁷ ¹⁸ as self-repairing materials,¹⁹ and for textiles.²⁰

Light-responsive and pH-responsive smart materials have attracted broad attention. As an external trigger, light can be localized in time and space. Therefore, optical stimuli can provide a local, fast, and non-contact approach to introduce energy and trigger either a chemical reaction or a conformational change. pH impacts physiological changes such as solubility and the generation of surface charges.²¹ Most biological systems possess pH gradients like those in blood, in gastrointestinal systems, and in normal/cancerous tissues.²¹ In these contexts, microbeads can be utilized for biomedical applications like pH-responsive targeted drug delivery systems.

The two case studies we present below demonstrate how pH and light can be utilized to trigger a stimulus-response in selectively modified cCNC microbeads. These modifications are

illustrated in the two routes depicted in Figure 3-1. pH responsive cCNC nanocomposite microbeads are made by combining the cCNC building blocks with a pH-responsive polymer, poly(acrylic acid) (PAA), as in Figure 3-1 (a). Light-responsive cCNC microbeads are synthesized by amidization chemistry by grafting a coumarin derivative onto cCNC (Figure 3-1(b)).

We selected poly(acrylic acid) because it is among several polymers that have been developed to deliver drugs across the mucous membrane. Crosslinked PAA is also used as a superabsorbent and soil amendment in agriculture. The efficacy of PAA for drug delivery and agricultural applications^{22, 23} depends on its response as a hydrogel. Hydrogels are adsorptive materials composed of hydrophilic component, usually a polymer, linked through chemical or physical means to enable absorption and retain large volumes of water within a three-dimensional network without dissolution. This efficacy is lost when using linear PAA because its high water solubility critically limits its use as a hydrogel medium unless combined and physically stabilized with other components like carboxymethyl cellulose,²⁴ or chitosan.²⁵

We show that we can make stable hydrogel composite microbeads of cCNC/PAA. Hydrogen bonding and crosslink formation during gas phase esterification confer pH stability against breakup of the microbeads. The hybrid microbeads swell in water, depending on pH and the ratio of cCNC to PAA. We extend our study of methylene blue dye uptake kinetics reported in Chapter 2 to show how pH regulates MB uptake in the microbeads. We then demonstrate uptake and release of MB gated by the reversible photodimerization of a coumarin derivative covalently bound to populations of cCNC in films and microbeads.

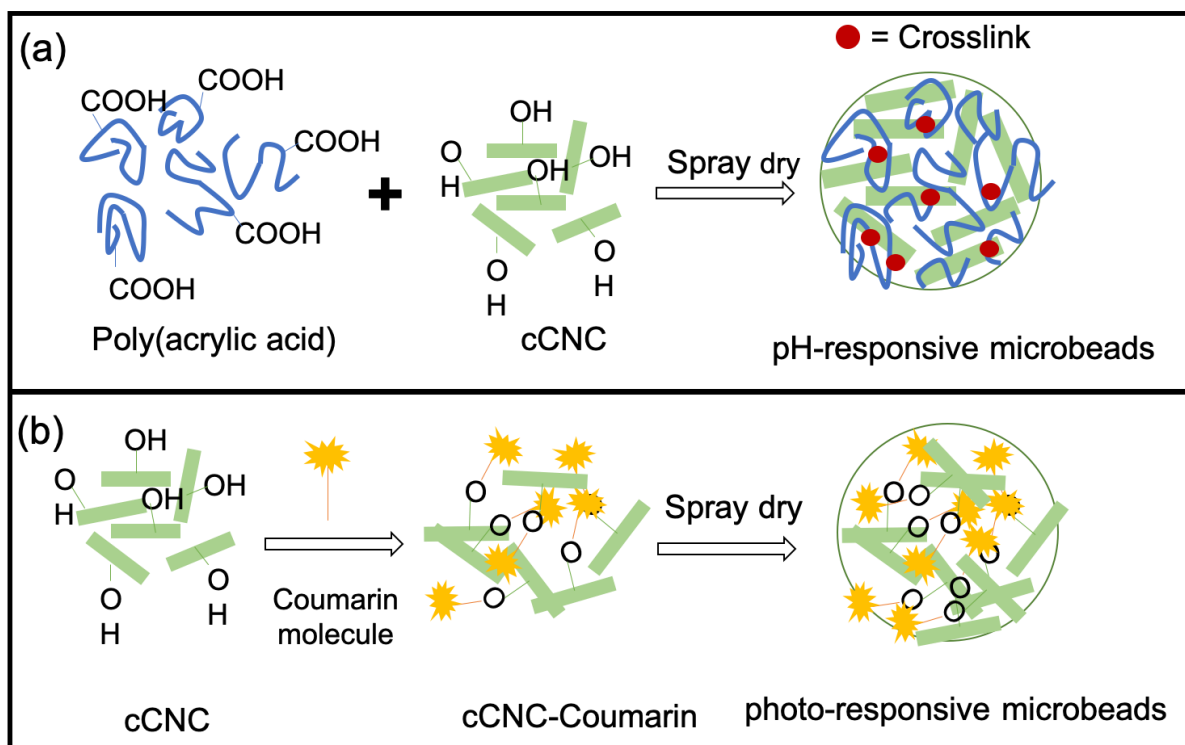


Figure 3-1 Two methods to make stimuli-responsive cCNC-based microbeads. (a) Hybridization with poly(acrylic acid) to make pH-responsive cCNC nanocomposite microbeads. (b) Chemical modification to graft a coumarin moiety to cCNC to make photo-responsive cCNC microbeads.

Case Study 1. pH responsive Carboxylated Cellulose

Nanocrystal – Poly(acrylic acid) Hybrid Microbeads

Introduction

pH-responsive microbeads can be made by emulsion solvent evaporation²⁶⁻³² and by coacervation,^{33, 34} but these processes can leave solvent residues in the final product. It is not straightforward to achieve industrial scale-up of these processes.³⁵ Spray-drying is a promising alternative. It is a one-step method that can be scaled without the use of organic solvents.³⁶ Basit *et al.*³⁷ report that spray-drying will yield pH-responsive microparticles from poly(methacrylic acid) alone. The method requires the use of ammonium hydrogen carbonate to make a soluble salt of the acidic polymer so that the enteric polymer can be spray-dried from water. The resulting microbead vehicle must be converted back to its acid form to prevent rapid release of the guest drug in the gastric tract. This approach has the disadvantage that the ammonium ion must be removed from the microbeads by heating to convert the carbonate salt to volatile ammonia and return the enteric acrylate polymer to its water-insoluble acid form. The strategy does not offer control over the release kinetics in response to changes in pH, since drug release from this microbead relies on total dissolution of the polymer.

Naturally derived polymers like polysaccharides can be used to make biodegradable, pH-responsive microbeads.^{38, 39} Cellulosic polymers are widely applied in medicine as biocompatible, non-toxic, and biodegradable drug excipients.⁴⁰ An excipient is defined as a substance formulated together with an active ingredient to enhance its long-term stability, to confer a therapeutic enhancement, or to aid in the handling of the drug during manufacturing.⁴¹ Because native cellulose is insoluble in water, it must be converted to a soluble form by dissolving in strong base (viscose process),⁴² or in ionic liquids.⁴³ These processes require

harsh conditions and consume a large amount of energy. Chitosan can be spray-dried⁴⁴ to make pH-responsive microbeads, but like cellulose, it must be prepared by complex steps of isolation from shrimp shells, followed by purification, de-acetylation, and dissolution in acidic media. pH-sensitive chitosan microbeads have been made using the oil-in-water emulsion method⁴⁵ and a microfluidic channel method.⁴⁶ Neither method is readily scalable to the industrial levels required to meet volume demand. Despite these drawbacks, there is sustained research to develop “smart” pH responsive hydrogels, particularly from combinations of alginate and chitosan.⁴⁷

pH-responsive hybrid microbeads comprising a nanoparticle component and a binder component can offer desirable mechanical properties, conferred by the choice of nanoparticle, combined with desirable chemical and physical attributes, conferred by the polymer.⁴⁸ As the nanocrystal component, cCNC provides mechanical strength, high surface area, and abundant surface functionalities for chemical transformation. Our paradigm of the polymer component is the well-known pH-responsive polymer, poly(acrylic acid). It plays a dual role as a binder and stabilizer for the nanoparticle matrix.⁴⁹ With changes in pH, the binder stabilizes the nanocrystals in the microbead against separating and fragmenting.

PAA is water-soluble. Films and microparticles of PAA are usually unstable in water. Crosslinking can improve stability of films and particles against dissolution while allowing them to swell. There is precedent for crosslinking by esterification among carboxylic acid polymers, like PAA.⁵⁰⁻⁵⁷ Lu *et al.*⁵⁰ showed that fibrous membranes can be made by electrospinning combinations of cCNC and PAA. Thermally induced esterification between the hydroxyl moieties of cCNC and the carboxyl units of PAA yields nanocomposite fibrous membranes that are insoluble in water, that are more thermally stable and superior in tensile strength. It is reported that poly(vinyl alcohol) can also be crosslinked by heat treatment when

combined with cCNC.⁵⁵ In the present article, we explore the potential of ester crosslinking between cCNC and PAA and the capacity of the resulting microbeads to behave as robust pH-responsive entities.

We first study crosslinking between cCNC and PAA. The reaction requires no anhydride and occurs simultaneous with spray-drying from water. Esterification creates a crosslinked nanocomposite microbead that resists breakup. We investigate some physical properties of the microbeads, including morphology, swelling, and small molecule uptake and release properties.

Experimental Method

2.1. Materials

cCNC suspension (2.5 wt % slurry) was obtained from Anomera Inc. Poly(acrylic acid) 63 wt % solution (average MW 1800), Poly(acrylic acid sodium salt) (MW ~2100), 5,10,15,20-*tetrakis*(1-methyl-4-porphyrin tetra(*p*-toluenesulfonate)) (dye content 97%), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), propargyl amine, and *N*-hydroxysuccinimide (NHS) were purchased from Millipore Sigma. Methylene blue (high purity) was obtained from Alfa Aesar (Heysham).

2.2. Preparation of 1:1 Poly (acrylic acid) cCNC Composite Microbeads

cCNC suspension was diluted to 0.5 wt % (total amount of cCNC 1 g, total volume of 200 mL) in an appropriately sized beaker. Poly(acrylic acid) 63 wt % solution (1 g) was diluted to 0.5 wt %. The PAA solution was mixed with cCNC suspensions while stirring until homogenous. The above mixture was spray-dried using a spray drier (Buchi model B191, 175 °C inlet temperature, 30% pump speed, and 70% aspirator), and the white free-flowing powder was collected in a sample vial. cCNC suspension, poly(acrylic acid) 0.5 wt %, and a

mixture of cCNC suspension (0.5 wt %) with poly(acrylic acid) sodium salt solution 0.5 wt % was spray-dried separately under the same spray-drying conditions. Free flowing white powders were produced.

2.3. Characterization of Esterification

^{13}C solid-state NMR spectra were acquired with a Varian VNMRS (now Agilent, Santa Clara, CA, USA) Spectrometer operating at 100.5 MHz for ^{13}C using a 4 mm double-resonance Varian Chemagnetics T3 probe. Approximately 70 mg of sample was centre-packed into rotors using Teflon inserts. The ^{13}C solid NMR spectrum was acquired in 512 scans with spinning at 8 kHz, using a recycle delay of 5 s with a cross polarization contact time of 1.5 s. Spectra were referenced to the carbon signal in glycine at 176.0 ppm with respect to TMS (tetramethylsilane).

Fourier Transform Infrared (FTIR) spectra were measured on a Spectrum Two FT-IR spectrometer (Perkin Elmer) equipped with single bounce diamond crystal, and standard, room-temperature LiTaO₃ (Lithium tantalite) MIR detector using ATR method. Spectra were obtained in the range of 4000 – 400 cm^{-1} with a resolution of 4 cm^{-1} by accumulating 64 scans.

2.4. Particle Characterization

Scanning Electron Microscopy

SEM was performed with a FEI Inspect F50 FE-SEM at McGill University on samples with 4 nm Pt coating. An FEI Helios Nanolab 660 DualBeam (Focused Ion Beam-Extreme High-Resolution Scanning Electron Microscope) was used to image cCNC microbeads in cross section. Samples were mounted on an aluminium stub and coated with a 4 nm layer of Pt to enhance electrical conductivity. Milling of the sample was carried out with the FEI Helios Nanolab 660 DualBeam instrument (Thermo Fisher Scientific, Hillsboro, OR USA). After

selecting a specific cCNC particle, a 2- μm thick of protective platinum stripe was deposited on the surface of the region of interest to protect it from ion beam damage. Microbead cross-sections were then prepared by gallium ion milling. A primary trench to clean the sample was milled at 30 kV ion beam accelerating voltage and 21 nA beam current. Following cleaning, cross-sections were obtained with beam current ranging between 9.3 nA to 0.79 nA. The cross-section was imaged with an electron beam at an accelerating voltage of 2 kV, a beam current of 0.40 nA, and a working distance of 4 mm in the secondary electron mode.

Optical Microscopy

Polarized optical microscope (POM) images were acquired with a Nikon Optiphot microscope connected to a SonyXCD-X7 10CR CCD camera equipped with iidec (Instrumentation & Industrial Digital Camera) image capture software to image (40 $\times$ objective) the microbeads at different pH. Samples were prepared by adding microbeads to a vial containing a quantitative amount of pH solution. The microbead suspension was immediately transferred to a glass substrate and protected by a cover slip for microscopy. Images were analysed with Fiji Image J open source image processing software (Copyright 2010-2020, Cambridge Astronomical Survey Unit). Particle diameters were measured with Image J and repeated for up to 100 particles. The particle diameter distribution was analysed using a Gaussian distribution function. Particle mean diameters were determined from the distribution analysis.

Third Harmonic Generation (THG) Microscopy

THG imaging was conducted with a customized upright multiphoton microscope (FV1200 MPE, Olympus Canada Inc., ON, Canada). The system is equipped with two ultrafast laser excitation sources that provide ~ 200 fs pulses: a Ti:sapphire laser (Mira 900F, Coherent, CA, USA) and an optical parametric oscillator (OPO) (Mira OPO, Coherent, CA, USA) configured

for excitation at 1150 nm. The OPO (1150 nm, 15-55 mW) for excitation was used to image THG light scattered from cCNC-PAA microbeads in water. A 400 nm bandpass filter (380 nm - 420 nm) was used to enhance detection. The microbeads for THG were prepared by dispersing them in water and depositing a droplet of suspension on a glass substrate. A coverslip over the sample was sealed with transparent nail polish to suppress evaporation and the motion of the microbeads by air currents.

2.5. Methylene Blue Dye Uptake and Release

500 mg of 1:1 cCNC-PAA microbeads were immersed in 45 ml of 75 mg/mL methylene blue at pH 7. The resulting suspension was immediately filtered under vacuum and washed with water and acetone. The dyed particles were dried under vacuum overnight.

10 mg methylene blue dyed cCNC-PAA microbeads were then equilibrated in aliquots of pH-adjusted water selected in the range 2-10. Methylene blue dye release kinetics were followed by UV-Vis absorption spectroscopy by monitoring the dye concentration at 665 nm every 5 hours for samples at pH=2, 4, 7, and 10. The amount of dye released over time was plotted against the different pH values.

2.6. Confocal Fluorescent Microscopy Study

10 mg of 5, 10, 15, 20-*tetrakis*(1-methyl-4-porphyrin tetra(*p*-toluenesulfonate)) was encapsulated in the cCNC-PAA matrix by dissolving it into an aqueous cCNC-PAA suspension (0.25 mg cCNC and 0.25 mg PAA), followed by spray-drying. A yellow free flowing powder was produced.

For confocal fluorescent microscopy, 3 mg dye bound microbeads was immersed in 3 mL water adjusted to pH 2.3 or 7.0. The suspension was immediately imaged using a confocal

fluorescent microscope (Zeiss LSM 880 ElyraPS1 laser scanning confocal and super-resolution microscopy) for a time study. Fluorescence from the microbeads was recorded and plotted versus time.

2.7. SIM Super-Resolution Fluorescent Microscope

A Zeiss LSM 880 ElyraPS1 laser scanning confocal spectrograph equipped with a Structured Illumination Microscopy (SIM) accessory was used to image the porphyrin encapsulated cCNC-PAA microbeads in sunflower oil with z-stackings (Molecular Imaging – the Research Institute of the McGill University Health Centre (RI-MUHC)).

2.8. Synthesis of cCNC-Alkyne

cCNC suspension (1 g, 0.15 mmol COOH) was diluted to 0.5 wt % in water and heated to 50 °C with stirring. The pH was adjusted to 5.5 by adding 0.1 M HCl solution dropwise. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (235 mg, 1.5 mmol) and *N*-hydroxysuccinimide (NHS) (231 mg, 2.0 mmol) were dissolved in 5 mL of water and added dropwise to the cCNC suspension. After 30 min, propargyl amine (96 µL, 1.5 mmol) was added dropwise, and the pH was adjusted to 7.5 – 8.0 with 0.1 M NaOH solution. The reaction was allowed to proceed overnight. The suspension was then purified *via* dialysis. FTIR and XPS were used to assess that chemical modification of the cCNC surface had occurred.

2.9. cCNC-Propargylated PAA Microbeads

A propargylated cCNC water suspension was mixed with a PAA suspension in 1:1 ratio and spray-dried using the same parameters given in section 2.2.

2.10. X-ray Photoelectron Spectroscopy (XPS)

A $K\alpha$ X-ray photoelectron spectrometer (Thermo Scientific, monochromatic Al $K\alpha$ X-ray source, flood gun at 10^{-8} mbar) was used to characterize the labelling of cCNC with alkyne *via* amide bond formation.

X-ray photoelectron spectroscopy depth profiling was performed on cCNC-Alkyne PAA hybrid microbeads with a Thermo Scientific K-Alpha XPS unit. An argon ion gun with an ion energy of 500 eV, at a current of 1.00 μ A was used to etch the surface. Etching was performed for 300 seconds per cycle with 10 cycles. The XPS was performed on each etching cycle with a flood gun on. The X-ray emission angle was 90 degrees with respect to the specimen surface. The diameter of the analysed area was 400 μ m. The survey spectrum and the high energy resolution spectra were collected for all elements assigned in the surface spectra: oxygen, carbon, and nitrogen. Spectra were deconvolved using Advantage Data System with a combination of Gaussian and Lorentzian functions after background subtraction by the Shirley method and the resulting curves attributed to the different types of bonds according to their binding energy. Integration of the obtained curves yielded the percent nitrogen content.

XPS depth profiling was associated with SEM to quantify the etching depth. cCNC-PAA microbeads attached by copper tape were first imaged using SEM. The copper tape with cCNC-PAA microbeads was then inserted into the XPS chamber. An argon ion gun with an ion energy of 500 eV, at a current of 1.00 μ A was used to etch the surface of the microbeads for 10 min. The samples were taken out of the XPS chamber and immediately transferred to the SEM sample chamber. The etched spot was identified and imaged to estimate the etched depth after 10 min. These steps were repeated for etching cCNC-PAA microbeads for 20 min.

Results and Discussions

3.1. Characterization of Spray-drying Induced Esterification of cCNC-PAA Hybrid Microbeads.

Publications report that cellulose can be esterified with PAA when heating films of the mixture to approximately 140 °C for about 30 min.^{52, 53, 56, 58-62} Figure 3-2 (b) shows that evaporation of water plays a significant role during this heating process as it pushes the reaction between carboxylic acid on PAA and the hydroxyl groups on cCNC in the direction of the ester. Simultaneously, heating promotes esterification. Esterified, crosslinked films show enhanced mechanical stability and better barrier properties.^{50,55} Figure 3-2 is a schematic overview of the events. A water droplet containing cCNC and PAA (at pH 3.3) generated from the spray dryer atomizer is formed in the drying chamber, which is preheated to 170 °C. The high temperature evaporates water rapidly, inducing esterification. At the same time, cCNC-PAA microbeads form and accumulate in the sample collector chamber.

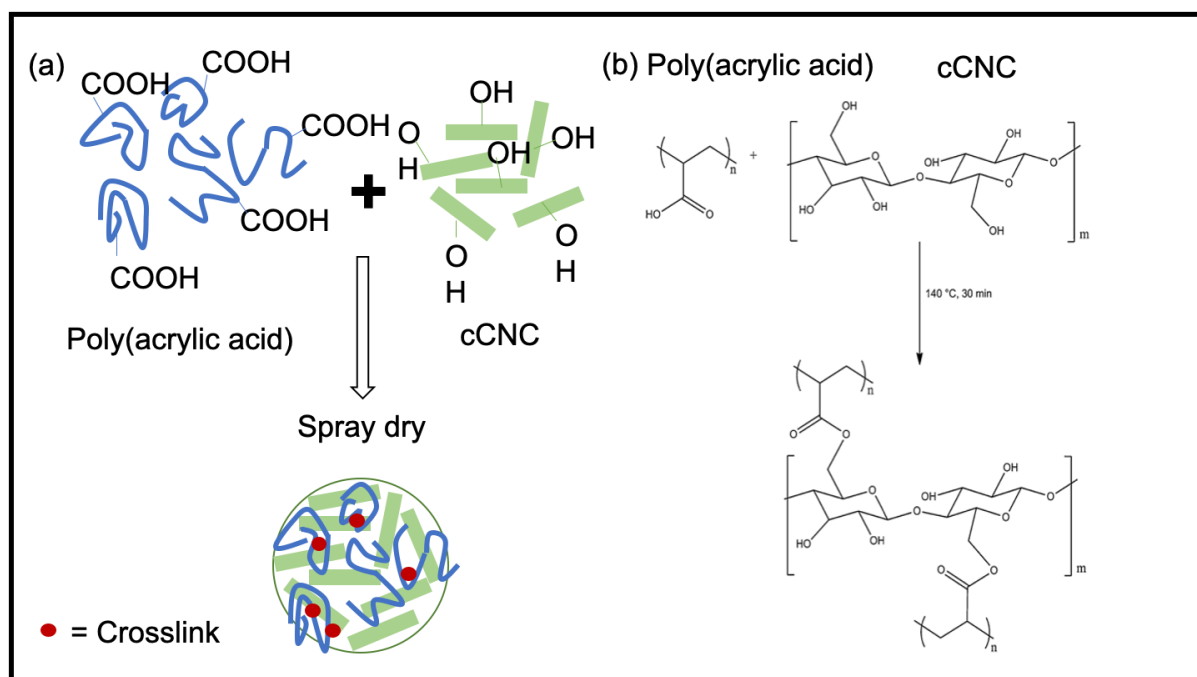


Figure 3-2 Crosslinked 1:1 cCNC-PAA microbead by gas phase spray-drying. a) Illustration of PAA and cCNC hybrid microbead formation *via* spray-drying. Red dots indicate crosslinks between cCNC and PAA. b) Reaction scheme of esterification between cCNC and PAA.

^{13}C solid-state NMR spectroscopy was used to characterize ester bond formation. Figure 3-3 shows the ^{13}C solid-state NMR spectra of cCNC, PAA, and cCNC-PAA microbeads, respectively. We observe a reduction in intensity of the shoulder on C6 (63 ppm), indicating a change in chemical environment for a portion of the C6 units of cCNC.⁶³ The peak shift from 183 ppm to 178 ppm provides direct evidence on ester bond formation.⁵⁹ The existence of a shoulder at around 183 ppm indicates that not all the carboxyl acid groups have been esterified. From this figure, cCNC C1-C5 peak positions are not affected by esterification since chemical reaction is restricted to the C6 position.⁶³

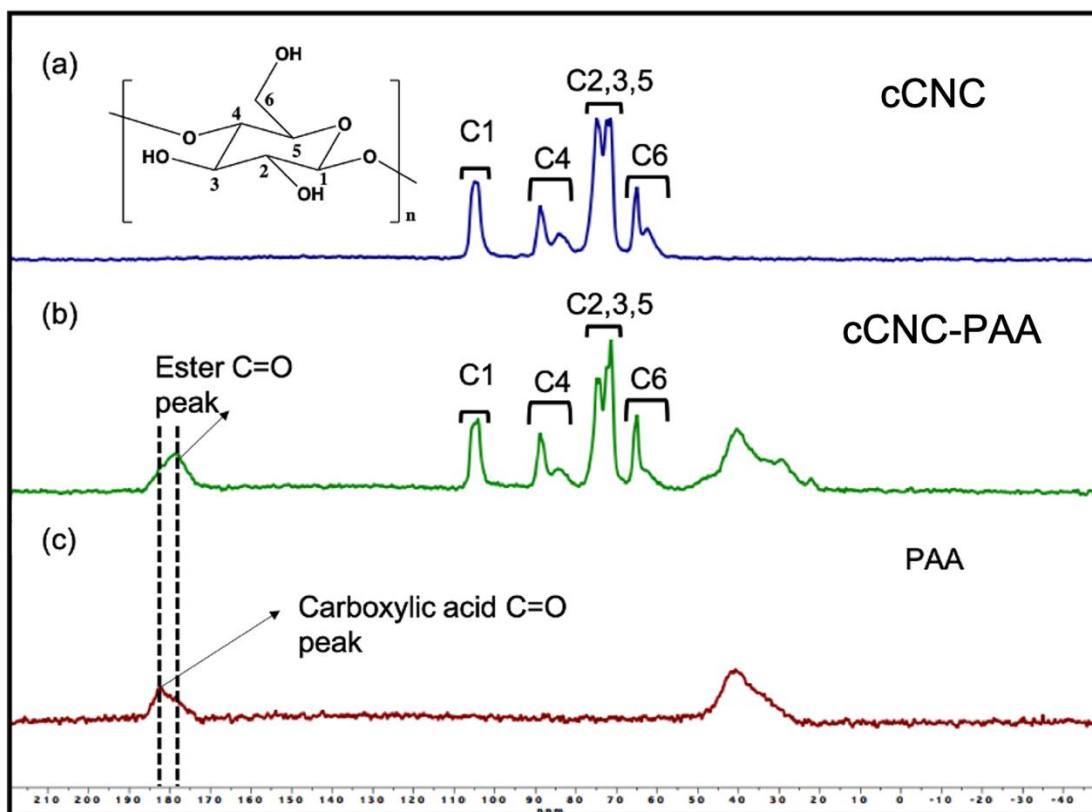


Figure 3-3 Solid state ^{13}C NMR spectra of cCNC in (a) cCNC-PAA microbeads, and (b) of neat PAA (c).

We conducted an FT-IR study on 1:1 cCNC-PAA microbeads for additional evidence of esterification. As shown in Figure 3-4, cCNC-PAA microbeads show a PAA carboxylic acid C=O stretching peak in the region of 1700 cm^{-1} . Peak overlap makes it difficult to identify the ester C=O stretch, which is expected between $1720\text{--}1750\text{ cm}^{-1}$. Formation of the sodium carboxylate salt shifts $\nu_{\text{C=O}}$ to 1600 cm^{-1} , which allows us to identify the ester stretch at 1750 cm^{-1} . Three peaks are evident between 1500 cm^{-1} and 1800 cm^{-1} : $\nu_{\text{C=O}}$ (carboxylic acid) at 1700 cm^{-1} , $\nu_{\text{C=O}}(\text{COO}^-)$ at 1600 cm^{-1} , and $\nu_{\text{C=O}}$ (ester) at 1750 cm^{-1} .

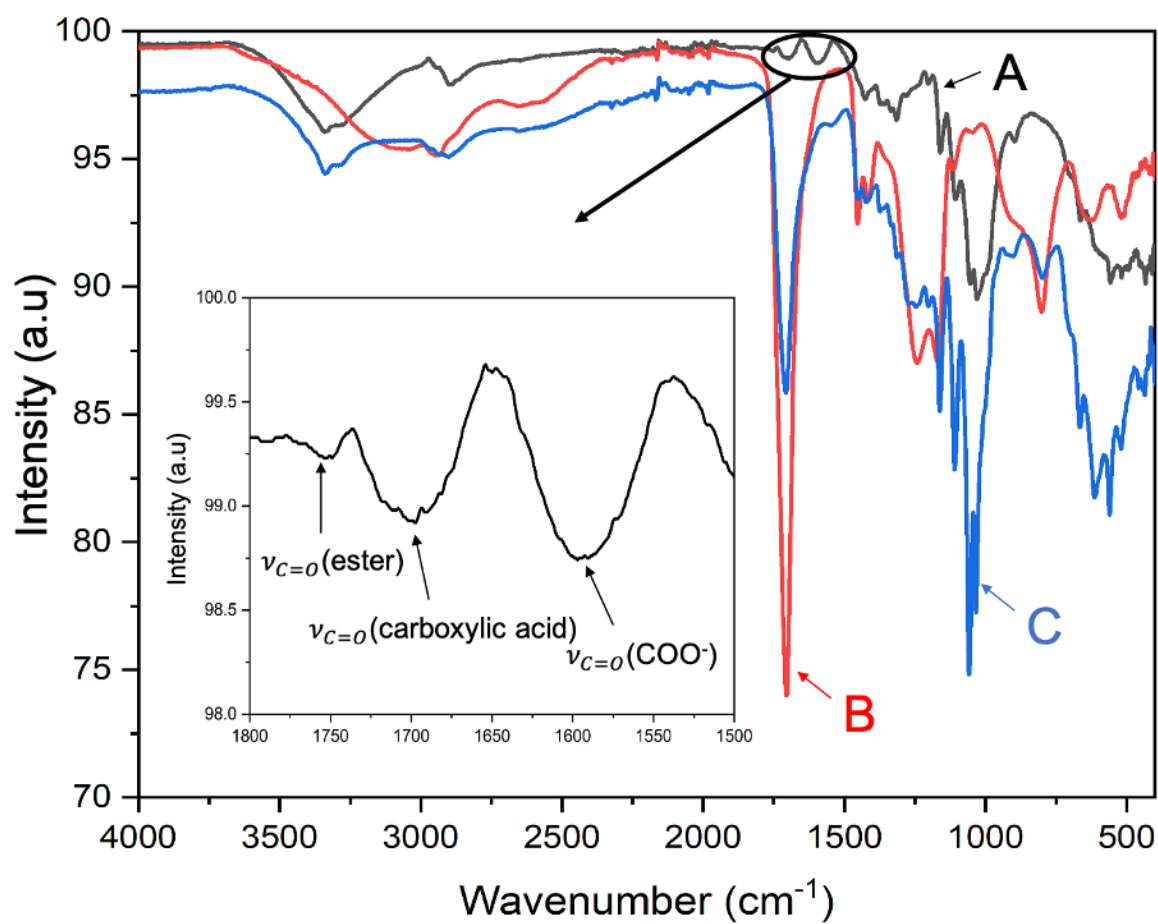


Figure 3-4 FT-IR spectra of basified cCNC-PAA microbeads (A), PAA (B), and cCNC-PAA microbeads (C).

Other evidence of esterification is obtained by studying the swelling behaviour of the esterified microbeads. Microbeads made from PAA sodium salt that are not crosslinked (not esterified) disintegrate when agitated in water, although they are stable under acidic conditions. By contrast, esterified cCNC-PAA microbeads are stable under both acidic and basic conditions when imaged by optical microscopy.

3.2. pH Responsive cCNC-PAA Microbeads

1:1 cCNC-PAA microbeads increase in mean diameter as the pH becomes increasingly basic. Optical microscope images are shown in Figure 3-5 (b)-(e). The beads show low swelling at acidic pH. Mean diameter then increases with increasing pH. The size distributions are symmetric (Gaussian) in Figure 3-5 (a), and the mean size not only increases with increasing pH, but that the size distribution broadens as the pH increases. For example, the D_{50} at pH = 0 is 2.46 μm , while the D_{10} and D_{90} are respectively, 0.53 and 4.41 μm . At pH = 13, the D_{50} is 4.25, while the D_{10} and D_{90} are respectively, 0.74 and 7.78 μm . The standard deviation at pH = 0 is 1.51 μm , increasing to 2.74 μm at pH = 13. We think the change in the width of the distributions occurs because there is a distribution in hybrid microbead composition around the putative value of 1:1 cCNC-PAA.

Figure 3-6 reveals how the diameter of the microbeads depends on composition. Clearly, the diameter increases with increasing PAA content at fixed pH of 13. When the PAA fraction in the microbead reaches 30%, the bead diameter remains unchanged thereafter. On a molecular level, the change in particle size is due to protonation/deprotonation of the carboxylic acid groups in the microbeads, *i.e.*, swelling or contraction of the PAA component. We observe that the negative surface charge of cCNC-PAA microbeads increases with the increase in pH according to the zeta potential measurement (Table S3-1). As shown in Figure 3-7 (a) and Figure 3-7 (b), a rise in pH results in deprotonation of carboxylic acid groups, increasing electrostatic repulsions that expand the polymer matrix to swell the microbead.

In some cases, we observe a 100% increase in expansion of the diameter of cCNC-PAA microbeads as the pH becomes increasingly basic. This is significant. The literature reports much smaller changes in the size of pH-responsive microbeads like PAA microbeads, or composite microbeads. For example, Sekido *et al.* demonstrated that poly(2-

(diethylamino)ethyl methacrylate) (PDEA) - latex microbeads increase from 2.62 μm to 3.32 μm in the pH range 3 to 10, a 26.7% expansion.⁶⁴ Hu *et al.* demonstrated that hollow PAA microbeads showed a 43.5% expansion, from 4.78 μm at pH =2, to 6.86 μm at pH=9.⁶⁵

Clearly, by changing the ratio of cCNC to PAA one can control dilation of the hybrid microbeads.

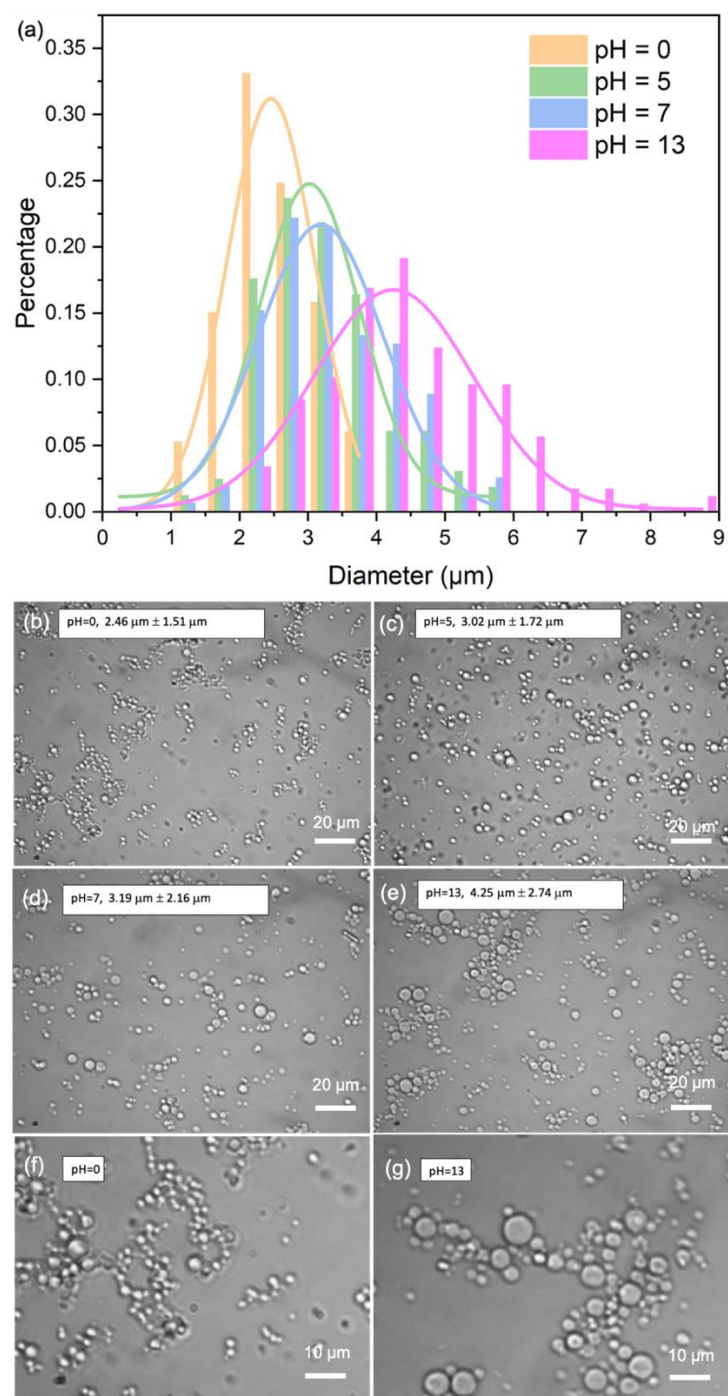


Figure 3-5 pH dependent size change in water. (a) cCNC-PAA microbead diameter distributions at pH = 0, 5, 7 and 13 measured from the optical microscope images shown in (b) - (e).

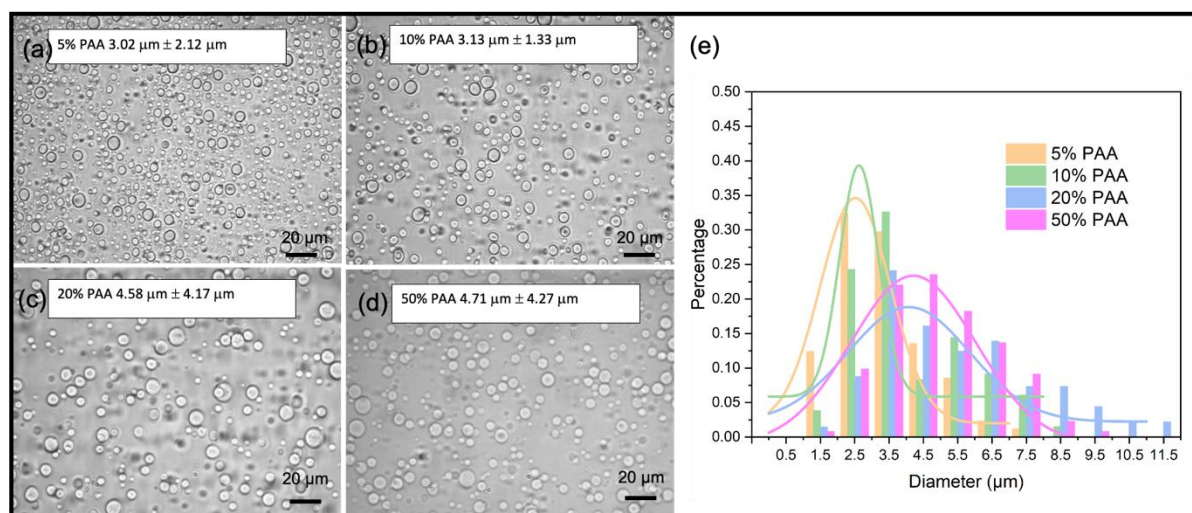


Figure 3-6 Particle diameter distribution at pH=13 as a function of PAA content in cCNC-PAA microbeads. (a) Optical microscope images of particles with different PAA content at pH=13. (b) Particle diameter distributions measured at different PAA content.

