UV-Absorbing Colloids for Advanced Photoprotection and Functional Materials

Douglas R. Hayden

Cover: The watermark shows a Scanning Electron Microscopy (SEM) image of the cross section of UV-blocking nanopaper functionalised with UV-absorbing ethyl cellulose nanoparticles (ECNPs). The left circled image shows a SEM image of UV-absorbing ECNPs. The right circled image shows a photograph of UV-blocking nanopaper functionalised with UV-absorbing ECNPs.

Invitation Bookmark: The top left and top right circled images are the same images shown on the front cover. The bottom left circled image is a photograph of a UV laser shined through UV-blocking nanopaper onto quantum dots which fluoresce under UV stimulation. The fluorescence of the quantum dots is largely suppressed. The bottom right circled image is a SEM image of a supraparticle composed of matchstick-shaped silica particles which contain a functional head.

PhD Thesis, Utrecht University, the Netherlands, September 2019.

ISBN: 978-90-393-7167-1

Printed by: ProefschriftMaken, www.proefschriftmaken.nl

A digital version of this thesis is available at http://colloid.nl/publications/theses/

UV-Absorbing Colloids for Advanced Photoprotection and Functional Materials

UV-Absorberende Colloïden voor Geavanceerde Zonbescherming en Functionele Materialen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 4 september 2019 des middags te 12.45 uur

door

Douglas Richard Hayden

geboren op 21 augustus 1993 te Bedford, Verenigd Koninkrijk

Promotoren:

Prof. dr. K. P. Velikov Prof. dr. A. van Blaaderen

Copromotor:

Dr. A. Imhof

This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs.

Table of Contents

Chapter	1. Introduction	1
1.1.	Photoprotection and UV Light	2
1.2.	A History of Photoprotection	3
1.3.	Nanotechnology in Photoprotection	5
1.4.	Scope of this Thesis	6
1.5.	Thesis Outline	7
1.6.	References	8
Part 1.	Biobased UV-Absorbing Nanoparticles for Photoprotection	11
Chapter	2. Biobased Nanoparticles for Broadband UV Protection with Photostal	bilized UV
Tilters		13
2.1.	Introduction	14
2.2.		15
2.3.	Conclusions	19
2.4. 2.5		20
2.5.	References	21
Chantor	References	or Organic
UV Filte	rs	27
3.1	Introduction	28
3.2	Results and Discussion	29
3.3	Conclusions	35
3.4	Materials and Methods	36
3.5	Acknowledgements	37
3.6	References	38
Chapter	4. Fully Biobased UV-Absorbing Nanoparticles from Ethyl Cellulose and	Zein for
Environ	mentally Friendly Photoprotection	49
4.1	Introduction	50
4.2	Results and Discussion	51
4.3	Conclusions	57
4.4	Materials and Methods	58
4.5	Acknowledgements	59
4.6	References	59
Chapter	5. Fully Biobased Highly Transparent Nanopaper with UV-Blocking Fund	ctionality71
5.1	Introduction	72

5.2	Results and Discussion	73
5.3	Conclusion	78
5.4	Materials and Methods	78
5.5	Acknowledgements	80
5.6	References	80
Chapter 6. Photochromic Nanoparticles from Ethyl Cellulose for Smart Materials		91
6.1	Introduction	92
6.2	Results and Discussion	93
6.3	Conclusions	96
6.4	Materials and Methods	97
6.5	Acknowledgements	98
6.6	References	98
Part 2.	UV-Absorbing Composite "Matchsticks" for Functional Materials	103
Chapter	7. Seeded-Growth of Silica Rods from Titania Particles and Self-Assembly	105
7.1	Introduction	106
7.2	Results and Discussion	106
7.3	Conclusions	109
7.4	Materials and Methods	110
7.5	Acknowledgements	111
7.6	References	111
Chapter 8. Seeded-Growth of Silica Rods from Silica-Coated Particles		115
8.1.	Introduction	116
8.2.	Results and Discussion	118
8.3.	Conclusions	122
8.4.	Materials and Methods	122
8.5.	Acknowledgements	124
8.6.	References	124
Summary for the Broad Public		131
Samenvatting voor het Brede Publiek		133
Acknowledgements		136
List of Publications		138
Conference Presentations		140
About the Author		141

Chapter 1. Introduction

In this Chapter we establish the background, challenges, and motivations behind this thesis.

1.1. Photoprotection and UV Light

Photoprotection is the protection of organisms and materials from sunlight. Sunlight consists of a broad spectrum of wavelengths of light before reaching earth, in which approximately 50% of the total energy lies in the infrared region (wavelength $\lambda = 1 \text{ mm}-700 \text{ nm}$), 40% in the visible ($\lambda = 700-400 \text{ nm}$), and 10% in the UV ($\lambda = 400-1 \text{ nm}$).¹ Shorter wavelengths are higher in energy, meaning that the term "photoprotection" more specifically refers to the protection of organisms against higher-energy UV light.

UV light can be categorised into "bands" based on the wavelength of the light: UVA (λ = 400-320 nm), UVB (λ = 320-280 nm), UVC (λ = 280-100 nm), and EUV (λ = 100-1 nm) (Figure **1.1a**). However, not all these bands of UV light from sunlight actually reach the earth. The UV light in sunlight is first significantly filtered by the largest photoprotective material on earth: the ozone layer. The ozone layer absorbs all high-energy UV light with wavelengths shorter than λ = 290 nm, allowing only UVB and UVA through. Next, UV light is also heavily scattered by clouds, fog, and pollutants in the atmosphere before finally reaching the earth's surface.^{2,3} If the human eye was able to "see" UV light, then the sky would no longer appear blue. The reason the sky appears blue is because blue is the highest energy radiation we can see, and blue light – being of a higher energy than other visible light – is scattered the most through our atmosphere (Figure 1.1b). If we could see UV light then the sky would appear UV coloured. Interestingly, if we could only see UV radiation then the world would appear rather hazy, with very low visibility. This is because higher energy wavelengths such as UV light are scattered considerably, where scattering is proportional to the inverse of the wavelength to the power of 4. Some insects can actually see UV light, which has even resulted in some flowers evolving pigments to attract these pollinators (Figure 1.1c-d). All in all, less than 5% of the total sunlight that reaches the organisms on earth is UV, where the ratio of UVA to UVB is 20:1.2

UV radiation has both positive and adverse effects for organisms on earth. For humans in particular, UVB radiation is required for the formation of bone-strengthening Vitamin D.³ Moreover, UVA radiation results in an often desirable skin-darkening (tanning). However, excessive exposure to UV radiation can have devastating effects. UVA and UVB radiation are both responsible for the generation of carcinogenic reactive oxygen species (ROS) in the skin which damages DNA. UVA is more penetrative than UVB and therefore can damage DNA deeper into the skin. UVA is also responsible for the degradation of collagen networks in the skin, leading to skin aging. UVB is responsible for sunburn.³ The harmful effects of overexposure to UV are serious, with more people diagnosed with skin cancer every year than all other cancers combined.⁴

The harmful effects of UV light have resulted in many organisms evolving natural photoprotection solutions. For example, the upper cell layers of plant leaves contain aromatic sinapate ester molecules which absorb harmful UV light whilst they absorb light for photosynthesis (**Figure 1.2a**).⁵ Hippopotamuses secrete a colourless, viscous sweat consisting of aromatic monomer compounds which polymerise on the surface of the skin resulting in a colour change to red and then brown (**Figure 1.2b**).⁶ As for humans, we have developed a natural solution through melanin (**Figure 1.2c**). Melanin is a broad term for a group of natural UV-absorbing pigments produced by the body in response to exposure to UVA and UVB radiation. However, melanin alone is not sufficient to keep up with modern-day habits, such as sunbathing holidays abroad. Moreover, the need to prolong the shelf life of our foods and





Figure 1.1. (a) Visual representation of the UV and visible light spectrum within the electromagnetic spectrum. (b) The sky appears blue to us because shorter light wavelengths are scattered more strongly, and blue is the limit which our eyes can detect. If we could see UV light then the sky would appear "UV". (c, d) Photographs of the *Potentilla Reptans* flower in visible (c) and UV (d) light. Hidden information about the plant becomes obvious when observing in the UV. Photo credit: "Flower in UV light Potentilla Reptans" by Wiedehopf20, licensed under CC BY-SA 4.0.

our desire to prolong the lifetime of our dearest material possessions means that photoprotection is an increasingly important industry. Therefore, humans are continuously developing novel methods of photoprotection.

1.2. A History of Photoprotection

Humans have developed solutions to photoprotection using clothes and sunscreens. Clothes have been used from antiquity, but perhaps surprisingly, so have sunscreens. The first known use of sunscreen by humans was with the ancient Egyptians, who used extracts of rice, jasmine, and lupine plants. The ancient Greeks would lather themselves with olive oil.⁷

The year 1928 saw the introduction of the first commercial synthetic product in the US market, which consisted of an emulsion of two UV-absorbing molecules: benzyl salicylate and benzyl cinnamate.⁷ The general term describing UV-absorbing molecules such as these later became "organic UV filters". Over the next two decades, products were introduced containing more organic UV filters, including phenyl salicylate, quinine oleate, quinine bisulfate, and most notably *para*-aminobenzoic acid (PABA, **Figure 1.3**). PABA is still used today and its discovery led to the development of several currently-used organic UV filters based on *para*-benzoates. During the second world war, the US military swore by "Red Vet



Figure 1.2. Natural solutions to photoprotection. (a) Leaves contain their own sunscreen, such as UVabsorbing molecules sinapic acid (left chemical structure) and sinapoyl malate (right chemical structure). (b) Hippopotamuses 'sweat' their own sunscreen, containing red and orange pigments aptly named "hipposudoric acid" and "norhipposudoric acid" respectively. (c) Darker skinned individuals contain more UV-absorbing melanin pigment in their skin.