3.5. pH Responsive Methylene Blue Dye Release Kinetics

In this section we describe the release of methylene blue dye loaded in 1:1 cCNC-PAA hydrogel microbeads. We offer MB as a primitive model for the release of a cationic drug like β -adrenoreceptor antagonists, calcium (Ca)-channel blockers, I_f channel inhibitors, antiarrhythmic drugs, or antibacterial drugs. MB release from the hydrogel microbeads depends mainly on their swelling behaviour, interaction of MB with the host, and the solubility of MB in the release medium, water. Implicitly, swelling depends on porosity, the quantity of water taken up by the hydrogel, the quantity of polyelectrolyte, the amount of charge and charge screening on the polyelectrolyte. These features are commonly explored in drug take-up and release studies, though it is not our purpose here to explore this complex domain of application. Methylene blue dye was loaded to ~ 0.8 mg/g in the cCNC/PAA microbeads. Beads were then exposed to increasing pH, beginning at 0 and ranging up to pH 12. We used electronic absorption spectroscopy (see Experimental) to quantify the amount of MB released after reaching equilibrium. Figure 3-8 (a) plots the frequency distribution of MB release in

mg/g versus pH. There is a clear, almost monotonic decrease in MB release with increasing pH. The time release of MB is given in Figure 3-8 (b) for different values of pH. Under acidic conditions, dye release reaches equilibrium in 50 hours. Under basic conditions, there is no significant dye release within this same time period, indicating a stable binding of the dye to the matrix. There is a small amount of “burst kinetics” (~ 0.1 mg/g), representing the release of MB from the surface of the microbeads, which is relatively negligible compared with release from the sub-surface and interior of the microbeads. This is a positive indicator that the hybrid hydrogel cCNC-PAA system should be explored further in drug loading and release studies.

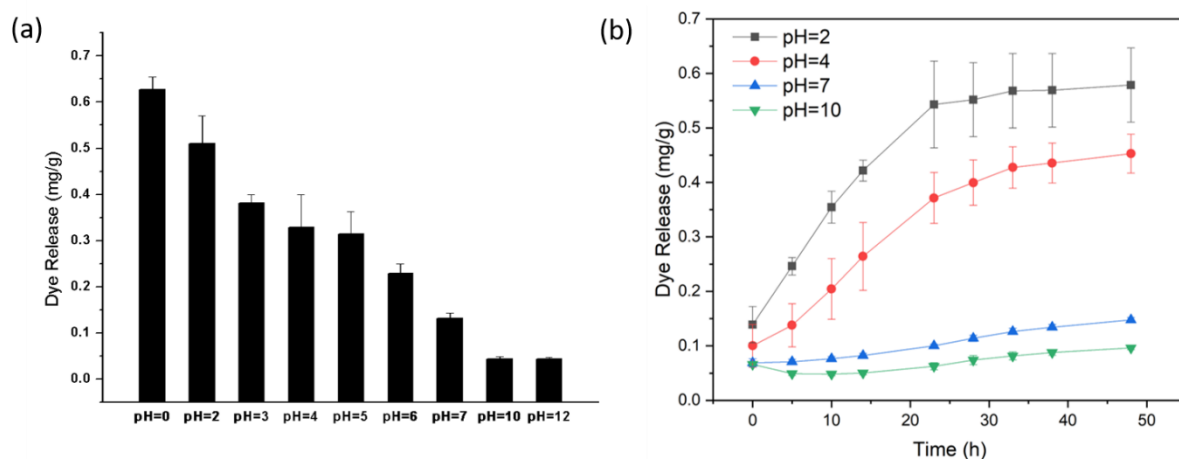


Figure 3-7 pH dependent methylene blue dye release from cCNC-PAA microbeads. (a) Amount of dye release at different pH (b) Dye release kinetics studies on different pH.

The mechanism behind the pH response is easy to rationalize. Figure 3-9 gives a simplified explanation. Hydrogel swelling is controlled by the ester crosslinks in response to pH and also probably significant H-bonding between PAA and the cCNC matrix particles, as well as chain adsorption and entanglements. At low pH, the COOH moieties are protonated. This chemistry causes the microbeads to contract and perhaps even form a skin on the surface of the beads. Increasing pH deprotonation leads to electrostatic repulsion that opens up the internal structure

of the beads, expanding the particles and increasing particle porosity, inviting ingress of water and solvation and liberation of MB. Thus, when cationic MB is encapsulated, its release can be controlled by the opening and closing of the microbead “gate” *via* pH, as in Figure 3-8 (c). This makes this nanocomposite microbead of potential interest for drug delivery applications. Besides introducing the pH response properties to the microbeads, the incorporation of PAA into cCNC microbeads matrix also affects its small molecule uptake properties. The MB dye *uptake* experiment on cCNC-PAA microbeads with different PAA molecular weight is reported in Table S3-2 to 4. Referring to Chapter 2, the cCNC-PAP MB adsorbs onto the cCNC-PAA microbeads according to a Freundlich Isotherm, whereas it adsorbs onto neat cCNC according to a Langmuir isotherm.

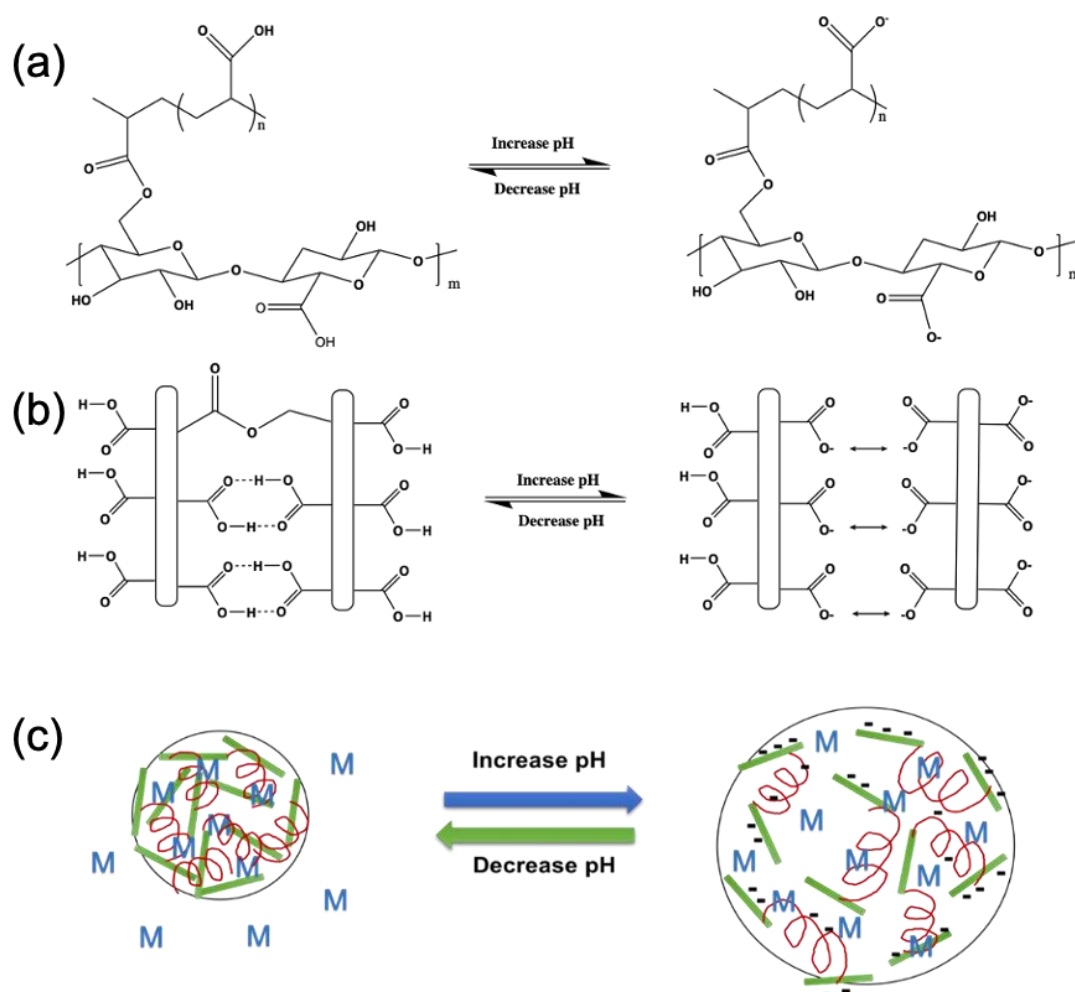


Figure 3-8 Illustration of pH dependent methylene blue release in cCNC-PAA microbeads. (a) The crosslinked cCNC-PAA network surface charge changes from neutral to negative by increasing the pH. (b) Demonstrations of the repulsive forces between the negative charges of carboxylic acid moieties among the cCNC-PAA network at basic pH. (c) Indication of methylene blue molecule has a stronger interaction with cCNC-PAA matrix when increasing the pH upon particle swelling *via* electrostatic forces.

More detail into the release process is provided by confocal fluorescence microscopy. This offers a perspective from the “inside out” of a fluorescently labelled microbead. In this case, we used the photostable porphyrin probe, 5, 10, 15, 20-*tetrakis*(1-methyl-4-porphyrin tetra(*p*-toluene sulfonate)). This porphyrin is water-soluble, positively charged, and fluorescence with

a maximum emission at 421 nm. The porphyrin probe was encapsulated by spray-drying (Figure 3-9). To map the distribution of the probe before its release, labelled cCNC-PAA microbeads were first immersed in oil. A separate sample of beads was immersed in water at pH 7.0 and pH 2, and the spatial distribution of the probe was determined (see later). Microscopic images were acquired every 1 min and the fluorescence intensity at the focal plane centre of the microbead was measured three times. The results are collected in Figure 3-9. In the oil phase, there was no significant change in emission intensity after 30 min. Brownian motion is responsible for the reconfiguration of the microbeads. The differences in fluorescence intensity at 5 min for pH 7.0 and pH = 2.3 media are the result of differences in sample preparation. The curves in Figure 3-9 (c) show that within experimental error there is no change in the amount of label released at pH = 7.0 after 30 minutes. At pH 2.3 we observe a gradual decline in emission as the label migrates away from the center of the microbead and molecules diffuse into the water medium. These observations correlate with our studies of MB dye release in Figure 3-8 (c).

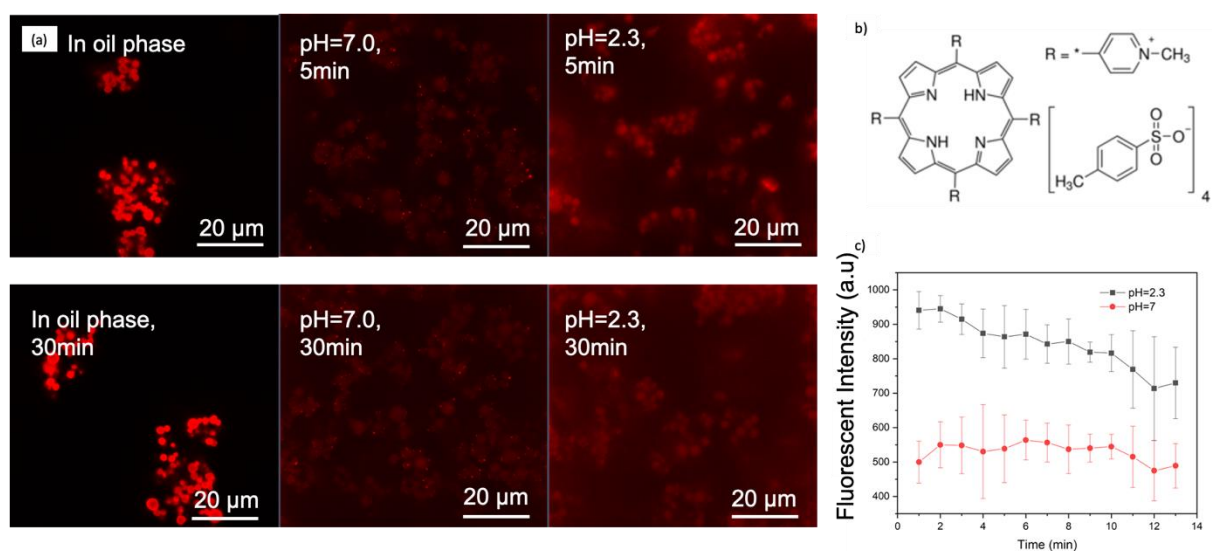


Figure 3-9 Confocal fluorescence microscopy of porphyrin dye release. (a) Labelled cCNC-PAA microbeads in oil phase, at pH=7.0, and at pH=2.3 imaged after 5 minutes and 30 minutes. Imaging was focused approximately at the centre of the microbeads. (b) Changes in fluorescence intensity measured at pH 2.3 and pH 7.0.

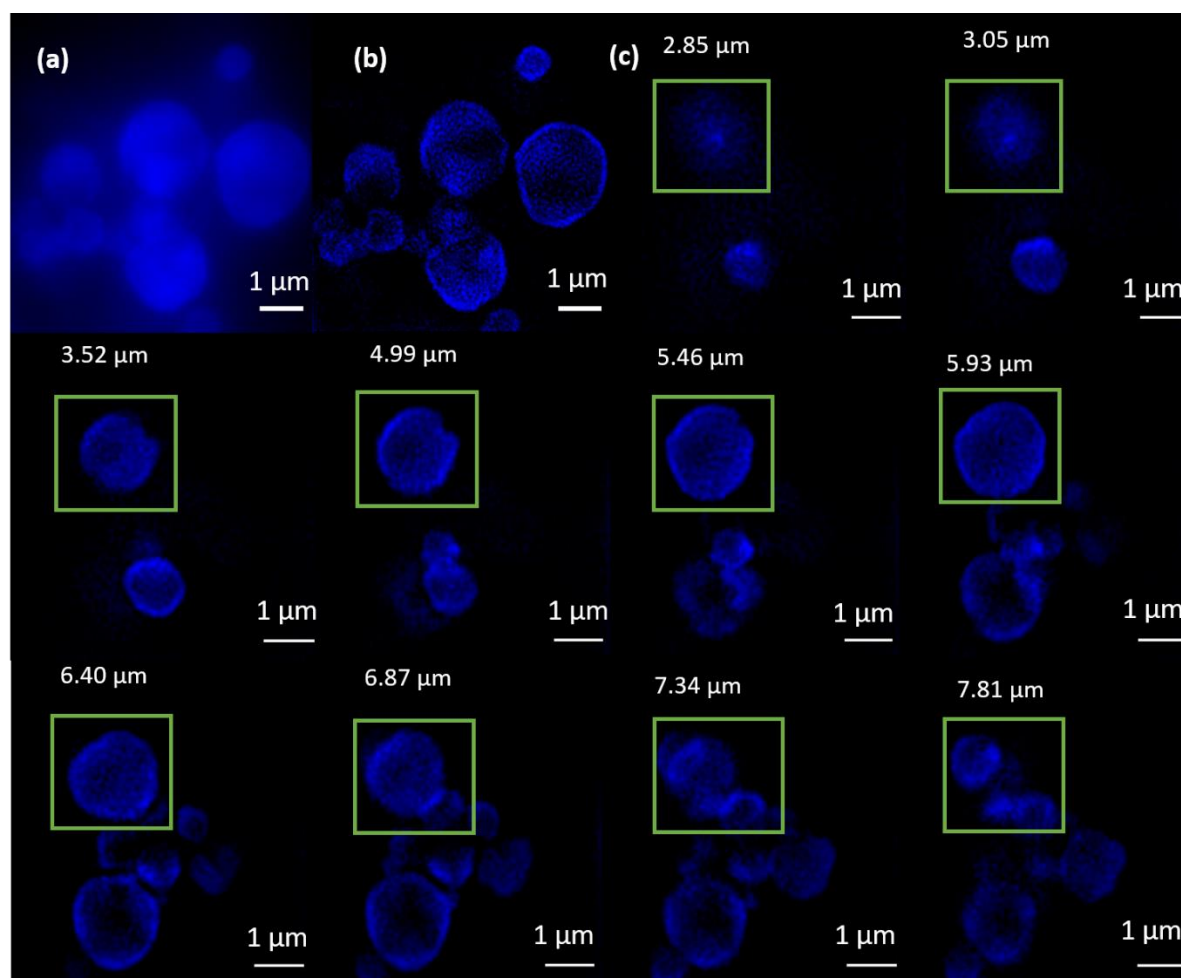


Figure 3-10 Structure illumination microscopy (SIM) images of cCNC-PAA microbeads in sun flower oil. (a) Confocal fluorescent microscope images of a cCNC-PAA microbead in sun flower oil. (b) SIM super resolution microscope images of cCNC-PAA microbeads in sun flower oil. Images (a) and (b) were acquired in the same focal plane. (c) SIM super resolution microscope images of cCNC-PAA microbeads Z-stacks with different light penetration layers.

While confocal microscopy is helpful to monitor the release of cationic porphyrin from the cCNC-PAA microbeads, it is still difficult to get a clearer picture of the spatial distribution of the dye label. To supplement the confocal experiments, we turned our attention to super-resolution structured illumination microscopy (SIM). We used 3D z-axis stacking to obtain depth resolution of the porphyrin label in a microbead. SIM fluorescence from microbeads in sunflower oil is shown in Figure 3-10 (b), where it is compared with that of the confocal data

for the porphyrin label in image (a). A series of z-stack images was taken to locate the dye molecules in the cCNC-PAA microbeads in three dimensions. These are shown in the green enclosures in the sequence of pictures in Figure 3-10 (c). The distance units (in μm) label the z-position focus. The SIM image z-stack capture begins from the top of the microbeads with penetration depth of 2.85 μm . This image is a slice through the microbead that shows what appears to be a relatively small disc with blue emission intensity highlighted near the center. The intensity distribution is non-uniform, suggesting a heterogeneous distribution of porphyrin dye molecules at this position in the microbead. On increasing the focusing depth to 5.93 μm , the disc expands, corresponding to a slice near the equator of the microbead. While blue emission is most intense at the outer edge of the slice, it is clear that the porphyrin is rather widely distributed in the microbead. The concentration of signal near the perimeter of the bead might be a reflection of the faster diffusion of porphyrin to the bead surface during evaporation of water from micro-droplet. Between focusing distances 6.40 μm to 7.81 μm , the disc shape shrinks as the focal plane approaches the bottom of the bead. The signal to noise decreases with increasing penetration depth because of contributions from the glass substrate. At 6.87 μm we observe images of overlapped microbeads. Signal intensity here indicates that porphyrin molecules are distributed these microbeads, although non-uniformly. More dye molecules accumulate near and at the surface of the microbead where they show more intense blue emission. SIM provides unusually intimate information about the location of the dye molecules encapsulated in cCNC-based hybrid microbeads.

This study suggests that SIM might be generalised to locate the spatial distributions of small molecules in polymer microbeads. SIM may also be significant to study encapsulation of pharmaceuticals. Direct visualization can help us to further predict small molecule release profiles. For example, if small molecules are enriched at the surface, then burst release is

expected. If small molecules are homogeneously distributed in the microbeads, then diffusion may be the rate-determining step in the release.

3.2. Morphology of cCNC-PAA Microbeads

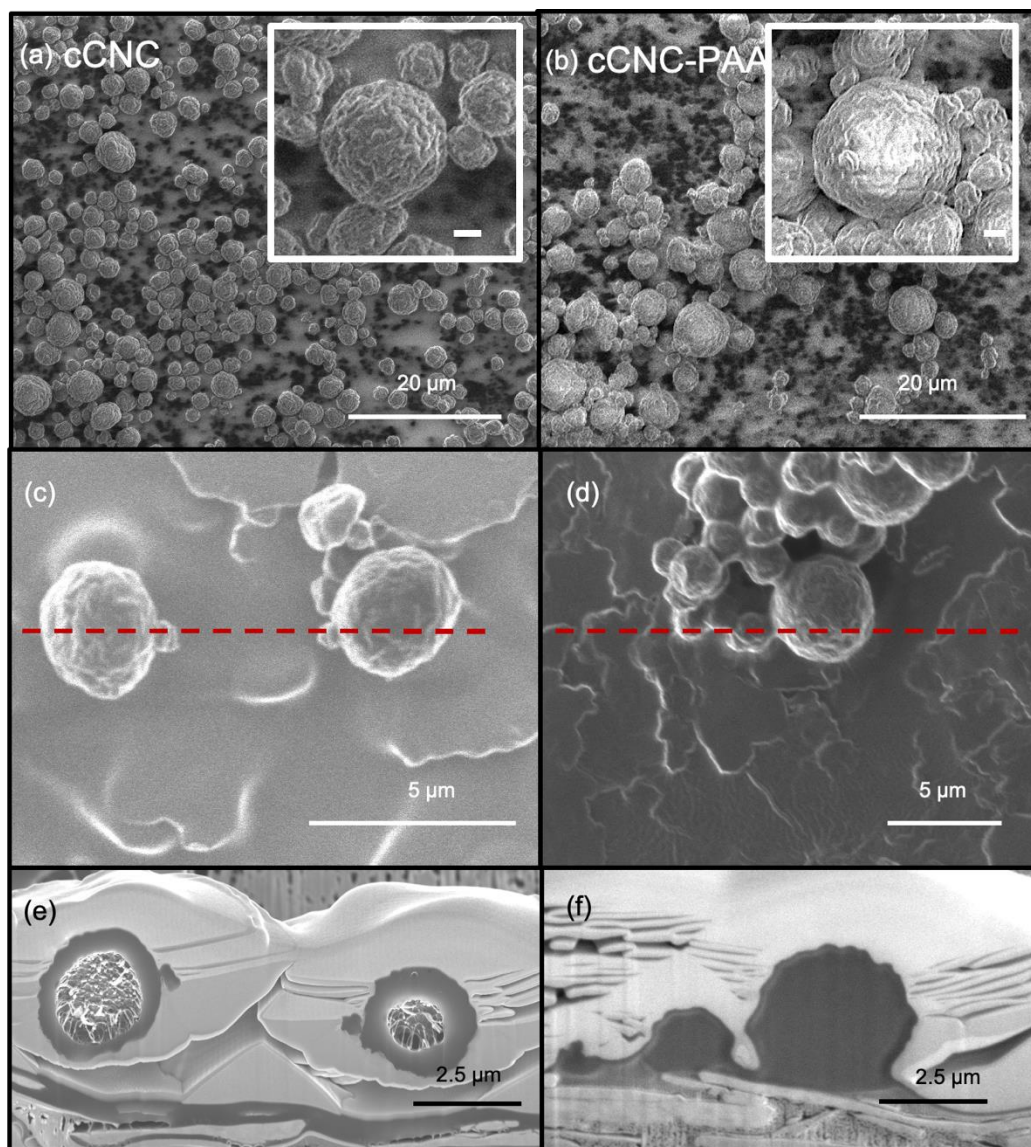


Figure 3-11 SEM images reveal surface and internal morphologies of 1:1 cCNC-PAA microbeads. (a) SEM images showing particle size and morphology of cCNC microbeads. (b) SEM images showing particle shape and surface texture of 1:1 cCNC-PAA microbeads. (c) and (e) FIB-SEM cross-section of cCNC microbeads. Red dotted line indicates the position of FIB milling section. (d) and (f) FIB-SEM cross-section of cCNC-PAA microbeads.

Particle size plays an important role in, for example, drug delivery. With relatively small particles, (nano and micro-sized) invasive delivery is reduced. Moreover, nano and micro-particles are much smaller than the inner diameter of most needles, reducing injection problems. A relatively small size also leads to a higher surface area for bioconjugation, facile natural clearance, and enhanced penetration through tissue barriers.⁶⁶ Microparticles smaller than 5 μm are often used in oral or pulmonary delivery.⁶⁷ The clearance of particles sized between 0.5 - 10 μm can be realised by phagocytosis. Besides particle size, deformability, shape, size distribution, and surface chemistry are other factors to consider for drug delivery vehicles.⁶⁸ Knowledge of particle morphology provides a starting point in the study of the drug delivery and release profiles, benefiting the design of drug delivery systems.

The SEM images of cCNC and 1:1 cCNC-PAA (MW 1800) are shown in Figure 3-11, where they offer striking evidence of differences. The SEM images of 1:1 cCNC-PAA with molecular weight of 5k and 90k are presented in Figure S3-1. No significant differences were observed for the morphologies of the other two molecular weight hybrids compared with cCNC-PAA (MW 1800) microbeads. The latter microbeads have a smaller average diameter and relatively low polydispersity (1.83 μm , 31.4% polydispersity) when compared with neat cCNC microbeads (average size 1.89 μm , 38.0% polydispersity) (Polydispersity was calculated as the ratio of the percent standard deviation to the average value.). Although the surface textures of cCNC and cCNC-PAA particles are similar, the internal structures differ significantly. This is evident from the FIB images of Figure 3-11 (e) and Figure 3-11 (f). cCNC microbeads exhibit a thick dense shell (dark grey region in Figure 3-1 (e)) with a fibrous cellular internal structure reminiscent of solid foams. In contrast, the cross-section of cCNC-PAA in Figure 3-11 (f) reveals a dense internal structure evident as the uniformly dark phase. Addition of PAA alters the particle internal structure. Clearly, the different internal structures reflect the dynamics of the spray-drying process. By comparison, in cCNC-PAA systems, cCNC and PAA are likely

to become entangled during the evaporation event, leading to a denser structure. Impeded diffusion in the hybrid system means that the solid phase will be more evenly distributed. This is discussed further below. To further understand the interaction of cCNC and PAA when mixing the two components for spray-drying, we used AFM to study the topographic images of cCNC-PAA and cCNC films. (Figure S3-2) The results show an increased nanocrystalline rod width and length, indicating a nano-scale mix of cCNC and PAA, suggesting that the cCNC-PAA microbeads are likely to be a nano-scale hybridization.

We turned to combinations of destructive and non-destructive techniques to build a picture of the distribution of PAA in the hybrid 1:1 cCNC-PAA microbeads. Next we examine the use of third harmonic generation (THG).

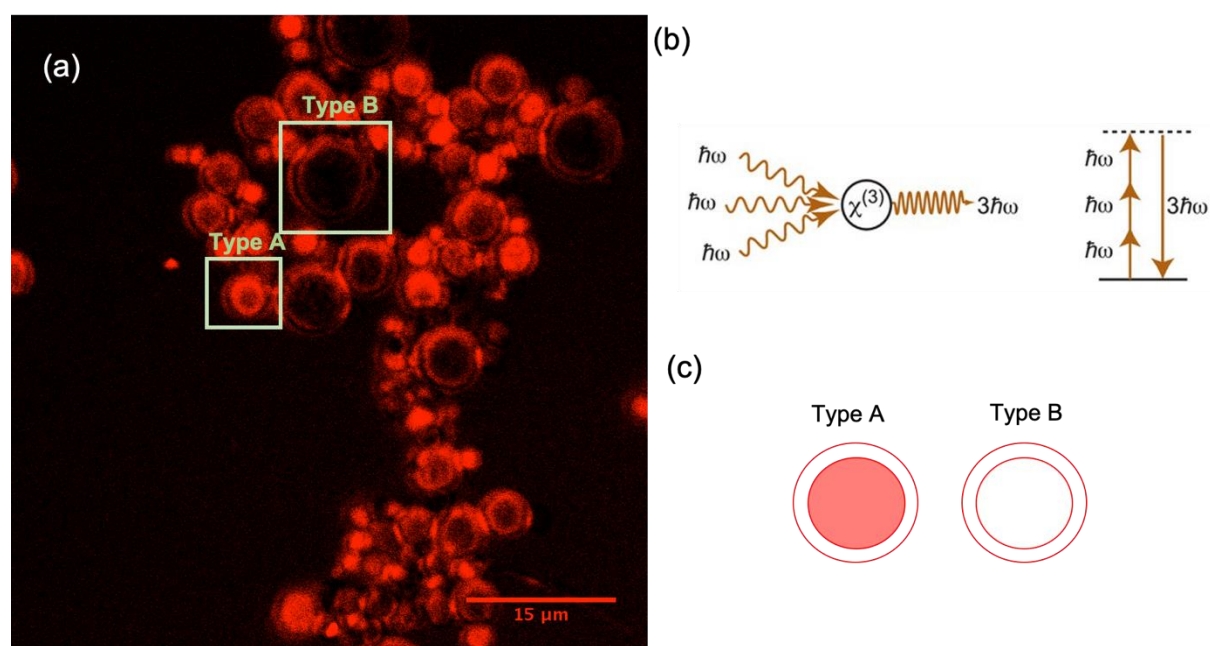


Figure 3-12 Potential polymer redistribution when cCNC-PAA microbeads are immersed in water. (a) Third harmonic generation (THG) images of cCNC-PAA microbeads in aqueous solution at neutral pH. (b) Definition of third-harmonic generation. (Reprint with permission from ref,⁶⁵ Copyright © 2013 Society of Photo-Optical Instrumentation Engineers (SPIE)). (c) Illustration of two types of cCNC-PAA structures observed from THG microscopy.

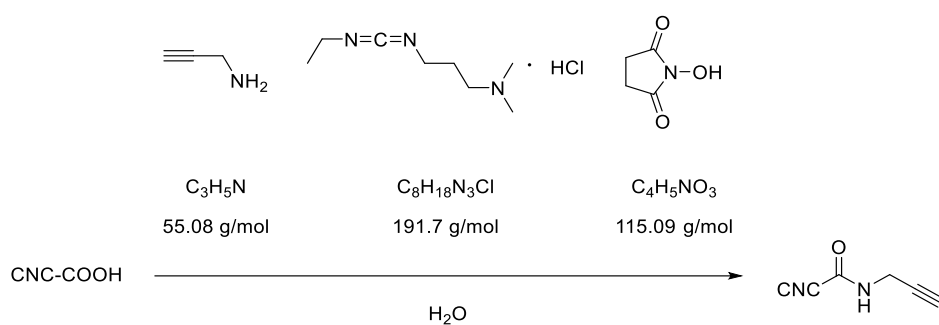
Neat cCNC microbeads that had imbibed water showed only weak THG signal. A Z-stack image of cCNC microbeads from the outer surface to the bottom of the bead revealed weak THG signal throughout, but signal from the interior was not uniform. (Figure S3-3) This suggests that the bead consists of cCNC that was not uniformly distributed, consistent with the FIB image in Figure 3-11 (e). CNC-PAA microbeads swell in water to an average diameter of 4.03 μm compared to the average dry state diameter of 1.83 μm . Figure 3-12 (a) shows a false color image of the THG signal from cCNC-PAA microbeads in water. The microbeads are not all in the same plane, so that the depth of focus in this Figure differs across the sample. From Figure 3-12 (a), we observe what appear to be “filled” and “unfilled” microbeads. The differences in red signal intensity are focusing artefacts and are not due some sort of preferential segregation of the PAA. This was proven by changing the depth of focus of the laser. Nevertheless, the stronger signal from the hybrid microbead suggests that PAA and cCNC are intermixed. This makes sense in view of the physics and thermodynamics of the spray-drying process. We suggest that the 2 components are “entangled” in the evolving micro-droplet and that diffusion of them to the surface of the drying micro-droplet is coupled: PAA and cCNC co-adsorb because of favourable H-bonded interactions and favourable negative enthalpy.⁶⁹ They are therefore likely to diffuse together to the surface of the microbead to form a skin through which water must evaporate. Diffusion of entangled polymer and particles is likely to be slow, and there should be a rapid onset of gelation. The result of this interaction should be rather uniform drying and therefore a rather dense microbead. This is what Figure 3-11 (f) suggests.

To collect more information on the distribution of PAA in the microbeads we turned to XPS depth profiling. This is discussed next.

3.3. Propargylation to Determine the Distribution of cCNC in Hybrid cCNC-PAA

Microbeads in the Dry State

XPS depth profiling was conducted on nitrogen-labelled cCNC in the hybrid microbeads to explore the spatial distribution of cCNC relative to PAA. To accomplish this, we propargylated cCNC *via* EDC coupling according to the reaction Scheme 3-1. Labelled cCNC-PAA hybrid microbeads were then produced by mixing propargylated cCNC with PAA, followed by spray-drying. XPS depth profiling was conducted with Ar⁺ projectiles at the lowest energy of 500 eV. Figure 3-7 illustrates the experiment and its outcome. To date, there are no reports of the relationship between etching depth and etching time for (nanocrystalline) cellulose or PAA. We determined that etching depth varies linearly with etching time at constant ion energy and current. This was calibrated from SEM images (Figure 3-13 (b)) acquired before and after etching the microbead sample for 10 and 20 minutes, respectively. By SEM, we estimate that 10 min of etching at 1.00 μ A and 500 eV Ar⁺ ion energy results in an etching depth of approximately 1 μ m. Figure 3-13 (b) reveals that the microbeads become smaller as the erosion process advances in time. The 2 μ m magnifications in Figure 3-13 (b) show damage tracks that are aligned, reflecting the angled and anisotropic etching by the Ar⁺ projectiles. Figure 3-13 (c) shows the XPS N1s binding energy near 400 eV over different etching times. Before etching (Level 0), two nitrogen peaks were observed, indicating two different nitrogen environments. One is assigned to the amide nitrogen at $\sim$ 399 eV, and the other to adsorbed, unreacted propargyl amine at $\sim$ 401 eV. After 300 s etching, only the N amide binding energy is apparent at 399 eV. From the depth profile, we conclude that nitrogen, and therefore cCNC, is homogeneously distributed throughout the hybrid microbead. XPS analysis of emission from the carbon and oxygen 2s cores after each etching cycle revealed no change in the chemical environment of these elements. This suggested that the etching process did not cause a redistribution of chemical bonding types (Figure 3-S2).



Scheme 3-1 Alkyne functionalization of carboxylated cCNC using EDC-Coupling.

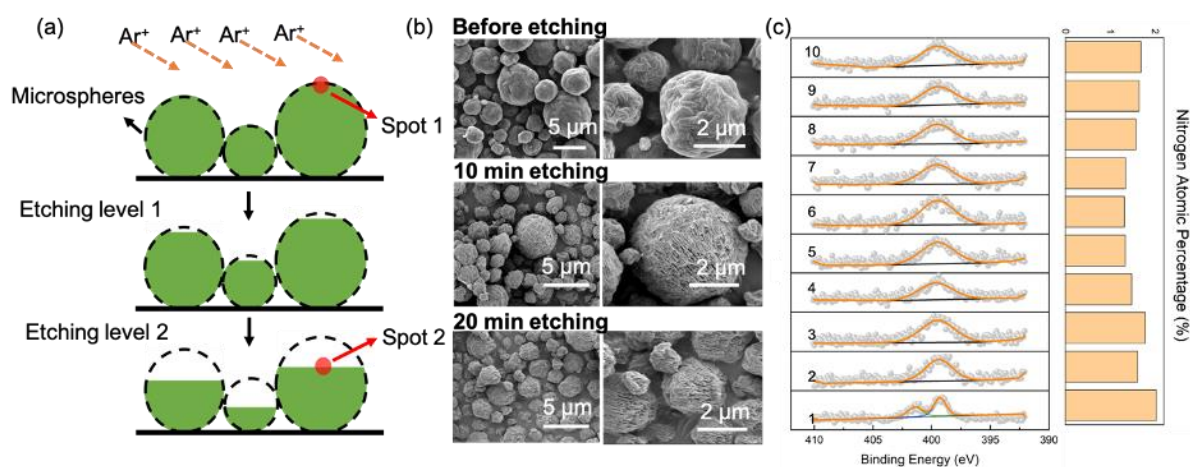


Figure 3-13 XPS depth profiling study on cCNC-alkyne PAA hybrid microbeads. (a) Demonstration of XPS depth profiling on microbeads. (b) SEM images of cCNC-PAA microbead surface texture before etching, after 10 min etching and 20 min etching. (c) XPS analysis of N binding energy for etching 5 min, 10 layers. The histogram image shows the percent nitrogen atomic after etching at different etching layers.

Conclusions

In conclusion, a type of pH-responsive nanocomposite microbead was made by hybridizing cCNC with PAA by spray-drying. We found that spray-drying is accompanied by esterification between the carboxylic acid group of PAA and the hydroxyl group on cCNC, which stabilizes the microbeads against fragmentation in aqueous solution. The hybrid microparticles exhibit pH-dependent swelling over a range of pH from 12 to 1. The change in diameters depends on the PAA content. Methylene blue was used as a prototypical small molecule to probe its pH responsive release profile. At a fixed cCNC-PAA ratio, we found that MB dye release depended on pH. Basic pH impeded release of MB dye molecules from cCNC-PAA microbeads, while acidic conditions promoted release. Confocal microscopy was used to monitor the *in situ* release of a porphyrin fluor from cCNC-PAA microbeads. Super-resolution fluorescent microscopy SIM was used to map the position of dye molecules in cCNC-PAA microbeads, providing a straightforward visualization tool. THG offers a label-free method to image the morphology of the microparticles when swelled in water. XPS depth profiling of propargylated cCNC-PAA microbeads revealed that cCNC and PAA are distributed homogeneously in the microbeads.

Case Study 2. Photo-gating Physical Response of Carboxylated Cellulose Nanocrystal Films and Microbeads

Introduction

In general, light responsive materials can be created by attaching photo-responsive entities to host structures by covalent binding,⁷⁰ co-polymerization,⁷¹ self-assembly,⁷² or the preparation of nanocomposites.⁷³ Coumarins are a family of fused pyrone and benzene rings, with the pyrone carbonyl group at position 2. The structure of 7-hydroxycoumarin is illustrated in Figure 3-1 (a). The photochemistry of coumarin and coumarin derivatives has been widely studied. Coumarin molecules are known to undergo photo-dimerization to form a cyclobutane ring *via* [2+2] cycloaddition upon irradiation near 365 nm. The reaction is reversible on exposure to light at 254 nm that causes the cyclobutane ring to cleave. This reaction has found broad applications in polymer science to make photo-responsive materials.^{74, 75}

Light-responsive materials have enormous potential in biomedical applications, especially in drug delivery systems.⁷⁶⁻⁷⁸ Biodegradability and non-toxicity are crucial for these applications.

cCNC is derived from cellulose, the most abundant biopolymer in the earth. Cellulose is well known as a drug excipient for controlled drug delivery due to its biodegradability, biocompatibility, and renewability. cCNC with its unique mechanical stiffness and surface properties, has emerged as a leading sustainable resource “platform” material, showing great potential in biomedical applications as scaffold materials, in wound healing, and in drug delivery systems,⁷⁹⁻⁸¹ as hydrogels, films, and microbeads. To date, our group has developed a method to produce carboxylated cCNC *via* hydrogen peroxide solution hydrolysis using 100%

Forest Stewardship Certified (100% FSC) pulp. This method eliminates the use of conventional sulfuric acid with only water and hydrolysed sugar unit as by-products.