Pet" (Red Petroleum Jelly), a sticky red substance that functioned as a physical barrier to the sun.⁸ Red Vet Pet was then developed after the war into a more consumer-friendly substance with the addition of cocoa butter and coconut oil and sold under the name Coppertone.⁹ Sunscreens at this time were not so popular largely because of a lack of awareness of the harmful effects of the sun, but also because of their unpleasant sticky feel, tendency to stain clothes, and because many of the organic UV filters present in the formulations were allergenic.¹⁰

The 1970s and 1980s were a crucial period in the development of sunscreen. The public was becoming more aware of tanning's "dark side". Scientists started to understand the difference between UVA and UVB in terms of their effects on the body.¹¹ They realised that UVA was not well protected against and so avobenzone was synthesized as a UVA only organic filter (Figure 1.3). Then, in further efforts to enhance UVA protection, large micronsized titanium dioxide (titania, TiO₂) and zinc oxide (ZnO) particles were used in formulations. The general term describing UV-attenuating particulates such as titania and zinc oxide later became "inorganic UV filters". Inorganic UV filters were attractive because they provided effective UVA and UVB protection whilst also being less allergy-inducing than many of the organic UV filters. Despite this, these micronized inorganic UV filters were cosmetically unappealing because of white appearance of the product when applied on skin, as well as having undesirable comedogenic properties.¹² Later, nano-sized titania and ZnO were introduced, which provide more effective protection against both UVA and UVB and, importantly, are transparent when applied as a coating because they scatter visible light less effectively than their micronized counterparts.¹³ Most current photoprotection products contain a mixture of organic and inorganic UV filters.¹²

Consumer goods use similar photoprotection ingredients and strategies. For example, plastics and many textiles often incorporate organic UV filters to protect against material disintegration, phototendering, and colour degradation which all occur as a direct result of extensive UV exposure.¹⁴ These adverse effects of UV light for both consumer goods and sunscreens is demanding increasingly advanced photoprotection solutions. Therefore, the desire for greater cosmetic appearance and texture, effectiveness, durability, safety,



Figure 1.3. The chemical structures of four commonplace organic UV filters used in photoprotection.

biocompatibility (less allergenic), water resistance, and environmental properties, has driven chemists towards further developments in nanotechnology.

1.3. Nanotechnology in Photoprotection

Nanotechnology is defined as the manipulation of matter between 1 nm and 100 nm. Nanotechnology has already significantly advanced photoprotection with the development of nanosized inorganic UV filters (titania and ZnO) and improved organic UV filters. The nanosized titania and ZnO are more effective UV attenuators than larger particles, have a transparent appearance when applied as a coating, and a low allergenic response.¹³ Improved organic UV filters have been developed which are less allergenic, more photostable, and can better protect across a broad spectrum of UV. However, although nanotechnology has progressed photoprotection to a certain extent, there are still improvements to be made.

The main concerns with current photoprotection components are effectiveness, stability, safety, and environmental impact. Ideal sunscreens, for example, must: (i) provide sufficient protection for long periods of time across the complete UVA and UVB spectrum, (ii) contain components that are not harmful to humans or the environment, and (iii) do not degrade to form by-products that are harmful to humans or the environment. However, current photoprotection does not satisfy all these criteria. Regarding point (i), some organic UV filters are unstable with respect to isomers that are less efficient UV absorbers, resulting in reduced UV attenuation as a function of time.¹⁵ Regarding point (ii), organic UV filters can be allergenic,¹⁶ and also penetrate through the skin to the bloodstream where they can undesirably act as endocrine disruptors.¹⁷ Additionally, the organic UV filter oxybenzone has been linked with coral reef bleaching,¹⁸ and the inorganic UV filter titania can accumulate in coastal waters resulting in negative effects to marine organisms after ingestion.¹⁹ These environmental concerns surrounding UV filters have even resulted in the US state Hawaii passing a bill banning the sale of sunscreen containing organic UV filters oxybenzone and octinoxate.²⁰ Regarding point (iii), both organic and inorganic UV filters generate carcinogenic reactive oxygen species (ROS) when exposed to sunlight which are undesirably in direct contact with skin.²¹

Further developments in nanotechnology have the potential to address these problems. For example, one effective method to address many issues in photoprotection is by encapsulation of organic UV filters into nanoparticles (**Figure 1.4**). Encapsulation of organic UV filters into nanoparticles has been shown to stabilise the UV filters against degradation.²² Moreover, the co-encapsulation of organic UV filters with antioxidant photostabilisers into nanoparticles is a potential method of enhancing the photostabilizing effects of the antioxidant which is confined in close proximity to the organic UV filters. Additionally, encapsulation of organic UV filters into nanoparticles reduces direct UV filter to skin contact, resulting in reduced allergenic properties and reduced amounts of carcinogenic ROS reaching



Figure 1.4. A cartoon to visually describe a potential future of sunscreens. Here, nanoparticles can be used to encapsulate multiple species, including organic UV filters/UV-absorbing plant extracts and antioxidants.

the skin.²³ Another effective method to address many issues in photoprotection is to replace the current inorganic and organic UV filters with UV-absorbing plant extracts.²⁴ UV-absorbing plant extracts can potentially enhance safety and environmental impact in photoprotection.²⁴ This leads us nicely into the scope of Part 1 of this thesis.

1.4. Scope of this Thesis

1.4.1 Part 1: Biobased UV-Absorbing Nanoparticles for Photoprotection

Part 1 of this thesis is about the development of biobased UV-absorbing nanoparticles for photoprotection. We investigate the encapsulation of commonplace organic UV filters, an antioxidant photostabilizer, and UV-absorbing plant extracts into biobased nanoparticles (NPs) from ethyl cellulose. We explore whether these biobased NPs can provide effective broadband UV protection, photostabilize the organic UV filters, and form coatings. Moreover, we investigate whether we can develop advanced functional materials from these biobased UV-absorbing NPs.

1.4.2 Part 2: UV-Absorbing Composite "Matchsticks" for Functional Materials

In Part 2, we investigate a UV-absorbing colloidal particle which we have already discussed in the context of inorganic UV filters, titanium dioxide (titania, TiO₂). Titania, alongside being UV-absorbing, also has other unique properties, such as a high refractive index and catalytic activity.²⁵ The high refractive index of titania makes it very interesting for the development of novel advanced materials with optical functionality, whilst the catalytic property makes it interesting for water-splitting²⁶ and self-propelling particles.²⁷

Titania's UV-absorbing and high refractive index properties have led to its extensive usage in sunscreens, paints, food colouring, paper, and personal care products.²⁵ Titania is therefore produced in considerable quantities, with the global production exceeding 9 million

metric tons per annum.²⁸ It is mined from ore and isolated as crystalline titania primarily in the form anatase or rutile by chemical reactions. These applications use titania across a wide range of sizes, from nanometers to micrometers. However, the use of titania in it's pure form limits its potential applications. Much research has therefore been devoted to the preparation of composite materials with titania.²⁵

Composite materials with titania have many advantages. As is the case with composite materials in general, titania-based composite materials allow the combination of the unique properties of titania with effective properties of other compounds. For example, combining titania with silica (SiO₂) has shown to improve the thermal stability, dispersibility, stability of NPs against aggregation and sintering,²⁵ and optical tenability.²⁹ Improvements in the thermal stability allow the use of titania catalysts at higher temperatures, improvements in the dispersibility allow easier cosmetic formulation preparations, and improved stability of NPs against aggregation allows maintenance of the desired NP properties. Moreover, combining titania with other compounds such as MgO and ZrO₂ can allow greater catalytic activity and selectivity,³⁰ whilst combining titania with magnetic iron oxide can significantly improve recoverability of titania from reaction media.³¹ Despite the effectiveness of many titania composite nanomaterials, one limitation of all these examples is that the structures are isotropic. The design of anisotropic composite materials which incorporate titania, whilst challenging, has the potential to open up completely new materials with new applications.³² Therefore, we investigate the synthesis of composite matchstick-shaped particles with titania in Part 2 of this thesis.

1.5. Thesis Outline

In this thesis, we develop UV-absorbing colloidal particles for advanced photoprotection and functional materials. In Part 1, we develop UV-absorbing nanoparticles (size range 1-100 nm) for advanced photoprotection and functional materials. In Part 2, we develop larger UV-absorbing particles (with at least one dimension on the size range 100-1000 nm) for functional materials. Therefore, we describe both sets of UV-absorbing particles under the umbrella term "colloids", which refers to the size range 1-1000 nm.³³

1.5.1 Part 1: Biobased UV-Absorbing Nanoparticles for Photoprotection

In Chapter 2, we develop ethyl cellulose nanoparticles (ECNPs) with encapsulated commonplace UV filters from sunscreens (oxybenzone, octinoxate, and avobenzone) and an antioxidant (α -tocopherol). We show that these ECNPs can absorb UV light across the entire UVA and UVB spectrum and that these ECNPs can form effective transparent flexible coatings. Significantly, we also show that the co-encapsulation of an antioxidant with the UV filters lowers the concentration of carcinogenic reactive oxygen species inside the ECNPs. These UV-absorbing ECNPs with encapsulated photostabilizing antioxidant are potentially very interesting for more effective and safer photoprotection.

In Chapter 3, we study ECNPs for their size tunability and encapsulation of oxybenzone, octinoxate, and avobenzone. We investigate the maximum loadings of each of the UV filters into the ECNPs, and the photodegradation of the UV filters upon incorporation into the ECNPs and when encapsulated with the antioxidant α -tocopherol.

In Chapter 4, we develop nanoparticles from ethyl cellulose (ECNPs) and zein (ZNPs) with encapsulated UV-absorbing plant extracts (retinol, p-coumaric acid, and quercetin). We show that these fully-biobased UV-absorbing nanoparticles from both ethyl cellulose and zein

can absorb across the entire spectrum of UVA and UVB. We then investigate the maximum loadings of the UV-absorbing plant extracts into the ECNPs, the ability for these ECNPs to form coatings, and the extent of leakage of these UV-absorbing plant extracts from the ECNPs. These fully biobased UV-absorbing ECNPs have great potential to satisfy many issues surrounding safety and environmental concerns in photoprotection.

In Chapter 5, we develop highly transparent UV-blocking "nanopaper" materials which have great potential as sustainable replacements for plastics in food packaging. The materials are prepared by combining the UV-absorbing ECNPs developed in Chapters 2-4 with a waste source of cellulose nanofibrils. We maximise the transparency of these materials to visible light whilst maintaining complete UV-blockage. Additionally, we investigate the photostability of these materials.

In Chapter 6, we develop ethyl cellulose nanoparticles with an encapsulated photochromic dye. This colourless photochromic dye changes molecular configuration upon the absorption of UV light into a structure which is coloured. We investigate the ability for these UV-absorbing ECNPs to form "smart materials", such as colour-changing coatings and colour changing nanopaper.

1.5.2 Part 2: UV-Absorbing Composite "Matchsticks" for Functional Materials

In Chapter 7, we develop composite matchstick-shaped particles with amorphous (noncrystalline) titania "heads" and silica "tails". We investigate the tunability of the "matchsticks" in terms of their aspect ratio, and the assembly of these particles into 2D arrays and 3D supraparticles.

In Chapter 8, we extend the synthesis from Chapter 7 for the preparation of matchsticks with potentially any functionality. This synthesis is interesting for the preparation of novel particle structures with a wide variety of functionalities, which could have a very wide range of applications and fundamental interest. Moreover, this extended synthesis technique should allow the incorporation of crystalline titania forms such as anatase and rutile, which have a higher refractive index and catalytic activity than amorphous.

1.6. References

- (1) Fu, Q. Radiation (Solar). In *Encyclopedia of Atmospheric Sciences*; 2003; pp 1859–1863.
- (2) Kullavanijaya, P.; Lim, H. W. Photoprotection. J. Am. Acad. Dermatol. 2005, 52, 937–958.
- (3) Bens, G. Chapter 25. In *Sunlight, Vitamin D and Skin Cancer*; Orleans, 2014; pp 429–463.
- (4) Cancer Facts and Figures 2019 https://www.cancer.org/content/dam/cancerorg/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-factsand-figures-2019.pdf.
- (5) Dean, J. C.; Kusaka, R.; Walsh, P. S.; Allais, F.; Zwier, T. S. Plant Sunscreens in the UV-B: Ultraviolet Spectroscopy of Jet-Cooled Sinapoyl Malate, Sinapic Acid, and Sinapate Ester Derivatives. J. Am. Chem. Soc. 2014, 136, 14780–14795.
- (6) Saikawa, Y.; Hashimoto, K.; Nakata, M.; Yoshihara, M.; Nagai, K.; Ida, M.; Komiya, T. Pigment Chemistry: The Red Sweat of the Hippopotamus. *Nature* **2004**, *429*, 363.
- (7) Shaath, N. A. Sunscreens: Regulations and Commercial Development; 2005.

- (8) Kocsard, E. "Red Vet Pet." Arch. Dermatol. **2010**, *89*, 902.
- (9) Svarc, F. A Brief Illustrated History on Sunscreens and Sun Protection. *Pure Appl. Chem.* **2015**, *87*, 929–936.
- (10) Nohynek, G. J.; Schaefer, H. Benefit and Risk of Organic Ultraviolet Filters. *Regul. Toxicol. Pharmacol.* **2001**, *33*, 285–299.
- (11) Urbach, F. The Historical Aspects of Sunscreens. J. Photochem. Photobiol. B Biol. 2001, 64, 99– 104.
- (12) Mancebo, S. E.; Hu, J. Y.; Wang, S. Q. Sunscreens. *Dermatol. Clin.* **2014**, *32*, 427–438.
- (13) Chen, L. L.; Tooley, I.; Wang, S. Q. Nanotechnology in Photoprotection. In *Nanotechnology in Dermatology*; 2013; pp 9-18.
- (14) Andrady, A. L.; Hamid, S. H.; Hu, X.; Torikai, A. Effects of Increased Solar Ultraviolet Radiation on Materials. *J. Photochem. Photobiol. B.* **1998**, *46*, 96–103.
- (15) Vellejo, J. J.; Mesa, M.; Gallardo, C. Evaluation of the Avobenzone Photostability in Solvents Used in Cosmetic Formulations. *Vitae Medellin* **2011**, *18*, 63–71.
- (16) Maier, T.; Korting, H. C. Sunscreens Which and What For? *Skin Pharmacol. Physiol.* **2005**, *18*, 253–262.
- (17) Janjua, N. R.; Mogensen, B.; Andersson, A. M.; Petersen, J. H.; Henriksen, M.; Skakkebæk, N. E.; Wulf, H. C. Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor after Whole-Body Topical Application and Reproductive Hormone Levels in Humans. J. Invest. Dermatol. 2004, 123, 57– 61.
- (18) Downs, C. A.; Kramarsky-Winter, E.; Segal, R.; Fauth, J.; Knutson, S.; Bronstein, O.; Ciner, F. R.; Jeger, R.; Lichtenfeld, Y.; Woodley, C. M.; Pennington, P.; Cadenas, K.; Kushmaro, A.; Loya, Y. Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. Arch. Environ. Contam. Toxicol. 2016, 70, 265–288.
- (19) Hund-Rinke, K.; Simon, M. Ecotoxic Effect of Photocatalytic Active Nanoparticles (TiO2) on Algae and Daphnids. *Environ. Sci. Pollut. Res.* **2006**, *13*, 225–232.
- (20) American Academy of Dermatology Association statement on sunscreen access https://www.aad.org/media/news-releases/aada-statement-on-sunscreen-access.
- (21) Damiani, E.; Baschong, W.; Greci, L. UV-Filter Combinations under UV-A Exposure: Concomitant Quantification of over-All Spectral Stability and Molecular Integrity. J. Photochem. Photobiol. B Biol. 2007, 87, 95–104.
- (22) Perugini, P.; Simeoni, S.; Scalia, S.; Genta, I.; Modena, T.; Conti, B.; Pavanetto, F. Effect of Nanoparticle Encapsulation on the Photostability of the Sunscreen Agent, 2-Ethylhexyl-p-Methoxycinnamate. *Int. J. Pharm.* 2002, 246, 37–45.
- (23) Deng, Y.; Ediriwickrema, A.; Yang, F.; Lewis, J.; Girardi, M.; Saltzman, W. M. A Sunblock Based on Bioadhesive Nanoparticles. *Nat. Mater.* **2015**, *14*, 1278–1285.

- (24) Cefali, L. C.; Ataide, J. A.; Moriel, P.; Foglio, M. A.; Mazzola, P. G. Plant Compounds as Active Photo Protectants in Sunscreens. *Int. J. Cosmet. Sci.* **2016**, *38*, 346–353.
- (25) Dahl, M.; Liu, Y.; Yin, Y. Composite Titanium Dioxide Nanomaterials. *Chem. Rev.* **2014**, *114*, 9853–9889.
- (26) Mor, G. K.; Shankar, K.; Paulose, M.; Varghese, O. K.; Grimes, C. A. Enhanced Photocleavage of Water Using Titania Nanotube Arrays. *Nano Lett.* **2005**, *5*, 191–195.
- (27) Palacci, J.; Sacanna, S.; Steinberg, A. P.; Pine, D. J.; Chaikin, P. M. Living Crystals of Light-Activated Colloidal Surfers. *Science* **2013**, *339*, 936–940.
- (28) Bedinger, G. M. 2014 Minerals Yearbook: Titanium; 2014.
- (29) Jannasch, A.; Demirörs, A. F.; van Oostrum, P. D. J.; van Blaaderen, A.; Schäffer, E. Nanonewton Optical Force Trap Employing Anti-Reflection Coated, High-Refractive-Index Titania Microspheres. *Nat. Photonics* **2012**, *6*, 469–476.
- (30) Burri, D. R.; Choi, K. M.; Han, S. C.; Burri, A.; Park, S. E. Selective Conversion of Ethylbenzene into Styrene over K2O/TiO2-ZrO2 Catalysts: Unified Effects of K2O and CO2. J. Mol. Catal. A Chem. 2007, 269, 58–63.
- (31) Lu, Z.; Duan, J.; He, L.; Hu, Y.; Yin, Y. Mesoporous TiO2 Nanocrystal Clusters for Selective Enrichment of Phosphopeptides. *Anal. Chem.* **2010**, *82*, 7249–7258.
- (32) Datskos, P.; Cullen, D. A.; Sharma, J. Step-by-Step Growth of Complex Oxide Microstructures. *Angew. Chemie - Int. Ed.* **2015**, *54*, 9011–9015.
- (33) Hunter, R. J. Foundations of Colloid Science, 2nd ed.; Oxford University Press, 2001.

Part 1

Biobased UV-Absorbing Nanoparticles for Photoprotection

Chapter 2. Biobased Nanoparticles for Broadband UV Protection with Photostabilized UV filters

Sunscreens rely on multiple compounds to provide effective and safe protection against UV radiation. UV filters in sunscreens, in particular, provide broadband UV protection but are heavily linked to adverse health effects due to the generation of carcinogenic skin-damaging reactive oxygen species (ROS) upon solar irradiation. Herein, we demonstrate significant reduction in the ROS concentration by encapsulating an antioxidant photostabilizer with multiple UV filters into biobased ethyl cellulose nanoparticles. The developed nanoparticles display complete broadband UV protection and can form transparent and flexible films. This system therefore shows significant potential toward effective and safe nanoparticle-based UV protective coatings.

2.1. Introduction

An essential issue within consumer products is protection against ultraviolet (UV) radiation. UV protection within food and packaging materials is required for retarding chemical degradation, whereas UV protection within personal care products (i.e., sunscreens) is imperative for the preservation of human health as excessive exposure to UV-radiation accounts for the vast majority of skin cancers.^{1–3}

The main requirements and challenges in the preparation of UV-protective coatings are (i) to provide broad protection over the entire UVA/UVB spectrum, and (ii) to maintain photo-stability after extended periods of irradiation.^{4–6} Currently, broad UV spectrum protection is realized via the use of multiple organic UV filters (e.g. avobenzone, octinoxate, oxybenzone, padimate-O, octocrylene) together in sunscreen formulations where the UV filters are solubilized via emulsions. There is, however, considerable concern regarding the production of carcinogenic reactive oxygen species (ROS) by organic UV filters because of photo-degradation when exposed to sunlight.^{7,8} To address the issue, antioxidants (i.e., α tocopherol) are added to sunscreen formulations in order to scavenge generated ROS, thus providing photostabilization.^{9–11} Minimising skin contact with UV filters is also desirable due to concern over adverse effects caused by systemic absorption of UV filters penetrating the skin.^{12,13} This has led to interest involving encapsulation into nanoparticles to reduce their (photo)-toxicity.^{14–17} Encapsulation into nanoparticles provides further advantages such as the amount of UV filter added to a formulation is no longer limited by its solubility in the solvent/vehicle,¹⁸ photodegradation can be stymied,¹⁵ and the need for unnecessary chemicals (i.e., surfactants, organic solvents) is reduced. So far, encapsulation has been most popular using materials such as solid–lipid nanoparticles (SLNs),¹⁹ poly(D,L-lactide) particles,^{12,15} and silica.^{14,16,20,21} Notably, SLNs have shown to be suitable carriers for encapsulating UV filter "couples", two UV filters into the same carrier, in order to provide broader UV spectrum protection.²² Nanoparticle-based UV-protective coatings still, however, need to meet the requirements currently faced by sunscreens. Therefore, there is a need to develop nanoparticles which can effectively encapsulate multiple UV filters and antioxidants all together, regardless of their initial physical states (liquid/solid), in order to provide broadband UV spectrum protection and vital photostabilization concerning protection against ROS. Furthermore, the developed nanoparticles should be biobased for maximum cosmetic appeal and also suitable for multiple solvent systems. Nanoparticles that can be dispersed in multiple solvent systems offer versatility for usage within many different sunscreen formulation types (e.g., oil, emulsion). SLNs for instance, although biobased, are not suitable for oil-based formulations as they will simply dissolve.

Herein, we therefore demonstrate the encapsulation of multiple UV filters together with an antioxidant into biobased and environmentally benign nanoparticles designed from ethyl cellulose (EC). EC is a material with potential for use within many solvent systems. We show that broadband UV spectrum protection can be achieved and that the concentration of ROS within the ethyl cellulose nanoparticles (ECNPs) is reduced upon the encapsulation of an antioxidant. Furthermore, considering the application of UV-protective coatings we show the ability to form uniform, transparent, flexible, UV protective coatings from the ECNPs.