Covalent binding of photo-active molecules like coumarin derivatives to the surface of cCNC can yield photo-responsive cCNC-based materials. The Argyropoulos group introduced a method to create fluorescent cellulose nanoparticles using a click reaction to graft both an anthracene and a coumarin onto cCNC. UV light was used to trigger photodimerization.¹⁴ The Foster group synthesized coumarin-functionalized cCNC *via* the 7-coumaryl-(6isocyanatohexyl) carbamate. This cCNC-Cou couple has been used as a filling to make photo-switchable nanocomposites. The mechanical properties of the nanocomposite have been measured and compared before and after UV-irradiation.⁷³ In contrast to the use of cCNC in solution and hydrogels, the preparation of light-responsive cCNC in bulk films remains a challenge.⁸²

Below we describe grafting of 7-hydroxycoumarin onto the surface of cCNC. The subsequent photo-induced gating effect (reversible crosslinking) is illustrated in Figure 3-14 (b). We developed a reduced pressure solvent exchange method to transfer cCNC from its water suspension to a DMF system. The product was characterized by ¹³C solid NMR, FT-IR, and XPS. PXRD was used to determine cCNC before and after coumarin functionalization. Following that, coumarin functionalized cCNC was cast as a thick film, where photogating effects were investigated. Figure 3-14 (c) demonstrates the photo-induced crosslinking effect in bulk films and microbeads.

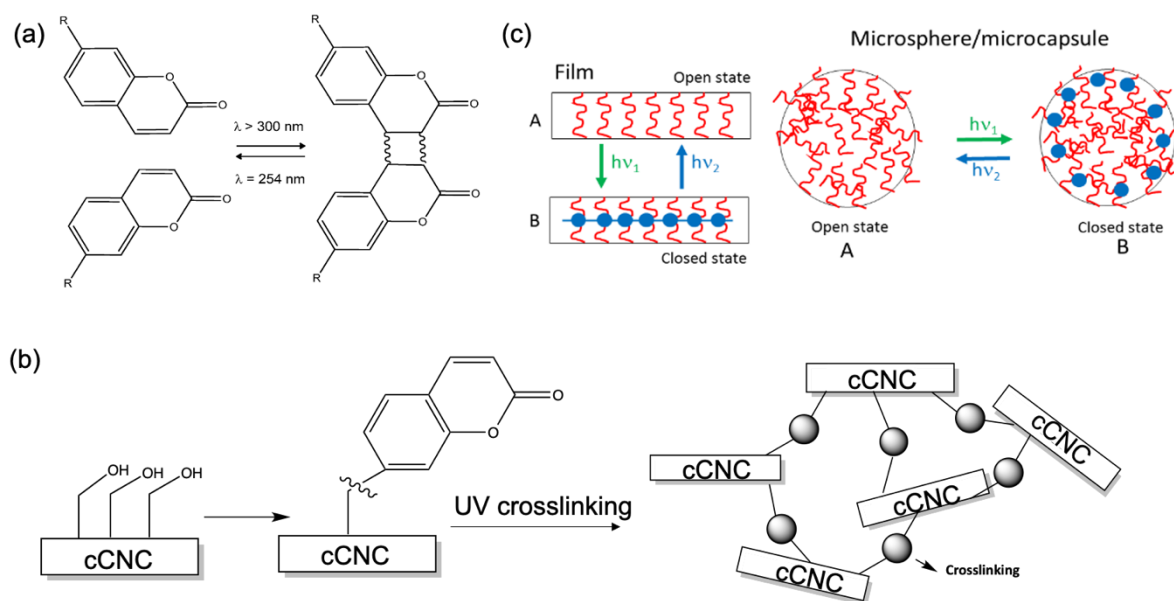


Figure 3-14 The design of photo-responsive cCNC films and microbeads. (a) Schematic of [2+2] cycloaddition of coumarin derivatives upon UV irradiation. (b) Grafting of coumarin onto cCNC followed by dimerization leads to crosslink formation in the cCNC-Cou matrix. (c) Photo-responsive cCNC films and microbeads with the open and closed states controlled by 2-color photons: 365 nm closes the gate, whereas 254 nm opens the gate.

Experimental section

2.1. Materials

The carboxylated cCNC suspension was provided by Anomera Inc. 7-hydroxycoumarin was purchased from Sigma Aldrich (ACS grade). Anhydrous DMF was purchased from Sigma Aldrich. Illumination at 365 and 254 nm was obtained using 100 W Hg arc lamp model number Q 60010 from Oriel with light filters (365 nm and 254 nm CWL, 10 nm FWHM, 25 mm Mounted Diameter filter) from Edmund Optics, USA.

2.2. Synthesis of 7-coumaryl-(6-isocyanatoheyl) Carbamate

7-hydroxycoumarin (4.0 g) and hexamethylene diisocyanate (28 ml) were charged into a two-necked air-free 150 ml round-bottom flask equipped with a magnetic stir bar, a reflux condenser, and a nitrogen inlet. The reaction mixture was stirred for 16 h at 100 °C in dark conditions by covering the round bottom flask with the aluminium foil. After the reaction, the mixture was cooled to room temperature and poured into 100 ml hexane. A light yellow precipitate was formed, and was further filtered and washed by hexane to remove any residues of hexamethyl diisocyanate. The product was dried at 50 °C under vacuum for 24 h and characterised using ¹H-NMR (DMSO-d₆), solid-state carbon NMR and FT-IR spectroscopy. ¹H-NMR (500 MHz-DMSO-d₆): δ=8.07(d,1H), 7.95(t, 1H), 7.73(dd, 1H), 7.21(d, 1H), 7.13(dd, 1H), 6.45(d, 1H), 3.35(q, 2H), 3.08(q, 2H), 1.60-1.29(m, 8H). FTIR: ν/cm⁻¹ split peak from 3300 amid N-H stretch, 3030 aromatic C-H stretch, 2950 - 2850 alkyl C-H stretch, 2250 N=C stretch, 1750 C=O stretch, 1625 Amide C=O stretch.

2.3. DMF Replacement of cCNC-Cou Water Suspension

The cCNC dispersion in DMF was obtained by replacing water with DMF *via* reduced pressure distillation. 20 mL of the cCNC suspension (2.4 wt %) was mixed with 100 ml anhydrous DMF in a round bottom flask, equipped with a reduced pressure distillation set up. The temperature was set to be 120 °C. The water was distilled out from the mixture until the volume of the mixture reached 20 ml, which indicated that the water was completely replaced by DMF. The quality of the cCNC-DMF dispersion was examined using atomic force microscopy (AFM) and transmission electron microscopy (TEM). To ensure the complete removal of water, the cCNC-DMF suspension was dried for two days by adding sodium sulfate to the suspension until it was no longer cloudy.

2.4. Synthesis of cCNC-Cou

0.5 g of the well dispersed cCNC (1 eq, 3.1 mmol) in anhydrous DMF was added to a three-neck round bottom flask equipped with a magnetic stir bar and an inert nitrogen atmosphere, containing 1.12 g already-dried 7-coumarin-(6-isocyanatohexyl) carbamate (1.1 eq, 3.4 mmol). 1 drop of DBTDL was added and the reaction mixture was stirred for 24 h at 100 °C under nitrogen in dark conditions by covering the flask with the aluminium foil. The formed product was purified by subsequent centrifugation (5000 rpm, 15 min). The supernatant was discarded and replaced with fresh anhydrous DMF. The process was repeated 5 times to completely remove the unreacted coumarin-NCO. The product was characterized using solid-state NMR, FT-IR, UV-VIS spectroscopy, XPS, and fluorescence microscopy.

2.5. Film Casting Method

Films were cast directly onto glass microscope slides from 3 mL cCNC-Cou DMF suspension and then allowed to evaporate to dryness. The morphology and surface properties of the film were characterized using SEM and AFM tapping mode.

2.6. Photo-irradiation

The experimental set-up is shown in Figure 3-11. Four films weighing $0.030 \text{ g} \pm 0.002 \text{ g}$ were separately irradiated with 365 nm light. Film 1 was not irradiated and was used as a control. Film 2 was irradiated at 365 nm for 5 hours. Film 3 was irradiated at 365 nm for 5 hours, followed by 3 hours at 264 nm to reverse the dimerization. Film 4 was irradiated at 365 nm light for 5 hours, followed by 3 hours of 264 nm light, and again with 365 nm light for 5 hours.

2.7. Methylene Blue Dye Uptake Kinetics

For dye uptake kinetics, the pre-treated films on the glass substrates were immersed in 100 mL methylene blue solution, and the concentration of the methylene blue remaining in solution was measured every 2 minutes by electronic absorption spectroscopy.

2.8. Spray-drying Method

Acetone was added to a cCNC-Cou DMF suspension to precipitate the cCNC-Cou particles from the DMF solvent. The cCNC-Cou precipitate was repeatedly washed with acetone and then dried under vacuum at room temperature. cCNC-Cou was then re-dispersed in water under sonication (80% output using a Sonics Vibra-cell VCX130) to yield a cCNC-Cou suspension. The aqueous cCNC-Cou suspension was spray-dried using a spray drier (Büchi B191, 175 °C inlet temperature, 30% pump speed, and 70% aspirator), and the powder was collected into a sample vial and stored in the dark.

2.9. Characterization Methods

FT-IR spectroscopy

Infrared spectra were acquired with a Spectrum Two FT-IR spectrometer (Perkin Elmer) equipped with single bounce diamond crystal, and standard, room-temperature LiTaO₃ (Lithium tantalite) MIR detector using ATR method. Spectra were obtained in the range of 4000 - 500 cm⁻¹ with a resolution of 4 cm⁻¹ by accumulating 16 scans.

Scanning Electron Microscopy (SEM)

A SEM-FEI Inspect F-50 FE-SEM was used to characterize the cCNC film surface morphology. The samples were sputter-coated with 4 nm Pt by a high-vacuum coater (Leica EM ACE 600). The images were taken at an accelerating voltage of 5 kV.

X-Ray Photoelectron microscopy (XPS)

A $K\alpha$ X-ray photoelectron spectrometer (Thermo Scientific, using a monochromatic Al $K\alpha$ X-ray source and a flood gun in a 10^{-8} mbar vacuum) was used to identify the amide bond between cCNC and the coumarin. Binding energies from nitrogen, carbon, and oxygen were measured. Curve was conducted.

Transmission Electron Microscopy (TEM)

CNC suspensions before and after chemical modification were diluted and sonicated to make sure the nanocrystals were well dispersed in water. TEM grids (ultrathin carbon films supported by fenestrated carbon films) were fixed at the edge of a TEM specific tweezer. A drop of CNC suspension was deposited on the TEM grid and dried under ambient condition. A drop of 2% uranyl acetate was deposited on the TEM grid and maintained for approximately 2 min until the droplet was absorbed by a paper tissue from the side of the droplet. The samples were dried under ambient condition for overnight and put into vacuum for continuously drying. The samples were then imaged with a FEI Tecnai 12 BioTwin 120 Kv TEM equipped with an AMT XR80c CCD Camera System.

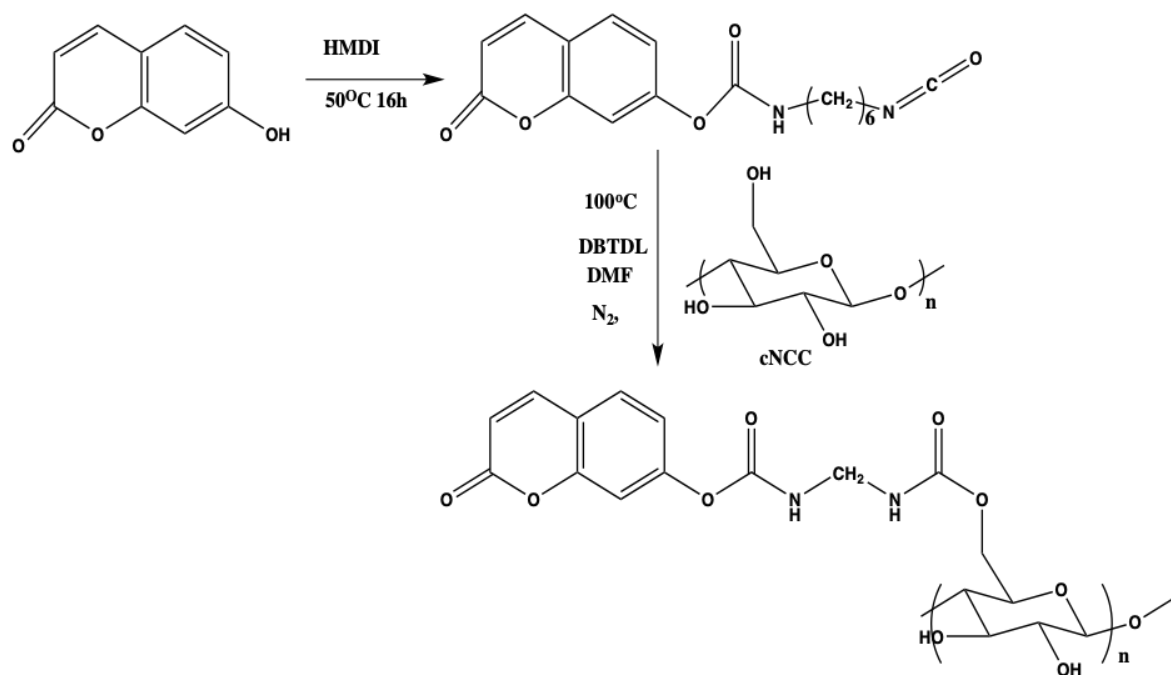
^{13}C Solid state NMR

^{13}C solid-state NMR spectra were acquired with a Varian VNMRS (now Agilent, Santa Clara, CA, USA) Spectrometer operating at 100.5 MHz for ^{13}C using a 4 mm double-resonance Varian Chemagnetics T3 probe. Approximately 70 mg of sample was centre-packed into rotors using Teflon inserts. The ^{13}C solid NMR spectrum was acquired in 512 scans with spinning at 8 kHz, using a recycle delay of 5 s with a cross polarization contact time of 1.5 s. Spectra were referenced to the carbon signal in glycine at 176.0 ppm with respect to TMS (tetramethylsilane).

Results and Discussion

3.1. Chemical Grafting of Coumarin Small Molecule on the Surface of cCNC

7-hydroxycoumarin was grafted to cCNC according to Scheme 3-1.⁷³ HMDI (hexamethyl diisocyanate) was used as a linker molecule to react both with the hydroxyl group on 7-hydroxycoumarin and the 6-position hydroxyl group of cCNC. The reaction was conducted in two steps. First, HMDI and 7-hydroxycoumarin were reacted to form the coumarin carbamate, cou-NCO (7-coumaryl-(6-isocyanatoheyl) carbamate). Figure S3-5 provides the ¹H NMR data on the formation of cou-NCO. In the second step, cou-NCO was reacted with cCNC, which was pre-dispersed in anhydrous DMF. All reactions were conducted under nitrogen.



Scheme 3-2 Surface modification of coumarin molecules onto cCNC through an amide bond formation

FT-IR spectra of cCNC, cou-NCO, and cCNC-Cou are shown in Figure 3-15. The complete reaction of the isocyanate group from cou-NCO is indicated by the disappearance of the band between 2500 cm^{-1} and 2000 cm^{-1} . This is evident by comparing the spectra of cCNC-Cou and NCO-Coumarin. The cCNC-Cou spectrum features a characteristic peak associated with the carbonyl group at 1660 cm^{-1} . This band is absent in the spectrum of pristine cCNC. The increase in intensity of the bands between $2800 - 2950\text{ cm}^{-1}$ corresponds to alkyl chain vibrations. This indicates covalent bonding between cCNC and cou-NCO.

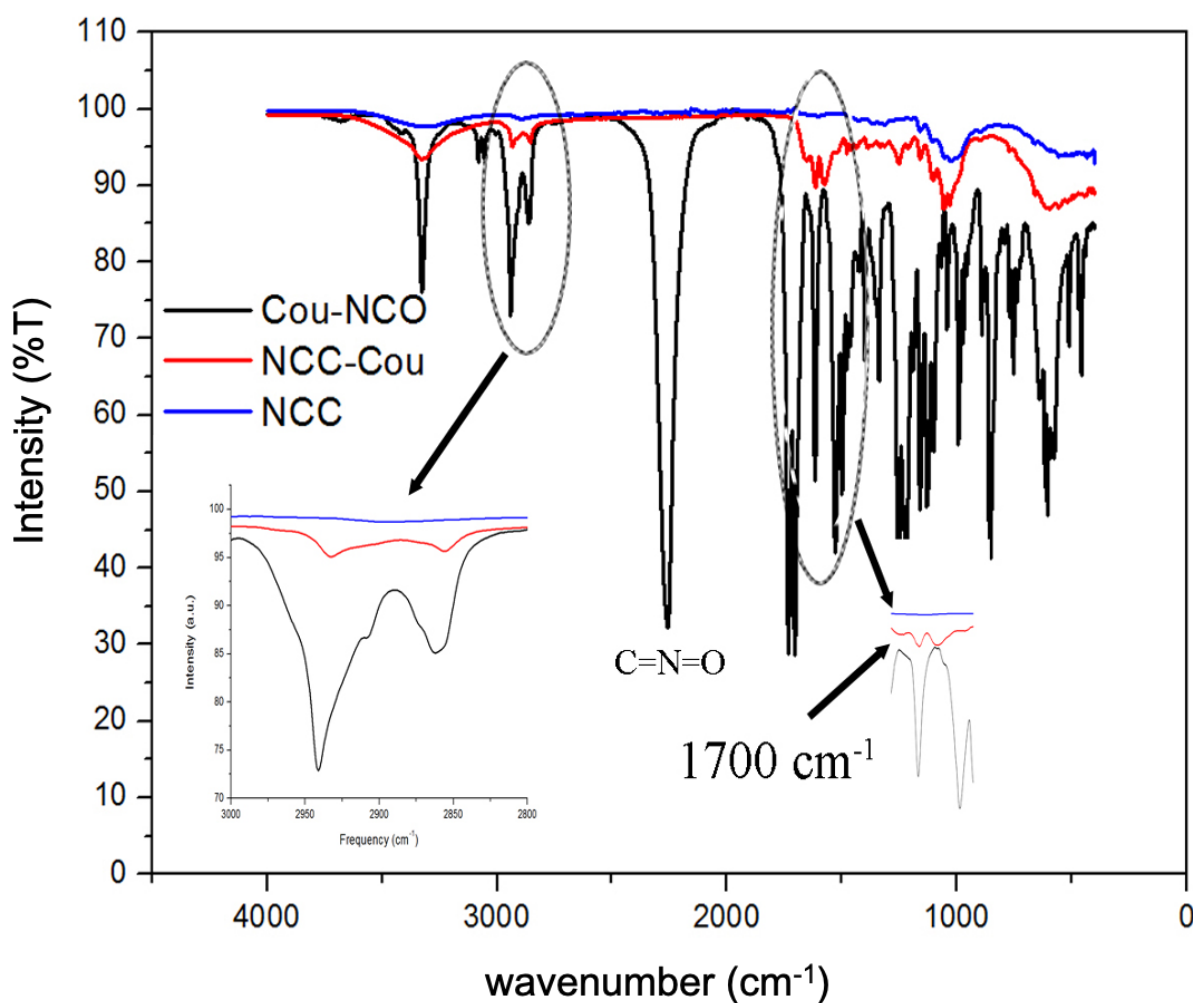


Figure 3-15 Comparison of infrared (FT-IR) spectra of unmodified cCNC, cou-NCO, and coumarin modified cCNC-Cou.

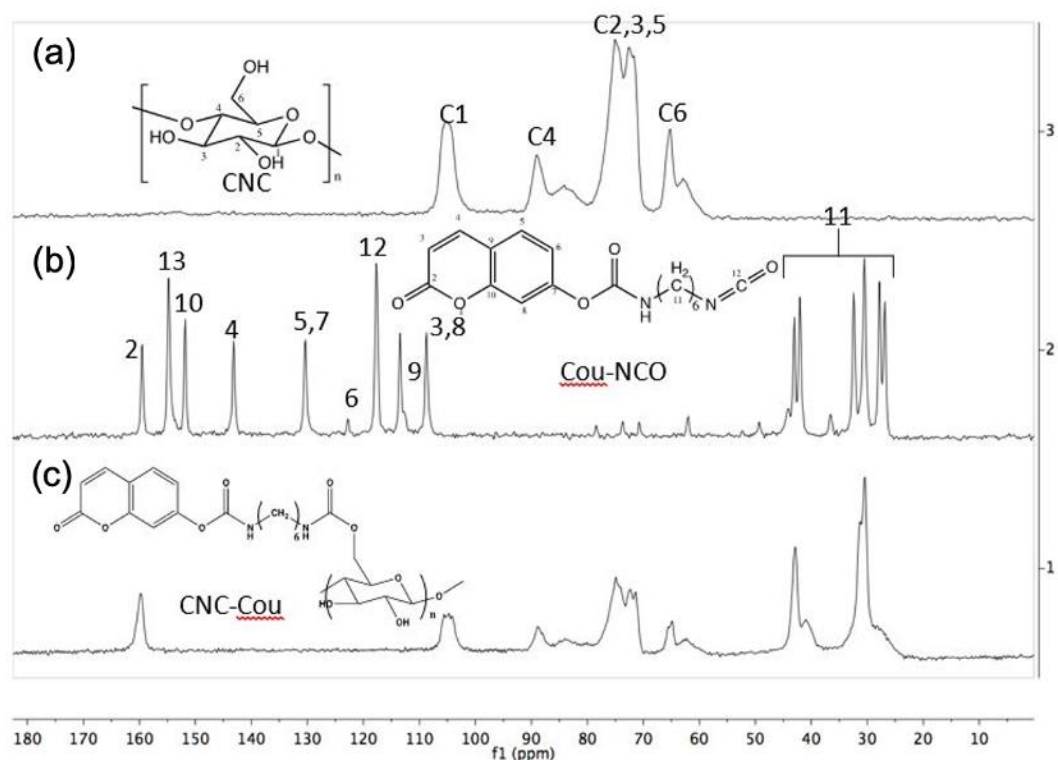


Figure 3-16 Solid-state NMR spectra of cCNC (a), cou-NCO (b), and cCNC-Cou (c).

^{13}C NMR solid-state spectra of cCNC, cou-NCO, and cCNC-Cou (Figure 3-16) gave further evidence of this chemical modification. We first assigned the chemical shifts of carbon atoms for the three samples. The chemical shifts of carbon atoms from cCNC were assigned to C1 (105 ppm), C4 (88 ppm), C2,3,5 (71-75 ppm), and C6 (65 ppm). The chemical shifts of cou-NCO were assigned, as C2 (159.6 ppm), C13 (158.0 ppm), C10 (153.1 ppm), C4 (142.7 ppm), C5,7 (130.0 ppm), C6 (123.6 ppm), C12 (122.3 ppm), C9 (118.1 ppm), C3, 8 (115.7 ppm), and C11 (25-45 ppm). For cCNC-Cou, C1-6 peaks between 110 ppm and 60 ppm show similar peak shifts as cCNC, indicating that the chemical environment of cCNC was not disturbed after the chemical modification. The peaks around 160 ppm and 50 - 20 ppm show the chemical shifts of the coumarin derivative. There could be several reasons why a broad peak was

observed at ~160 ppm. Firstly of all, due to the low loading rate of coumarin molecules on CNC (see section 3.3), the aromatic coumarin carbon peak might be broadened into one peak positioned at 160 ppm. The appearance of 160 ppm peak might also be related to the residues of DMF on CNC during chemical modification. However, the existence of peaks at position 11 shows the appearance of alkyl group on CNC, indicating the successful covalent attachment of coumarin molecules on CNC.

XPS shows another evidence of the grafting of coumarin to cCNC. Figure 3-17 shows the XPS spectra of cCNC, cCNC-Cou and the starting material Cou-NCO. All the spectra reveal that C and O are the predominant species. These have respective binding energies at 285 eV and 532 eV. The cCNC-Cou sample shows a new XPS peak at 400 eV, corresponding to the N 1s peak. Deconvolution of the C 1s peak yields four types of carbon bonds: C-C/C-H (C 1sa, 285 eV), C-O (C1sb, 286.7 - 286.8 eV), C=O (C 1sc, 288.2 - 288.3 eV) and O-C=O (C 1sd, 289.2 - 289.9 eV). The shift to lower binding energy of the C1s peak on going from cCNC to cCNC-Cou is due to the long alkyl chain from the grafted nanocrystal, cCNC-Cou. Figure 3-17 (c) identifies the evolution of the nitrogen binding energy to yield the amide N on going from cCNC to cCNC-Cou. The nitrogen content in CNC-Coumarin exceeds what is anticipated based on the reaction conditions; therefore, we conjecture that the enhanced signal may be due to residual DMF in the sample.

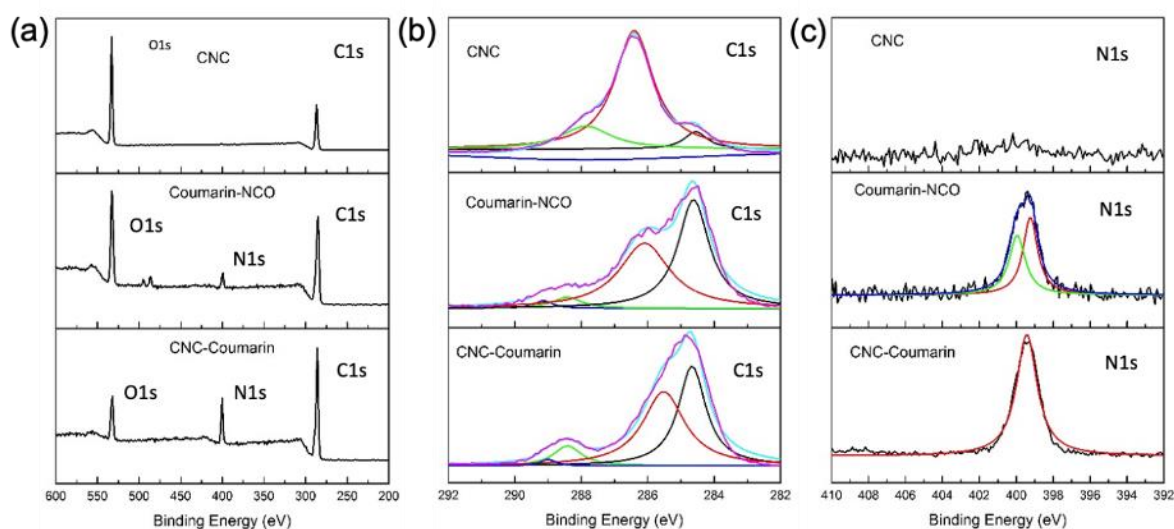


Figure 3-17 XPS of cCNC, Coumarin-NCO, and cCNC-Cou. (a) Survey scan of cCNC, Coumarin-NCO and cCNC-Cou. (b) Carbon 1s peak deconvolution. (e) Nitrogen 1s peak scan and deconvolution for cCNC, coumarin-NCO, and cCNC-Cou. The amide N is visible at 399 eV.

3.2. Morphology of cCNC after Chemical Modification

TEM images of cCNC, cCNC-Cou are displayed in Figure 3-18. The images show that after reaction with the isocyanate to give cCNC-Cou, there is some aggregation of nanoparticles, including formation of what appear to be nanofilaments.

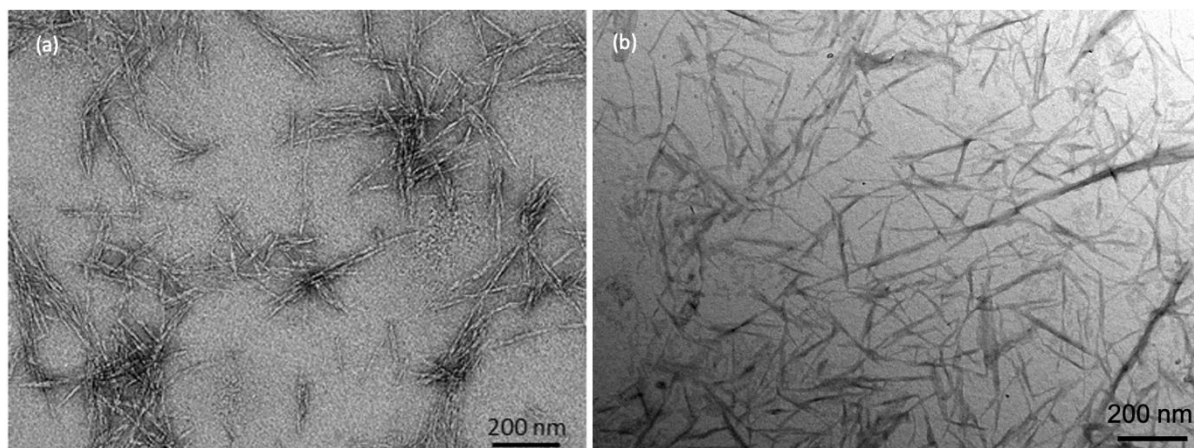


Figure 3-18 TEM micrographs of cCNC before (a) and after isocyanation (b).

3.3. Determination of Coumarin Molecule Grafting Ratio

The electronic absorption spectra of the starting material Cou-NCO in different concentrations in DMF, along with the cCNC-Cou in DMF were measured from the intensity of the adsorption peak at 318 nm. The UV-vis spectra of cou-NCO and cCNC-Cou are shown in SI-Figure S3-6. The absorption peak of cCNC-Cou shifted to the higher energy due to solvatochromism. From the calibration curve, the coumarin loading was determined to be 0.044 mmol/g. This yields a grafting ratio of 1 coumarin unit per every 126 glucose units.

3.4. CNC-Coumarin Film Characterization

cCNC-Cou films were prepared by drop casting from DMF. The film thickness was adjusted by changing the amount of cCNC-Cou in suspension. Figure 3-19 shows the surface morphology and cross-section images of the films. SEM indicates that the films have a mat-like texture, clearly evident in 3-19 (b). The cross-sections reveal that the films are stratified. The stratification is reminiscent of what is observed from the cross-sections of chiral nematic films made by slow evaporation of water from self-assembling *sulfated* CNC. This observation

is intriguing, suggesting that slow drying of cCNC-Cou from DMF proceeds with some degree of self-assembly.

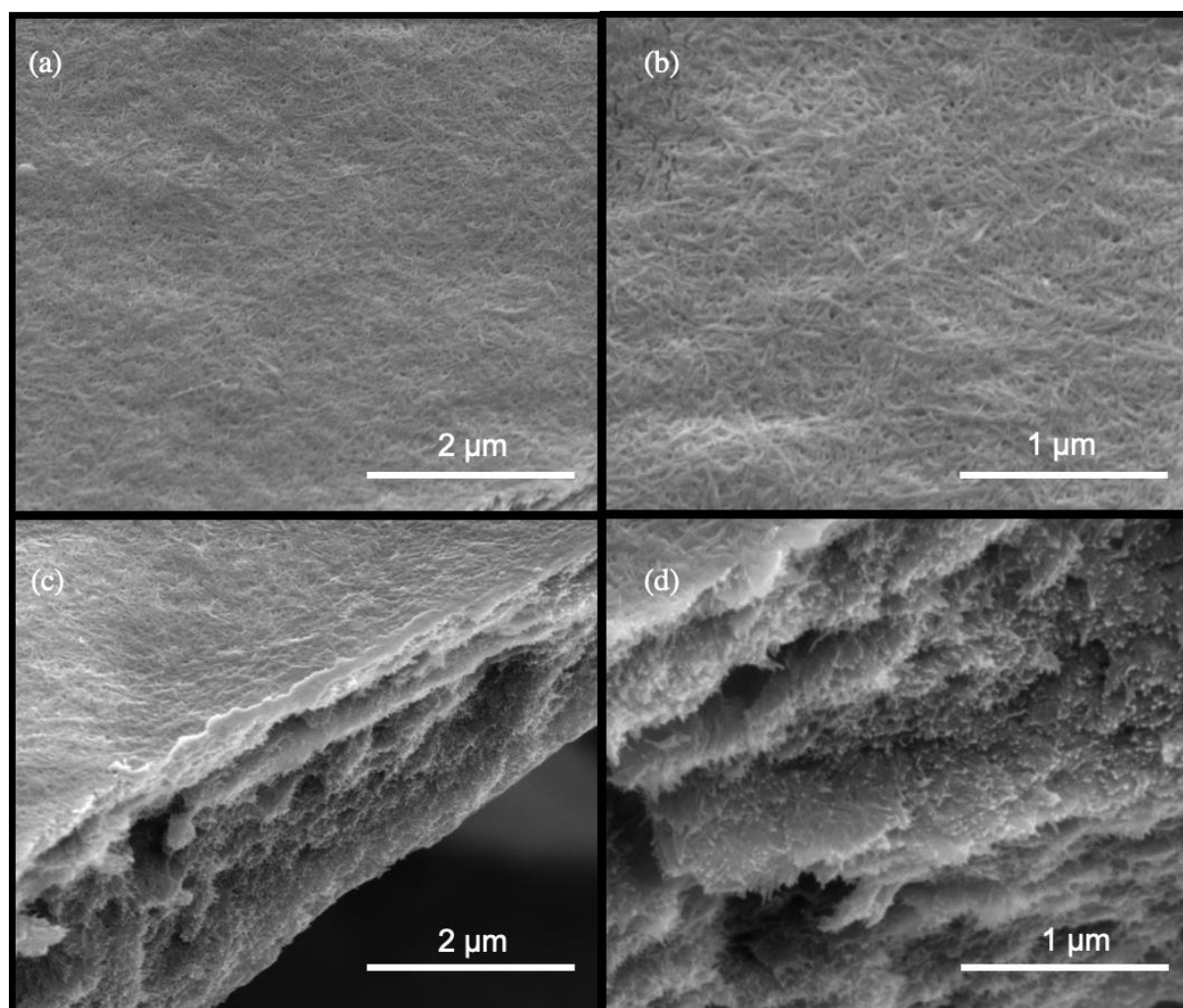


Figure 3-19 SEM images at different magnifications of cCNC-Cou films cast from DMF on a glass substrate. (a) Surface morphology of cCNC-Cou film at low magnification, and at twice the magnification in (b); (c) cCNC-Cou film cross-section at low magnification and twice the magnification in (d).

3.5. Methylene Blue Dye Uptake Kinetics Show a Reversible Photogating Effect

We first demonstrate the photo-gating effect in cCNC-Cou gels. Figure S3-7 shows the cCNC-Coumarin in DMF suspension before and after 365 nm irradiation. After chemical modification

and purification by centrifugation, the product obtained in DMF was gel-like. Dilution in DMF yielded a stable colloidal suspension. The suspension was exposed to 365 nm light in a fused silica cuvette. In the right-hand image above, dimerization of cCNC-coumarin caused the cCNC-Cou particles to aggregate, observable as the phase separated white features in the cuvette. A control sample, shielded from incident radiation with foil, but exposed to infrared heating from the photolysis lamp, showed no evidence of phase separation. Accordingly, phase separation was induced by crosslinking and not temperature under the conditions of the photolysis experiment.

To demonstrate the photo-gating effect, we used methylene blue dye to probe uptake kinetics. Figure 3-20 shows the uptake in two films, one irradiated at 365 nm and a control that was not exposed to light. The irradiated film showed reduced dye uptake compared with the control. We attribute the suppressed uptake to hindered diffusion of MB due to densification at the surface of the film, the result of crosslinking *via* dimerization of coumarin moieties. The effect is emphasized by reversing the cycloaddition with 245 nm light. Figure 3-21 shows three cycles of photo-induced crosslinking and uncrosslinking. Thus, exposure of the crosslinked film to 245 nm light results in an increase in MB uptake. In other words, dye uptake is reversible because the molecular gates can be opened and closed.

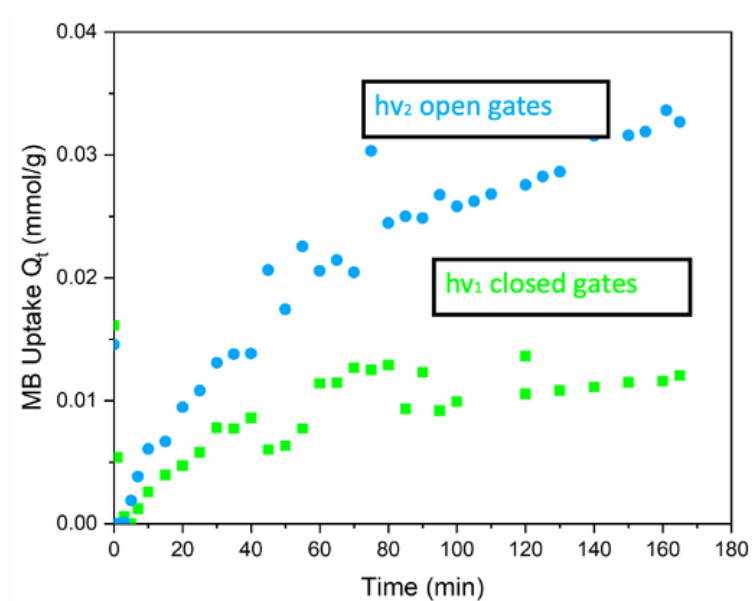


Figure 3-20 Methylene blue dye uptake kinetics by photo-crosslinked film and uncrosslinked film. $h\nu_1$ refers to 245 nm light. $h\nu_2$ refers to 365 nm light

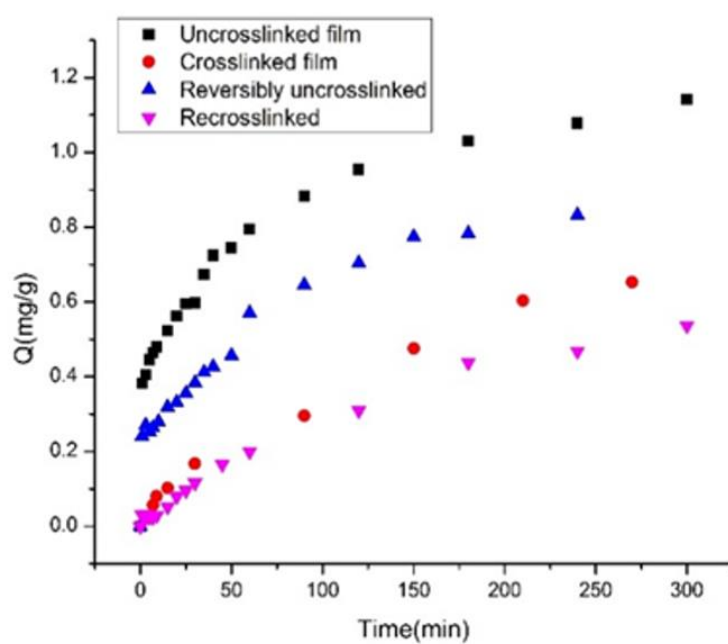


Figure 3-21 Methylene blue dye uptake kinetics of control (unexposed), photo-crosslinked, reversibly uncrosslinked, and re-crosslinked cCNC-Cou films. $h\nu_1$ refers to 245 nm light. $h\nu_2$ refers to 365 nm light.