2.2. Results and Discussion

ECNPs, with a desirable size for cosmetic applications (<100 nm) were prepared using a modified "anti-solvent precipitation" procedure of that from literature.²³ This was chosen primarily for its up-scalable potential. Here, ethyl cellulose was dissolved in ethanol before being poured into a large volume of water resulting in spontaneous formation of ECNPs. Removal of the ethanol (and some water) by rotary evaporation resulted in a stable aqueous dispersion of ECNPs with an average size of 50 nm and narrow distribution (**Figure S2.1**).

We then investigated the encapsulation of three commonplace UV filters separately, which together span the entire UVA/UVB spectrum (oxybenzone, avobenzone, and octinoxate), into ECNPs. The encapsulation procedure relies on the coprecipitation of the hydrophobic UV filters together with the EC. To physically encapsulate UV filters into ECNPs, the individual UV filter was dissolved with the ethyl cellulose in ethanol before undergoing the antisolvent precipitation to form ECNPs with encapsulated UV filter. For each of the three model UV filters the encapsulation efficiency and the maximum amount of UV filter encapsulated into ECNPs were explored. To explore this, we prepared a series of dispersions



Figure 2.1. (a-c) Absorption spectra for a series of ECNPs prepared in which varying amounts of UV filter (a) octinoxate, (b) oxybenzone, and (c) avobenzone have been added to the synthesis. The concentration of all aqueous ECNPs dispersions measured here are equal $(5 \times 10^{-2} \text{ g L}^{-1})$. "50%" is a weight percentage indicating, for example, 0.5 g UV filter was added to 1 g of EC. (d) TEM image of ECNPs with encapsulated octinoxate. Scale bar 500 nm.

2



Figure 2.2. (a, b) Absorption spectra of ECNPs with equivalent amounts (by weight) of encapsulated UV filters octinoxate, oxybenzone, and avobenzone, (a) individually and (b) together. (c, d) Absorption spectra of ECNPs containing these three UV filters and an antioxidant (α -tocopherol (λ = 288 nm)) all encapsulated together, (c) in equivalent amounts (by weight) and (d) with more α -tocopherol relative to the UV filters (mass ratio 1:1:1:10). Increasing the mass ratio as in (d) accentuates the presence of the α -tocopherol.

for each UV filter in which increasing amounts of the UV filter were added to the synthesis along with the EC. To clarify, the amount of EC used in the synthesis was always kept constant but the amount of UV filter was varied. **Figure 2.1** shows three absorption spectra, one for each UV filter series, for the resulting aqueous dispersions of ECNPs. The spectra clearly show the efficient encapsulation of all UV filters tested. There is a general trend that the more UV filter initially added to the synthesis the more encapsulated, which is intuitive. However, in the two series involving encapsulating avobenzone and oxybenzone the maximum absorbance appears to level off for the addition of greater amounts of UV filter. This leveling off indicates that the ECNPs become completely saturated with UV filter to a point in which they cannot encapsulate any greater amounts. This result is consistent with the experimental observation that increasing amounts of nondispersed aggregates were seen. Transmission electron microscopy (TEM) imaging (**Figure 2.1d**) and dynamic light scattering (DLS) measurements (**Figure S2.1**) indicated no change in morphology/size of the nanoparticles upon encapsulation of the UV filters.

To provide full UV spectrum coverage with the ECNPs, we prepared ECNPs with multiple UV filters encapsulated together. To achieve this, the same antisolvent precipitation technique used before to encapsulate an individual UV filter was implemented. Thus, equal

2

amounts (by weight) of the three UV filters (oxybenzone, avobenzone, octinoxate) were dissolved with EC in ethanol before pouring into water. After evaporation of the ethanol, an aqueous dispersion of ECNPs with multiple UV filters encapsulated was prepared. From the spectra in **Figure 2.2a-b**, it is clear that by encapsulating all three UV filters together the ECNPs provide protection across the entire UVA/UVB spectrum ($\lambda = 290-380$ nm).

To address the issue of large concentrations of ROS generated by UV filters when irradiated by sunlight, we encapsulated an antioxidant along with the UV filters. We chose α -tocopherol as the model antioxidant to be encapsulated as a result of the many independent studies demonstrating the photostabilizing effect when used in combination with UV filters.^{9,10,24} α -tocopherol has an absorption maximum at λ = 288 nm, which overlaps with that of octinoxate and oxybenzone but is far less prominent. Despite this, however, its presence can be seen by these absorption spectra since there is a slight absorbance increase at λ = 288 nm (**Figure 2.2c**), which becomes more prominent upon the addition of more α -tocopherol (**Figure 2.2d**). We found these ECNPs with encapsulated UV filters and antioxidant maintained a high level of stability with respect to the loss of absorbance as a function of time when irradiated by artificial sunlight (**Figure S2.2**).

We devised an experiment to test whether the encapsulated antioxidant reduced the concentration of carcinogenic ROS within the ECNPs by using the ROS scavenger 9,10-diphenylanthracene (DPA). DPA is well-known as a ROS – in particular, singlet oxygen species (${}^{1}O_{2}$) – scavenger, used for the quantification of ${}^{1}O_{2}$.²⁵ DPA absorbs UV light whereas its reaction product with ${}^{1}O_{2}$ (DPA-endoperoxide) does not (**Scheme 2.1**), so monitoring the degradation of the absorbance of DPA at λ_{max} = 376 nm indirectly provides an indication of the amount of ${}^{1}O_{2}$ species produced.

Scheme 2.1. Reaction of Diphenylanthracene (DPA) with a Singlet Oxygen Species to Form DPA-Endoperoxide.



An aqueous dispersion of ECNPs with encapsulated DPA was irradiated by artificial sunlight. DPA was found to degrade significantly under UV light itself. We found that encapsulating the UV-filter octinoxate – known to produce ${}^{1}O_{2}{}^{26}$ – along with DPA did result in even greater degradation, although this was a relatively small increase. We demonstrated that the encapsulation of the antioxidant significantly suppresses this degradation (**Figure 2.3a-b**), and that a greater amount of encapsulated antioxidant results in slightly greater suppression (**Figure 2.3c**). Therefore, we demonstrated that the encapsulation of an antioxidant reduces the concentration of carcinogenic ${}^{1}O_{2}$ within the ECNPs.



Figure 2.3. (a, b) Absorption spectra showing the degradation of the absorbance of singlet oxygen (${}^{1}O_{2}$) scavenger DPA encapsulated into ECNPs as a function of time when irradiated by artificial sunlight. DPA itself degrades under UV-irradiation to a certain extent but also due to the reaction with ${}^{1}O_{2}$ to form DPA-endoperoxide (**Scheme 2.1**) which unlike DPA does not absorb in the 500-300 nm range. The decrease in absorbance at $\lambda = 376$ nm therefore is an indication for the amount of ${}^{1}O_{2}$ in the ECNPs. (a) ECNPs contain DPA and octinoxate only (mass ratio 1:1) and significant degradation at $\lambda = 376$ nm is observed. (b) ECNPs contain DPA, octinoxate, and α -tocopherol (mass ratio 1:1:1) and this degradation at $\lambda = 376$ nm is considerably suppressed as a result of the α -tocopherol presence reducing the concentration of ${}^{1}O_{2}$. (c) displays these degradations from a and b as a percentage as a function of time, as well as cases not shown in which the degradation was monitored for (i) ECNPs with DPA only and (ii) ECNPs with DPA, octinoxate, and α -tocopherol but with a greater amount of α -tocopherol than in b.

Finally, transparent, flexible and uniform UV-protective coatings were prepared by spin coating a concentrated dispersion (290 g L⁻¹) of the ECNPs containing encapsulated UV filters avobenzone, oxybenzone, and octinoxate in equal amounts onto plasma-cleaned glass microscope coverslips at 1500 rpm for 1 min (**Figure 2.4a**). We found that coating subsequent layers upon the original layer – a well-established technique in spin coating – allowed complete flexibility to tune the coating UV protection that we desired (**Figure 2.4b**). We observed that the coating remained transparent to visible light when the glass was coated with multiple layers, but was effective in blocking out UV light (**Figure 2.4c** and **Figure 2.4d**). Furthermore, the coating is completely flexible (**Figure 2.4e**) in contrast with sol–gel inorganic coatings,²¹ rendering it attractive for applications in, for example, UV protective food



Figure 2.4. (a) Photo of a coated glass coverslip with a transparent, uniform, UV-protective coating. (b) Absorption spectrum showing the absorbance exhibited when multiple layers are spin coated. Wavelengths lower than λ =290 nm are not shown as they are absorbed by the glass coverslip. (c, d): Three UV-induced (by a λ =375 nm LED) fluorescent squares in which the middle square has either: (c) a plain glass cover slip or (d) our multiple-coated glass coverslip on top of it. The middle square with the UV-protective coated glass slide (border indicated by the dashed line) appears considerably darker than that of the plain glass slide because the coating – containing encapsulated UV filters in the ECNPs – blocks UV light from passing through it and stimulating the square to fluoresce. The top and bottom squares are references. (e) Photo of a coated rectangular glass coverslip with an intact coating upon bending, showing that the coating is flexible.

packaging materials. By SEM (scanning electron microscopy) imaging we observed that a three-layer coating was 235 ± 18 nm thick (**Figure S2.3**); therefore, we deduced that each coating layer was 78 ± 6 nm thick, assuming each layer contributes an equal amount to the overall coating. Thus, to get a coating that filters 90% of UVA-light (sun protection factor (SPF) =10) at, for example, λ = 320 nm (Absorbance = 0.68 A.U. for three-layer coating), a coating thickness of 391 nm is required.

2.3. Conclusions

In conclusion, the encapsulation of multiple UV filters along with a photostabilizing antioxidant into nanoparticles designed from ethyl cellulose (ECNPs) was demonstrated. Importantly, the addition of this antioxidant photostabilizer (α -tocopherol) showed significant reduction of the concentration of carcinogenic ROS within the ECNPs, known to be produced by UV filters. These nanoparticle carriers effectively encapsulated all the UV filters

2

(oxybenzone, avobenzone, octinoxate) and the antioxidant tested resulting in ECNPs with complete UVA/UVB protection and photostabilized UV filters. Moreover, considering that these nanoparticles are interesting for the application of UV protective coatings, the ability for these nanoparticles to form transparent, uniform, flexible, UV-protective coatings with tunable thicknesses and SPF were also demonstrated. As interest for nanoparticle use within sunscreens grows because of the substantial concerns associated with UV filter–skin contact, it must be demonstrated that suitable options exist that fulfill the requirements. Here, we present a step toward realizing this goal.

2.4. Materials and Methods

Materials

Ethyl cellulose (EC) degree of substitution 2.1-2.6 (100 cP, lot number MKBT0521V), oxybenzone (98%, solubility in water 69 mg/L at 25°C (PubChem database)), avobenzone (\geq 99%, solubility in water 2.2 mg/L at 25°C (PubChem database)), octinoxate (98%, solubility in water 0.4 mg/L at 24°C (PubChem database)), antioxidant α -tocopherol (\geq 95%), and diphenylanthracene (DPA, 97%) were all purchased from Sigma Aldrich. Ethanol (100%) was purchased from Interchema and pure water was used from a Millipore system.

Preparation of ECNPs

ECNPs were synthesised via a modified anti-solvent technique as reported by Bizmark *et al.*²³ A 5 g L⁻¹ solution of ethyl cellulose in ethanol was prepared and decanted into three times as much water. Rotary evaporation removed ethanol and some water resulting in an aqueous dispersion of ECNPs. The concentration was then set at 5 g L⁻¹ by gravimetric analysis and then adjustments with water. This was then passed through a 2 μ m filter to remove any aggregate.

Loading of UV-filters and an Antioxidant into ECNPs

The UV-filters oxybenzone, avobenzone, octinoxate and the antioxidant α -tocopherol were encapsulated by dissolving them into the original ethanol solution with ethyl cellulose, before repeating the anti-solvent precipitation procedure.

Measurement of ROS reduction in ECNPs

ENCPs with encapsulated: (i) DPA; (ii) DPA and UV-filter; (iii) DPA, UV-filter and antioxidant; were all prepared via the anti-solvent precipitation as described above. The dispersions were diluted and added to a quartz cuvette sealed with a Teflon stopper. The cuvette was subjected to irradiation by a 75 W Xenon lamp at a distance of 20 cm (a flux of 3 mW cm⁻² between 300 and 400 nm). The absorbance was measured hourly for four hours. The total UV dose was 432 kJ m⁻²; equivalent to 2 hours 24 minutes of summer sunlight in Nice at noon.²⁷

Preparation of UV-Protective Coatings

A concentrated dispersion of the ECNPs containing encapsulated avobenzone, oxybenzone, and octinoxate (42 g L^{-1} , 300μ L) was spin-coated onto plasma-cleaned glass microscope cover slips (No. 1, 22mm) at 1500 rpm for one minute. Additional layers were spin coated onto the original coating layer to increase the coating thickness. Coating thickness was determined by breaking the coated cover slip in half and imaging the broken slide placed vertically in the SEM.

Measurements and characterizations

Aqueous ECNP dispersions were characterized by: TEM (Philips TECNAI12 electron microscope), spectrophotometry (HP 8452a), SEM (FEI XL30FEG) and DLS (Zetasizer Nano ZS, Malvern). Coatings were prepared with a spin-coater (SCS P6700) and the coating thickness images were analyzed with ImageJ. Photographs of fluorescent squares were taken using a DigiEye (VeriVide). The photographs of the cover slips with transparent and flexible coatings were taken with a Nikon D70.

2.5. Acknowledgments

This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs. We thank Chris Schneijdenberg and Dave van den Heuvel for technical assistance. We also thank Wiebke Albrecht and Tonnishtha Dasgupta for careful reading of the manuscript.

2.6. References

- (1) Corbyn, Z. Lessons from a Sunburnt Country. *Nature* **2014**, *515*, S114.
- (2) Stavros, V. G. Photochemistry: A Bright Future for Sunscreens. *Nat. Chem.* **2014**, *6*, 955–956.
- Viros, A.; Sanchez-Laorden, B.; Pedersen, M.; Furney, S. J.; Rae, J.; Hogan, K.; Ejiama, S.; Girotti, M. R.; Cook, M.; Dhomen, N.; Marais, R. Ultraviolet Radiation Accelerates BRAF-Driven Melanomagenesis by Targeting TP53. *Nature* 2014, *511*, 478–482.
- (4) Morabito, K.; Shapley, N. C.; Steeley, K. G.; Tripathi, a. Review of Sunscreen and the Emergence of Non-Conventional Absorbers and Their Applications in Ultraviolet Protection. *Int. J. Cosmet. Sci.* **2011**, *33*, 385–390.
- (5) Bens, G. Chapter 25. In *Sunlight, Vitamin D and Skin Cancer*; Orleans, 2014; pp 429–463.
- (6) Mancebo, S. E.; Hu, J. Y.; Wang, S. Q. Sunscreens: A Review of Health Benefits, Regulations, and Controversies. *Dermatol. Clin.* **2014**, *32*, 427–438.
- (7) Biba, E. The Sunscreen Pill. *Nature* **2014**, *515*, S124-125.
- (8) Damiani, E.; Baschong, W.; Greci, L. UV-Filter Combinations under UV-A Exposure: Concomitant Quantification of over-All Spectral Stability and Molecular Integrity. J. Photochem. Photobiol. B Biol. 2007, 87, 95–104.
- (9) Oresajo, C.; Yatskayer, M.; Galdi, A.; Foltis, P.; Pillai, S. Complementary Effects of Antioxidants and Sunscreens in Reducing UV-Induced Skin Damage as Demonstrated by Skin Biomarker Expression. *J. Cosmet. Laser Ther.* **2010**, *12*, 157–162.
- (10) Kockler, J.; Oelgemöller, M.; Robertson, S.; Glass, B. D. Photostability of Sunscreens. J. *Photochem. Photobiol. C Photochem. Rev.* **2012**, *13*, 91–110.
- (11) González, S.; Fernández-Lorente, M.; Gilaberte-Calzada, Y. The Latest on Skin Photoprotection. *Clin. Dermatol.* **2008**, *26*, 614–626.
- (12) Deng, Y.; Ediriwickrema, A.; Yang, F.; Lewis, J.; Girardi, M.; Saltzman, W. M. A Sunblock Based

on Bioadhesive Nanoparticles. Nat. Mater. 2015, 14, 1278-1285.

- (13) Janjua, N. R.; Mogensen, B.; Andersson, A. M.; Petersen, J. H.; Henriksen, M.; Skakkebæk, N. E.; Wulf, H. C. Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor after Whole-Body Topical Application and Reproductive Hormone Levels in Humans. J. Invest. Dermatol. 2004, 123, 57– 61.
- (14) Tolbert, S. H.; McFadden, P. D.; Loy, D. A. New Hybrid Organic/Inorganic Polysilsesquioxane– Silica Particles as Sunscreens. *ACS Appl. Mater. Interfaces* **2016**, *8*, 3160–3174.
- (15) Perugini, P.; Simeoni, S.; Scalia, S.; Genta, I.; Modena, T.; Conti, B.; Pavanetto, F. Effect of Nanoparticle Encapsulation on the Photostability of the Sunscreen Agent, 2-Ethylhexyl-p-Methoxycinnamate. *Int. J. Pharm.* **2002**, *246*, 37–45.
- (16) Lapidot, N.; Gans, O.; Biagini, F.; Sosonkin, L.; Rottman, C. Advanced Sunscreens: UV Absorbers Encapsulated in Sol-Gel Glass Microcapsules. *J. Sol-Gel Sci. Technol.* **2003**, *26*, 67–72.
- (17) Jiménez, M. M.; Pelletier, J.; Bobin, M. F.; Martini, M. C. Influence of Encapsulation on the in Vitro Percutaneous Absorption of Octyl Methoxycinnamate. *Int. J. Pharm.* **2004**, *272*, 45–55.
- (18) Herzog, B.; Hüglin, D.; Borsos, E.; Stehlin, A.; Luther, H. New UV Absorbers for Cosmetic Sunscreens - A Breakthrough for the Photoprotection of Human Skin. *Chimia* 2004, 58 (7–8), 554–559.
- (19) Wissing, S. A.; Muller, R. H. Solid Lipid Nanoparticles as Carrier for Sunscreens: In Vitro Release and in Vivo Skin Penetration. *J. Control. Release* **2002**, *81*, 225–233.
- (20) Kim, S. S.; Kim, V.; Kim, Y. B. Preparation and Characterization of Polysilsesquioxane Particles Containing UV-Absorbing Groups. *Macromol. Res.* **2012**, *20*, 437–446.
- (21) Cui, H.; Zayat, M.; Parejo, P. G.; Levy, D. Highly Efficient Inorganic Transparent UV-Protective Thin-Film Coating by Low Temperature Sol-Gel Procedure for Application on Heat-Sensitive Substrates. *Adv. Mater.* **2008**, *20*, 65–68.
- (22) Niculae, G.; Badea, N.; Meghea, A.; Oprea, O.; Lacatusu, I. Coencapsulation of Butyl-Methoxydibenzoylmethane and Octocrylene into Lipid Nanocarriers: UV Performance, Photostability and in Vitro Release. *Photochem. Photobiol.* **2013**, *89*, 1085–1094.
- (23) Bizmark, N.; Ioannidis, M. a.; Henneke, D. E. Irreversible Adsorption-Driven Assembly of Nanoparticles at Fluid Interfaces Revealed by a Dynamic Surface Tension Probe. *Langmuir* 2014, 30, 710–717.
- (24) Abdassah, M.; Aryani, R.; Surachman, E.; Muchtaridi, M. In-Vitro Assessment of Effectiveness and Photostability Avobenzone in Cream Formulations by Combination Ethyl Ascorbic Acid and Alpha Tocopherol Acetate. *J. Appl. Pharm. Sci.* **2015**, *5*, 070–074.
- (25) Steinbeck, M. J.; Khan, A. U.; Karnovsky, M. J. Extracellular Production of Singlet Oxygen by Stimulated Macrophages Quantified Using 9,10-Diphenylanthracene and Perylene in a Polystyrene Film. J. Biol. Chem. 1993, 268, 15649–15654.

- (26) Allen, J. M.; Gossett, C. J.; Allen, S. K. Photochemical Formation of Singlet Molecular Oxygen
 (102) in Illuminated Aqueous Solutions of p-Aminobenzoic Acid (PABA). J. Photochem.
 Photobiol. B Biol. 1996, 32, 33–37.
- Séite, S.; Moyal, D.; Richard, S.; De Rigal, J.; Lévêque, J. L.; Hourseau, C.; Fourtanier, A. Mexoryl[®]
 SX: A Broad Absorption UVA Filter Protects Human Skin from the Effects of Repeated Suberythemal Doses of UVA. *J. Photochem. Photobiol. B Biol.* **1998**, *44*, 69–76.

Appendix

Characterization of ECNPs

DLS measurements (Figure S2.1a) showed a monodal size distribution with z-average 50 nm. TEM (Figure S2.1b) imaging was consistent with this observation. Cryo-TEM imaging was performed to capture a snapshot of the particles as they would exist in dispersion (Figure S2.1c). The cryo-TEM images were also consistent with the size measurements from the DLS.