3.6. Characterization of cCNC-Cou Microbeads

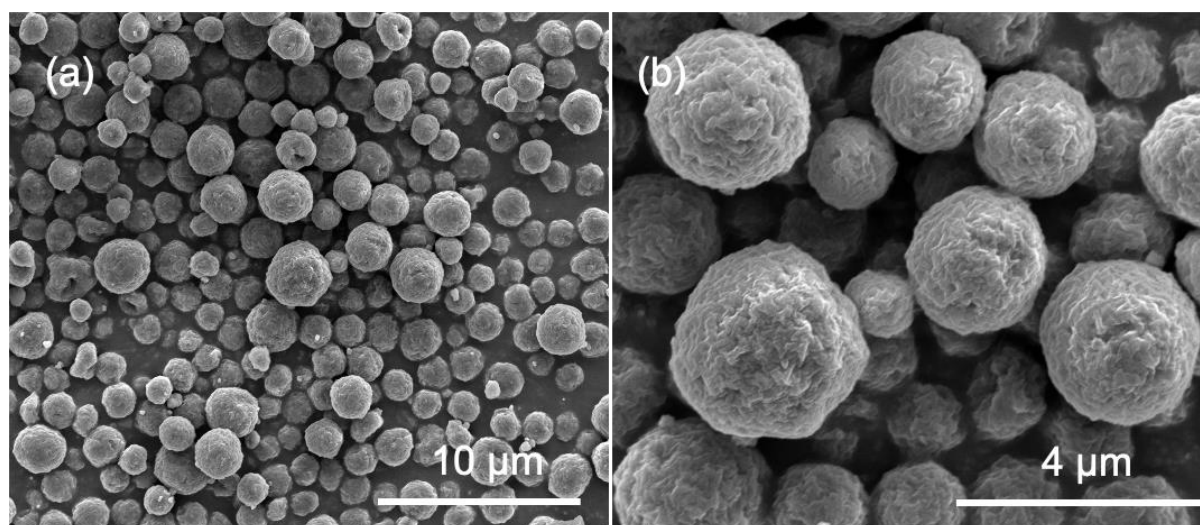


Figure 3-22 SEM images of cCNC-Cou microbeads at different magnifications reveal the morphology and surface texture of the particles.

Spray-drying yielded coumarin functionalized cCNC microbeads with a mean particle size of 1.69 μm. From Figure 3-22 (a), two kinds of particles are observed. Trace amounts of “red-blood cell” shaped particles are seen among the predominant population of spherical microbeads with wrinkled surfaces. Compared with neat cCNC microbeads (SEM images are shown in Chapter 2 and Chapter 4), cCNC-Cou microbeads shows a greater sphericity and lower polydispersity. The surface wrinkle texture is also unique compared both with cCNC microbeads and cCNC-based hybrid beads like cCNC-PAA microbeads (Chapter 3 Case study 1). The “red-blood cell” shaped microbeads resemble some SF-cCNC microbeads. These are described in Chapter 5. The formation of the particle shapes is likely related to subtle changes in the surface energy of cCNC-Cou as compared with unfunctionalized cCNC. Coumarin is somewhat hydrophobic. Particle shape by spray-drying is sensitive to the chemical and physical properties of the solute in water.

Conclusions

This work describes the preparation of molecular photo-gates in cCNC-coumarin composite films and microbeads. The gates were made by grafting 7-hydroxycoumarin by isocyanate coupling to yield cCNC-Coumarin (cCNC-Cou). A solvent exchange method was developed to conduct the grafting process in DMF. Chemical grafting was characterized by, electronic absorption, XPS, infrared, and ^{13}C solid-state NMR. A grafting ratio of 0.044 mmol coumarin molecules per 1 g of cCNC was achieved. Films of cCNC-Cou were cast from gel-like suspensions of the nanoparticles. cCNC-Coumarin microbeads with excellent sphericity were also prepared by spray-drying.

The coumarin gate was closed and opened by two-color reversible dimerization. Dimerization is associated with crosslinking and densification of the surface of cCNC-Cou. The photo-gating effect was demonstrated by following the uptake of methylene blue (MB). Uptake was inhibited when the gate was closed with 365 nm photons and activated when the gate was opened with 245 nm photons.

Supplementary

This supplementary section contains information and experiments in support of the text for Case 1 and Case 2.

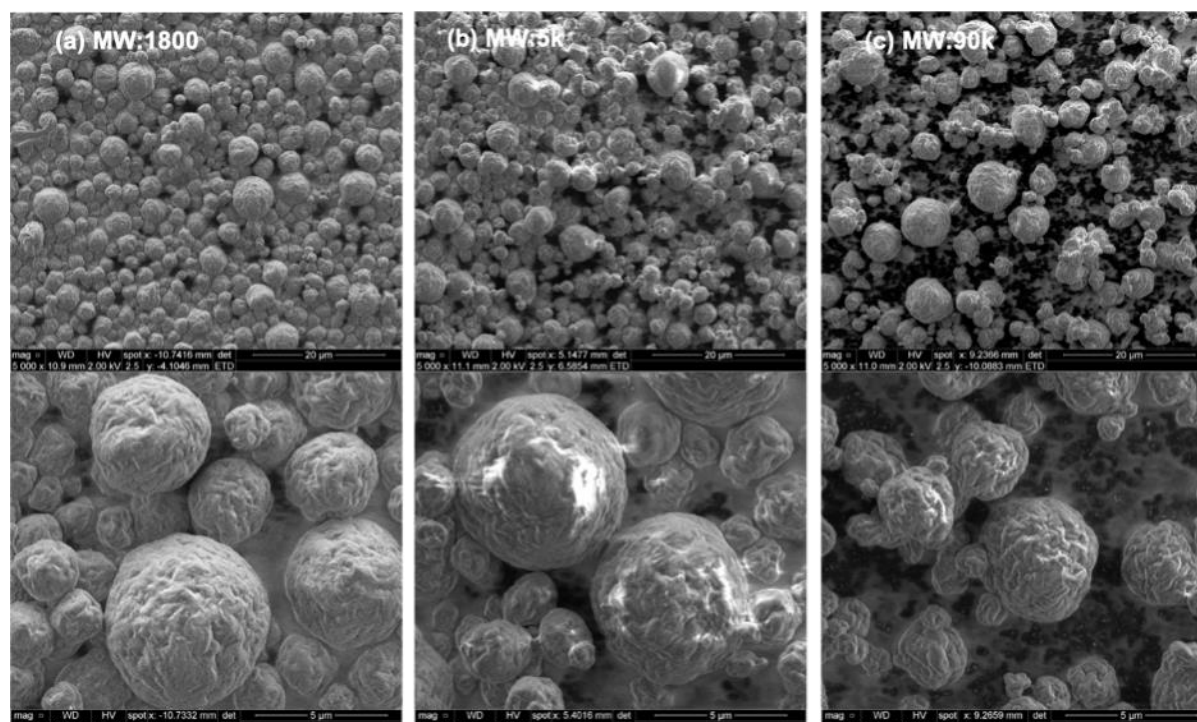


Figure S 3-1 SEM images of cCNC-PAA microbeads combined with PAA with molecular weight of 1800, 5k and 90k. All compositions are 1:1 cCNC-PAA.

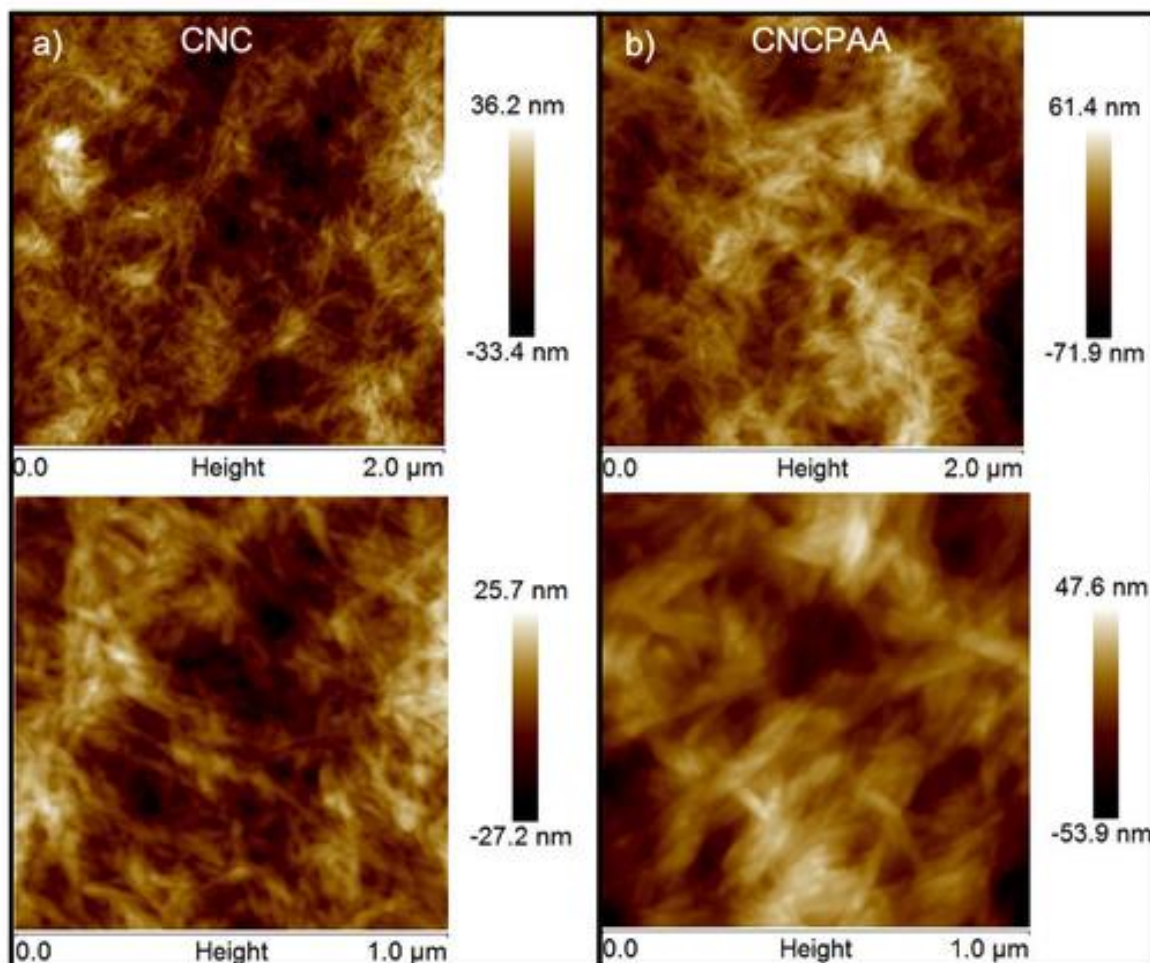


Figure S 3-2 Atomic Force Microscopy (AFM) topography images of cCNC (a) and cCNC-PAA (b) films. The increase of the width and length of the nanorods after hybridizing with PAA indicates a nano-scale association of the two components. Therefore, we suggest that cCNC-PAA can be described as a nanocomposite, at least in film form.

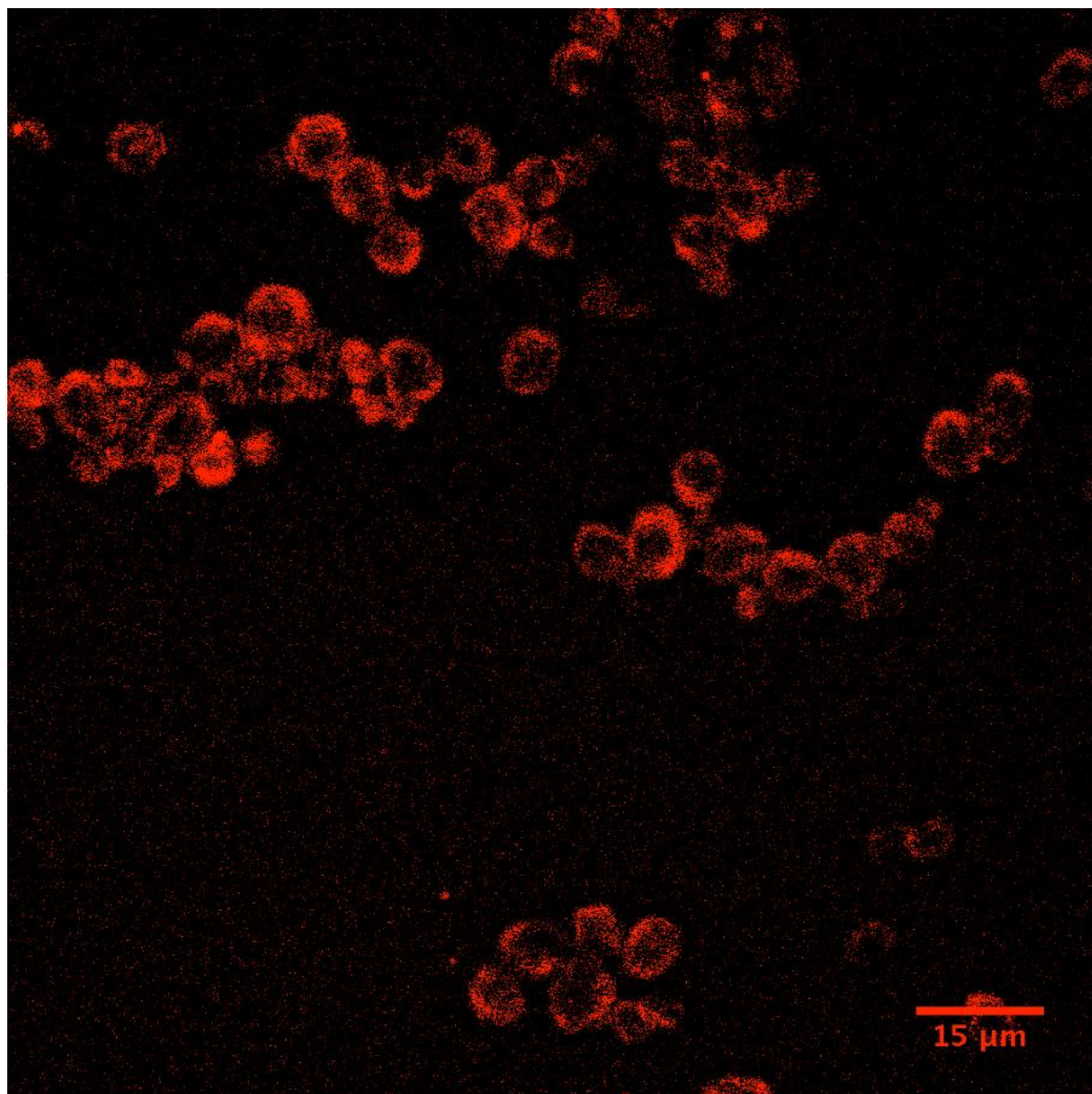


Figure S 3-3 THG microscopy images of neat cCNC microbeads in water.

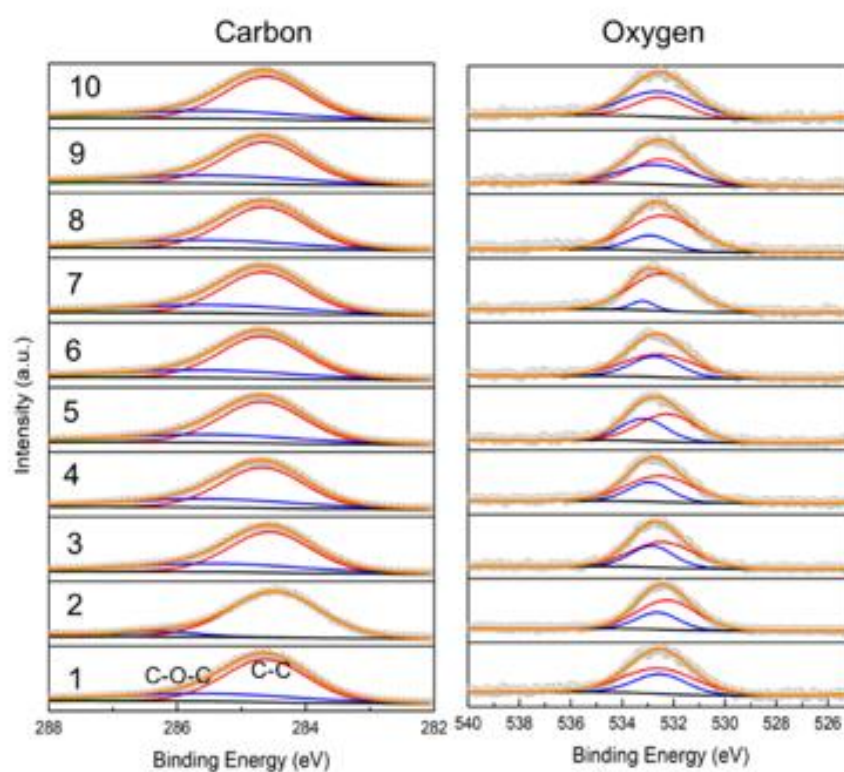


Figure S 3-4 XPS depth profiling analysis of C, O peaks for etching 5 min per layer with 10 layers.

Table S 3-1 Zeta potential measurements of cCNC-PAA microbeads

	H ₂ O	pH=2	pH=7	pH=12
cCNC	-32.10±4.30	-0.58±0.43	-18.00±6.65	-27.53±2.77
PAA(1800)-cCNC	-32.03±0.80	-2.83±0.43	-16.90±3.18	-24.33±0.50
PAA(5k)-cCNC	-43.53±2.14	-3.22±0.29	-17.50±3.04	-26.73±1.89
PAA(90k)-cCNC	-46.03±0.71	-2.29±0.15	-40.23±4.41	-36.67±2.59

Table S 3-2 Methylene blue dye uptake isotherms of cCNC-PAA microbeads with different PAA molecular weight.

C_o(mg/L)	MW=1800	MW=5K	MW=90k
75	48.63	37.4	55.81
50	30.28	22.94	37.16
25	13.86	8.36	20.32
10	5.34	5.73	6.44
5	1.80	1.44	3.18

Table S 3-3 The parameters of the methylene blue adsorption isotherms.

	cCNC microsphere			cCNC-PAA (1800)microsphere		
Langmuir	k _L	Q ₀	R ²	k _L	Q ₀	R ²
	479	43.48	0.7176	0.0119	39.53	0.8791
D-R		q _d	R ²		q _d	R ²
	0.0034		0.7559			0.8709
Temkin	k _T	B ₁	R ²	k _T	B ₁	R ²
	0.1867	19.45	0.9316	0.2301	408.58	0.9398
Freundlich	k _F	n	R ²	k _F	n	R ²
	0.9565	1.04	0.9981	0.4944	1.32	0.9904
H-J	B ₂	A	R ²	B ₂	A	R ²
	1025.06	294.12	0.8877	1.96	3.55	0.9622

Table S 3-4 Fruendilich isotherms of cCNC, and cCNC-PAA microbeads with different PAA molecular weight.

	cCNC	MW=1800	MW=5k	MW=90k
K_F	0.9565	0.3668	0.2938	0.5741
n	1.04	0.84	0.90	0.93
R^2	0.9981	0.9948	0.9593	0.9967

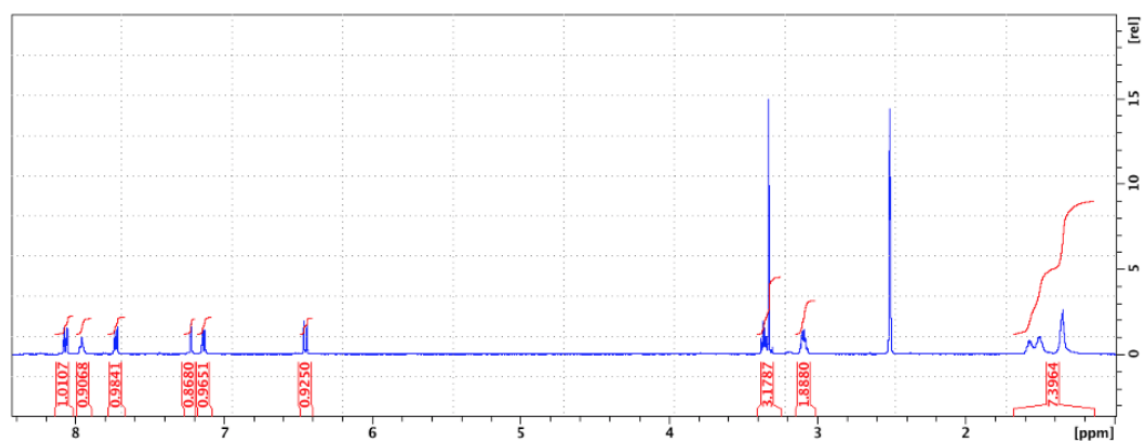


Figure S 3-5 ^1H -NMR spectrum of 7-coumaryl-(6-isocyanatohexyl) carbamate (Cou-NCO) in DMSO- d_6 .

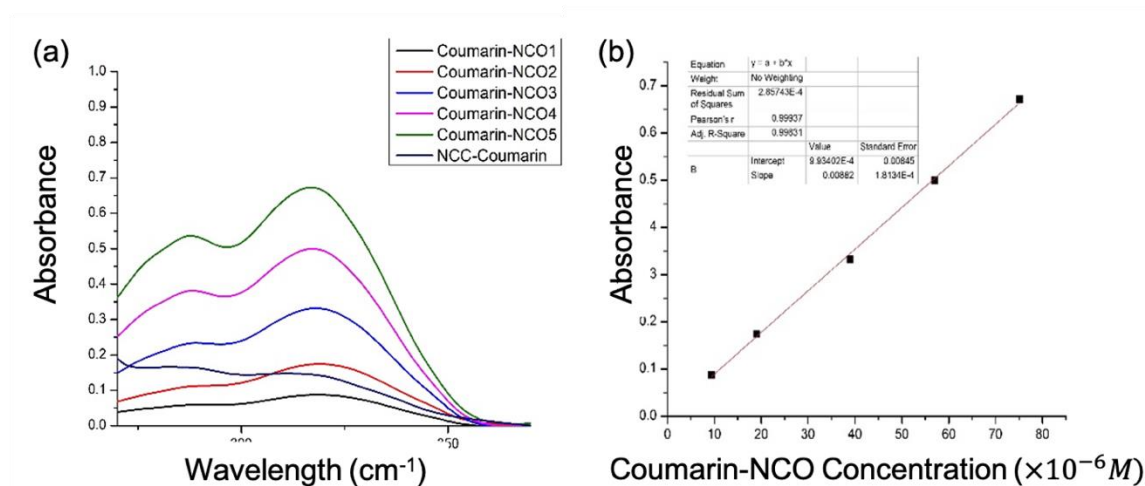


Figure S 3-6 UV-vis spectroscopy of different concentrations of Coumarin-NCO in DMF and the calibration curve derived to show the relationship between absorbance and Coumarin-NCO concentration.

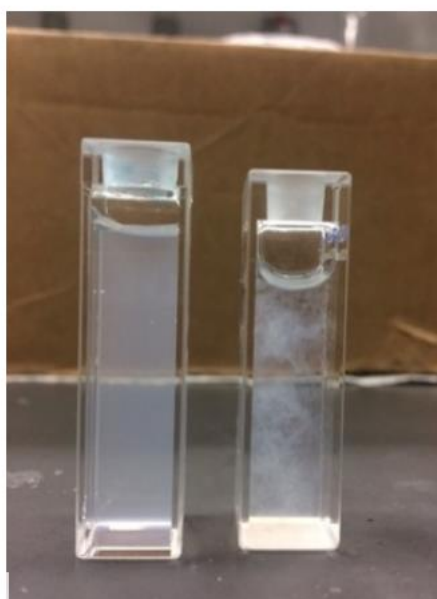


Figure S 3-7 cCNC-Cou suspension, before (left) and after 365 nm exposure (right), result of photo irradiation in DMF.

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| 4 |

**Measuring Young's Modulus of Carboxylated Cellulose
Nanocrystal Microbeads *via* Atomic Force Microscopy**

Chapter 4 Measuring Young's Modulus of Carboxylated Cellulose Nanocrystal Microbeads *via* Atomic Force Microscopy

This chapter includes one manuscript that is ready to submit. The bibliographic details of the paper are:

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My contribution to the paper involved:

4. Experimental design and implementation
5. Data collection and analysis
6. Preparation of the manuscript

Preface

In the previous two chapters, we explored small molecule transport properties of cCNC-based microbeads. We demonstrated how to use pH to regulate small molecule uptake and release, selecting methylene blue as a model compound. In this chapter, we explore the mechanical properties of cCNC and cCNC-based microbeads.

Mechanical properties of microbeads like the elastic modulus in particular, are critical in applications like flow control, in drug delivery, as smart materials, and in cosmetics. Measurement of the elastic modulus of microbeads, especially those smaller than 5 μm , remains a challenge. In Chapter 1, section 3.2., we introduced mechanical properties of microbeads, including the definitions and principles. We then reviewed the predominant

methods to measure mechanical properties of microbeads. In this chapter, we show how atomic force microscopy (AFM) can be used as a reliable nanoindenter to measure force-displacement curves, provided the tip is equipped with microsphere colloidal probe. The elastic modulus of individual microbeads can be obtained reliably with this modification. We compare the benefits of this method to the use of conventional nano-sized AFM tips.

With the microsphere colloidal probe method, we show how small molecule crosslinking agents like citric acid can enhance the elastic modulus of microbeads. We then correlate the measured elasticity with spray-drying parameters, in particular, the concentration of cCNC suspensions. Chapter 5 describes how we use this method to measure the outcome of adjusting mechanical properties by incorporating silk fibroin into cCNC microbeads. We show how mechanical properties can be systematically adjusted by controlling methanol-induced β -pleated sheet conformational transitions in the guest silk fibroin-host cCNC hybrid microbeads.

Measuring Young's Modulus of Carboxylated Cellulose Nanocrystal (cCNC) Microbeads *via* Atomic Force Microscopy

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Young's modulus, cellulose nanocrystal, crosslink, AFM, nanoindentation, spray dry, microbeads, plastic microbeads replacement

Abstract

The mechanical properties of carboxylated cellulose nanocrystal (cCNC) microbeads made by spray-drying were measured by Atomic Force Microscopy (AFM). We introduce a microsphere colloidal probe method to determine the elastic modulus from force-displacement curves obtained by indenting individual microbeads. Use of a microsphere probe to measure the deformation of cCNC microbeads is shown to eliminate spurious contributions from localized mechanical responses that plague conventional cantilever probe tips. The colloidal probe method is shown to be especially beneficial to examine porous cellulose beads. Data were interpreted within the framework of the Hertz model. The cCNC microbeads show a Young's modulus of 18.02 MPa. We established that incorporating citric acid into the cCNC

matrix raises the elasticity of the microbeads to 27.97 MPa. An increase in the particle density of a cCNC suspension before spray-drying increases the elastic modulus to 24.55 MPa.

Introduction

Microbeads are commonly used as carriers for drug delivery,^{1, 2} in cosmetics and personal care industries,³ as catalysts,⁴ and as fillers for cement and concrete industries.⁵ The mechanical properties of microbeads play important roles in several aspects. For instance, knowledge of mechanical properties can be used for quality control when manufacturing the beads.⁶ Mechanical properties can be used to identify structure-function-property relationships in microparticles.^{7, 8}

In drug delivery systems, the elasticity of a microbead determines if it can survive the stress in the needle tract during the injection.⁹ Mechanical stability also controls the reusability of microbeads as catalysts for heterogeneous reactions. As concrete fillers, the elasticity of microbeads and the change of elasticity when immersed in water are the keys to the strength and stability of the concrete.¹⁰ In cosmetics and personal care industries, microbeads are extensively used to enhance sensory properties in formulations and to provide protection to, or amelioration of, the skin.¹¹ Microbeads impart a variety of benefits, including exfoliation, improved product blending, and improved skin feel. All of these rely on microbeads to be mechanically strong and stable.

Cellulose is the most abundant natural polymer in the world.¹² With its non-toxicity, biodegradability, and renewability, it is widely used as an absorbent for water treatment and as an excipient in the pharmaceutical industry. Recently, cellulose microbeads have emerged as viable candidates to replace existing plastic microbeads used in cosmetics.¹³ In our lab, we have developed a method to make cellulose microbeads from cCNC *via* spray-drying.

cCNC is a nanomaterial with a strong Young's moduli up to 100 GPa,¹⁴ and high surface functionality (abundant amount of hydroxyl and carboxylic acid groups forming stable agglomerates *via* hydrogen bonding and van der Waals interactions). cCNC has a potential for use in new types of cellulose microbeads. Measurement of their mechanical properties is important to understand their structure-function relationships and to design strategies for tailoring the mechanical properties to fit specific applications.

Mechanical property measurements include the measurements of elasticity, viscoelasticity, hardness, and fatigue limit. Among them, the Young's modulus measures the stiffness of materials. The modulus is defined as the ratio of stress along an axis to strain along the same axis in response to uniaxial loading.¹⁵ It can be used to measure the resistance of a microbead to elastic deformation under load; however, measurement of the Young's modulus of microbeads remains a challenge.

The force generated from traditional mechanical testing machines is too large to probe microbead deformation: for microbeads, a reasonable deformation requires the forces within the micro-newton range.¹⁶ Scott *et al.* used a compression test to measure the compressibility of non-crosslinked and crosslinked cellulose microbeads. Crosslinked cellulose microbeads show an increase in mechanical strength, but also an increase in brittleness.¹³ The Scott method measures the mechanical properties of loosely-packed powders. In this case, interactions among microbeads may compromise the measurement results. Several methods have been developed to measure single microbeads. Micromanipulation provides mechanical perturbation to small structures using microplates or probes connected to force transducers. The method enables the real-time observation of specimen deformation. But the set-up can be complicated.¹⁷ Micropipette aspiration,^{18, 19} and optical tweezer²⁰ have been used to measure the mechanical properties of micro-sized objects. But these are used mainly for cell

deformation measurement because the force range is often insufficient to induce any significant deformation in other types of microbeads. Ngan *et al.* proposed a microplate compression method to probe the viscoelastic mechanical properties of a hydrogel collagen microbead.¹⁶ Shimadzu Corporation has commercialized the micro-compression test machine to measure softness and elasticity.²¹

Nanoindentation is one approach to measure the mechanical properties of small spheres. Nanoindentation is usually performed with a nano-indenter or by AFM. AFM offers a quantitative, programmable, and repeatable method for microbead mechanical properties measurement over a wide range of particle sizes.²²⁻²⁵ But several concerns have been raised about the measurement of only local mechanical properties rather than bulk mechanical properties, since the indenter size is significantly smaller than the size of the microparticles.^{7,}

16

We have adapted a method to measure cellulose microbead stiffness by AFM. We used a colloidal microbead tip to enable a more accurate fitting of the Hertz model. This method provides more accurate Young's modulus when compared with the standard procedure, which is to use a conventional sharp tip. Section 3.2 below compares the two methods. In this chapter we measure how small molecule crosslinks affect the mechanical properties of cCNC microbeads. cCNC microbeads with porous internal structure and solid internal structure are also compared. Finally, we compare the elasticity of cCNC microbeads to commercial cellulose and other classes of microbeads. We show that AFM mechanical measurements are a promising approach to quantify the elastic response of soft microbeads.

Experimental Procedures

2.1. Materials

cCNC (5.3 wt-% suspension in water) and ChromaPur Neige® 2B cCNC microbeads were obtained from Anomera Inc., Montreal, Canada. ChromaPur Neige 2B is a product spray-dried from CNC >4 wt % water suspension (American Custom Drying, Burlington, New Jersey). Cellulose D-5 cellulose microbeads prepared by emulsion precipitation, were obtained from Kobo Products, Inc. Marsh Mallow Powder (Average size: 9.8 μm) was obtained from Sunjin Beauty Science (INCI (International Nomenclature of Cosmetic Ingredients) Name: Hexamethylene diisocyanate/Trimethylol Hexyllactone Crosspolymer).

2.2. Spray-drying

Spray-drying was carried out with a Büchi Mini Spray Dryer B-191 (Büchi Corporation, New Castle, USA) with inlet and outlet temperatures of 175 °C and 104 °C, respectively, 30% pump speed and 70% aspirator. cCNC (0.5 wt %) microbeads were made by spray-drying cCNC prediluted in water (0.5 wt %). 10% citric acid (CA) microbeads were made by spray-drying the aqueous cCNC suspension (0.5 wt %) mixed with 10% CA.

2.3. SEM

SEM was conducted using an FEI Quanta 450 Environmental Scanning Electron Microscope on samples with 3 nm platinum coating.

An FEI Helios Nanolab 660 DualBeam (Focused Ion Beam-Extreme High-Resolution Scanning Electron Microscope) was used for cCNC-based microbead cross-section imaging. The samples were mounted on an aluminum SEM stub and coated with a 4 nm layer of Pt to

enhance electrical conductivity. Milling of the sample was carried out with an FEI Helios Nanolab 660 DualBeam instrument (Thermo Fisher Scientific, Hillsboro, OR USA). After selecting a specific particle, a 2 micron thick of protective platinum stripe was deposited on the surface of the region of interest to protect the surface from ion beam damage. Microbead cross-sections were then prepared by gallium ion milling. A primary trench to clean the sample was milled at 30 kV ion beam accelerating voltage and 21 nA beam current. Following cleaning, cross-sections were obtained with beam current ranging between 9.3 nA to 0.79 nA. The smooth cross-section was imaged with an electron beam at an accelerating voltage of 2 kV, a beam current of 0.40 nA, and a working distance of 4 mm in the secondary electron mode.

2.4. Sample Preparation

cCNC (0.5%), 10% CA, marshmallow powder, ChromaPur Neige 2B were dispersed individually in water, and the mixture was cast on a freshly cleaved mica substrate and dried at ambient conditions overnight before conducting AFM nanoindentation.

2.5. Calibration of the AFM Cantilever Spring Constant k

The spring constant of the cantilever was measured following the procedure of thermal tune computation.²⁶ A force curve is first performed on a glass substrate, and the slope of the approaching curve is measured to determine the sensitivity of the cantilever (The definition and detailed explanation of force curves is introduced in Chapter 1, Figure 3.2.3.). Then the AFM tip was taken far from the surface so the cantilever oscillation was only contributed by the thermal oscillation. In this way, the power spectra was collected. Other noise sources contribute to the background to this thermal response, which is able to be subtracted since they are not likely to have a resonance at the resonant frequency of the cantilever. The power spectra density of the fluctuations in the spring displacement was fit to Lorentzian model, where the

resonance frequency can be extracted. The calculation of the spring constant follows Equation (1).

$$k = k_B T / P \quad (1)$$

Where P is the area of the power spectrum of the thermal fluctuations alone, T is the Temperature, k_B is Boltzmann's constant.

2.6. Mechanical Properties Measurement by AFM

The mechanical property analysis was conducted in air under ambient conditions with a commercial AFM MFP - 3D - BIO AFM instrument (Asylum Research, Santa Barbara CA). FMV-A cantilevers (Bruker Nano Inc.) with an average spring constant of $k = 2.8$ N/m and a tip radius of 8 nm were used for force measurement and microbead surface morphology imaging (coated with reflective Aluminium on the backside). Biosphere B500-FM cantilevers (Nanotools USA LLC, Henderson, NV) with an average spring constant of $k = 2.8$ N/m and a tip radius of 500 nm were used for force measurement. Topographic images were acquired firstly to identify the center positions of the microbeads and measure the sizes of individual microbeads. To investigate the mechanical properties of individual microbeads at the right location, the point and shoot view was used. After scanning an area with individual microbeads presented in the area, around 100 microbeads were selected from the topographic image. A crosshair was used to select the specific point (the center of a microbead) for force curve collection. The mechanical properties of the microbeads were obtained by analyzing the force-distance curves. For force measurement, a contact mode was used with a trigger point of 2 V, force distance of 1 μ m, scan rate of 0.99 Hz, and velocity of 1.98 μ m/s. For the topographic images, the AC mode was conducted with 20 μ m scan size, 0.8 Hz scan rate. We excluded force curves in those curves where the Young's modulus was as high as the substrate. In this

case, the force curve was conducted on the substrate rather than on the microbead surface. We also excluded force curves with extremely low stiffness. This happens when the probe is not indenting on the microbeads, but creating a lateral motion rather than a sphere deformation. Samples of microbeads were provided from a batch of cosmetic grade material by Anomera, Inc.

2.7. Elastic Modulus Modelling

Three theoretical models are commonly used to model tip-sphere contact: the Hertzian elastic contact theory,^{22, 23, 25} the Johnson-Kendall-Roberts (JKR) model,^{23, 25, 27-29} and the Derjagui-Muller-Toporov (DMT) model.³⁰ The Hertzian elastic model is the most widely used method. It describes the perfect elastic deformation between two spherical bodies in contact, without considering the adhesion force. JKR model and DMT model describe an elastic adhesive contact. They are used mainly when adhesion plays a role in the elastic response. The mathematical models are shown in Table 4-1.

Table 4-1 Mathematical Models for Hertz, DMT and JKR for Calculation of Young's Modulus Using Atomic Force Microscopy

	Mathematical model	F_{adh}
Hertz Model	$F_{sphere} = \frac{4}{3} \frac{E}{1 - \nu^2} \sqrt{R} \delta^{\frac{3}{2}}$ $E = \frac{3}{4} \left(\frac{\Delta(F_{sphere})^{2/3}}{\Delta\delta} \right)^{3/2} \frac{1 - \nu^2}{\sqrt{R}}$	0
DMT (Derjagui, Muller and Toporov)	$F_{sphere} - F_{adh} = \frac{4}{3} \frac{E}{1 - \nu^2} \sqrt{R} \delta^{\frac{3}{2}}$ $E = \frac{3}{4} \left(\frac{\Delta(F_{sphere} - F_{adh})^{2/3}}{\Delta\delta} \right)^{3/2} \frac{1 - \nu^2}{\sqrt{R}}$	$2\pi RW$
JFK (Johnson, Kendall and Roberts)	$F' = F_{sphere} + 3W\pi R + \sqrt{6WRF_{sphere} + (3W\pi R)^2}$ $E = \frac{3F'(1 - \nu^2)}{4\sqrt{R}\delta^{3/2}} \left[1 - \frac{2}{3} \left(\frac{6\pi RW}{F'} \right)^{1/2} \right]^{3/2}$	$\frac{3\pi RW}{2}$

Where, F_{sphere} is the force applied to the sample surface, R is the generalized radius, δ is indentation depth, E is Young's Modulus (elastic modulus), ν is the Poisson ratio of the sample, and W is the adhesion energy.

The calculation of Young's modulus follows the Hertz Model equation in Table 4-1. We used the Poisson's ratio ν of bulk cellulose (0.38) for the calculation.³¹ The AFM silicon tip has an elastic modulus of 130 - 160 GPa and Poisson's ratio of 0.27. The tip deformation is negligible due to the significantly higher stiffness of the tip compared with cellulose materials.³²

2.8. Data Processing

Force-displacement curves were converted into the force-indentation curves to quantify the elastic modulus. This was accomplished with AFM MFP-3D-BIO AFM instrument software. MATLAB code (in supporting information) was written to automatically calculate elasticity and fitting parameters based on the Hertz Model, using a series of force curves of the retracing part (up to 100 curves) collected using the method described in 2.6.

Results and discussion

3.1. The Contact Model for Young's Modulus

We first calibrated the microsphere colloidal probe method with polystyrene microbeads attached to the cantilever tip. The calculated Young's modulus is within the range of values reported in the literature (Figure 4-S1).

We justify our use of the Hertzian model as follows: Figure 4-1 (a) presents a force-indentation curve for cCNC microbeads. The average indentation depth is inside 25 nm. This agrees with the literature consensus that the indentation depth should fall within 10% of the total depth so that the substrate does not influence the deformation measurement.³³ The adhesion force can play a crucial role in the elastic response of microbead mechanical property measurements, especially at low indentation depth. Sarrazin *et al.* have shown that the adhesion force influences the measured Young's modulus for indentations < 2 - 5 nm. Increasing the indentation depth to more than 5 nm ensures that elastic bulk effects dominate, and the adhesion force can be interpreted as negligible.²³ For these reasons we are justified in choosing the Hertzian model, which depends on a linear fit to the indentation depth expressed as $\delta^{3/2}$.³⁴ As shown in Figure 4-1 (b), $\delta^{3/2}$ is linear with respect to force (coefficient of determination of

0.997), justifying our choice of the Hertz model. Comparing the loading and unloading curves, we observe a “holding phase” starting at 17 nm indentation depth, which may indicate that the CNC microbeads are somewhat viscoelastic. If this is the case, the Young’s modulus will depend on the loading rate in AFM settings. In our experiment, we applied the same loading rate to make sure Young’s moduli from different samples were comparable. Future work will explore the viscoelastic response of the CNC microbeads, especially after moisture uptake.

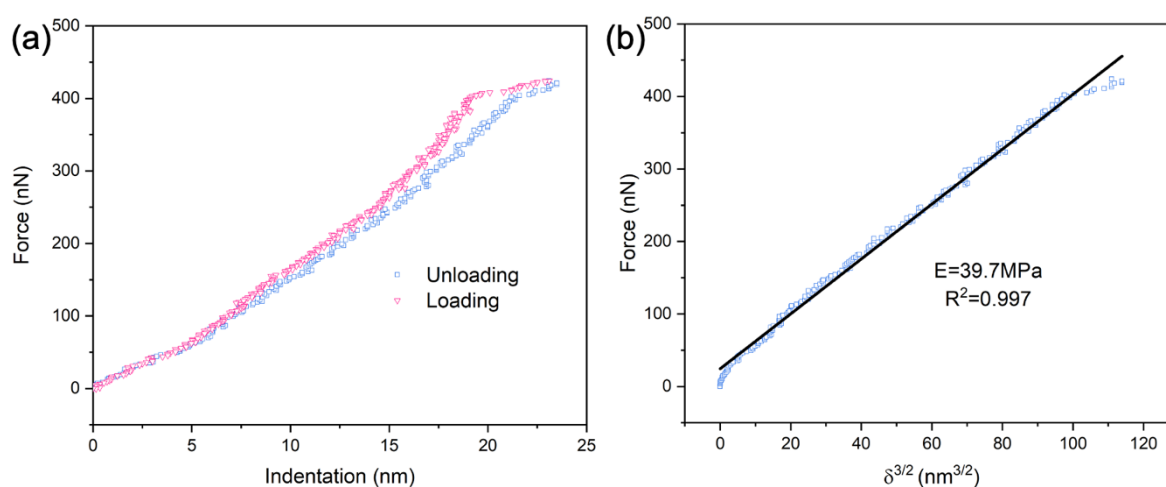


Figure 4-1 Hertz model to determine the Young’s modulus of cCNC microbeads using a nanoprobe. (a) One representation of force-indentation curve for cCNC microbeads using the FMV-A cantilevers (Bruker Nano Inc.). (b) The fitting of hertz model to determine the Young’s modulus.

We deposited cCNC microbeads on a freshly-cleaved mica (phyllosilicate) substrate to measure mechanical properties. The mica stabilizes microbeads through van der Waals and hydrogen bonding interactions with the substrate. A topographical scan was repeated in the measurement area to ensure the particles remain at the same spot after indentation (Figure 4-S2). With the mica substrate, the microbeads indeed remained fixed after indentation. No damage to the microbeads, such as a surface depression, was detected after indentation,

indicating no viscoplastic response. This is important since plastic deformation of particles is inconsistent with assumptions in the Hertz model.

3.2. Comparison of AFM Indentation on cCNC Microbeads using AFM

Nanoprobes and Microsphere Probes.

We first measured the Young's modulus by conventional AFM in order to compare our findings with those obtained by the colloidal probe method. Figure 4-2 (a) shows the results from a conventional tip, a nano-scale probe. With the method described in the experimental section, we extracted the force curves by indenting more than 100 microbeads. After fitting the force curves to the Hertz model, the Young's modulus was calculated. Figure 4-2 (c) shows the results accumulated from 50 force curves after rejecting the spurious curves. With a conventional tip, the Young's moduli vary widely, from 20 MPa to 2 GPa (Figure 4-2 (c)).

AFM nanoindentation is well-known to measure local mechanical properties. With nano-scale probes, a nano-sized region is indented to obtain the Young's modulus. But measurement of local mechanical properties with a sharp tip does not represent the mechanical deformation of a microbead as a whole. As shown in Figure 4-2 (a), when approaching the surface of a cCNC microbead with an AFM tip of 8 nm radius of curvature, the force curves generated are location-dependent due to the porous structure of cCNC microbeads. For example, location 1 in Figure 4-3 (a) probes cCNC crystals, giving a significantly higher value of elasticity. Location 2 probes the gaps between the crystals, showing a relatively low elasticity. This results in a broad range of elasticity outcomes, as shown in Figure 4-2 (c). This makes AFM indentation for porous microbeads unreliable because one cannot predict where the tip lands on a microbead surface.

A second drawback when using a nano-radius tip is damage to the probe that changes its radius. In Figure 4-2 (a), we imaged the tip radius of nano-scale probes before and after nanoindentation. The radius R of the tip increased from 8 nm to ~ 20 nm. This change is significant when calculating the Young's modulus, as the modulus depends on $\sqrt{R}$. The change in tip size produces significant error in determining the modulus. Guo *et al.*²⁵ have studied tip wear and its effect on measured Young's modulus values. They concluded that a tip with a smaller radius of curvature is more likely to induce location offset on the substrate, giving rise to tip wear.³⁵ Therefore, a larger radius of curvature, like a colloidal sphere, is recommended for mechanical measurement.

Thirdly, the Hertz model describes elastic deformation between two perfect spheres. It is most accurate when the two spheres have the same radius. Using a nano-sized probe gives less accurate Hertz fitting because of the significant difference in radius between the radius of AFM tip and cCNC microbeads.³⁶

When indenting microbeads with micro-sized colloidal probes, we overcome the limitations of nano-sized tips described above. Figure 4-2 (d) shows the Young's modulus measured using this colloidal probe tip. The tip yields a good Gaussian distribution of elasticity results with decreased measurement errors. The average Young's modulus is 18.02 MPa with standard deviation of 1.13 MPa. This method is not only able to provide average mechanical properties, but the ability to measure the elasticity of each particle means it also yields the deviation from the mean of mechanical properties. This is significant because most current indentation methods fail to measure the Young's modulus of single particles, especially for microparticles that are $\sim 1\text{-}2\text{ }\mu\text{m}$ in size. Philippe *et al.*³⁷ compared their results with colloidal probe with literature data and concluded that colloidal probe indentation gives a Young's modulus lower than that measured using sharp tips. Our results align with their conclusion. The results from

colloidal probes are likely to be more accurate. Since the radius of the nano-sized tip is two orders of magnitude smaller than a colloidal probe, the pressure on a microbead from a nano-scale tip is much higher. The nano-scale tip can also cause local strain hardening,³⁷ making the Young's modulus results less accurate.

Figure 4-3 (a) shows the phase image of a cCNC microbead surface. The surface is porous like a structured foam. The texture is likely the result of the rapid drying and water loss from the aqueous microdroplet during spray-drying. The thickness of the ridges is on the order of the width of 1 to several cCNC rods that are overlapped end to end to yield a fibrous network. The pores have an average value of ~50 nm. Microbeads produced from 0.5 wt % cCNC are ~ 2 μm in diameter, hence, a 500 nm microsphere colloidal probe is appropriate for mechanical measurement. We used a 500 nm radius Si probe from Nanotools for the measurement. The probe was fabricated from a silicon microsphere that was eroded to about 1000 nm in diameter electron beam. A probe of this size reduces tip wear and the potential of microsphere tip damage during indentation.³⁸

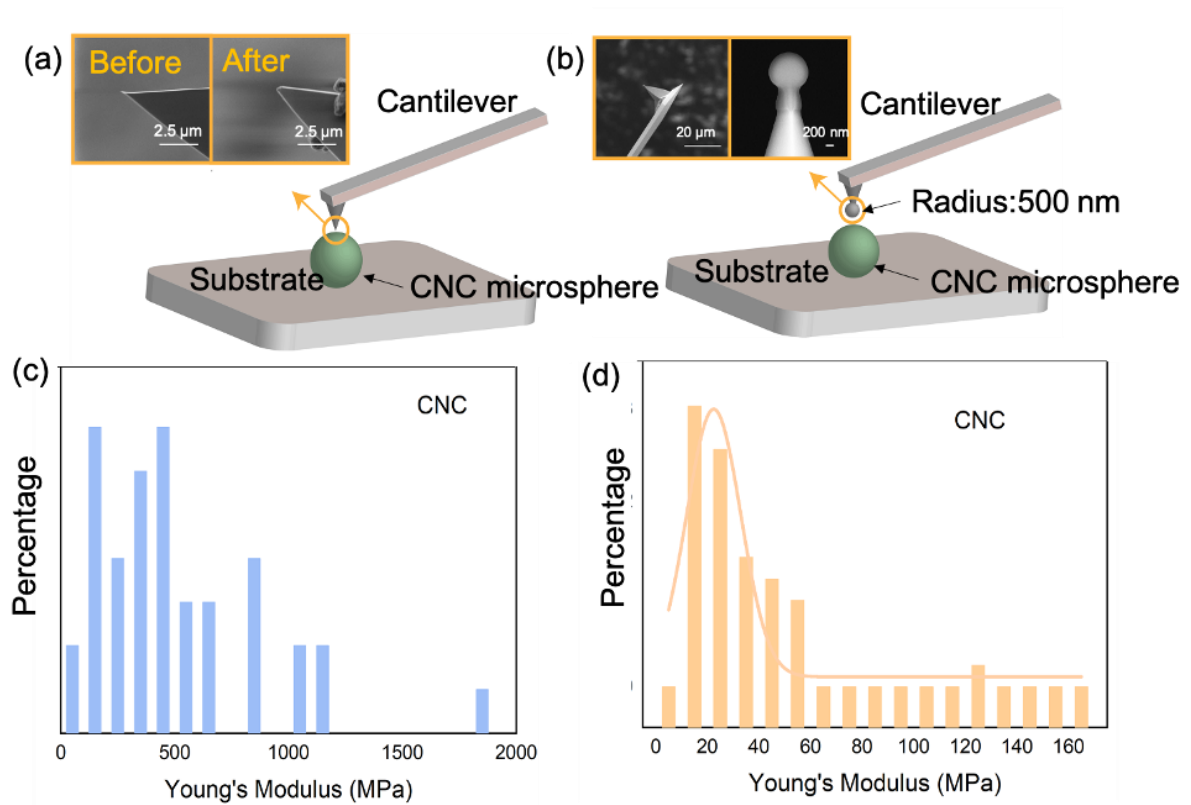


Figure 4-2 Comparison AFM indentation with a conventional nano-scale tip and micro-sized colloidal tip. (a) AFM nanoindentation set-up illustration and SEM images of tip used for indentation before and after. (b) AFM indentation set-up illustration with micro-sized tip. (c) Young's modulus of cCNC microbeads calculated from the Hertz model for a nano-scale tip. (N=96) (d) Young's modulus of cCNC microbeads calculated from the Hertz model for a microsphere probe. N=40 (N is the number of sample size)

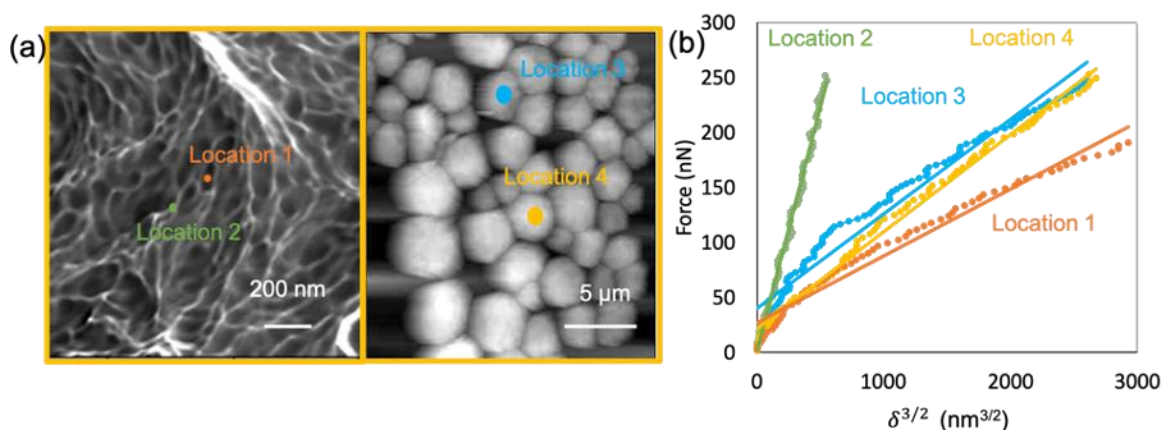


Figure 4-3 Comparison of AFM indentation using conventional nano-sized tips and microbead colloidal tips. (a) AFM phase image of cCNC microbead surface porous structure with two locations identified for force curve measurement (image on the left) and AFM topographic scanning image of cCNC microbeads with two microbeads identified for two force curves measured (image on the right). (b) Plots of force versus $\delta^{3/2}$ for the locations identified in Figure 4-2 (a). δ is the indentation depth.

3.3. Enhanced Young's Modulus from Citric Acid Crosslinked cCNC Microbeads

With the colloidal microsphere method, we explored the effect crosslinking among cCNC particles in microbeads and its impact on the elastic modulus. As described in experimental section, we first scanned to produce a topographic AFM images of both cCNC microbeads and 10% CA microbeads to identify the positions of the microbeads. The outcomes are shown in Figure 4-4 (a) and Figure 4-4 (b). The red “X” marks the targeted indentation positions. From the topographic image, cCNC microbeads and 10% CA crosslinked microbeads show similar shapes and particle sizes. The force curves were collected from which the Young's moduli were calculated. The accumulated results are plotted in Figure 4-4 (c), where it is evident that citric acid increases modulus to 27.97 MPa compared with that of 18.02 MPa for undoped cCNC. The increase in the modulus is 55% with a loading of 10% CA. For comparison, Reddy

et al. reported that citric acid crosslinked starch film exhibited a 150% increase in tensile strength compared with non-crosslinked films.³⁹ Similarly, Shao *et al.* showed that the tensile strength of citric acid crosslinked hemicellulose-based films was increased by 67% tensile on reacting with 20% CA.⁴⁰ Arne *et al.* used citric acid to crosslink nanocellulose to develop robust paper filters.⁴¹ In our case, the vibrational spectra reported in the supplementary sections, SI Figure S4-4 and Figure S4-5 prove that crosslinking occurs between CA and the cCNC microbeads. The ester crosslink is illustrated in Figure 4-4 (d). The implications of the findings are significant. They demonstrate a) that ester bond formation can take place in one step in the gas phase in the micro environment of an atomized water droplet of CA and cCNC particles; (b) that the process does not require ancillary agents like anhydrides; (c) that the chemical outcome increases the Young's modulus of the microbead; (d) that a hybrid microbead can be made that comprises "naturally sourced" components; and (e) that the process uses scalable spray-drying. In the following section, we turn to a qualitative interpretation of how some spray-drying parameters determine particle morphology and other features of cCNC powders.

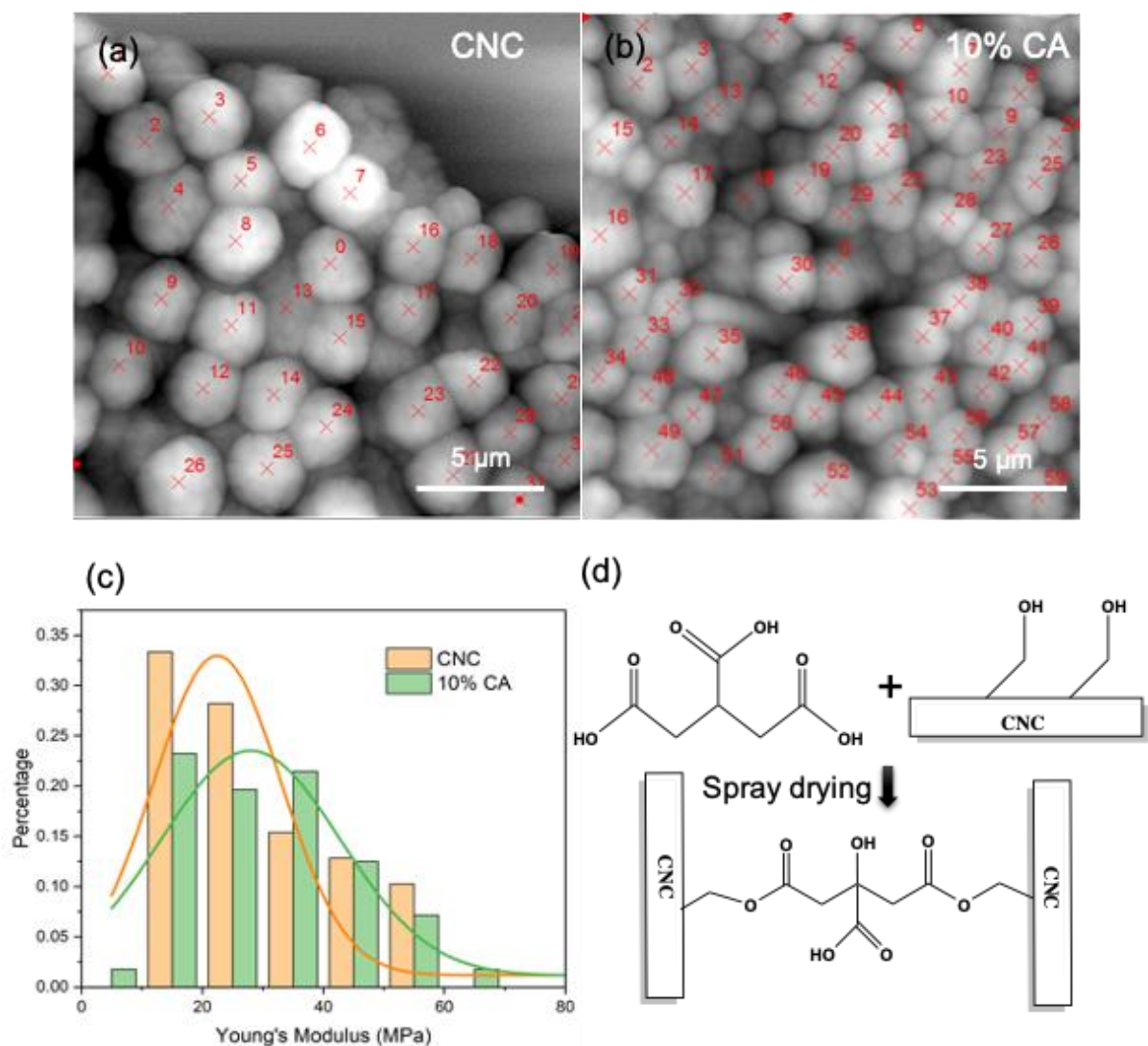


Figure 4-4 Comparison of Young's modulus of cCNC microbeads and 10% citric acid crosslinked cCNC microbeads. (a) AFM topographic images of cCNC microbeads with red cross marks to identify the center of the indentation. (b) AFM topographic images of 10% CA crosslinked microbeads with red cross marks to identify the center of the indentation. (c) Young's modulus measured for cCNC microbeads (N=40) and 10% CA microbeads (N=54). (d) Illustration of chemical crosslinking between cCNC and citric acid by esterification.

3.4. Mechanical Properties of Porous cCNC Microbeads and Dense cCNC

Microbeads

In our hands, spray-drying is a one-step, water-based method to make cCNC microbeads. Spray-drying involves multiple parameters that can influence the properties of the microparticles.⁴² Some of these parameters were discussed in Chapter 1. For example, the viscosity of the suspension determines particle size and size distribution. The inlet and outlet temperatures affect the morphology of the particles. In our lab, we have found that the concentration of cCNC feed suspension correlates with particle sphericity and particle density. Figure 4-5 (a) and Figure 4-5 (b) are AFM tapping mode images of the particle morphologies of cCNC microbeads prepared from 0.5 wt % suspensions and a commercial sample of cCNC microbeads prepared from 4.5 wt % suspensions (ChromaPure Neige 2B). The microbeads were produced from a similar type of two-fluid nozzle, and the spray-drying inlet and outlet temperatures are similar. Only the throughput differs, since the commercial sample was prepared from a large chamber, high throughput dryer. Nevertheless, microbeads from both suspensions are roughly spherical. But shape does not tell the whole story. The FIB cross-sections of Figure 4-5 (c) and Figure 4-5 (d) for beads from the two concentrations of suspensions reveal entirely different internal structure. cCNC microbeads from the 0.5 wt % suspension have a porous internal core and a densely packed shell structure, while microbeads from 4.5 wt % suspensions are dense. It seems that spray-drying a higher wt % cCNC suspension produces cCNC microbeads with higher density. In Chapter 1, we calculated the Péclet number of cCNC to be about 10^{-3} based on a drying time of 6 s, particle radius of 180 nm, and diffusion coefficient $D \sim 0.542 \times 10^{-11} \text{ m}^2/\text{s}$. The Péclet number here is misleading because it assumes that the cCNC particle are non-interacting, when in fact they gel rapidly as water evaporates, meaning that the diffusion coefficient increases rapidly because it is not that of an isolated cCNC particle. The effect is to cause a rapid increase in the Péclet number. A high

Péclet number promotes spherical shell formation. We theorize that for the 4.5 wt % cCNC suspension, the microbeads are densely packed because the initial Péclet number is already higher to start with. According to Equation (1) in Chapter 1, the spray-drying time plays a major role for determining the density of the particles. With higher concentration of cCNC suspensions, the drying time increases, leading to a longer time for nanocrystal precipitation during the water loss phase. Pre-aggregation of the particles in the higher wt % cCNC suspension also contributes to the increase in density of the spray-dried particles.

By colloidal probe AFM nanoindentation the Young's modulus of ChromaPure Neige 2B is 24.55 MPa, compared with a value of 18.02 MPa for microbeads produced from 0.5 wt % suspension. The results are shown in Figure 4-5 (e) and (f). As expected, densely packed cCNC microbeads have a higher Young's modulus than porous particles when other parameters are controlled.

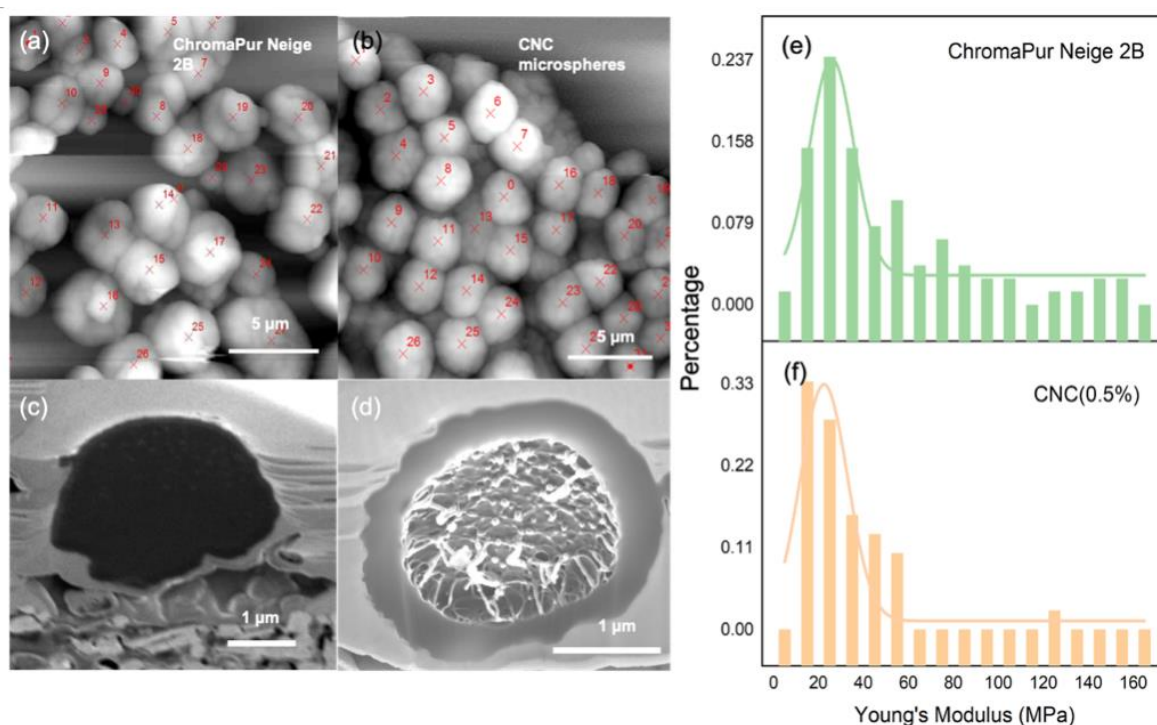


Figure 4-5 Dependence of Young's modulus on cCNC suspension concentration for spray-drying. (a) ChromaPur Neige 2B AFM AC mode topographic image. (b) cCNC microbeads from 0.5% slurry AFM AC mode topographic image. (c) FIB-SEM image of cross-section of a ChromaPur Neige 2B. (d) FIB-SEM image of cross-section of a cCNC microbead. (e)-(f) Histogram distribution for Young's modulus of cCNC (N=40) microbeads and ChromaPur Neige 2B (N=75) microbeads.

3.5. Comparison with Commercial Beads Made from Soft Materials

We compared the elasticity of cCNC microbeads with available biodegradable microbeads. cCNC microbeads have the highest Young's modulus at 18.02 MPa. This value contrasts with the much lower moduli for collagen microbeads (132 Pa)⁴³, hyaluronic acid microbeads (2 kPa),⁴⁴ alginate microbeads (0.012 MPa),⁴⁵ dextran microbeads (4.5 MPa)⁴⁶ and pectin microbeads (< 6 MPa).⁴⁷ This comparison was based on the assumption that all the beads are

measured at ambient conditions. We expect the Young's modulus of cCNC microbeads decreases with higher water content.

Several commercial beads are available to replace microplastic beads used in cosmetics. Examples are the Marsh Mallow powder from Sunjin Beauty Science and Cellulose D-5 from Kobo Products Inc. We now compare the Young's modulus (elasticity) of our cCNC microbeads with the commercially available ones. Figure 4-6 shows the Young's modulus of cCNC 0.5 wt %, ChromaPure Neige 2B, 10% CA, cellulose D-5 and Marsh Mallow powders. cCNC-based microbeads have the lowest values of Young's modulus. Of course, measures of the Young's modulus tell only part of the story when it comes to the properties of microbeads. We have shown elsewhere in this thesis that "smart" cCNC-based microbeads can be produced and suggested that the use-value of cCNC microbeads lies in adjusting their physical and chemical properties according to the role that the beads are required to play. In Chapter 5, we introduce a new type of microbead made by incorporating silk fibroin (SF) into the cCNC matrix. We show how increasing the amount of SF and polar solvent treatments to regulate SF conformational transitions can modify the Young's modulus.

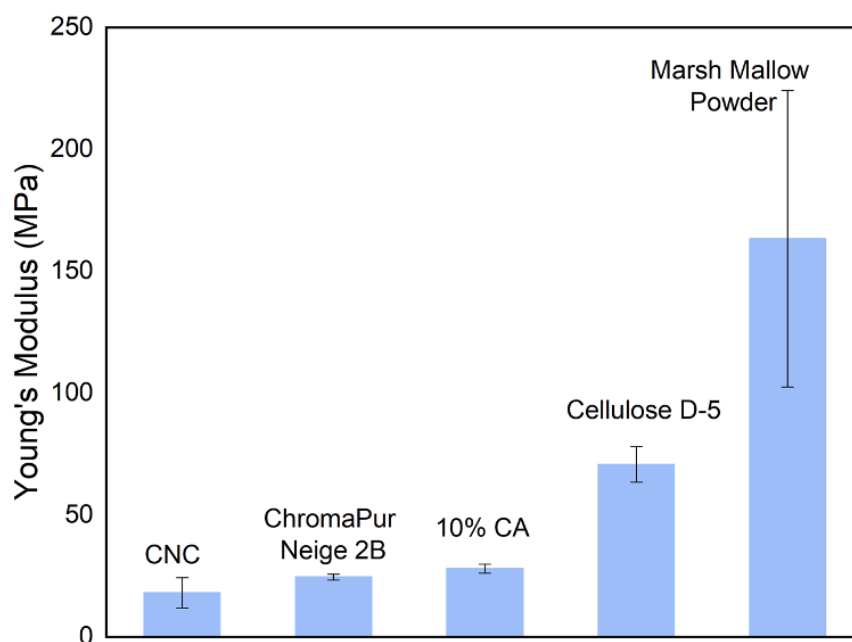


Figure 4-6 Young's Modulus of available commercial beads Cellulose D-5 and Marsh Mallow Powder comparison with cCNC-based microbeads. ($N_{\text{cellulose D-5}} = 23$, $N_{\text{marsh mallow powder}} = 42$)

Conclusions

In conclusion, we have introduced a AFM colloidal probe nanoindentation method that has been shown capable of measuring the elasticity (Young's modulus) of individual cCNC-based microbeads. The method is an improvement over conventional AFM nanoindentation. The Hertz model was shown to yield reliable data over samples of many individual microbeads. While neat cCNC microbeads show a Young's modulus of ~18.02 MPa, the modulus was shown to depend on factors like chemical crosslinking with citric acid and spray-drying parameters. Gas phase esterification opens a new pathway to making naturally sourced microbeads with a range of mechanical properties.

Supplementary

This supplementary section contains information and experiments in support of the text for Chapter 4.

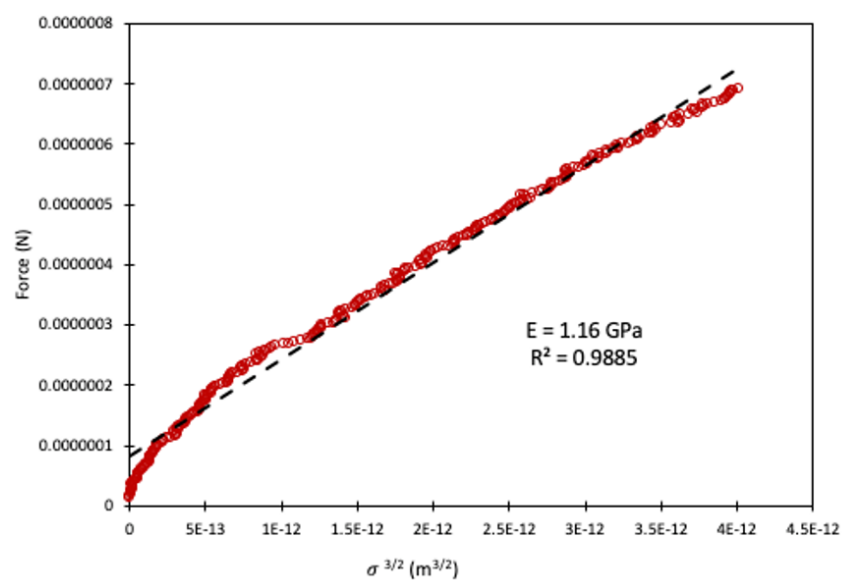


Figure S 4-1 Young's Modulus of polystyrene microbeads.

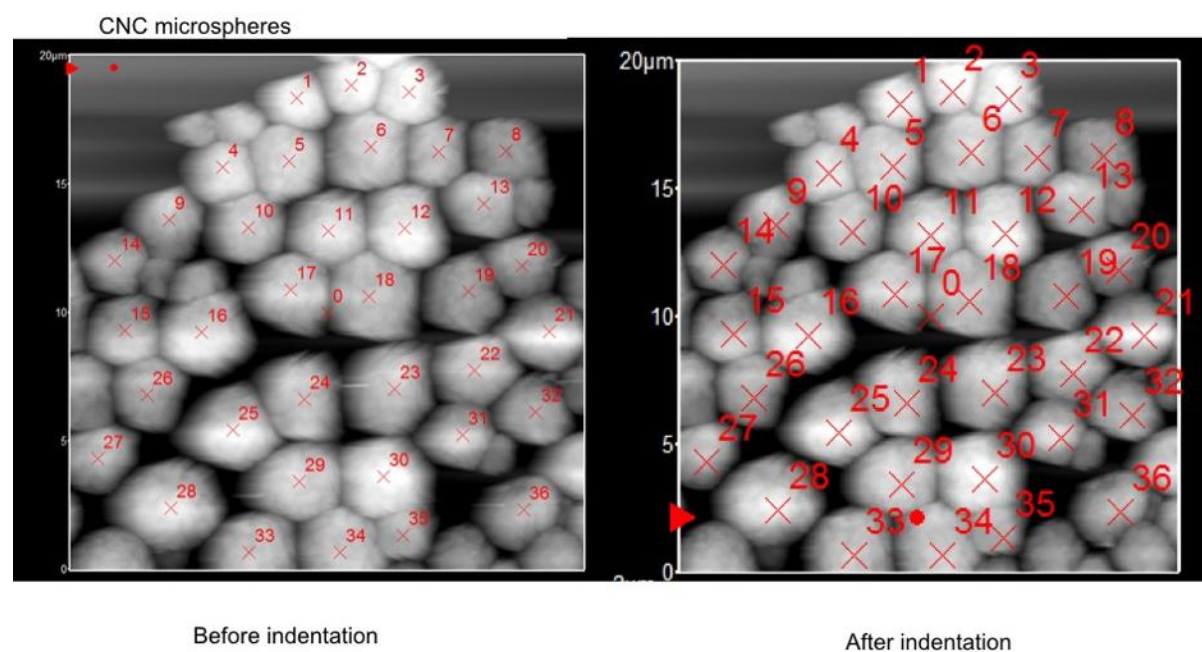


Figure S 4-2 cCNC microbead AFM topographic image before and after indentation.

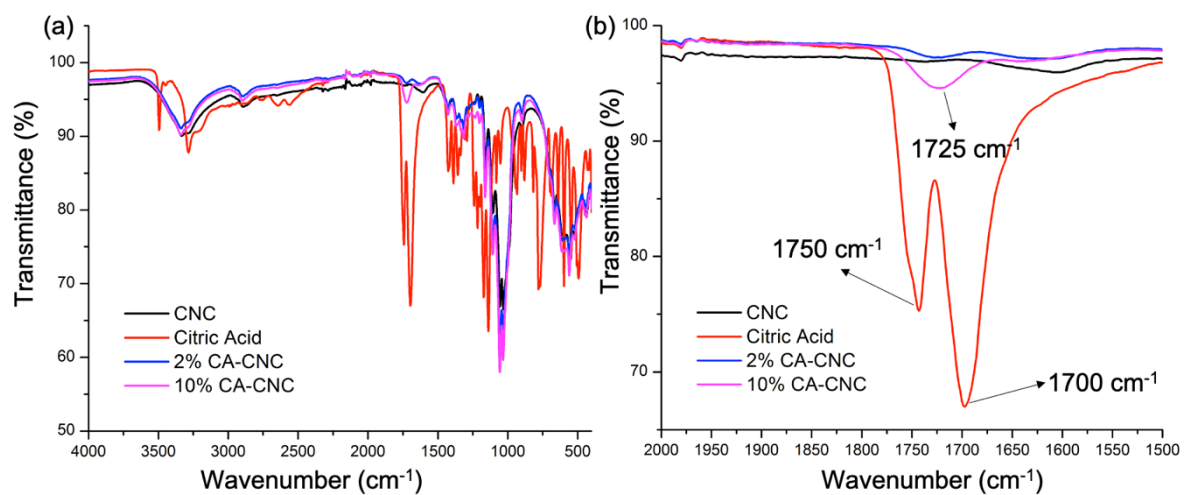


Figure S 4-3 FT-IR spectra comparing cCNC microbeads, cCNC microbeads doped with 2% citric acid, cCNC microbeads doped with 10% citric acid, and citric acid.

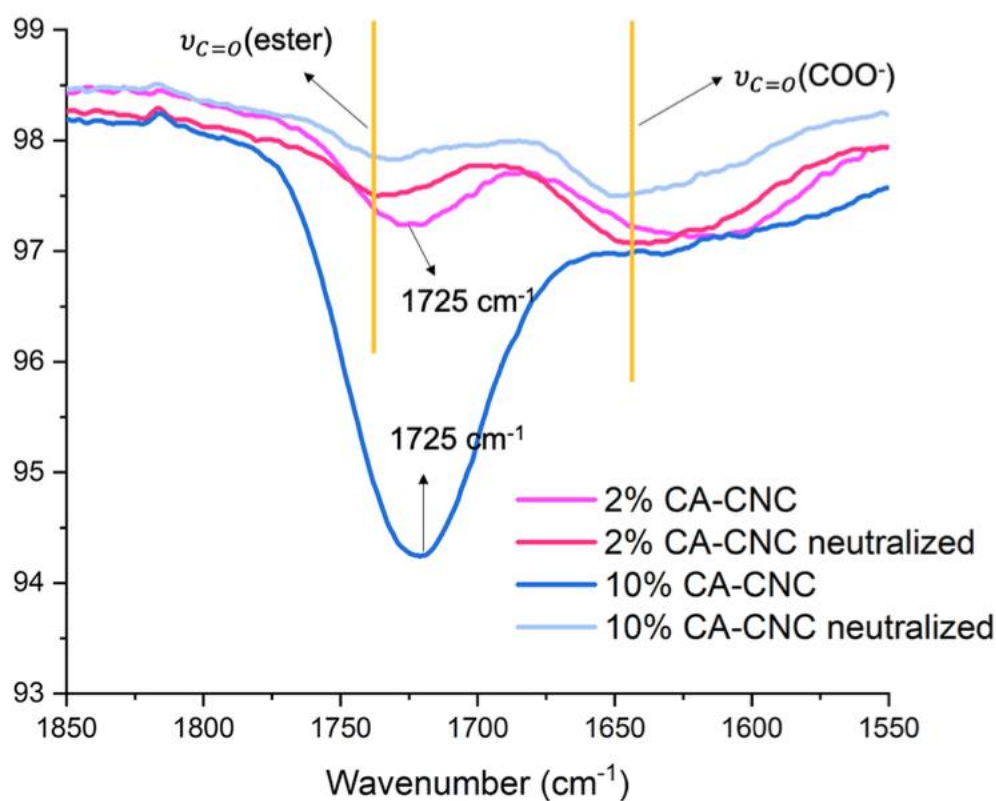


Figure S 4-4 FT-IR spectrum showing the ester bond formation ($\sim 1740\text{ cm}^{-1}$) for 2% and 10% CA-cCNC microbeads after immersing the microbeads at basic solution to shift carboxylic acid $\nu_{C=O}$ stretch from $\sim 1700\text{ cm}^{-1}$ to sodium carboxylate at $\sim 1650\text{ cm}^{-1}$.