Figure S2.1. (a) DLS measurement of the aqueous dispersion of ECNPs, (b) TEM image of the ECNPs. Scale bar 500 nm. (c) cryo-TEM image of the ECNPs. Scale bar 200 nm.

Photostability measurements of ECNPs with encapsulated UV-filters and antioxidant

ECNPs with encapsulated UV-filters and antioxidant (oxybenzone, avobenzone, octinoxate, α -tocopherol, (1:1:1:1 mass ratio)) were irradiated by artificial sunlight in an identical way as for the ROS measurements. We found that the absorbance degrades slightly but only by 20% (measured from the degradation at 292 nm, **Figure S2.2**) after 4 hours of irradiation under artificial sunlight (equivalent to 2 hours 24 minutes of summer sunlight in Nice at noon²⁷).



Figure S2.2. Absorption spectrum showing the degradation of the absorbance of the three UV-filters and antioxidant encapsulated together into ECNPs as a function of time when irradiated by artificial sunlight.

Analysis of coating thickness

The thickness of the coating was measured via SEM imaging, where the coated microscope slide was broken in half and the broken edge was imaged from directly above. The edge of the glass was visible and a small coating layer upon it (**Figure S2.3a**). **Figure S2.3b-c** show the surface of the broken coated microscope slide at greater magnification and a small coating layer is visible. The thickness of the layer was determined by ten measurements at different points across the layer using ImageJ. **Figure S2.3d** is an image of the surface of the coated microscope slide. At this magnification some artefacts become apparent but to the human eye the coating is uniform.



Figure S2.3. SEM images of the microscope slide with ECNP coating broken in half in which the coating thickness was determined. (a) The broken edge of the microscope slide is imaged from directly above at low magnification. (b) The edge is imaged at higher magnification and a thin coating layer is present. (c) The edge at a different area along the broken slide edge is imaged at high magnification. (d) The surface of the coating is imaged at low magnification and appears uniform despite some artifacts.

Chapter 3. Size and Optically Tunable Ethyl Cellulose Nanoparticles as Carriers for Organic UV Filters

Optically active nanoparticles (NPs) are potential building blocks for bottom-up functional technology in applications such as displays, sensors, and sunscreens. For sunscreens in particular, NPs can be used as delivery systems for organic UV filters in order to minimise skin exposure to these molecules. Here, we investigate the synthesis of size-tunable ethyl cellulose NPs (ECNPs) and their application as carriers for multiple organic UV filters. We prepared ECNPs with sizes of 50 to 165 nm via an antisolvent precipitation technique and investigate the incorporation of three commonplace organic UV filters – oxybenzone, avobenzone, and octinoxate – into the ECNPs. We found the particle loading varied greatly with each UV filter. Photodegradation of the UV filters remained unchanged upon incorporation into ECNPs and was not affected by co-encapsulating the antioxidant α -tocopherol. These results can significantly advance the development of environmentally friendly functionalized nanoparticles and UV-protective coatings.

3.1 Introduction

Nanoparticles are becoming increasingly popular as building blocks for novel functional materials via a bottom-up approach. Adding functionality to nanoparticles allows for specialization for particular applications, and this functionality is most often achieved either via surface modification or incorporation of materials into the nanoparticles. The incorporation of optically active molecules into nanoparticles, in particular, has significant importance in applications such as displays,^{1,2} sensors,^{1,3} pigments,⁴ and sunscreens.^{5–8}

Sunscreens can potentially utilize nanoparticles to address the issues surrounding possible direct skin contact with the organic UV filtering compounds. These organic UV filters – for example oxybenzone, avobenzone, octinoxate, padimate-O, and octocrylene – are often highly conjugated aromatic molecules and work by absorbing UV radiation, therefore filtering the amount that reaches the substrate (i.e. skin) which they are protecting. Despite providing vital protection against harmful UV light, there are many adverse health effects associated with the direct contact of skin with organic UV filters. Multiple studies have raised concern that organic UV filters can penetrate through the skin and enter the blood stream^{7,9}. Some such UV filters, oxybenzone in particular, have also been identified as potential endocrine disruptors and potent skin allergens.^{5,10–14} Moreover, many organic UV filters are also well-known for their photo-instability resulting in the production of carcinogenic reactive oxygen species (ROS) upon solar irradiation.^{15–18} One prospective method to improve the stability of organic UV filters whilst also minimizing skin contact is to incorporate them into nanoparticles.

The incorporation of individual organic UV filters into nanoparticles has been demonstrated with nanoparticles designed from materials such as: silica,^{8,19–21} solid-lipid nanoparticles,²² poly-lactide particles,^{7,23} gelatin,²⁴ cyclodextrins,²⁵ and ethyl cellulose²⁶ (EC). Nanoparticles from EC, in particular, are very appealing as carriers for organic UV filters because: i) they are biobased and biocompatible, ii) the particles can be prepared in a simple precipitation process using acceptable solvents, and iii) they are suitable for use in various solvent systems thus multiple sunscreen formulation types i.e., emulsion, oil based and even formulations containing ethanol – EC is soluble only in pure ethanol and EC nanoparticles are perfectly stable in 25% v/v ethanol.²⁶ Although EC nanoparticles (ECNPs) are promising carriers for UV filters in sunscreen applications, their synthesis and optical properties are not well studied.²⁷ In this study, we investigate the size and optical tunability of ECNPs.

Herein, we initially explore the preparation of ECNP dispersions using a simple antisolvent precipitation method and explore the range of particle sizes that can be prepared. Thereafter, we encapsulate three commercial organic UV filters (oxybenzone, avobenzone, and octinoxate) into the ECNPs and determine the maximum particle loadings for each UV filter. We then investigate the incorporation of the UV filters into the ECNPs – the particle morphological changes upon incorporation, the effect of incorporating UV filters on the ECNP dispersion stability, and the physical state (amorphous or crystalline) of the UV filters when incorporated. Finally, we explore the photostability of the UV filters upon encapsulation when exposed to artificial sunlight. We compare the photodegradation of the UV filters before and after encapsulation into the ECNPs, and furthermore investigate whether the photodegradation can be suppressed by co-encapsulating an antioxidant photostabilizer.



Figure 3.1. (a-h) Size distributions determined by DLS for ECNPs at various EC concentrations in ethanol. (i) Average particle size (values from the DLS measurements) as a function of the concentration of EC used in the antisolvent precipitation.

Our results provide a vital insight into the preparation of ECNPs and the incorporation of organic molecules into ECNPs to give optically functionalized nanoparticles.

3.2 Results and Discussion

3.2.1 Preparation of ECNPs and Investigation of the Particle Size Tunability

We investigated the size range that we could prepare ECNPs using a modified antisolvent precipitation method from literature.²⁸ The ability to prepare particles of very small sizes (<100 nm) is advantageous for use in sunscreens to enhance cosmetic appeal – sunscreen formulations appear transparent when applied on skin due to the reduced scattering of visible light. We found that we could easily tune the average size of the ECNPs between 50 nm and 165 nm in diameter by varying the amount of EC used in the synthesis (see dynamic light scattering (DLS) measurements in **Figure 3.1(a-h)** and **Figure 3.1i**.

Attempts to prepare particles smaller than 50 nm by using lower concentrations of EC resulted in dispersions where the particle concentration was too low to be measured by DLS. We therefore did not probe lower concentrations than 1.6×10⁻³ g mL⁻¹ of EC in ethanol. Aqueous dispersions of ECNPs of smaller sizes (42 nm) have actually been reported in literature by using lower EC concentrations and a different solvent system (isopropyl alcohol instead of ethanol).²⁹ Attempts to prepare particles greater in size than 165 nm resulted in bimodal, very polydisperse distributions of particle sizes (Figure S3.1). Moreover, there was a large amount of macroscopic aggregates formed during the precipitation. We actually found this in general, that greater amounts of macroscopic aggregates of precipitated EC formed during the antisolvent precipitation as a function of greater initial EC concentrations in ethanol. This can be explained by the decrease in the yield of particles upon increasing concentrations of EC (Figure S3.2, raw data in Table S3.1). Smaller particles were prepared with very high yields – close to 100% – but the larger particles (>100 nm) had lower yields. Although we expect that the yield of larger particles could be enhanced by introducing a steric stabilizer, we did not use one because we wanted to keep the formulation as simple as possible.

The ECNP size shows an almost perfect linear dependence on the initial concentration of EC in ethanol **Figure 3.1i** (raw data in **Table S3.1**), a phenomenon which has previously



Figure 3.2. TEM (a) and SEM (b) image of ECNPs. Scale bars 500 nm.

been reported with other materials using an antisolvent precipitation method.^{30–32} TEM and SEM imaging showed particle sizes consistent with the DLS measurements (**Figure 3.2**).

We have therefore demonstrated tunability of particle sizes in the range very appealing for sunscreen applications (<100 nm), where these very small sizes are particularly interesting because such formulations appear transparent when applied on skin.

3.2.2 Loading of UV Filters into ECNPs and Investigation of the Maximum Particle Loadings

We chose to investigate the ECNPs with size 71 nm (corresponding to the dispersion in **Figure 3.1d**) because these are the largest ECNPs that can be prepared with a very high yield (>80%) (**Figure S3.2**). We then explored the optical tunability in terms of the maximum possible loadings of UV filters into the ECNPs. UV filters can be incorporated into ECNPs by performing the antisolvent precipitation with both EC and UV filter dissolved together in ethanol before pouring into water.²⁶ The antisolvent precipitation procedure results in aqueous dispersions of nanoparticles with incorporated UV filters, because of the coprecipitation of hydrophobic EC along with the hydrophobic UV filters. We chose to investigate the three UV filters oxybenzone, avobenzone, and octinoxate, because they are commonplace in sunscreen formulations and because of their solubility properties (soluble in ethanol and insoluble in water).

We found that all UV filters could be encapsulated efficiently and that more UV filter used in the synthesis generally resulted in more incorporated (**Figure 3.3**, data in **Table S3.3**). Interestingly, the UV filter octinoxate was efficiently incorporated to very high maximum particle loadings (54.5 wt%), whereas the UV filters avobenzone and oxybenzone show lower maximum particle loadings of 7.8 wt% and 13.8 wt% respectively. To put these loading values into context, similarly-sized ECNPs (<100 nm) have been explored as drug carriers, showing maximum particle loadings of 17 wt% for the drug Repaglinide³³. As an experimental observation, this lower encapsulation efficiency of oxybenzone and avobenzone is evident during the synthesis because larger amounts of macroscopic precipitate are observed after the antisolvent precipitation when larger amounts of UV filter are used. Although the exact reason for this large discrepancy in loading between the UV filters is not known, we hypothesise that this may be a result of a higher solubility of octinoxate in EC (thus a greater


Figure 3.3. Amount of UV filter loaded into the ECNPs as a function of the amount of UV filter dissolved in the solvent phase in the synthesis. Actual values are reported in **Table S3.3**.

partition coefficient) compared with oxybenzone and avobenzone, analogous to the loading of SLNs with lypophilic drugs³⁴ and polycarbonate NPs with highly hydrophobic drugs.³⁵ Avobenzone and oxybenzone apparently have a lower solubility limit in the EC than octinoxate and therefore the ECNPs are saturated at lower loadings.