MATLAB code for the Hertz Model fitting

```
clc;
close all;
clear all;

%separate x and y, create a=x^1.5,x is indentation, y is force

load('plot.mat');
hasMatch=~cellfun('isempty', regexp(plot.Properties.VariableNames,
'Ind_Ret','once'));
x=plot(:, plot.Properties.VariableNames (hasMatch));

hasMatch=~cellfun('isempty', regexp(plot.Properties.VariableNames,
'Force_Ret','once'));
y=plot(:, plot.Properties.VariableNames (hasMatch));
x=x{:, :};
```

```

y=y{:, :};
a=x.^1.5;

%create a loop for xdata and ydata higher than 0, i equals to the number of
force curves

for i=1:27

    y1{i}=y(:, i);

    a1{i}=a(:, i);

    a2{i} = a1{i}(a1{i}>0);

    y2{i}= y1{i}(a1{i}>0);

end

%create a loop for polyfitZero

for i=1:27

    [p{i}, S{i}, mu{i}]=polyfitZero(a2{i}, y2{i}, 1);

    [y_fit{i}, delta{i}] = polyval(p{i}, a2{i}, S{i}, mu{i});

    k{i}=y_fit{i}./a2{i};

    Rsq{i}=1-sum((y2{i}-y_fit{i}).^2)/sum((y2{i}-mean(y2{i})).^2);

end

%extract k data from cells, M is the slope of the linear fitting

k2=cellfun(@(x) reshape(x, [], 1), k, 'un', 0);

M=padcat (k2{:})

```

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| 5 |

**Tuning the Physical Properties of Carboxylated
Cellulose Nanocrystal Microbeads by Hybridizing with
Silk Fibroin**

Chapter 5 Tuning the Physical Properties of Carboxylated Cellulose Nanocrystal Microbeads by Hybridizing with Silk Fibroin

This chapter includes one manuscript that is ready to submit, and one filed patent.

Patent “Proteinaceous Cellulose Microparticles”, Mark Andrews, Timothy Morse, Monika Rak Junqi Wu, Zhen Hu and Mary Bateman, US provisional application No. 62/846, 281

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1. Experimental design and implementation
2. Data collection and analysis
3. Preparation of the manuscript

Preface

Chapter 5 describes how spray-drying can be used to incorporate silk fibroin protein (SF) into carboxylated cellulose nanocrystal (cCNC) microbeads. Silk fibroin is known to undergo a β -sheet transition when amorphous SF is exposed to a polar solvent. The β -sheet transition is accompanied by crystallization and stiffening of SF protein aggregates. Spray-drying SF kinetically traps a portion of the protein in the amorphous state when it is combined with cCNC. Post exposure of the hybrid SF-cCNC microbeads to methanol induces the β -sheet phase transition to the Silk II polymorph, the progress of which is followed by monitoring

characteristic amide bands by infrared spectroscopy. The phase transition is used to adjust the stiffness of the microbeads. By microsphere colloidal probe nanoindentation atomic force microscopy, the hybrid microbeads were found to become stiffer below 20 % incorporation of SF. The ratio of SF to cCNC at fixed spray-drying conditions leads to significant changes in the morphology of the particles. These changes are briefly discussed in relation to the empirical Péclet number that distinguishes regimes of mass transport within an evaporating droplet. Depth profiling of the SF-cCNC microbeads by X-ray photoelectron spectroscopy reveals that SF is more concentrated at the microbead surface. Surface segregation has significant impact on both the surface energy of the microparticles and diffusive transport of methylene blue (MB) probe molecules. When methanol is used to induce the β -sheet phase transition, microparticles containing 2% SF become hydrophobic and penetration of MB is hindered, meaning that not only the hydrophobic surface, but also the crystallized protein are barriers to mass transport. We show that hydrophobic SF-cCNC microbeads do not destabilize water-in-oil emulsions, making them potentially viable alternatives to classical surface functionalized microbeads.

Tuning the Physical Properties of Carboxylated Cellulose Nanocrystal Microbeads by Hybridizing with Silk Fibroin

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silk fibroin, cellulose nanocrystal, hybridization, microbeads, hydrophobic microbeads, plastic microbeads replacement

Abstract

Cellulose-based microbeads derived from cellulosic polymers like regenerated cellulose, carboxymethyl cellulose and others, have been widely explored for use as drug delivery vehicles, excipients, cosmetic ingredients, chromatographic supports, aids for water purification and other applications. The versatility of the cellulose microbead is linked both to a rather mature carbohydrate functional group transformation chemistry and to methods developed to form microbeads from solubilized cellulosic polymers. In this paper, we show how a class of hydrophobic/lipophilic cellulose microbeads can be made without covalent bond chemistry by combining carboxylated cellulose nanocrystals (cCNC) together with silk fibroin (SF) protein in the course of spray-drying from aqueous suspension. Spray-drying SF kinetically traps a portion of the protein in the amorphous state when combined with cCNC. Post exposure of the SF-cCNC hybrid microbeads to methanol induces a β -sheet phase transition to the Silk II structure, the progress of which is followed by monitoring the vibrational modes of characteristic amide bands. The Silk II phase transition is used to adjust

the mechanical properties of the microbeads. By colloidal microsphere nanoindentation atomic force microscopy, the hybrid microbeads are found to become stiffer below 20% incorporation of SF. Ar⁺ depth profiling of the SF-cCNC microbeads coupled with N 1s X-ray photoelectron spectroscopy reveals that SF is distributed throughout the hybrid cCNC, but is more concentrated at the surface. Surface localization has significant impact on both the surface energy of the microparticles and diffusive transport of water soluble ions like methylene blue (MB) probe molecules. When methanol is used to induce the β -sheet transition, microparticles containing 2% SF become hydrophobic and penetration of MB is hindered, meaning that not only the hydrophobic surface, but also the crystallized protein are barriers to mass transport. We show that the ratio of SF to cCNC, under fixed spray-drying conditions, leads to significant changes in the morphology of the particles. We relate these changes to the empirical and dimensionless Péclet number. We discuss the implications of our findings to the broader area of functional cellulosic microbeads.

Introduction

In this paper, we document the impact of combining silk fibroin with carboxylated cellulose nanocrystals (cCNC) to adjust morphology, mechanical and surface energy properties in hybrid SF-cCNC microbeads. Microbeads in general play important roles in drug delivery,¹ cosmetics and skincare,² in florescent immunoassay,³ as micro-carriers in biotechnology,⁴ as viscosity modifiers,⁵ stationary phases in chromatography,⁶ and as abrasives.⁷ The diversity of applications can be attributed to the many attractive features that can be built into microbeads: variations in size, volume and specific surface area, versatility in microencapsulation techniques, regulated diffusion characteristics for molecule release and uptake, control over dispersibility, unique surface chemistry, and morphological complexity.⁸ Microbeads made from plastics like poly(methylmethacrylate), nylon, and poly(ethylene) are used extensively in

the cosmetic and personal care industries to enhance sensory properties in formulations and to provide protection to, or amelioration of, the skin.⁹ There is compelling evidence that plastic microbeads cause harm to the environment, including damage along the food chain.^{9, 10} Increased consumer concerns regarding personal health and environmental health have stimulated searches for sustainable and biodegradable alternatives; but it is not straightforward to replace plastic microbeads with those derived from natural sources like proteins, cellulose, chitosan, starch, or silica. This is because the mechanical, optical, and surface properties of these materials differ from those of conventional plastics, which were designed for functions that hydrophilic biopolymers were not. Plastic microbeads are generally hydrophobic and lipophilic. This makes them compatible with water-in-oil emulsions.^{11, 12} Microbeads made from proteins,¹³ starches,¹⁴ cellulose,¹⁵ chitosan,¹⁶ and silica,^{17, 18} are generally hydrophilic. In hydrous media, the polarity of protein, polysaccharide and silica surfaces can lead to weak interfacial compatibility, emulsion instability and poor moisture barrier properties. To tune the hydrophobicity of the materials, researchers treat the material with polystyrene nanoparticles,¹⁹ lauryl lysine,²⁰ alkylsilane,²¹ methicone, dimethicone, and polyaniline.²² For cellulose microbead separation science, alkylation, arylation and nitrogen heterocycle covalent chemistry have been developed for hydrophobic interaction chromatography and hydrophobic charge induction chromatography.²³

Cellulose is a widely recognized starting point among the polysaccharides to make microbeads. When appropriately solubilized or chemically modified, cellulose can provide desirable mechanical and chemical properties, reinforced by its attractive features as a bioresource: abundance, biocompatibility, and sustainability. Preparation and applications of cellulose and cellulose-derived microbeads have been reviewed in several publications.²⁴⁻³² Characteristically, cellulose microbead production is preceded by solubilization and derivatization of the native cellulose by mercerization, conversion to the xanthate (viscose

process), or dissolution in ionic liquids. Through multi-step covalent chemical transformations, the solubilized cellulose is converted to cellulose acetate, cellulose acetate butyrate, cellulose xanthate, trimethylsilyl cellulose, various cellulose ethers, and other derivatives.

The principles of Green chemistry and Green engineering³³ are strong incentive to develop new entries into cellulose microbeads that use only water as the solvent, that conserve energy, reduce waste and hazards, exhibit atom economy, are sourced from renewable feedstocks, and that are biodegradable and recyclable. cCNC produced by catalyst-free aqueous hydrogen peroxide hydrolysis are alternatives to the specialty cellulose derivatives described above. The process³⁴ developed to make cCNC has the advantages that H₂O₂ is used at concentrations below 10%, is fully consumed, simultaneously introduces the carboxylic acid group, produces primarily dilute sugars as easily disposable waste, conserves entropy (does not break up the cellulose crystal lattice), and yields cCNC that can be assembled into a biodegradable microbead.³⁴

To elaborate the physical, chemical and functional properties of cCNC microbeads beyond the homogeneous cCNC composition, we chose to combine cCNC with silk fibroin (SF). The idea was to investigate the well-known amorphous-to- β -pleated sheet transition to control the mechanical and surface properties of the microbead. We used methanol to mediate the hydrophilic-lipophilic balance (HLB)^{35, 36} that is known to stimulate the organization of the silk protein chains. Amphiphilic SF consists of large hydrophobic H (heavy) chain and small hydrophilic L (light) chain regions, connected by a disulfide bond. The H chain comprises 12 repetitive domains consisting of clusters of oligo-peptides, Gly-Ala-Gly- Ala-Gly-Ser, [Gly-Ala]_n-Gly-Tyr, and [Gly-Val]_n-Gly-Ala (n = 1-8) separated by 11 amorphous peptide regions, mainly as Gly-Ala-Gly-Ser and Gly-Ala-Gly-Ala-Gly-Ser. The H chain contains considerable amounts of hydrophobic amino acid residues. Nevertheless, Ser and Tyr residues along the H chain are hydrophilic, while the charged glutamic acid (Glu) and aspartic acid (Asp) residues,

are attributes that may confer polyelectrolyte character. We used spray-drying to produce the hybrid microbeads over a wide range of composition, with a view to rendering the microbead both mechanically tunable and hydrophobic. This marks a new entry into hydrophobic microbeads made entirely from natural sources, and only by non-covalent interactions.

SF can be isolated from the cocoons of the *Bombyx mori* silkworm.³⁷ Like cellulose, it is a renewable, biodegradable, and biocompatible material.³⁸ While SF has been combined with cellulose and other polysaccharides,³⁹⁻⁴⁵ we know of only one report that describes the combination of (sulfated) CNC with SF.⁴⁶ In this case, it was observed that low SF loadings improved mechanical properties.

According to Griffin,^{35, 36} the hydrophilic-lipophilic balance value (HLB-value) is defined as the percentage ratio between the molecular weight of the hydrophilic group and the total molecule. In general, HLB values below 9 indicate surfactants in which lipophilicity dominates. For surfactants with HLB values above 11, hydrophilicity is larger than the lipophilicity. Surfactants with HLB values between 9 and 11 exhibit intermediate character. Tsukada *et al.* were among the first to document how aqueous methanol (HLB_{methanol} ~10.6) induces $\alpha \rightarrow \beta$ conformational transitions in neat silk fibroin films.⁴⁷ This was followed by studies with ethanol⁴⁸ and small-molecule polyalcohols with different HLB values.⁴⁹ Alcohol induced structural transitions in SF have been reviewed.⁵⁰ The transformation of hydrophilic SF films to hydrophobic films can be induced by glycerol,⁵¹ by blending with cellulose,^{42, 52} by tuning the casting temperature,⁵³ and by crosslinking.⁵⁴ The transformations result from β -sheet formation, though the products can be diamorphic Silk I or II depending on processing conditions and solvent medium.^{55 56}

SF has a tensile strength of about 0.5 GPa, an elongation to break of up to 15%, and toughness of 60 MJ·kg⁻¹.⁵⁷ These values vary, depending on the formation conditions. Strength and

stiffness are determined mainly by β -sheet crystallites present in the fibroin,⁵⁰ which make SF an attractive material for tissue engineering, drug delivery, diagnostics, as surgical suture material, and applications in the textile and optics fields.⁵⁸ The formation of the β -sheet can enhance the mechanical properties (modulus of elasticity and strength) of the material.⁵⁹ This has been widely used as a post processing method to tune the physical properties of SF films.

In the following vibrational spectroscopic study, we examine structural transformations induced by methanol to delineate how the transformations are influenced by combining SF with cCNC in hybrid microbead form *via* spray-drying. N1s XPS depth profiling reveals the distribution of SF in the cCNC in the microbeads. We evaluate how mechanical properties depend on the SF-cCNC ratio, evidence for enhanced hydrophobicity over the native cCNC microbead, and the functional property of small molecule uptake and release.

Experimental method

2.1. Materials

Silk cocoons from Chinese *B. mori* silk were provided by Treenway silk (Lakewood, USA). Carboxylated cellulose nanocrystal (cCNC, 5.3 wt % solution in water) was obtained from Anomera Inc., Montreal, Canada. Na₂CO₃ (anhydrous) was from Fisher Scientific (Ottawa, Canada). LiBr ($\geq 99\%$) was purchased from Sigma Aldrich (St. Louis, USA). Methylene blue (high purity) was obtained from Alfa Aesar (Heysham, UK). Methanol (HPLC grade) was purchased from Fisher Chemicals (Fair Lawn, USA), and acetone (99.6%) was purchased from Anachemica (Mississauga, Canada).

2.2. Degumming SF

SF was produced from silk cocoons following the procedure of *Rockwood et al.*³⁷ Cut cocoons (5 g) were added to a boiling solution of Na_2CO_3 (0.02 M, 2 L) where they were degummed for 30 mins. Afterward, degummed fibroin was rinsed in ultrapure water, and excess water was squeezed out. The residue was rinsed in water (1 L) for 20 mins (3 times), pressed and dried on aluminum foil overnight.

A solution of SF in LiBr (20 wt %) was prepared by pouring LiBr solution (9.3 M) on degummed SF and leaving the protein in an oven at 60°C for 4 h to dissolve. The resulting solution was dialyzed against water for 48 h, with a total of six times water changes. The solution was centrifuged twice at 4°C (9000 rpm, 20 min), decanted and stored at 4°C.

2.3. Spray-drying

Spray-drying was carried out with a Büchi Mini Spray Dryer B-191 (BUCHI Corporation, New Castle, USA) with inlet and outlet temperatures of 175 °C and 104 °C respectively, 30% pump speed and 70% aspirator. SF solution (SF, 2 wt %) was added to an aqueous suspension of cCNC sodium salt with vigorous stirring to obtain a homogenous suspension for spray-drying. cCNC microbeads containing 2 wt %, 5 wt %, 10 wt %, 20 wt %, and 50 wt % SF-cCNC were isolated as white free flowing powders.

2.4 Methanol Treatment

For methanol treatment, SF-cCNC microbeads (100 mg) were left overnight in an aqueous methanol solution (80 wt %, methanol 100 mL), filtered and then dried under vacuum.

2.5. SEM Imaging

SEM was conducted using an FEI Quanta 450 Environmental Scanning Electron Microscope on samples with 3 nm platinum coating.

An FEI Helios Nanolab 660 DualBeam (Focused Ion Beam-Extreme High-Resolution Scanning Electron Microscope) was to image cCNC microbeads in cross-section. Samples were mounted on an aluminum SEM stub and coated with a 4 nm layer of Pt to enhance electrical conductivity. Milling of the sample was carried out with the FEI Helios Nanolab 660 DualBeam instrument (Thermo Fisher Scientific, Hillsboro, OR USA). After selecting a specific particle, a 2 μm thick layer of protective platinum stripe was deposited on the surface of the region of interest to protect the surface from ion beam damage. Microbead cross-sections were then prepared by gallium ion milling. A primary trench to clean the sample was milled at 30 kV ion beam accelerating voltage and 21 nA beam current. Following cleaning, cross-sections were obtained with beam current ranging between 9.3 nA to 0.79 nA. The smooth cross-section was imaged with an electron beam at an accelerating voltage of 2 kV, a beam current of 0.40 nA, and a working distance of 4 mm in the secondary electron mode.

2.6. Contact Angle Measurement

cCNC microbeads and 10% SF-cCNC microbeads powders were pressed into 1 cm diameter pellets. ~ 0.5 g of the cCNC or 10% SF-CNC microbead powder was transferred into a recessed pellet mould. Microbeads were hydraulically pressed at 27.58 MPa load for 5 min. Pressure was applied slowly to prevent cracking of the pellet. Pellets of either cCNC or 2% SF-cCNC microbeads were used for sessile drop contact angle measurement. These were conducted with a Data Physics OCA 15EC goniometer and recorded with SCA software under ambient

conditions. Under computer control, a water droplet of 5-7 μL was deposited on the pellet surface, and the contact angle measured within five seconds.

2.7. Water-in-Oil Emulsion

For water-in-oil emulsion preparation, the water phase was prepared by mixing water (6.2 g), with ethanol (95%, 0.4 g), NaCl (0.1 g), and Butylene Glycol (0.3 g). The oil phase was prepared by mixing C12-15 Alkyl Benzoate (2 g), KP 578 (silicone acrylate, 0.4 g), and KF-6038 silicone emulsifier, which comprises Lauryl PEG-9 polydimethylsiloxylethyl dimethicone (0.4 g). The oil phase was blended at 400 rpm until a homogeneous mixture was formed. The stirring rate was then adjusted to 500 rpm, and 0.2 g powder (cCNC microbeads or 10% SF-cCNC microbeads) was added and stirred until all the microbeads were dispersed well in the oil phase. The stirring rate was then adjusted to 800 rpm. The water phase was added dropwise to the oil phase. Once finished, the stirring rate was adjusted to 2500 rpm, and the mixture was stirred for 5 min until an emulsion formed. A blank sample was also prepared by replacing the powder by 0.2 g of water in the water phase. The final emulsions were cast as films on glass microscope slides. The dried films were observed under an optical microscope to determine the homogeneity of the emulsion system.

2.8. FTIR Spectroscopy and Deconvolution

Fourier transform infrared spectra were measured from SF-cCNC spray-dried powders using ATR method in a Bruker ALPHA FTIR spectrometer (Bruker Optics Inc., Billerica, USA) with single bounce diamond crystal and standard room-temperature LiTaO₃ MIR detector in the spectral region of 400 cm^{-1} to 4000 cm^{-1} using 60 scans with a nominal resolution of 4 cm^{-1} . Peak fitting was done using *OriginPro 2018b* software (OriginLab, Northampton, USA). Fourier self-deconvolution (FSD) was performed in the amide-I region (1595-1705 cm^{-1}). The

second derivative was obtained from the original spectra in the amide I region with a nine-point Savitsky-Golay smoothing filter. The baseline correction is created based on the end points, both start and end to detect the baseline followed by application of the adjacent average smoothing method to reduce the noise. A simple linear interpolation is used to generate the baseline. Deconvolution was performed using Gaussian line shape with a half-bandwidth of 25 cm^{-1} and a noise reduction factor of 0.3. The band position of the deconvoluted spectra were chosen based on the band minima in the second derivative of the undeconvoluted spectra. The eleven bands was reasoned from literature references.⁶⁰ The resolved peaks were attributed to the different secondary structures of SF as follows: β -sheet at 1616-1637 cm^{-1} (two peaks) and 1697-1703 cm^{-1} (one peak); side chain aggregated strands: 1590-1615 cm^{-1} (two peaks); random coils: 1638-1654 cm^{-1} (two peaks); α -helices: 1655-1662 cm^{-1} (one peak); and β -turns and bends: 1663-1696 cm^{-1} (three peaks). Percentage contributions of the different secondary structures were calculated from the sum of the integrated peaks divided by the integral of the cumulative curve.

2.9. UV-Visible Spectroscopy Uptake and Release of Methylene Blue

Absorbance measurements to determine methylene blue uptake and release in SF-cCNC microbeads were conducted with a Thermo ScientificTM EvolutionTM 260 Bio UV-Vis spectrophotometer (Fisher Scientific Company, Ottawa, Canada) and performed in the same way for both non-methanol treated and methanol-treated microbeads. For dye uptake, SF-cCNC microbeads (5 mg) were immersed in methylene blue solution (10 mg/L, 3 mL). The absorbance measurement was performed for 16 h, measuring every 10 min at the wavelength of 665 nm and a reference wavelength of 750 nm. For release monitoring, samples were prepared by immersing SF-cCNC microbeads (100 mg) in methylene blue solution (78 mg/L, 45 mL) overnight. The sample was filtered and washed with acetone. The measurement was

conducted by immersing the dyed microbeads (5 mg) in water (3 mL). The release of methylene blue was measured by UV-vis spectroscopy for 72 h at a wavelength of 665 nm and a reference wavelength of 750 nm.

2.10. X-ray Photoelectron Spectroscopy (XPS)

XPS measurements were performed with a Thermo Scientific K-Alpha spectrometer. Binding energy values were calculated relative to the C (1s) photoelectron peak at 285.0 eV. An argon ion gun with an energy of 500 eV and the low current (1.00 μ A) was used for surface etching, which was performed for 300 s with 10 observation cycles. XPS was performed on each etching level with the flood gun on. The X-ray emission angle was 90 degrees with respect to the specimen surface. The diameter of the analyzed area was 400 μ m. It was estimated that 10 min of etching corresponded to an etching depth of 1 μ m. Spectra were deconvoluted using Advantage Data System software with a combination of Gaussian and Lorentzian functions after background subtraction by the Shirley method, and the resulting curves attributed to the different types of bonds according to their binding energy. Integration of the obtained curves yielded the percent nitrogen content.

2.11. Mechanical Properties Measurement *via* AFM

A quantity of 10% SF-cCNC microbeads was dispersed in acetone and the mixture was cast on freshly cleaved, highly-oriented pyrolytic graphite (HOPG) substrate. The coating was dried under ambient conditions. Analysis was conducted under ambient conditions with a commercial AFM MFP-3D-BIO AFM instrument (Asylum Research, Santa Barbara CA). Biosphere B500-FM cantilevers (Nanotools, München, Germany) with an average spring constant of $k = 2.8$ N/m (determined by the thermal tune method) and a silicon colloidal bead tip radius of 500 nm were used for force measurement. Topographic images were acquired first

to identify the SF-cCNC microbead center positions and individual microbead size. To investigate the mechanical properties of individual microbeads at well-defined location, the point-and-shoot method was used. After scanning an area coated with individual microbeads, approximately 100 of them were selected from the topographic image. A crosshair was used to select the center of a target microbead for force curve collection. The mechanical properties of the microbeads were obtained by analyzing the force-distance curves according to the Hertz model. For force measurement, the contact mode was used with a trigger point of 2 V, a force distance of 1 μm , a scan rate of 0.99 Hz, and a velocity of 1.98 $\mu\text{m/s}$. For the topographic imaging, the AC mode was selected with a 20 μm scan size and 0.8 Hz scan rate. Some force curves were excluded when the results exhibited a Young's modulus indistinguishable from the substrate, meaning that the force curve was not measured for the microbead surface. Similarly, points were rejected if the measurement yielded a stiffness too low, usually the result of lateral motion of the bead caused by a tangential force on the microbead. This was tested by comparing topographic images of beads before and after indentation.

Results and Discussion

3.1. Characterization of Hybrid Microbeads

Figure 5-1 shows SEM images of cCNC microbeads, SF-cCNC hybrid microbeads, and SF microbeads. We spray-dried particles over the range 100% cCNC, 2%, 5%, 10%, 20%, 50% SF in cCNC, to 100% SF. All suspensions were fixed at 0.5 wt % solids. From Figure 5-1, unadulterated (neat, 100%) cCNC microbeads emerged as roughly spherical particles that exhibited wrinkled surface texture. In this case, the average microbead size was 1.89 μm , with broad 38.0% polydispersity. Incorporating 2% SF produced elongated, horseshoe and irregularly shaped particles. At 10% SF, we observed some donut shaped, collapsed

hemisphere and irregular particles, but few elongated species. When the SF component was increased to 50%, we observed two strikingly different particle populations – smooth spherical particles (possibly inflated), collapsed blistered particles, set against a large population of much smaller irregularly shaped particles of considerable morphological variation.

The average particle size increased over the range 2% SF-cCNC to 100% SF-cCNC. We believe that particle size correlates with the size of water droplets before spray-drying, *i.e.*, is related to the viscosity of water suspension when mixing different amounts of SF to cCNC. Equation 1⁶¹ expresses the relation between droplet diameter and the feed solution properties such as surface tension, viscosity, and density.

$$D_d = K_f \cdot Q^n [\rho^a \cdot \gamma^b \cdot \mu^c] \quad (1)$$

Here D_d is the droplet diameter, Q is the feed solution volumetric flow rate, ρ is the density, γ is the surface tension, μ is the viscosity, K_f is an equipment constant, n is the flow rate, and a , b , and c represent the so-called power constants of solution properties. From the equation, the significant increase in the particle size for 100% SF may be related to increases in droplet diameter dominated by the high viscosity of the silk fibroin suspension, compared with that of cCNC. Spray-drying neat SF suspensions resulted in collapsed and broken shell particles. These may be due to internal bubble nucleation and collapse (see later).

FIB-SEM was conducted on SF-cCNC microbeads with 2% and 20% SF content (Figure S5-1 and Figure S5-2). FIB milling reveals the porous internal structure of the microbeads.

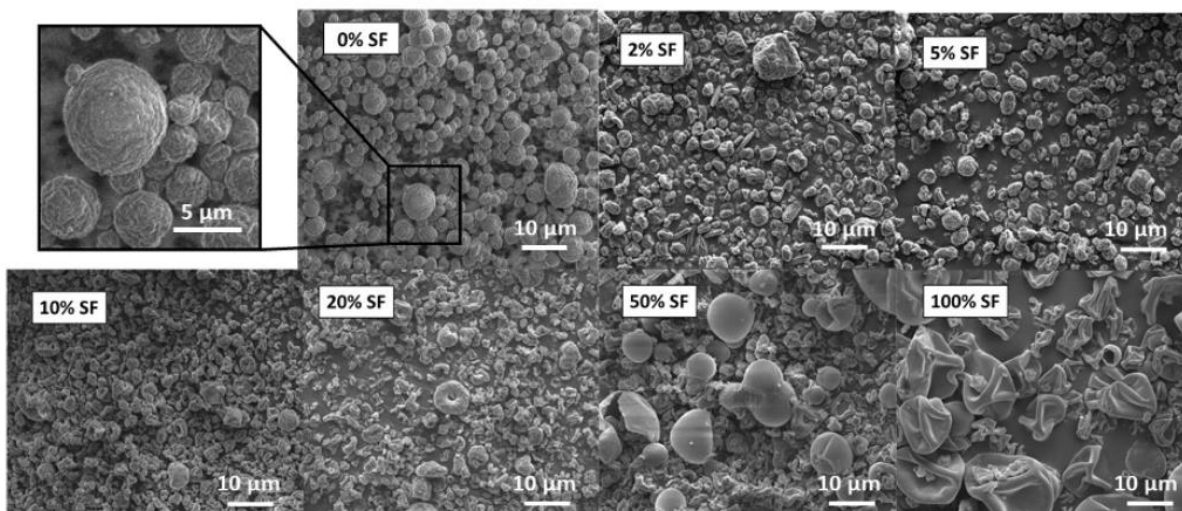


Figure 5-1 SEM images of cCNC microbeads. From left to right and top to bottom, increasing SF content.

3.2. How Spray-drying May Lead to Morphological Changes Depending on SF Content in cCNC Microbeads

Figure 5-1 emphasizes the broad range in particle morphology that arises when spray-drying cCNC, SF, and mixed suspensions of cCNC and SF. The morphology and size distribution of spray-dried particles depends in complex ways on heat and mass transfer-related parameters. These have been elaborated in several publications concerned with spray-drying suspensions of solids.⁶²⁻⁶⁹ Indeed, the parameter space for spray-drying suspensions of solids that gives rise to the diversity of particle shape and size distribution is an independent and lengthy study in itself. In common practice, spray-drying process development is often empirical and experimentally driven. Nevertheless, given our own *fixed* spray-drying conditions (see Experimental), it is worth conjecturing about possible origins of the morphologies shown in Figure 5-1. We first note that particle formation is a non-stationary state event. The processes involving evaporation and solidification must be described by coupled nonlinear heat and mass

transfer equations in the gas and the droplet, for which there are no analytical solutions. Our system has the added complexity that translational diffusion of cCNC rods differs from that of the protein, and that mixing and segregation of the two solidifying components must reflect non-covalent cCNC-cCNC, cCNC-SF and SF-SF interactions as the droplet concentrates. In general, the structural categories for spray-dried particles will depend on the drying mechanism. Nevertheless, empirically, particle morphology can be related to process parameters through the dimensionless Péclet number, $P_e = \frac{R^2}{Dt_{dry}}$, where R is the droplet radius, t_{dry} is the drying time and D is the diffusion constant.⁶⁸ P_e can be used to predict when a skin is likely to form. For low P_e , the diffusional velocity of the solute is faster or on the same order as the recession rate of the surface. In other words, diffusive transport is comparable to convective transport. Spherical or irregularly textured spherical particles can emerge. For large P_e , convective transport dominates over diffusive transport and spherical shell (internal void) particles and collapsed apricot-like particles can emerge. In other words, large Péclet numbers are associated with enrichment of the solute at the surface, which may lead to shell or skin formation. With this brief introduction, we turn to Figures 5-1 and 5-2 for discussion.

Figure 5-5 (a) connects with Figure 5-1 for 100% SF. SF is poorly soluble in pure water. In the range 0.1 to 0.7 wt %, the diffusion coefficient of silk fibroin varies from 2.4 to 1.3×10^{-12} m²/s. As water evaporates upon spray-drying, SF diffuses to the water-air interface where the local viscosity increases, the protein reaches supersaturation, and SF precipitates, driving the droplet system from low Péclet number to larger Péclet. The high Péclet number system means that recession of the surface is fast compared with diffusion of the dissolved protein. Initially, we assume that SF is distributed homogeneously at the instant of water droplet formation. This creates a skin/shell structure as evaporation progresses. A sufficiently thick skin can maintain the shape of the particle while the remaining water diffuses through the shell or evaporates

through openings or less dense regions of the shell. The resulting particles are hollow, and may collapse or wrinkle, depending on the thickness and mechanical properties of the skin. Indeed, the outlet (drying) temperature for the entire range of samples was maintained at 104 °C, *i.e.*, above the boiling point of water. Since the outlet temperature exceeds the boiling point of water in the neat SF system, the pressure inside the evolving particle can exceed external pressure and the particle may inflate, burst and collapse. This is evident from Figure 5-1 and Figure 5-2 (a). Our finding for SF echoes that for spray-drying the small molecule, trileucine (L-Leucyl-L-Leucyl-L-Leucine).⁶⁶ The poor solubility of trileucine pushes the otherwise low Péclet number to a value larger than 1. There is rapid surface accumulation of the phase-separated trileucine, resulting in a wrinkled, low-density particle resembling our SF microbeads. Under our spray-drying conditions, Figure 5-1 also reveals that spherical, textured cCNC particles are produced by evaporating suspensions containing only cCNC. In Figure 5-2 (b) the FIB cross-section of a neat cCNC microbead reveals a dense shell that circumscribes a porous or fibrous interior. Despite the large Péclet number for this system, collapse of the microbead may be prevented by the hard, cCNC crust of the outer shell. Moreover, water evaporation may be impeded by formation of a cCNC gel phase as the cCNC nanocrystal concentration rapidly rises, a transition that might inhibit inflation and collapse of the particle. It is interesting that Peng *et al.*⁶⁵ observe only donut shaped and irregularly shaped particles when *sulfated* CNC is evaporated from 1-2 wt % water suspensions at outlet temperatures between 93 and 84 °C, *i.e.*, below the water boiling point.

Figure 5-1 shows that multicomponent spray-drying, by combining cCNC with 2 - 50% SF content, produced particles of heterogeneous morphology. It is tempting to attribute the morphological diversity to the addition of SF, but this glosses over the complex interplay of diffusion and aggregation of the two components. The literature on the origins of the morphological complexity that results from spray-drying multicomponent systems is just

emerging.⁶² There is some evidence that addition hydrophobic lignin to sulfated cCNC increases the evaporation rate of water, but we see only scant evidence of the annular shaped particles reported by Zhu *et al.*⁷⁰ XPS evidence for accumulation of SF at the microbead surface makes sense in view of the fact that during evaporation the hydrophobic protein will be driven to sequester at the air-water interface rather than remain homogeneously distributed within the particle. The literature on spray-drying frequently cites how diffusion (through the Péclet number, for example) impacts morphology when spray-drying mixtures of compounds, but interpretation of the data is difficult. The diffusion coefficients of sulfated CNC and SF are of the same order of magnitude in dilute regimes. The diffusion coefficient of SF is reported above. The translational diffusion coefficient of sulfated CNC varies with concentration⁷¹ from about 0.2 to 7×10^{-12} m²/s. Moreover, the cCNC diffusion coefficient can exhibit a significant entropic contribution should it enter the anisotropic liquid crystalline phase. XPS depth profiling (section 3.4) indicates that SF and cCNC appear to be intimately mixed within the 2 wt % and 20 wt % SF-cCNC microbeads. Rather than attribute homogeneity in the distribution of the cCNC and SF components to similar diffusion coefficients, we suggest that strong H-bonded interactions and component self-association may govern spatial uniformity. Figure 5-2 (c) illustrates these remarks for the case that the multicomponent system yields a donut morphology.

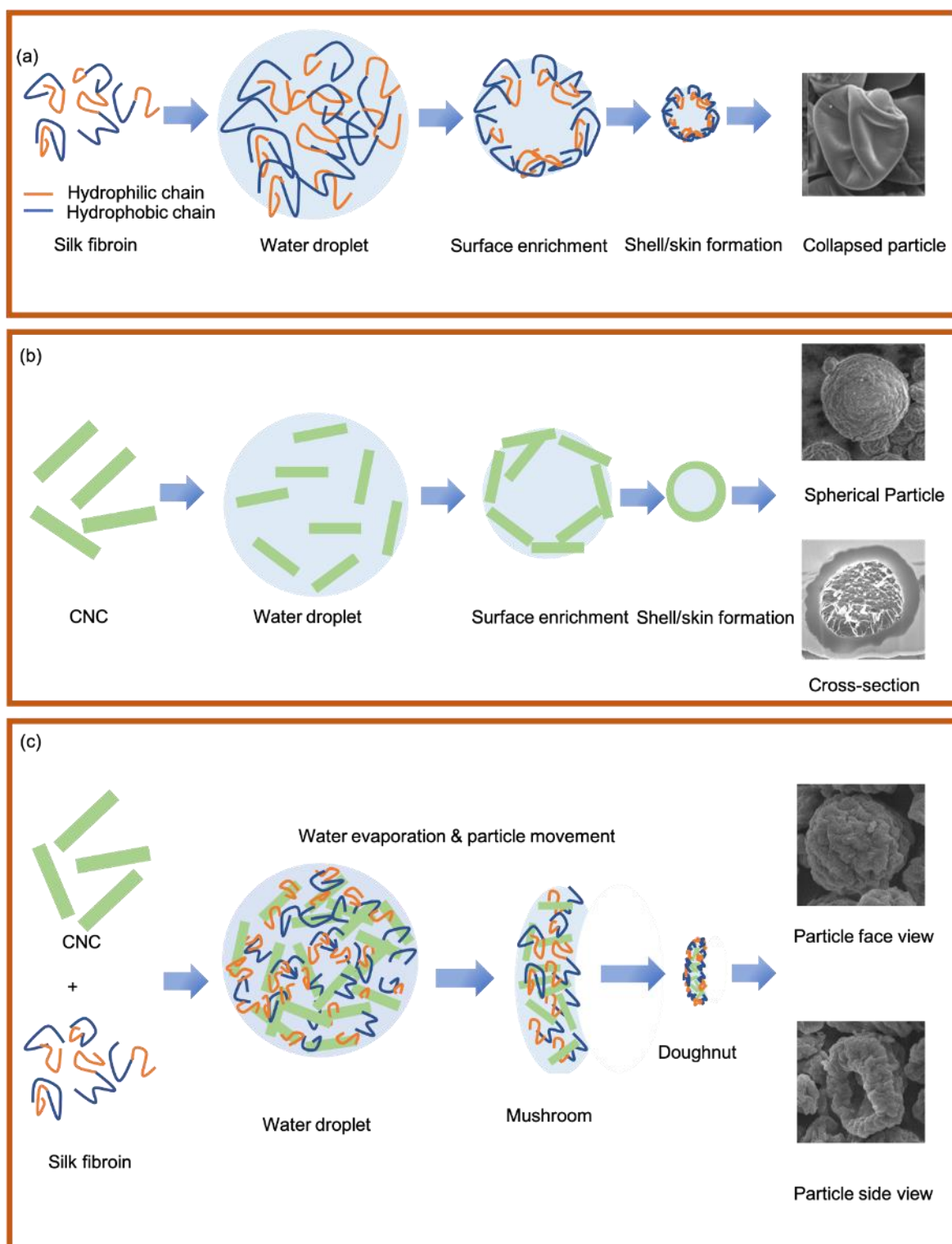


Figure 5-2 Illustrations of possible microbead formation mechanisms *via* spray-drying. (a) Spray-drying SF water suspension leads to collapsed particles. (b) Spray-drying cCNC water suspension leads to spherical particles with a dense shell and porous interior. (c) Spray-drying cCNC and SF mixtures may lead to donut-shaped particles.