Despite the increasing amounts of precipitating material between dispersions, the loadings were still relatively low which meant that the particle sizes mostly did not change upon encapsulation of the UV filters and remained constant (~70 nm) for all dispersions except for the ECNP dispersion containing the highest loading of octinoxate (54.5 wt%), where the average particle size increased to ~90 nm (see **Figure S3.3**).

Attempting additions of large amounts of avobenzone (50 wt%) resulted in the formation of a bimodal particle distribution where micron sized particles were also observed along with the smaller ECNPs (Figure S3.4). This is why there is no data point in Figure 3.3 for avobenzone at 50 wt%. These micron sized particles are either pure avobenzone particles or avobenzone particles stabilized with some EC at the interface: similarly-sized particles form when the antisolvent precipitation procedure is carried out with an equal amount of only UV filter (no EC, Figure S3.5). The addition of very large amounts of octinoxate (>50 wt%) also resulted in a bimodal particle distribution of micron-sized particles observed along with the smaller ECNPs (Figure S3.6) - similar to what we witnessed previously with large amounts of avobenzone. Similar to the case with avobenzone, we hypothesize that the larger micronsized particles were pure octinoxate particles or octinoxate particles stabilized with some EC at the interface: similarly-sized particles form when the antisolvent precipitation procedure is carried out with an equal amount of only UV filter (no EC, Figure S3.7). The larger micronsized particles form along with the ECNPs only when there is enough UV filter added. We hypothesize that these larger micron-sized particles only form beyond a critical point when there is enough excess unencapsulated UV filter to form stable particles.

Oxybenzone

a

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b

b
</tr



Figure 3.4. TEM/SEM images of ECNPs with encapsulated oxybenzone (a-b), encapsulated avobenzone (c-d), encapsulated octinoxate (e-f). All scale bars 200 nm.

3.2.3 Insight into the ECNPs with Incorporated UV Filters

We gained an insight into the incorporation of UV filters into the ECNPs with TEM imaging, zeta potential measurements, and powder X-Ray diffraction measurements.

To explore whether the particle morphology changed upon the incorporation of UV filters, TEM imaging was performed on the ECNPs from the dispersions with the highest loadings of the UV filters (13.8 wt% for oxybenzone, 54.5 wt% for octinoxate, 7.8 wt% for avobenzone). In **Figure 3.4** we see that the ECNPs remain roughly spherical upon incorporation of the UV filters and that the particle size of the ECNPs remains consistent with the DLS measurements.

Using the zeta potential measurements we investigated two factors associated with the incorporation of UV filters into ECNPs: i) whether the dispersion was made more/less colloidally stable (and therefore prone to particle aggregation) by the encapsulation of UV filters, and ii) whether the UV filters exist surface bound, inside the ECNPs, or both upon encapsulation. With respect to the former point, **Figure 3.5a** shows that the zeta potential gets more negative as a function of greater loadings for all UV filters, which is desirable as it



Figure 3.5. (a) Zeta potential as a function of the amount of the loaded UV filter for oxybenzone, octinoxate, and avobenzone. (b) X-Ray diffraction measurements for: dried ECNPs with encapsulated avobenzone (ECNPs contained 13.8 wt% avobenzone), dried ECNPs with encapsulated oxybenzone (ECNPs contained 7.8 wt% oxybenzone), pure ECNPs, pure ethyl cellulose, pure oxybenzone, and pure avobenzone.

indicates improved stability. This could be considered a surprising result because the UV filters are all neutral molecules and therefore should have little effect on the surface charge, however, it is known that even nonpolar surfaces can acquire a considerable negative potential by adsorption of hydroxide ions released by the self-dissociation equilibrium of water.³⁶ A similar charging mechanism is also commonly seen with emulsions and particles stabilized by non-ionic surfactants.^{32,37} With respect to the second point, it is desirable that UV filters are *less* surface bound because the intended application is for cosmetic UV protection – therefore skin contact is minimized. The change in zeta potential as a function of particle loading in **Figure 3.5a** indicates that all the three UV filters are likely present on the ECNP surface, and that higher loadings result in greater surface presence. Despite this, the UV filters are likely not *exclusively* present on the ECNP surface, and this hypothesis is supported by our findings in **Figure S3.10** which show that the maximum loadings of the UV filters into larger ECNPs is considerably greater than would be expected if the UV filters were exclusively incorporated on the particle surface.

We gained further insight into the incorporation of the UV filter into the ECNPs using X-Ray diffraction measurements. Upon encapsulation, it is necessary that the UV filters are distributed evenly and amorphously amongst the ECNPs and therefore do not exist in crystalline clusters. Crystalline clusters would hypothetically result in an uneven distribution of UV filter across a coating prepared from these ECNPs, which is obviously undesirable. We performed the X-Ray diffraction measurements on the dried particles from the dispersions which contained the largest amount of encapsulated UV filter (13.8 wt% for oxybenzone, 7.8 wt% for avobenzone). In **Figure 3.5b** we see the crystalline nature of both UV filters oxybenzone and avobenzone in their pure form (octinoxate is a liquid and therefore was not measured) before incorporation into the ECNPs and how this crystalline nature is no longer



Figure 3.6. Absorbance measurements of the three UV filters (octinoxate, avobenzone, and oxybenzone) taken at hourly intervals when irradiated by artificial sunlight. (a, b, c) correspond to oxybenzone, (d, e, f) correspond to octinoxate, (g, h, i) correspond to avobenzone. (a, d, g) The UV filters are dissolved in ethanol, (b, e, h) the UV filters are encapsulated into ECNPs, (c, f, i) the UV filters are encapsulated into ECNPs along with an antioxidant which is co-encapsulated (mass ratio 1:1).

exhibited when incorporated. These UV filters therefore exist in the desired state inside the ECNPs for coating applications such as sunscreens.

3.2.4 Photodegradation Studies of UV Filters in ECNPs and the Effect of Co-Encapsulation with an Antioxidant Photostabilizer

Finally, an effective nanoparticle carrier for UV filters must allow the UV filters to remain photostable, where 'photostability' is quantified by the extent of the degradation of absorption of UV radiation as a function of time when irradiated by sunlight (a.k.a. photodegradation). Therefore, the ECNPs should preferably have little effect on the ability of the UV filters to absorb UV radiation as a function of time. Nanoparticles prepared from certain materials, such as poly-D,L-lactide-co-glycolide, have even been reported to actually provide a stabilizing effect on UV filters resulting in reduced photodegradation²³.

In order to investigate the photodegradation of the three UV filters (oxybenzone, octinoxate, and avobenzone) encapsulated into our ECNPs, we irradiated diluted aqueous dispersions of ECNPs with encapsulated UV filters (loading 8.0 wt%, 7.9 wt% and 7.8 wt% for oxybenzone, octinoxate, and avobenzone respectively) by artificial sunlight and measured the absorption profiles at hourly intervals for three hours in total. We found that the

photodegradation of the UV filters is identical when incorporated into the ECNPs to when dissolved in ethanol (**Figure 3.6** farthest left column compared with the middle column, no significant difference is observed in the degradation profiles). It is noteworthy that octinoxate shows a large degradation after the first hour. This is because octinoxate initially exists primarily of the high-absorbing cis isomer and irradiation of sunlight causes an isomerism of the octinoxate molecule to a racemic mixture.³⁸ The identical photodegradation profiles of the UV filters when both incorporated and not incorporated therefore show that ECNPs fulfill the requirement of allowing the UV filters to remain photostable when incorporated.

The photostability of UV filters is known to be influenced by the presence of antioxidants, which are commonly added to sunscreen formulations for this reason. The antioxidants – in particular α -tocopherol – are primarily known to neutralize the degradation products generated as a result of irradiation by sunlight.^{39–41} Some studies, although less common, have also reported α -tocopherol to provide a beneficial effect towards suppressing the photodegradation of UV filters.^{41,42} We therefore investigated whether co-encapsulating α -tocopherol along with UV filters in ECNPs would suppress the photodegradation of the UV filters.

In previous work, we observed that the incorporation of octinoxate and α -tocopherol in a 1:1 ratio into ECNPs results in a significant suppression of the reactive oxygen species produced upon irradiation by artificial sunlight.²⁶ Here, we therefore prepared three ECNP dispersions with this same 1:1 ratio of UV filter to antioxidant but this time investigated the effect of the antioxidant α -tocopherol on the *photodegradation* of the three UV filters oxybenzone, avobenzone, and octinoxate. Dispersion 1 consisted of ECNPs with oxybenzone and α -tocopherol incorporated, dispersion 2 consisted of ECNPs with octinoxate and α tocopherol incorporated, and dispersion 3 consisted of ECNPs with avobenzone and α tocopherol incorporated. The incorporation of the α -tocopherol is evident from the slightly increased absorbance peak at λ =288 nm (see **Figure S3.11** for absorption spectrum of ECNPs with *only* α -tocopherol incorporated), as can be seen in **Figure 3.6** (most noticeable when comparing **Figure 3.6h** with **Figure 3.6i**). The dispersions were stable and the particles were sized ~70 nm.

Interestingly, we observed no photo-stabilizing effect of the antioxidant α -tocopherol on any of the three UV filters. This can be seen in **Figure 3.6**, in which the photodegradation profiles where an antioxidant is co-encapsulated with the UV filter (in the farthest-right column) show no lesser photodegradation than when no antioxidant is incorporated (middle column). In fact, we found that the photodegradation of octinoxate was actually marginally greater in the presence of the antioxidant α -tocopherol (**Figure 3.6f** shows a marginally greater degradation than **Figure 3.6e**). It is conceivable that this marginally greater degradation is not actually the octinoxate photodegrading faster, but the α -tocopherol – which absorbs in the same region as octinoxate and is known to photodegrade⁴³ – also photodegrading. These results imply that the antioxidant α -tocopherol does *not* suppress photodegradation of UV filters, and thus the antioxidant's stabilizing effect comes only in neutralizing the skin-damaging decomposition products which we have shown in previous work.²⁶

3.3 Conclusions

We demonstrated the preparation of ECNPs with tunable size (50–165 nm) via an upscalable antisolvent precipitation technique. We then investigated the loading of ECNPs with the three commonplace commercial UV filters: oxybenzone, avobenzone, and octinoxate.