3.3. Hydrophobic/lipophilic properties of microbeads hybridized with silk fibroin

cCNC powders disperse well in water because they are hydrophilic, a property that originates from the abundance of hydroxyl, carboxylate or the carboxylic acid groups on the cCNC surface. Previous studies (Chapter 2 SI Figure 2S-1) showed that cCNC microbeads will also swell in water. Incorporation of SF to make SF-cCNC composite microbeads renders them hydrophobic and lipophilic. We compared the water contact angle of cCNC and 2% SF-cCNC composite microbeads to measure the effect, and then examined their compatibility with an oil-in-water emulsion.

Contact angle measurement of powdered materials remains a challenge. Researchers have proposed several techniques to acquire contact angles on powders, frequently noting measurements on compressed materials.⁶²⁻⁶⁴ Procedures include the sessile drop method on compacted pellets, and the Wilhelmy plate, AFM, and capillary pressure methods.⁶⁴ We chose the sessile drop method for simplicity, since we were interested in a comparative measure of hydrophilic/hydrophobic character rather than quantitative determination of surface energy. Figure 5-3 (a) and 5-3 (b) show the contact angles of compacted cCNC and 2% SF-cCNC films by this method. We observed a significant increase in the contact angle from 27.5° for the cCNC pellet with no SF, to 60.4° for the 2% SF-cCNC pellet. This shows that incorporation of SF confers hydrophobic character.

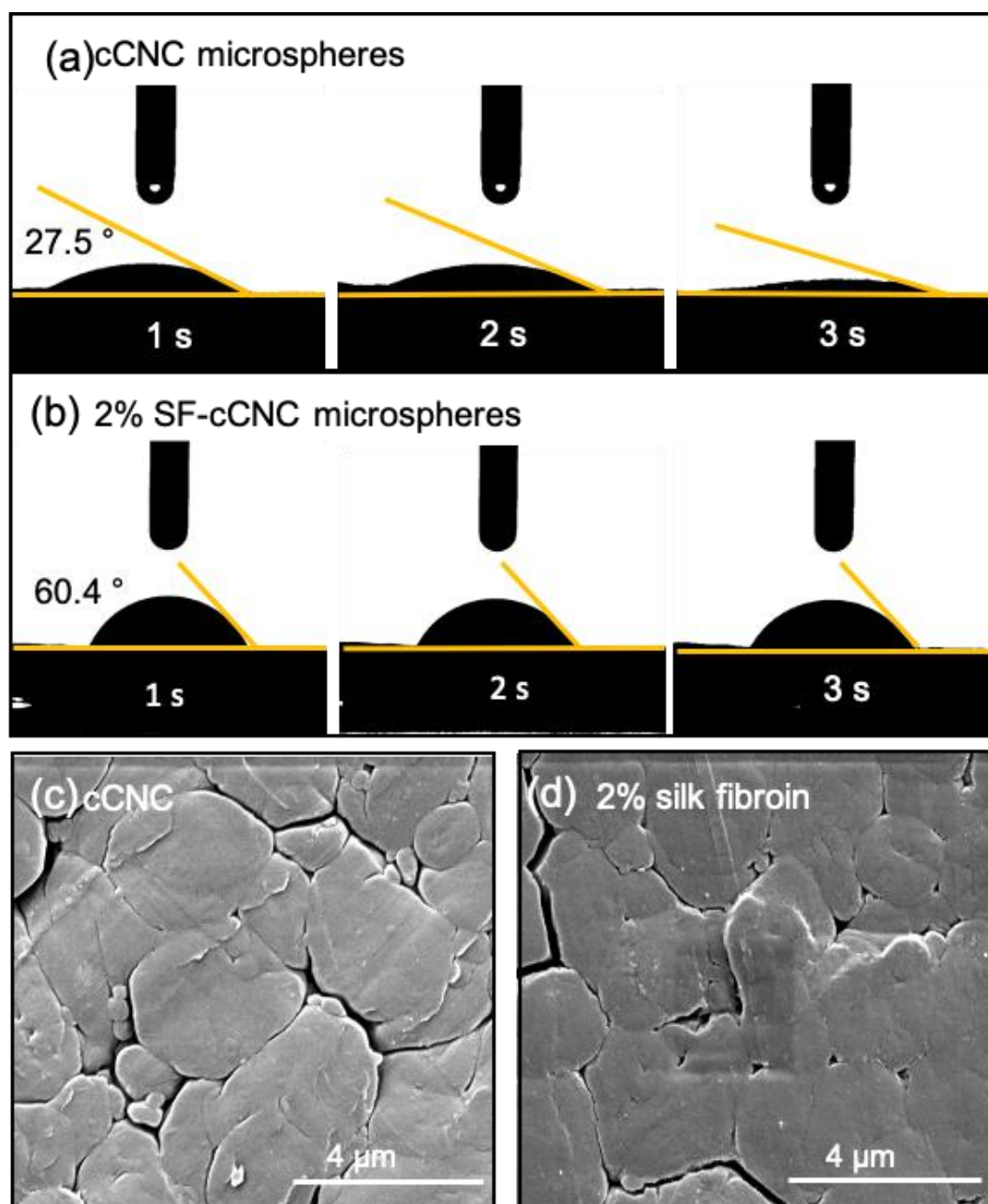


Figure 5-3 Sessile drop contact angle measurement of pelletized cCNC and 2% SF-cCNC microbeads. The needle tips are visible poised above the water droplets that are delivered under computer control. The microbeads pressed into flat pellets, visible in (a) and (b) as the black rectangular substrate where the water droplet sits. (a) Contact angle on pellet of cCNC microbeads at 1 s, 2 s, and 3 s elapsed time. (b) Contact angle of pellet from 2% SF-cCNC microbeads at 1 s, 2 s, and 3 s elapsed time. (c) SEM image of the surface of the pelletized cCNC microbeads. (d) SEM image of the surface of the pelletized 2% SF-cCNC microbeads.

Figure 5-3 (a) also reveals a degree of “creep” in the decreasing contact angle for cCNC as the pellet continually absorbs surface water. In contrast, the contact angle of the 2% SF-cCNC pellet was able to hold the water droplet for a comparatively longer period. We imaged the top of the pellet surface by SEM (Figure 5-3 (c) and Figure 5-3 (d)). As expected, the microbeads were “pancaked” by compression. The images reveal irregular open boundaries and holes between the flattened beads. These openings, and the process of crushing the microbeads, will change the surface energy and hence the wetting properties. While the results may not be a true reflection of the uncompressed powder, the contact angles measured for the SF doped and undoped samples are outside experimental error. The hydrophobic response of the SF-containing microbead is consistent with their behavior in water-in-oil emulsions. This is described next.

3.3. Water-in-oil emulsion – Test of the Hybrid SF-cCNC Microbead

Water-in-oil (W/O) emulsions are widely used in the cosmetics and food industries. When introducing microbeads in W/O emulsions, the microbeads need to be not only lipophilic to be stable in the oil phase, but they must also be hydrophobic so that they do not aggregate inside water droplets. In a cosmetic product, when water evaporates, the residual oil phase needs to be homogeneous in order to produce a fine film on the skin. When microbeads aggregate in the water phase, the homogeneity of the dried film is interrupted.

SF-cCNC microbeads in the range 2-10% SF in cCNC are both hydrophobic and lipophilic, making them candidates to replace microplastic beads that are used in W/O emulsion applications. The concept of hydrophobicity and lipophilicity was introduced in Chapter 1 Section 3.1. Figure 5-4 (b) shows that when pure cCNC microbeads are added to a W/O emulsion, the cCNC microbeads immediately transfer to the water phase where they form large

agglomerates. In contrast, the SF-cCNC microbeads of Figure 5-4 (c) disperse uniformly and are stable over time in the oil phase.

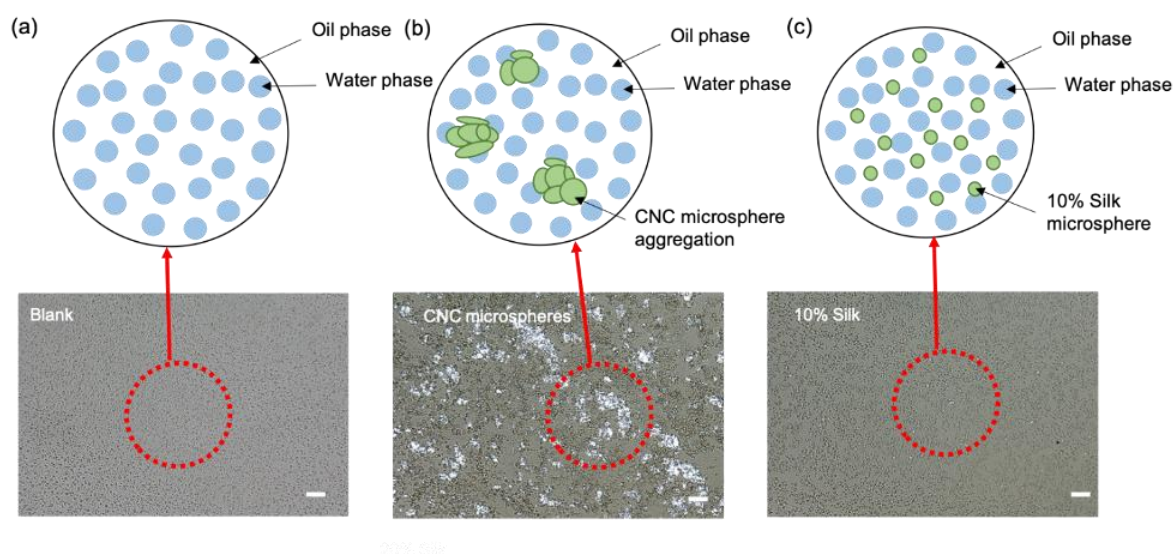


Figure 5-4 Comparison of cCNC microbeads and SF-cCNC microbeads in water-in-oil (W/O) emulsions. (a) W/O emulsion and optical microscope image of the emulsion as a film on a glass substrate. (b) Optical micrograph of cCNC microbead aggregation in the water phase in a film on a glass substrate. (c) Optical micrograph of 10% SF-cCNC microbeads homogenously distributed in the same W/O emulsion as a film on a glass substrate. Scale bar: 30 µm

The hydrophobicity of the SF-cCNC microbeads originates from the hydrophobic heavy (H) chains of the silk proteins. The amino-acid sequence of the L-chain is non-repetitive, forming hydrophilic and more elastic domains.⁵⁰ We now show that the SF-cCNC composite microbeads exhibit hydrophobic character because of the enrichment of hydrophobic domains on the surface of the microbeads.

3.4. Spatial Distribution of SF in SF-cCNC Hybrid System

We explored the spatial distribution of SF and cCNC in the microbeads *via* depth resolved XPS. A survey spectrum of SF revealed the major photoelectron peaks located at binding

energy values of 531 eV, 400 eV and 285 eV, corresponding to O (1s), N (1s), and C (1s) atomic orbitals, respectively. Argon-ion projectiles were then used to remove successive layers between etching cycles.⁷² We first calibrated etching depth and time. For cCNC microbeads, 10 min of etching with a current of 1.00 μ A, 500 eV ion energy results in an etching depth of $\sim$ 1 μ m, or 0.1 μ m/min. Calibration of the etching process was described in Chapter 3. 2% SF-cCNC and 20% SF-cCNC microbeads were profiled, focusing on the elemental distribution of carbon, oxygen, and nitrogen. Then 10 cycles of etching were completed, gathering the element spatial profile after each etching cycle to collect subsurface information. Each etching cycle lasted $\sim$ 5 min since the first measure did not include etching. Figure 5-5 (a) - (c) and (e) - (g) show the carbon, oxygen, and nitrogen peaks for 10 etching sequences of 2% and 20% SF in cCNC, respectively. The etching process starts from the surface of the microbeads, and then penetrates $\sim$ 5 μ m into the densely packed microbead sample over the total etching time. From Figure 5-5 (d) and (h), it is evident that the surface of the microbeads is enriched in nitrogen, while the internal nitrogen content remains almost constant over the etching time. But the constant signal is due to etching through probably more than one layers of microbeads.

Published XPS data for neat silk fibroin powder reveals that registers nitrogen content typically on the order of 15.1%.⁷³ Figure 5-5 (h) shows that we measure nitrogen about 14% N at the surface of a 20% SF-cCNC microbead. This indicates that we have about 93% coverage by SF on the bead surface. Similarly, incorporation of 2% SF in cCNC results a surface nitrogen content of $\sim$ 8%, showing a surface enrichment of $\sim$ 53%.

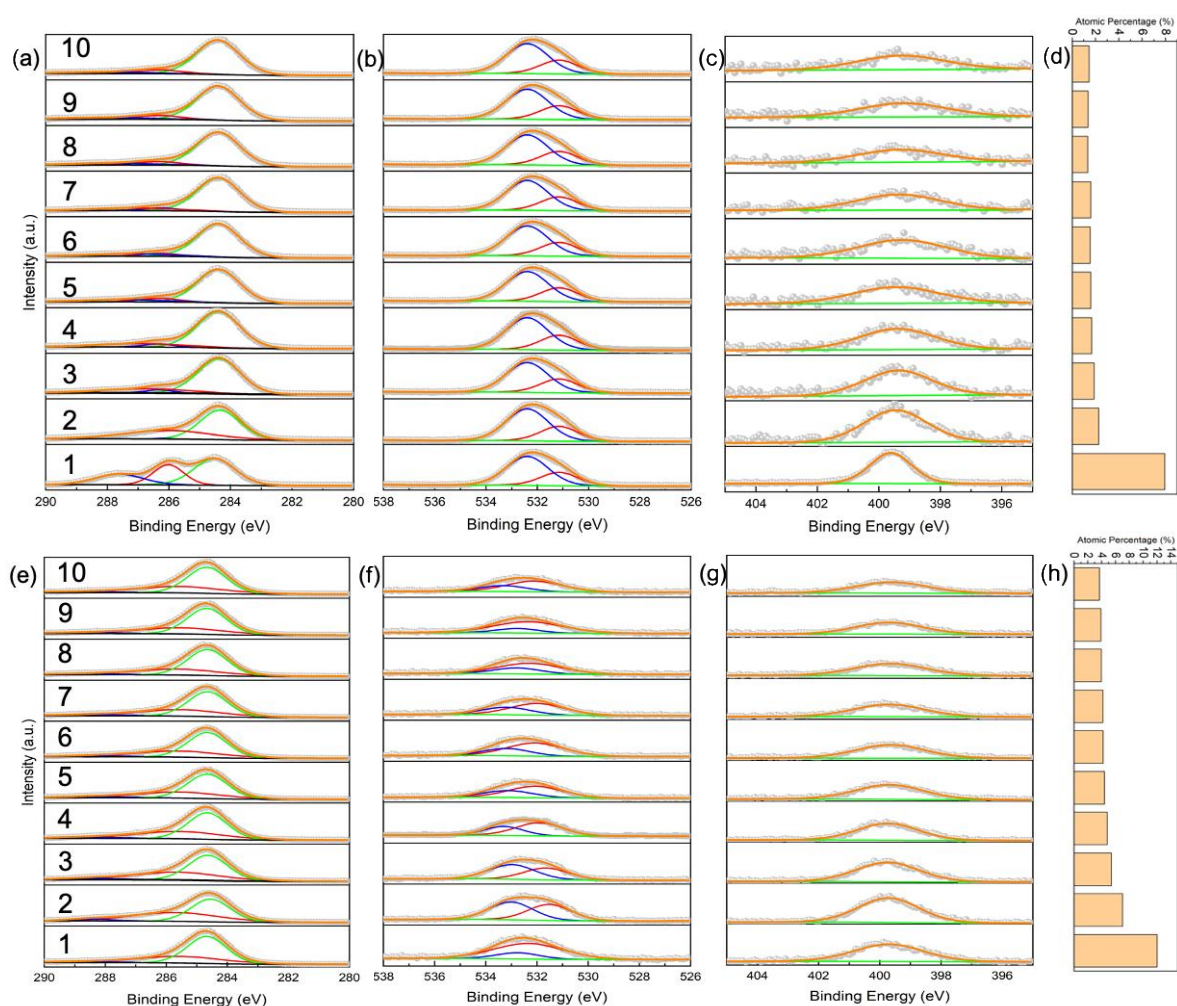


Figure 5-5 XPS depth profile of 2% SF-cCNC microbeads (a-c) and 20% SF-cCNC microbeads (e-g) for C 1s (a), O 1s (b) and N 1s (c) peaks for 10 etching sequences over a total etching time of 5 min. The histogram (d) shows the decreasing % nitrogen located from the bead surface to the interior over 10 successive etching sequences for 2% SF-cCNC microbeads. The histogram (h) shows the % nitrogen located from the bead surface to the interior over 10 successive etching sequences for 20% SF-cCNC microbeads.

The spatial distribution of SF in cCNC means that spray-drying creates a type of hybrid core-shell microbead, with SF enrichment in the shell layer. The hydrophobic shell (and the interior (see below)) provides a barrier to transport of aqueous solutes to the surface and then to the

interior of the hybrid microparticle. In the next section we amplify the hydrophobic character by inducing conformational transitions of the SF *via* methanol treatment.

3.5. Structural transitions in SF-cCNC microbeads by methanol treatment

Applications of silk fibroin rely widely on the formation of highly periodic crystalline regions in the protein.^{38, 42, 50, 74} Adoption of the β -sheet conformational state changes mechanical and transport properties of materials made from silk fibroin and its composites. Infrared spectroscopy is one of the primary tools to interrogate the conformational transitions leading to β -sheet crystallinity in silk materials. The fractions of secondary structural components including random coils, α -helices, β -pleated sheets, turns, and side chains can be evaluated by careful spectral deconvolution. Here, we investigated how SF secondary structure is affected by spray-drying 2-component SF-cCNC suspensions, and how methanol treatment stimulates additional conformation relaxations. We selected Fourier spectral self-deconvolution signal processing to resolve the band contours that comprise the overlapped bands. With a high pass filter, broad and indistinct bands, like those of the amide I region of SF, are narrowed synthetically to yield deconvoluted spectrum with enhanced peak resolution.

The key carriers of spectroscopic information about chain conformation are absorption bands in the following regions: the amide I band ($1600\text{-}1700\text{ cm}^{-1}$, C=O stretching), amide II ($1480\text{-}1575\text{ cm}^{-1}$, N-H bending and C-N stretching) and amide III ($1270\text{-}1230\text{ cm}^{-1}$, C-N stretching and C=O vibration). In particular, the amide I peak in the range $1644\text{ -}1658\text{ cm}^{-1}$ can be used to confirm the extent to which fibroin adopts the silk I conformation. The amide I region comprises overlapping of the stretching vibrations ($\nu_{\text{C=O}}$) of pure C=O bonds in the protein backbones. The frequency of these vibrations is determined hydrogen bonding with the carbonyl groups, which directly reflect a particular secondary structure. It has been reported that organic solvents, mechanical stress, temperature, humidity, or the addition of other

polymers can stimulate the conformational transition from silk I to silk II.⁵⁰ Silk II typically shows an amide I peak in the range 1620-1640 cm⁻¹.

Figure 5-6 (a) shows the vibrational spectra for different compositions of SF-cCNC microbeads before and after methanol treatment. Before methanol treatment, all compositions of SF-cCNC adopt the silk I structure. These transform to silk II with methanol treatment. Figure 5-6 (b) confirms that in the amide I region, methanol treatment shifts the peak from 1651 cm⁻¹ to 1627 cm⁻¹. Therefore, spray-drying and adding cCNC to the silk structure are not the only factors that determine conformational distribution. Methanol treatment plays a crucial role in this transformation.

We quantified the β -sheet formation before and after methanol treatment by Fourier self-deconvolution (FSD) of the FT-IR peak in amide I region.^{38, 60, 75} The FSD curves contain tyrosine side chain, beta-sheet, random coil, alpha-helix, turns and beta aggregated strands in the amide I region. These are indicated in Figure 5-6 (d). The peaks are marked as different colors: tyrosine side chains as orange, beta-sheet as green, random coils as blue, turns as cyan blue and alpha-helix as red. The bands between 1590-1615 cm⁻¹ marked as orange are mostly from tyrosine side chains, which is a characteristic band for *B. mori* fibroin with ~ 4.8% content. Arg side chains contribute less to this band because *B. mori* fibroin only contains ~ 0.5% of them.

We used the ratio of each peak area to the total amide I band area to quantify the amount of secondary structure in silk fibroin. Figure 5-6 (c) shows the results of the percentage of β -sheet in SF-cCNC hybrid microbeads for different SF content from 2% to 50%, before and after methanol induced protein crystallization. Before methanol treatment, increasing the content of cCNC in the SF-cCNC microbeads is associated with higher higher β -sheet contribution to the spectra. Spray drying 100% silk fibroin quenches silk fibroin into its amorphous phase. It is

possible that cCNC with SF in aqueous suspension prior to spray drying leads to mutual association between the two materials and some degree of β -sheet conformational change of the SF as it adsorbs onto nanocrystals. Not expectedly, methanol treatment of samples with increasing the SF content yielded greater content of the β -sheet.

We found that the duration of methanol treatment did not affect the maximum conversion to β -sheet, but the concentration of methanol solution did give a difference of β -sheet formation. (SI Figure 5-3, and Figure 5-4): exposure 20% aqueous MT induced a higher yield of β -sheet than did 80% aqueous MT. This effect is more significant for SF-cCNC microbeads than neat cCNC microbeads. The methanol exposure time was also monitored for β -sheet transition (SI Figure S5-5 and Figure S5-6). The data shows a very fast β -sheet transition time (within 1 s) when treated with 80% methanol.

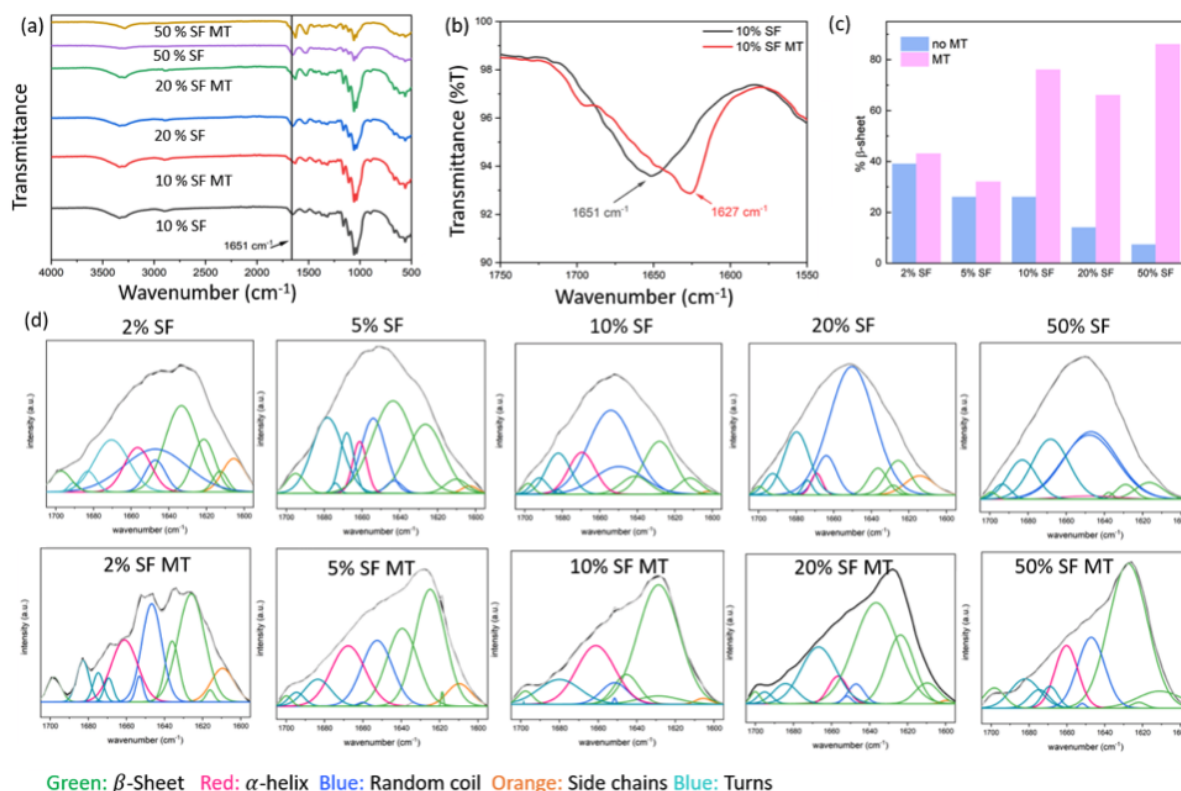


Figure 5-6 FT-IR spectra of SF-cCNC microbeads. (a) FT-IR spectra of SF-cCNC microbeads as functions of SF content in the microbead, before and after methanol treatment. The solid vertical line locates the position of the amide I band. (b) FTIR spectra in the amide I region for 10% SF-cCNC microbeads before and after methanol treatment showing the shift of the amide I peak. (c) Percentage contribution of the β -sheet conformation, before and after methanol treatment, as a function of the composition of SF-cCNC microbeads. (d) Fourier self-deconvoluted spectra of SF-cCNC microbeads in the amide I region. The colored peaks are identified by their spectral contributors beneath the spectra in (d).

3.6. Small Molecule Uptake and Release

Modification of the cCNC microbeads with SF should affect small molecule uptake and release. We used the methylene blue (MB) dye molecule as a model compound to probe diffusion in the SF-cCNC microbeads. We compared the uptake/release (expressed as Q_t in mg dye/g

microbead) to those of neat cCNC microbeads to explore the impact of adding SF, before and after methanol treatment.

Figure 5-7 (a) shows the time evolution of MB uptake for 2% SF-cCNC microbeads. With neat cCNC microbeads, MB uptake is nearly instantaneous, and equilibrium is reached within 400 s (Figure S5-7). After combining cCNC with SF, the rate of dye uptake decreased, requiring up to 1000 min to reach equilibrium. Notably, we observed no dye uptake before 200 min for either untreated and methanol treated SF-cCNC samples, for which the onset of uptake occurred at 200 min or approximately 300 min, respectively.

Methanol treated microbeads show slower dye uptake compared with the untreated SF-cCNC beads. This is consistent with enrichment in β -sheet conformations, increased crystallinity of the SF aggregates and enhanced hydrophobicity of the methanol treated particles. Our findings for the treated and untreated hydrophobic beads are in line with what we might expect by interpreting the transport of MB in the context of our reported film-pore diffusion model (Chapter 2). In this two-resistance model, the first diffusion step requires that the dye overcome a diffusion barrier at the surface of a microbead. This barrier regulates adsorption, which can be expressed in terms of an appropriate isotherm. We suppose that this barrier is dominated by the hydrophobic surface and that this is what delays the onset of diffusion compared with diffusion in the neat hydrophilic cCNC microbead. The longer induction period for uptake in the hydrophobic particles may be related to the requirement for water diffuse into hydrophilic defects at the microbead surface to render the bead more hydroplastic for penetration by MB. The second step can be described by Fickian diffusion of MB inside the pores of the SF-cCNC microbead, but in this instance, the dye must move against the resistance created by the interior distribution of hydrophobic and crystalline domains of silk fibroin.

For MB release studies, we loaded neat cCNC, untreated 2% SF-cCNC, and methanol treated 2% SF-cCNC each with ~ 3.2 mg/g of MB. Near $t=0$, the release kinetics are very rapid for all three types of microbead. This indicates a burst effect when MB molecules that are saturated on the surface of the microbeads are rapidly released to the solution. Figure 5-7 (b) shows that over time neat cCNC is most reluctant to release the dye. Hence, incorporating SF increased both the rate of release and the amount of dye released. We explain this as follows: cationic methylene blue dye may bind to cCNC particles in the microbead through combinations of H-bonding and electrostatic interactions with the carboxylate anion. Controlled release of methylene blue from cCNC-based microbeads is regulated by the diffusion of MB through the porous interior and surface, a process that we have shown depends markedly on pH and ionic strength. The neat cCNC microbead swells in neutral and high pH environments, an indication that water penetrates well into the structure. From the Figure 5-5 XPS depth profiling study, SF creates a hydrophobic cage outside of the microbeads, which is more likely to hinder dye release when the particles are immersed in water. The contradictory results may suggest a redistribution of the SF and cCNC in water, leading to the surprisingly increase of the dye release.

Both the release rate and amount of release decrease after methanol treatment. This may be due to increases both to β -sheet crystallinity (physical barrier) and hydrophobicity.

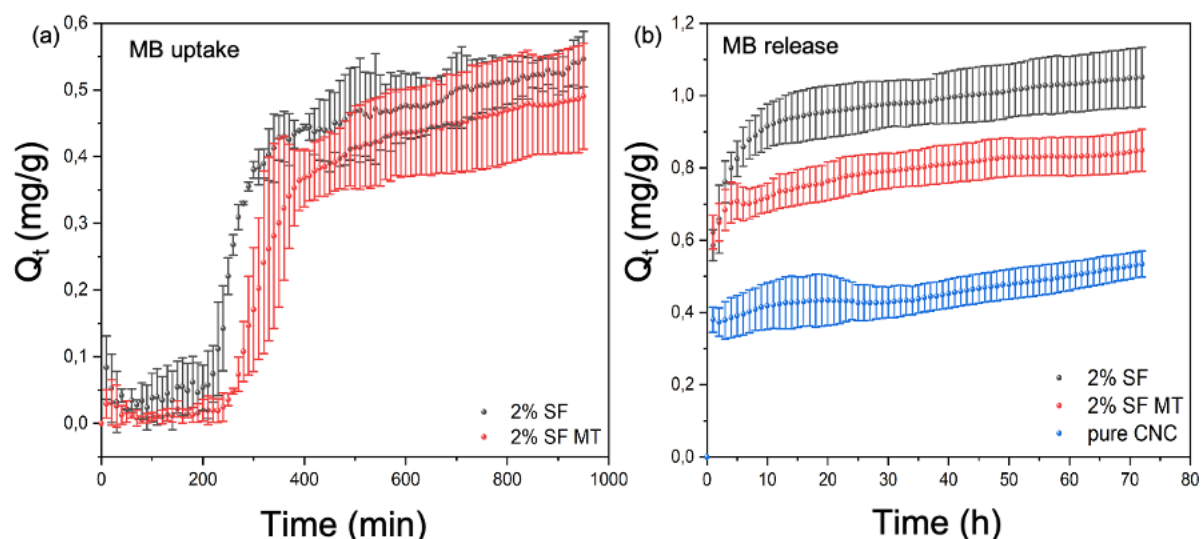


Figure 5-7 Methylene blue dye mass transfer in cCNC and SF-cCNC microbeads. (a) Methylene blue dye adsorption kinetics for 2% SF-cCNC microbeads before and after methanol treatment (MT). (b) Methylene blue dye release kinetics for 2% SF-cCNC microbeads before and after methanol treatment compared with neat cCNC microbeads.

3.7. Mechanical properties

Several methods are available to measure mechanical properties of microbeads. These include nanoindentation,⁷⁶ AFM force measurement,⁷⁷ and dynamic mechanical analysis compression.⁷⁸ Here we used microsphere colloidal probe nanoindentation to measure the Young's Modulus of the microbeads by AFM. We compared the mechanical properties of cCNC and SF-cCNC microbeads with those of other types of microbead. For petroleum-based beads, the Young's modulus varies from 2-8 GPa for polystyrene beads,⁷⁷ to 1-2 GPa for latex particles,⁷⁹ to 0.25-2 kPa for poly(acrylamide) microbeads.⁸⁰

Figure 5-8 (a) plots the Young's modulus determined from the AFM force curve by fitting to the Hertz model. It is clear that with methanol treatment, there is a redistribution of the population of the microbeads such that a portion of them manifest larger Young's modulus

compared with the untreated SF-cCNC beads. Neat cCNC microbeads have the lowest Young's modulus. Addition of 10% SF increases the stiffness of the SF-cCNC. This is visible as a shift in the bar graph to higher modulus. 10% SF microbeads exhibit a bimodal distribution of the Young's modulus with two maximum at approximately 30 MPa and 70MPa. This may indicate non-uniform distributions of silk fibroin and CNC within the microbeads, resulting in roughly two types of microbeads with different stiffness. 20% SF shows a similar bimodal distribution with two maximum at around 15 MPa and 50 MPa. Methanol treatment induces a shift in the Young's modulus to higher values, an effect that is not surprising given the way that methanol enhances β -sheet conformations. This effect is observable for both 10% and 20% SF methanol treated samples. Beyond $\sim$ 10% SF loading, further increases the amount of silk fibroin, say to 20%, decreases the stiffness of the microbeads. This may be the result of a plasticizing effect by the protein, making the microbeads more deformable.

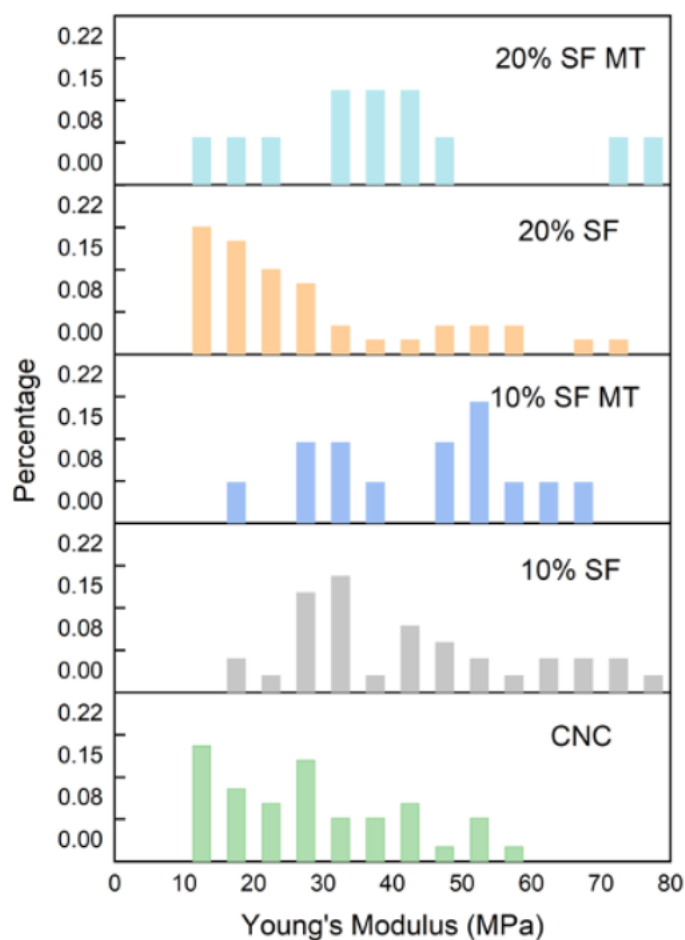


Figure 5-8 Young's Modulus comparison of cCNC, 10% SF-cCNC, and 20% SF-cCNC before and after methanol treatment. ($N_{\text{CNC}}=46$, $N_{10\% \text{SF}}=52$, $N_{10\% \text{SFMT}}=31$, $N_{20\% \text{SF}}=50$, $N_{20\% \text{SFMT}}=24$)

Conclusions

A new type of hybrid microbead has been developed by incorporating SF protein into cCNC *via* spray-drying. SF-cCNC microbead morphology depends strongly on SF content under fixed spray-drying conditions. XPS depth profiling indicates that SF is concentrated on the surface of the microbeads, introducing hydrophobic and lipophilic character, attributed to the heavy chain hydrophobic domains in SF. AFM nanoindentation reveals that inclusion of SF up to 20% enhances the Young's modulus of the microbeads. The enhancement is linked to the

quantity of β -pleated sheet conformations in the composite microbead. Exposure of the hybrid beads to aqueous methanol increases the quantity of β -pleated sheet SF and further raises the stiffness of the particles. The quantity of β -pleated sheet conformations was determined by Fourier spectral deconvolution in the region of the amide I band of SF. Uptake and release of methylene blue probe molecule is inhibited by SF, compared with neat cCNC microbeads. The SF-cCNC microbeads exhibit hydrophobic and lipophilic properties, making them stable in water-in-oil emulsions and suggesting that the hybrid microbeads might be candidates to replace microplastic beads used in water-in-oil emulsions in cosmetics and personal care.

Supplementary

This supplementary section contains information and experiments in support of the text for Chapter 5.

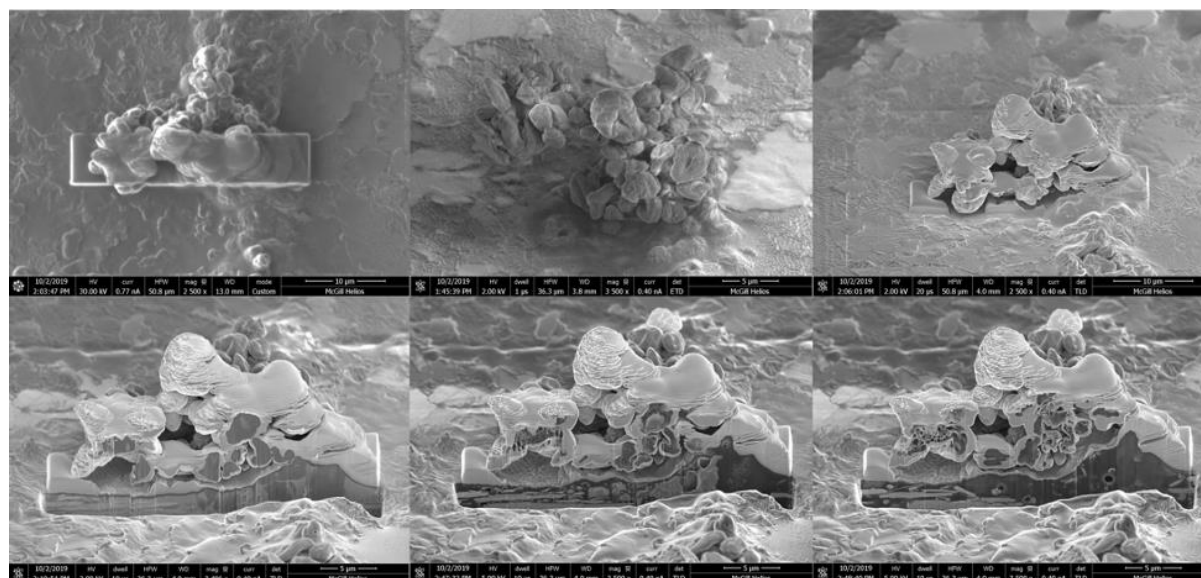


Figure S 5-1 FIB-SEM milling to reveal internal structure of 2% SF-cCNC microbeads.

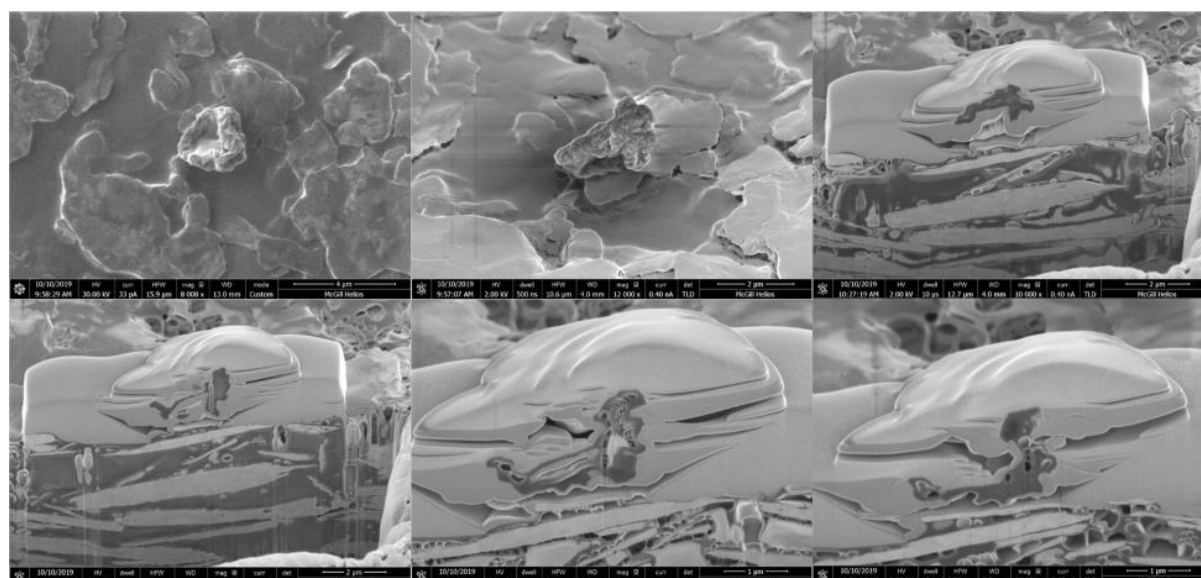


Figure S 5-2 FIB-SEM milling to reveal internal structure of 2% SF-cCNC microbeads

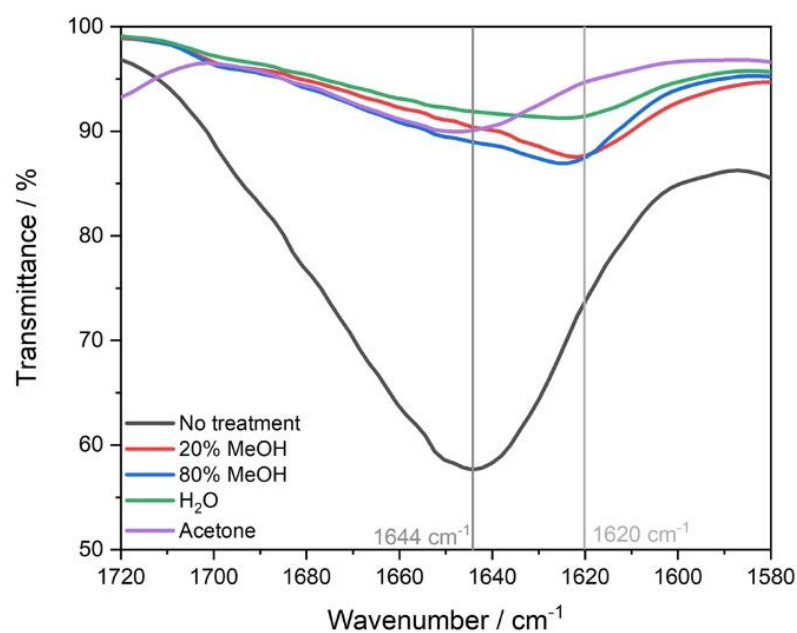


Figure S 5-3 Evolution of amide bond stretching region of 10 % SF-cCNC microbeads after treatment with aqueous methanol, water and acetone compared with an untreated sample.

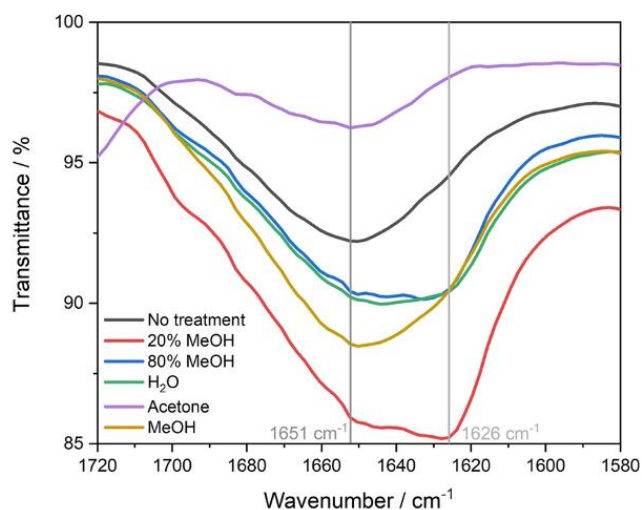


Figure S 5-4 Evolution of amide bond stretching region of 20 % SF-cCNC microbeads after treatment with neat methanol, aqueous methanol, water and acetone compared with an untreated sample.

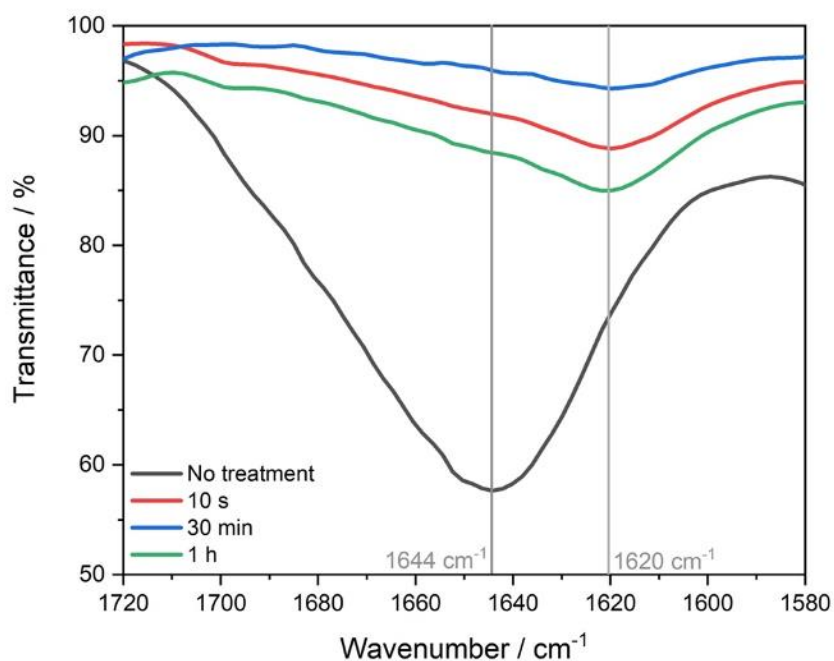


Figure S 5-5 Time evolution of amide bond stretching region of 10 % SF-cCNC microbeads after treatment with 80% methanol compared with an untreated sample.

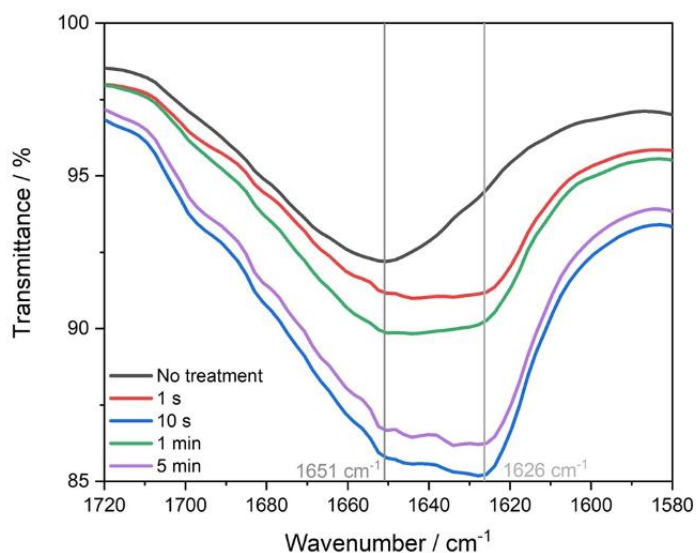


Figure S 5-6 Time evolution of amide bond stretching region of 20 % SF-cCNC microbeads after treatment with 80% methanol compared with an untreated sample.

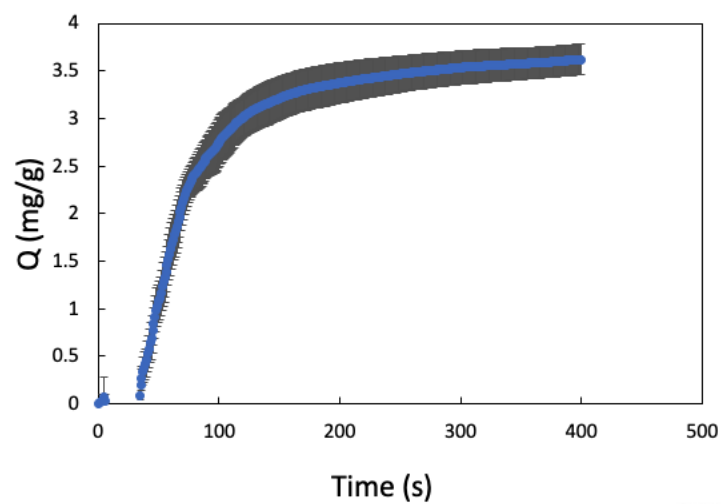


Figure S 5-7 Dye uptake kinetics of cCNC pristine microspheres at room temperature in methylene blue solution.

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| 6 |

Conclusions and Future Work

Chapter 6 Conclusions and Future Work

6.1. Conclusions

The research for this thesis started with a search for a performing alternative to plastic microbeads whose use are now under scientific, legislative and consumer pressure to be banned. This pressure creates incentive to adopt naturally sourced materials that are biodegradable and manufactured according to sustainable practices. Cellulose as a naturally sourced biopolymer, is a good candidate due in part to its abundance, biodegradability, non-toxicity, and sustainability. But naturally sourced microbeads like those from cellulose are not a “drop-in” solution to the microplastic dilemma. There is a need to quantify the performance characteristics of replacements. Moreover, despite the fact that microbeads derived from cellulose had already been widely explored, it was clear to us that there were opportunities for new developments if new types of cellulose to be introduced.

Research in our laboratory had discovered a new entry into crystalline nanocellulose based on rather simple dilute hydrogen peroxide oxidation of 100% Forest Stewardship Certified wood pulp. The catalyst-free process yields carboxylated nanocrystals, cCNC, in one step (Chapter 2). While the majority of research into CNC focused on properties intrinsic to the nanoscale (and predominantly on sulfated CNC), we envisioned an approach that involved rescaling the CNC back into microscale objects, namely microbeads. Our approach intended to conserve the native crystalline structure of cellulose whilst elaborating the microscale objects. Indeed, it became evident to us that diverse properties might result. These properties were developed in the 4 research chapters of this thesis. Chapter 2 focused on learning how cCNC microbeads uptake and release small molecules. We collected information about the mobility of a charged probe molecule paradigm, methylene blue (MB) We developed a film-pore diffusion model that considered transport of the dye across a resistance boundary (external

diffusion layer) and intraparticle transport (internal diffusion). The external-film mass transfer coefficient k_f was found to be $\sim 10^{-6}$ m/s. Effective internal diffusion coefficients D_{eff} , were on the order of 10^{-11} m²/s. pH and ionic strength regulate the movement and adsorption of MB in the microbeads, giving us the first insight into how the beads might respond as “smart” materials: changes in pH and ionic strength could be used to control the uptake of the dye. Measurement of free energy, enthalpy and entropy revealed the uptake to be spontaneous and slightly endothermic. Our study is one of the rare instances where dye uptake kinetics are treated as coupled with diffusion in cellulose microbeads. This is despite the fact that conventional specialty cellulose polymers are widely used for time-release of pharmaceuticals, where well-known kinetic models dominate.

With some knowledge of MB dye diffusion and adsorption in the cCNC microbeads, we then turned to study how external stimuli might regulate (gate) small molecule movement in and out of the microbeads. Our experiments and findings are realized in two case studies detailed in Chapter 3. Case Study 1 explored nanocomposite microbeads made by hybridizing cCNC with PAA by spray-drying the two components simultaneously. We discovered that esterification and crosslinking occurred in the gas phase during evaporation from the microdroplets. The nanocomposite microparticles exhibit reversible swelling over a range of pH varying from 12 to 1. Methylene blue was again used to probe the pH response of MB release profiles. We showed that uptake and release depended on the pH of the surrounding medium and the ratio of PAA to cCNC, at the fixed molecular weight. The cellulose nanocrystal is hygroscopic, but insoluble in water. Therefore, the microbead tends to swell in water but has no tendency to fragment because it is held together by ester bond crosslinks between cCNC and PAA. XPS depth profiling suggested that cCNC and PAA are distributed more or less together in the nanocomposite beads. But SEM-FIB milling indicated that the nanocomposite beads were denser than the neat cCNC beads. Third harmonic generation

experiments gave morphology information of cCNC-PAA microbeads when immersed and swelled in water. Confocal microscopy was recruited to monitor the release of a porphyrin fluorophore probe in real time. Super resolution microscopy (SIM) provided a 3-D map of the porphyrin, revealing that it preferentially accumulates at the periphery of the microbead, perhaps as a consequence of the different diffusion rates of the dye and the cCNC in the drying microdroplet. Case study 2 in Chapter 3 discussed the synthesis of light-responsive cCNC microbeads and bulk films by grafting a photo-responsive coumarin molecule onto the surface of cCNC. We have achieved a chemical grafting ratio of 0.044 mmol coumarin molecules per 1 g of cCNC. Bulk films cCNC-Coumarin underwent two-color photo-reversible crosslinking. 365 nm photons closed the “gate” by [2+2] cycloaddition between coumarin tags. We proposed that dimerization densified the film surface to suppress, but not eliminate MB dye uptake. This effect was reversed by irradiating the crosslinked film with 254 nm photons. cCNC-Coumarin microbeads were made by spray-drying method.

Mechanical properties microbeads are relevant measures of the cCNC microbeads. In Chapter 4, we focus on the Young’s modulus obtained from force-distance curves in the framework of the Hertz model. We adapted a silicon bead (microcolloid) tip attached to an AFM tip to get a more accurate picture of the elastic properties of the microbeads. The results show that neat cCNC microbeads have a Young’s modulus of ~18.02 MPa. Two new methods are described in this chapter to adjust the stiffness of the microbeads. Citric acid doped into aqueous suspensions of cCNC underwent gas phase esterification resulting in crosslinks within the CA-cCNC microbeads. The Young’s modulus increased to ~27.97 MPa. Dense, neat cCNC microbeads were obtained by adjusting spray-drying parameters. Compared with the modulus of “foamed” neat cCNC microbeads, the stiffness of the dense beads increased by almost 9 MPa to ~24.55 MPa.

Chapter 5 expanded the Chapter 3 hybrid microbead fabrication method to include silk fibroin (SF) protein. The SF-cCNC microbeads are hydrophobic and lipophilic and stable in water-in-oil emulsions. We quench-condensed SF in the hybrid beads by spray drying, and then showed how the β -pleated sheet conformational transition of the protein in the microbead can be used to adjust mechanical properties and small molecule transport. Conformational transitions were enhanced by perturbing the protein with aqueous methanol. Fourier self-deconvolution vibrational spectroscopy gave a detailed picture of the conformational transitions, and therefore a window into the coupling of protein crystallization with changes in Young's modulus. SF has large domains of hydrophobic amino acids. XPS depth profiling indicated that SF is enriched at the surface of the microbeads, consistent with its water-repellent activity.

6.2. Suggestions for Future Work

6.2.1. Spatial distribution of two polymers in hybrid microbeads in water

In Chapter 3, XPS depth profiling provided some insight into the spatial distribution of cCNC and PAA in the hybrid microbeads in powder. We showed that cCNC and PAA are distributed intimately *in the dry state*. But THG studies may suggest that PAA might preferentially redistribute when the beads are swelled in water, although we believe the ring structure generated from THG imaging is highly likely a result of technical artifact. Should redistribution occur, the surface energy of the microbeads will change, as will the capacity of the beads to bind small molecules and ions. Accessibility of functional groups is an important concept in the mixing of polymers in heterogeneous phases, where accessibility involves factors like chain connectivity, steric shielding, and functional group distribution, etc. We suggest further dye-labelling experiments with super-resolution microscopy (STED) to image the microbeads to locate the two cCNC and PAA components separately. Figure 6-1 illustrates the idea. PAA and cCNC can be tagged by the usual click chemistry or by EDC-coupling. Scheme 6-1 to 6-3 offer

some pathways. For example, Alexa Fluor™ 594 Cadaverine can be linked to PAA (Scheme 6-1) and Huisgen cycloaddition (Scheme 6-3) can bind STAR 635P dye to cCNC.

The spray-dried labelled cCNC-PAA microbeads would be imaged by STED. Two-channel detection will locate the labels in the microbeads. If a redistribution of PAA and cCNC happen when immersing the dye-labelled microbeads in water, a color-separation should be expected, where Dye 1 from PAA will appear on the outside layer of the beads, whereas Dye 2 from cCNC will stay inside of the beads. This method might also be used to study SF-cCNC microbeads in both dry state and in solvents to study the potential of SF protein redistribution when solvent environment is changed.

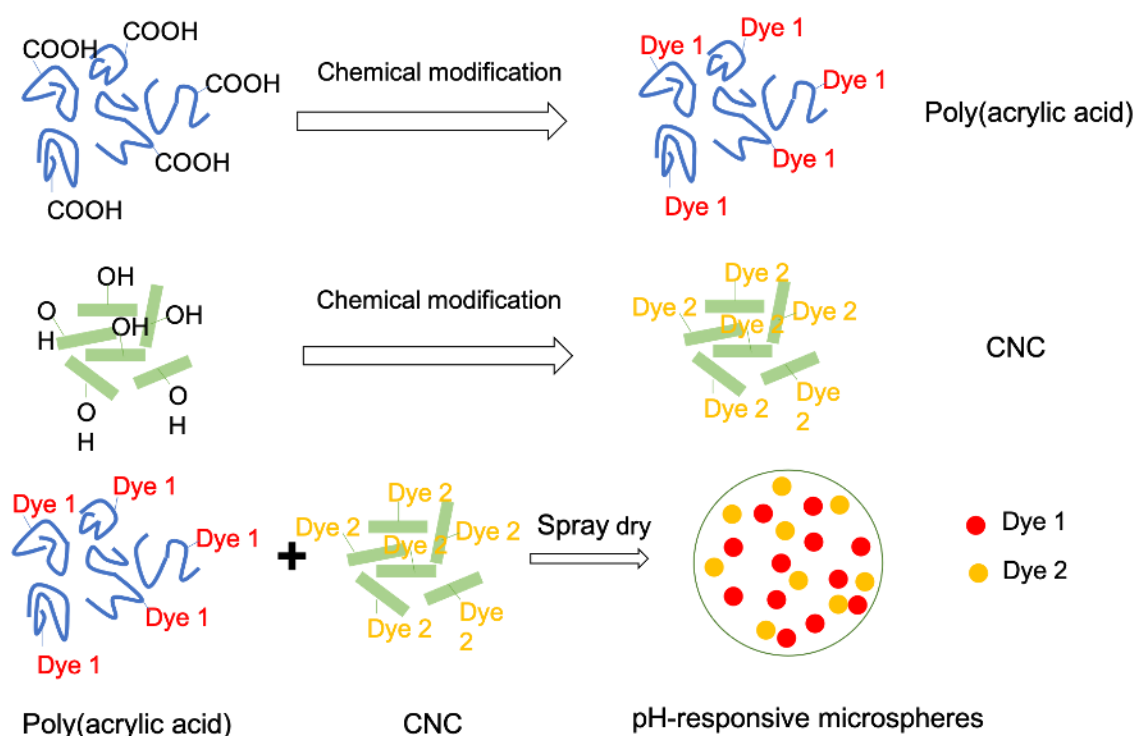
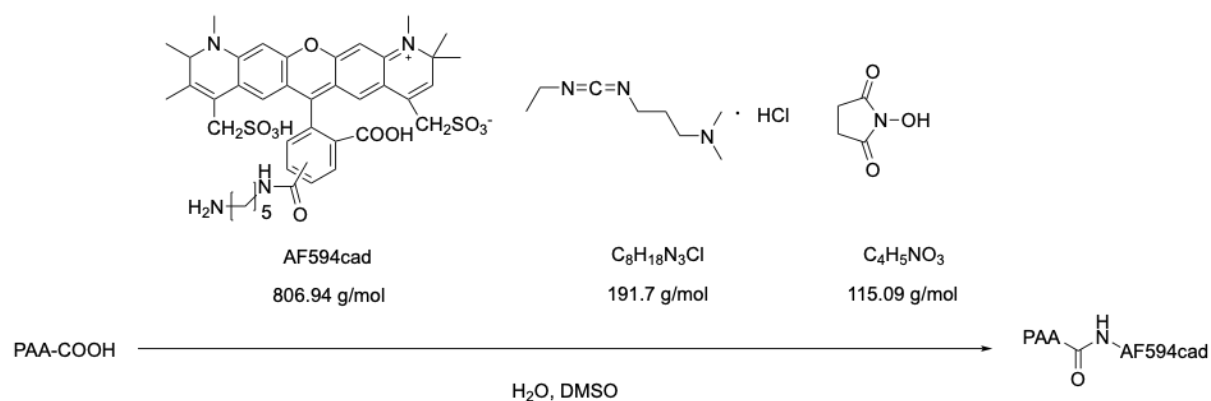
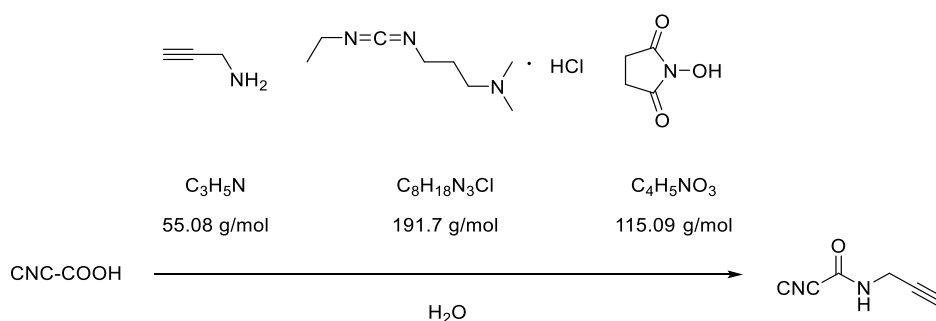


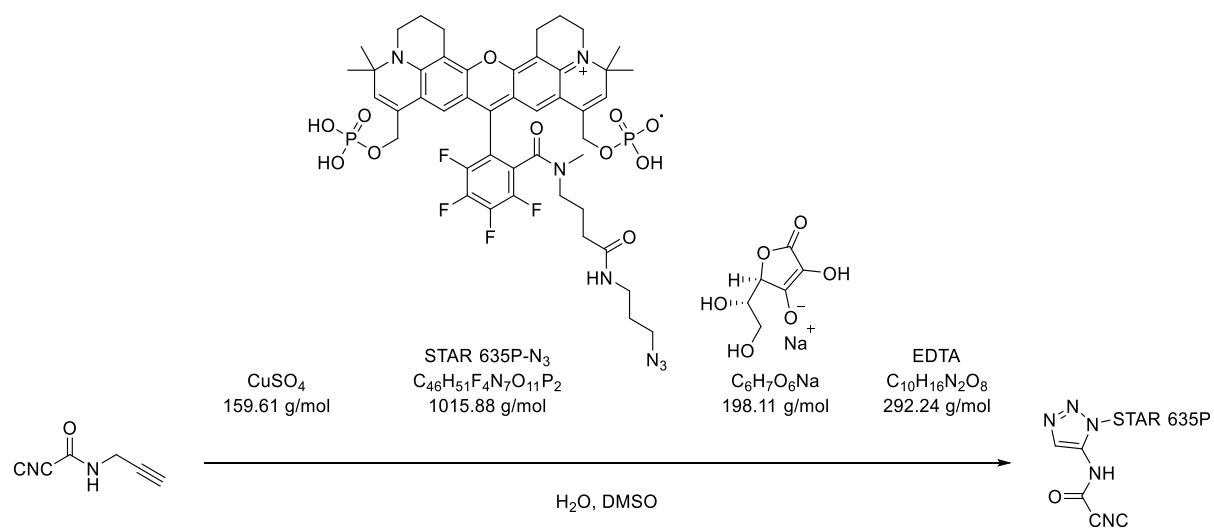
Figure 6-1 Proposed method to make cCNC-PAA microbeads with two different dye labelling.



Scheme 6-1 Functionalization of PAA with Alexa Fluor™ 594 Cadaverine.



Scheme 6-2 Alkyne functionalization of cCNC using EDC-coupling.



Scheme 6-3 Dye labelling of alkyne-functionalized cCNC with STAR 635P using Huisgen cycloaddition.

6.2.2. Response of microbead mechanical properties to local changes in environment

The time-dependence of the photo-gating [2+2] cycloaddition of coumarin in films and beads of CNC is of interest, especially as these can be linked to the time evolution of the Young's modulus. We suggest an *in situ* atomic force microscopy nanoindentation experiment, as indicated in Figure 6-2 below.

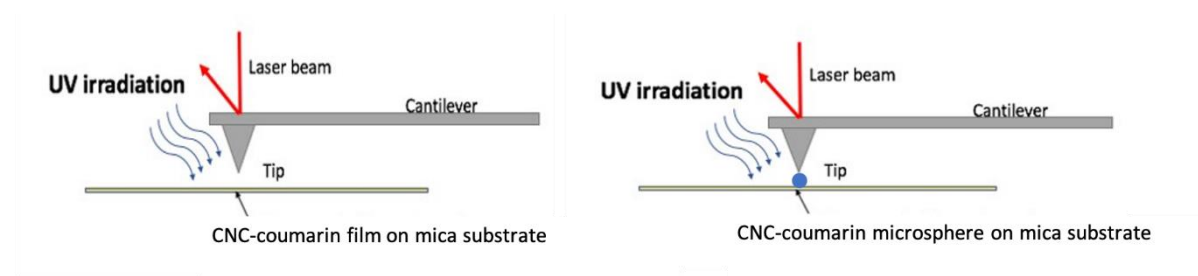


Figure 6-2 Proposed experimental set up for *in situ* AFM nanoindentation upon UV irradiation.

The microbeads are made by spray nozzle atomization and quench-condensation/evaporation. The distribution of void volume in the microbeads should be explored. Void volume will affect elasticity. The cCNC microbeads may relax over time, and it will be useful to understand how relaxations impact mechanical properties. Moreover, there is a need to understand plasticization effects and how these are linked to mechanical properties when different polymers and small molecules are combined with cCNC by spray drying. The micro-colloid AFM nanoindentation experiment should be further developed to study these effects, and others, like the time evolution of the modulus as a function of solvent exposure.

6.2.3. Microscopic level *in situ* dye mixing via optical microscopy

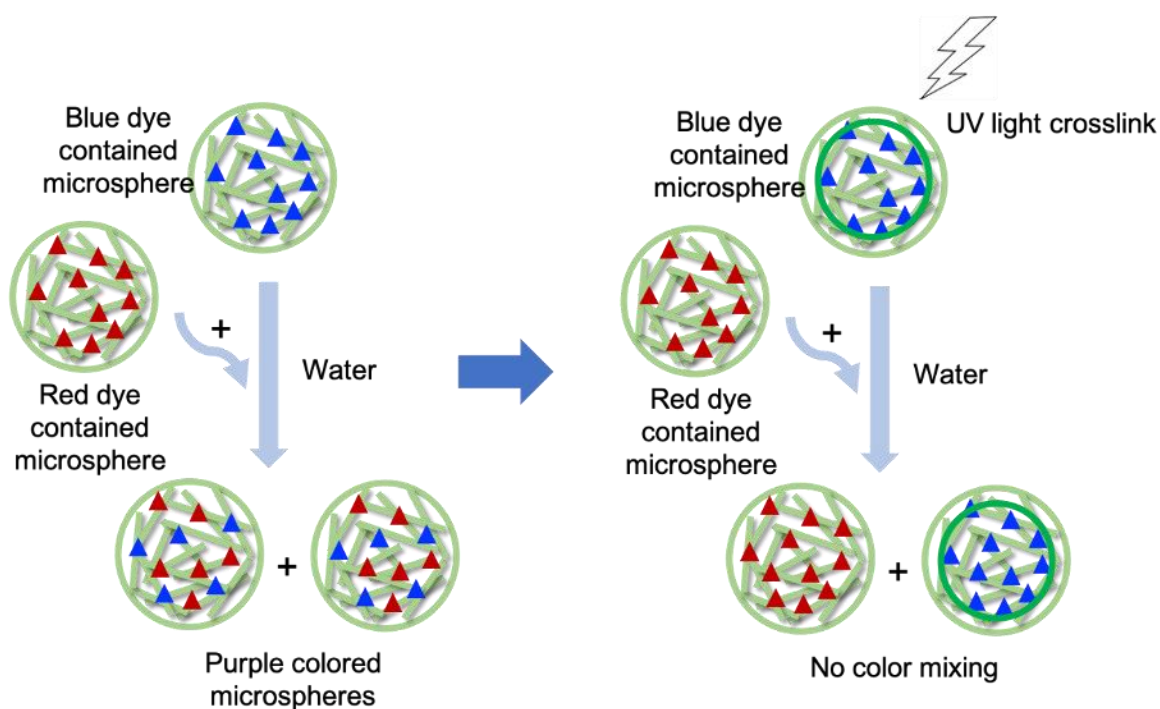


Figure 6-3 Proposed color mixing behaviors of dye contained cCNC-Cou microbeads before and after UV irradiation.

We suggest advancing the film-pore diffusion model, and possible variants, to a study of dye molecule exchange and color evolution in the cCNC microbeads. Again, one is invited to examine the photo-gating effect. Figure 6-3 offers a pathway. It is interesting to note that the method might also be viewed as an avenue to “molecularly mixed” pigment particles. The long range impact of such a study may be to quantify or suggest how the microbeads might be used as “micro-reactors” to study the impact of delivering “packaged” reagents and then having the reagents interdiffuse, react and confer new properties on the microbeads.

6.2.4. Citric acid crosslinked microbeads

Future work might examine how citric acid crosslinking affects the small molecule diffusion properties by studying methylene blue uptake kinetics following the experimental procedure in Chapter 2.

6.2.5. Iron chelation of cCNC-PAA microbeads

Cellulose finds broad application in water purification. In separate studies, we have that cCNC-PAA microbeads will bind Fe^{3+} . Figure 6-4 shows the XPS depth profile for ferric ion in neat cCNC and in cCNC-PAA microbeads. Departing from a focus on applications in water purification, we suggest expanding the research on metal ion and dye binding, not only to the cCNC-PAA microbeads but to other cCNC hybrids. Indeed, more detailed study to quantify depth profiling in the microbeads is warranted.

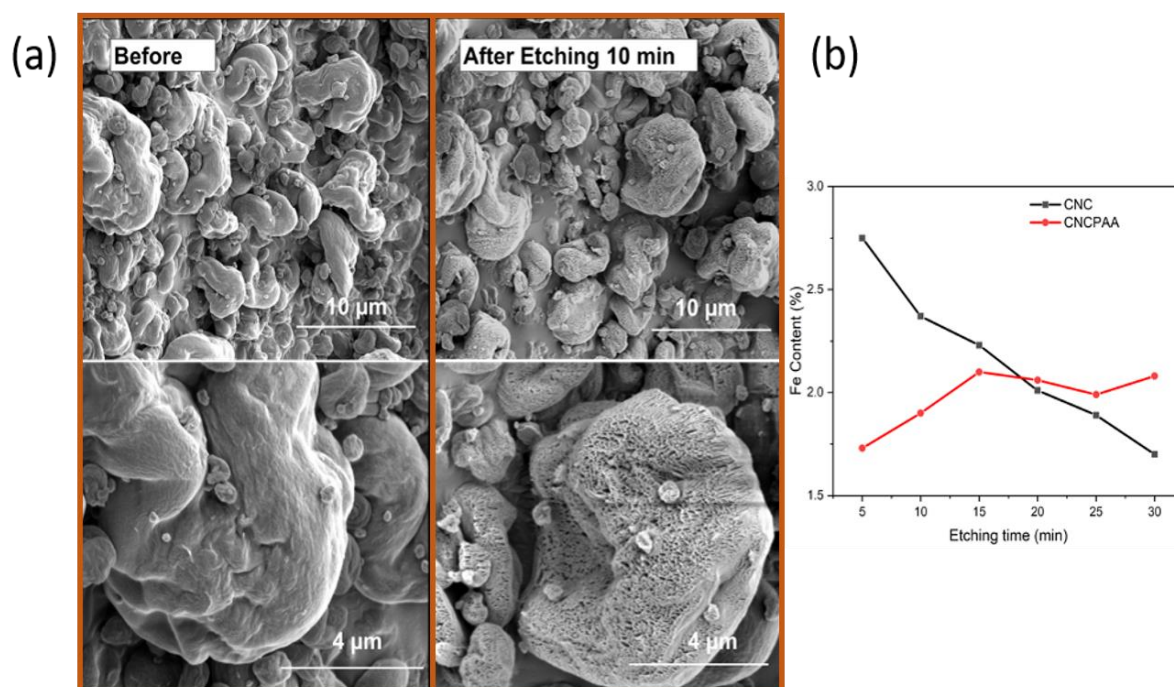


Figure 6-4 Iron chelation experiments. (a) SEM image reveals the morphology and etching texture. (b) XPS depth profiling shows distribution of Fe^{3+} in cCNC microbeads, and in cCNC-PAA microbeads.

6.2.6. Contact angle measurement using AFM

Contact angles from the sessile drop method described in this thesis give only qualitative information. We suggest extending the micro-colloid AFM experiment to measure contact

angles from individual microbeads. Ecke *et al.* describe this method as “microbead tensiometry”.¹ Figure 6-5 shows an annotated schematic AFM force curve and how it might be used to measure the contact angle of a single microbead. For this experiment, a water droplet is placed on a glass substrate. The cCNC or SF-cCNC, or some other hybrid cCNC microbead is glued to the bottom of a tipless cantilever for AFM. When approaching the the water droplet, the force curves change. At large distance, the cantilever is not deflected (Figure 6-5(A)). When the particle contacts the air-water interface, the jumps into the drop and a three-phase contact (TPC) is formed, as shown in Figure 6-5 (B). The jump-in originates from the capillary force. Moving the particle further towards the drop shifts the TPC line over the particle surface (C). Retracting the particle gives position (D), and at some point the force is high enough to draw the particle off the air-water interface (E). According to the force curve presented in Figure 6-5, the contact angle can be derived from: $\cos\theta_a^{sphere} = \frac{D_{drop}-R}{R}$.

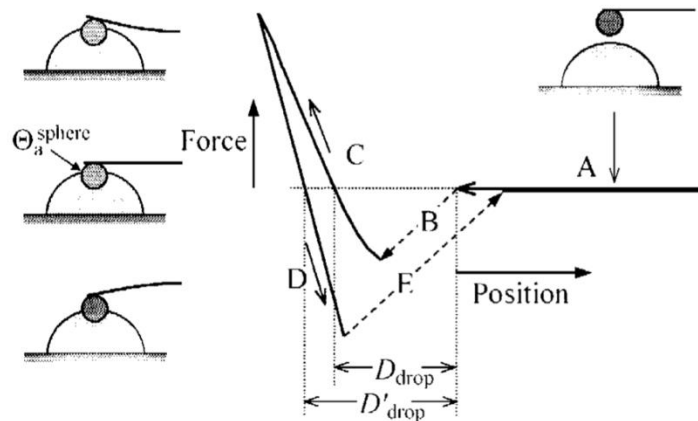


Figure 6-5 A typical force-position curve between a spherical colloidal particle and a liquid drop. (Reprint with permission from ref ¹, Rights managed by Taylor & Francis)

We have a method to attach a single microbead to the tipless cantilever. We describe the procedure with respect to a SF-cCNC microbead.

Beading experiment

SF-cCNC microbeads were glued onto tipless cantilevers using a small amount of epoxy resin with the following steps: A small amount of cCNC microbeads were dispersed in acetone. One drop of the mixture was deposited on a cover glass and dried by blowing air on the cover slip to ensure a complete disperse of microbeads on the cover glass. The cover slip with microbeads were attached to a glass substrate using one drop of water. Besides the cover slip another cover slip was attached the same way and a tiny amount of glue was deposited on the surface. The glue was dispersed as thin as possible by using a spatula. The glass substrate with two cover slips on the top was transferred to the Atomic Force Microscope. The beading experiment was conducted in a commercial AFM MFP-3D-BIO AFM instrument (Asylum Research, Santa Barbara CA) mounted on an Olympus IX-71 inverted optical microscope. A tipless cantilever was mounted in the AFM head. The cantilever back face is usually coated with a thin metallic layer (Au typically) in order to enhance the reflectivity and signal to noise. The AFM head was mounted on the stage of the inverted optical microscope so the sample and the glue can be simultaneously probed from above with the AFM tip while optically viewed from below. The AFM probe was firstly moved above the surface of the glue, and a tiny amount of glue was picked up by conducting a force indentation. Extra amount of glue was eliminated by dragging the cantilever on the surface in contact mode. Following that, the AFM probe was extracted from the surface and moved to the area containing microbeads. By moving the cantilever on the top of one targeted microbead, the microbead was picked with and glued by the resin already on the cantilever. The microbead attached cantilever was removed from the AFM head and placed in one of the containers and dried from 48 h. Attachment of the bead to the cantilever was determined both by optical and scanning electron microscopy.

The implications of perfecting the bead-tip protocols will then open manifold studies of bead–interface interactions akin to the breakthrough chemical force microscopy experiments that have been introduced in the past.

6.2.7. cCNC-based Microbeads for Drug Delivery

Through Chapter 2 to Chapter 5, we explored small molecule uptake and release properties on cCNC-based microbeads, including neat cCNC, cCNC-PAA, cCNC-cou, and SF-cCNC microbeads. These studies provided insight into how small molecules diffuse and transport in host microbeads and how external stimuli such as pH and light can regulate transport. The film-pore diffusion study provided a model for the diffusion process. Together, these studies suggest that cCNC-based microbeads may have potential as drug delivery vehicles. Future work should focus on encapsulation of hydrophobic, charged and hydrophilic drug molecules in cCNC hybrid microbeads. Procedures to regulate drug release should be developed, together with detailed pharmacokinetic and pharmacodynamic studies. Such research can quantify the potential of CNC-based drug excipients and uptake/release vehicles.

Reference

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