We found that the maximum loadings varied strongly depending on the encapsulated UV filter (avobenzone 7.8 wt%, oxybenzone 13.8 wt%. octinoxate 54.5 wt%). TEM imaging showed the composite particles remained spherical with no significant morphological changes upon incorporation of the UV filters. The incorporation of greater amounts of UV filters resulted in moderate (avobenzone, oxybenzone) to strong (octinoxate) increases in the zeta potential. X-Ray diffraction measurements showed that the UV filters was not affected after incorporation into the ECNPs. Photostability of the UV filters was not affected after incorporation into the ECNPs. Moreover, the addition of an antioxidant didn't result in better maintenance of absorbance by the UV filters, contrary to some other studies.^{41,42}

Here, we studied the incorporation of three commonplace UV filters into ECNPs and conclude that ECNPs have great potential in photoprotection applications because of their simple size and optical tunability, upscalable potential and biobased nature. UV-absorbing ECNPs can significantly advance the utilisation of biobased functionalized nanoparticles for many industrial applications where photoprotection is required, but further studies are needed for this to become a commercial reality, such as the extent of leakage, the incorporation of biobased UV filters and photostabilisers, and tests on the compatibility of ECNPs with other formulation components.

3.4 Materials and Methods

Materials

Ethyl cellulose (EC) degree of substitution 2.1-2.6 (100 cP, lot number MKBT0521V), oxybenzone (98%, solubility in water 69 mg/L at 25°C (PubChem database)), avobenzone (\geq 99%, solubility in water 2.2 mg/L at 25°C (PubChem database)), octinoxate (98%, solubility in water 0.4 mg/L at 24°C (PubChem database)), antioxidant α -tocopherol (\geq 95%) were all purchased from Sigma Aldrich. Ethanol (100%) was purchased from Interchema and pure water was used from a Millipore system.

Preparation of ECNPs

ECNPs were prepared via a modified 'antisolvent precipitation' technique from literature²⁸. We prepared a series of various sizes of ECNPs by dissolving various masses of EC (0.08 g, 0.1 g, 0.1375 g, 0.275 g, 0.5 g, 0.75 g, 1 g, 1.125 g, 1.25 g) in ethanol (50 mL) before pouring into the antisolvent water (150 mL, pH 5-6) under fast magnetic stirring, resulting in the spontaneous formation of ECNPs. Rotary evaporation removed the ethanol and some water until the dispersion was 50 mL. If too much was evaporated, the dispersions were topped up to 50 mL with water to keep particle concentrations constant. The dispersions were then passed through a 1.2 μ m filter to remove any large aggregates that formed. The dispersions were prepared two-fold.

Loading of UV Filters into ECNPs

UV filters were encapsulated into ECNPs via a coprecipitation in the same antisolvent synthesis described above. We prepared a series of various amounts of encapsulated UV filter in ECNPs, where each series contained six dispersions for each UV filter oxybenzone, avobenzone, and octinoxate. Here, EC (0.275 g) and UV filter (6.9×10^{-3} g, 0.014 g, 0.021 g, 0.028 g, 0.055 g, 0.14 g, see full data in **Table S3.2**) were both dissolved in ethanol (50 mL) before pouring into antisolvent water (150 mL). The same antisolvent precipitation procedure was then followed (evaporation, top up to 50 mL, pass through a filter) resulting in 50 mL aqueous dispersions of ECNPs with encapsulated UV filters.

Determination of Particle Loadings

Loadings of UV filter in ECNPs were determined via a spectrophotometric method. Here, approximately 40 mL of the ECNPs with encapsulated UV filter dispersions was dried in a glass 100 mL beaker with magnetic stirrer at 80°C overnight. The following morning, the dried solid was removed from the beaker and weighed. This known mass of ECNPs with encapsulated UV filter was then completely dissolved in ethanol (10 mL) in a small glass vial sealed with a cap. Once completely dissolved, a certain amount of this solution was diluted by a known amount (usually 100 times further – 0.1 mL solution made up to 10 mL with water) and the absorption spectrum was measured using a HP 8452a spectrophotometer. The absorbance value at the peak in the spectrum was compared with a pre-prepared calibration curve comprised of multiple concentrations of UV filter dissolved in ethanol. The measurements were performed in triplicate.

Particle Size Characterisation

ECNPs were characterised with TEM (Philips TECNAI12 electron microscope) in which the samples were prepared by pipetting a drop of the ECNP dispersion onto a Butvar-coated TEM grid, SEM (FEI XL30FEG), and DLS (Malvern Zetasizer Nano ZS, particle size distributions were obtained by using a CONTIN fitting).

Powder X-Ray Diffraction Measurements

Powder X-Ray Diffraction measurements were performed with PW 1729 Philips diffractometer, equipped with a Cu K α X-ray source (λ =1.5418 Å), by drying the dispersions overnight at 80°C under stirring and taking the resultant solid material for the XRD measurements.

Zeta potential Measurements

Zeta potential measurements were performed with a Malvern Zetasizer Nano ZS, at equal particle concentrations, equal dilutions, and in the presence of a background salt (10 mM NaCl). The pH was also kept constant (that of milliQ water, pH 5-6). Keeping the pH constant therefore allowed us to directly compare the particles from dispersion to dispersion. Typically, a sample of the ECNP dispersion was taken and this was diluted 40 times (i.e., 0.1 mL dispersion topped up to 4 mL with water which contained 10 mM dissolved NaCl). All measurements were performed one day after preparation of the ECNP dispersion.

Photodegradation Studies of UV Filters in ECNPs and the Effect of Co-Encapsulation with an Antioxidant Photostabilizer

ECNPs with encapsulated UV filters were prepared as described above. Encapsulating an antioxidant was also done in the same manner, where EC (0.275 g), antioxidant α -tocopherol (0.028 g) and UV filter (0.028 g) (therefore mass ratio 1:1) were dissolved in ethanol before performing the antisolvent precipitation as described above. In order to quantify the photodegradation, the dispersions were diluted with water and added to a quartz cuvette sealed with a Teflon stopper. The cuvette was subjected to irradiation by a 75 W Xenon lamp at a distance of 20 cm (a flux of 3 mW cm⁻² between 300 and 400 nm). The absorbance was measured hourly for three hours. The total UV dose was thus 324 kJ m⁻²; equivalent to 1 hour 48 minutes of summer sunlight in Nice at noon⁴⁴.

3.5 Acknowledgements

This project was performed in collaboration with Heleen V. M. Kibbelaar. This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs. We thank Chris Schneijdenberg and Dave van den Heuvel for technical assistance and thank Wiebke Albrecht and Tonnishtha Dasgupta for careful reading of the manuscript.

3.6 References

- (1) Shipway, A. N.; Katz, E.; Willner, I. Nanoparticle Arrays on Surfaces for Electronic, Optical, and Sensor Applications. *ChemPhysChem* **2000**, *1*, 18–52.
- (2) Bell, N. S.; Piech, M. Photophysical Effects between Spirobenzopyran Methyl Methacrylate-Functionalized Colloidal Particles. *Langmuir* **2006**, *22*, 1420–1427.
- (3) Ren, Y.; Chen, M.; Zhang, Y.; Wu, L. Fabrication of Rattle-Type TiO2/SiO2 Core/Shell Particles with Both High Photoactivity and UV-Shielding Property. *Langmuir* **2010**, *26*, 11391–11396.
- (4) Patel, a. R.; Heussen, P. C. M.; Dorst, E.; Hazekamp, J.; Velikov, K. P. Colloidal Approach to Prepare Colour Blends from Colourants with Different Solubility Profiles. *Food Chem.* 2013, 141, 1466–1471.
- (5) Bens, G. Chapter 25. In *Sunlight, Vitamin D and Skin Cancer*; Orleans, 2014; pp 429–463.
- (6) Wang, S. Q.; Balagula, Y.; Osterwalder, U. Photoprotection: A Review of the Current and Future Technologies. *Dermatol. Ther.* **2010**, *23*, 31–47.
- (7) Deng, Y.; Ediriwickrema, A.; Yang, F.; Lewis, J.; Girardi, M.; Saltzman, W. M. A Sunblock Based on Bioadhesive Nanoparticles. *Nat. Mater.* **2015**, *14*, 1278–1285.
- (8) Tolbert, S. H.; McFadden, P. D.; Loy, D. A. New Hybrid Organic/Inorganic Polysilsesquioxane– Silica Particles as Sunscreens. *ACS Appl. Mater. Interfaces* **2016**, *8*, 3160–3174.
- (9) Janjua, N. R.; Mogensen, B.; Andersson, A. M.; Petersen, J. H.; Henriksen, M.; Skakkebæk, N. E.; Wulf, H. C. Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor after Whole-Body Topical Application and Reproductive Hormone Levels in Humans. J. Invest. Dermatol. 2004, 123, 57– 61.
- (10) Krause, M.; Klit, A.; Blomberg Jensen, M.; Søeborg, T.; Frederiksen, H.; Schlumpf, M.; Lichtensteiger, W.; Skakkebaek, N. E.; Drzewiecki, K. T. Sunscreens: Are They Beneficial for Health? An Overview of Endocrine Disrupting Properties of UV-Filters. *Int. J. Androl.* 2012, 35, 424–436.
- (11) Kullavanijaya, P.; Lim, H. W. Photoprotection. J. Am. Acad. Dermatol. 2005, 52, 937–958.
- (12) Mancebo, S. E.; Hu, J. Y.; Wang, S. Q. Sunscreens: A Review of Health Benefits, Regulations, and Controversies. *Dermatol. Clin.* **2014**, *32*, 427–438.
- (13) Hayden, C. G. J.; Cross, S. E.; Anderson, C.; Saunders, N. A.; Roberts, M. S. Sunscreen Penetration of Human Skin and Related Keratinocyte Toxicity after Topical Application. Skin Pharmacol. Physiol. 2005, 18, 170–174.
- (14) Hayden, C. G. J.; Roberts, M. S.; Benson, H. A. E. Systemic Absorption of Sunscreen after Topical Application. *Lancet* **1997**, *350*, 863–864.
- (15) Wu, H.; Song, Q.; Ran, G.; Lu, X.; Xu, B. Recent Developments in the Detection of Singlet Oxygen with Molecular Spectroscopic Methods. *TrAC Trends Anal. Chem.* **2011**, *30*, 133–141.
- (16) Hanson, K. M.; Gratton, E.; Bardeen, C. J. Sunscreen Enhancement of UV-Induced Reactive Oxygen Species in the Skin. *Free Radic. Biol. Med.* **2006**, *41*, 1205–1212.
- (17) Chrétien, M. N.; Migahed, L.; Scaiano, J. C. Protecting the Protectors: Reducing the Biological

Toxicity of UV Sunscreens by Zeolite Encapsulation. *Photochem. Photobiol.* **2006**, *82*, 1606–1611.

- (18) Buchalska, M.; Kras, G.; Oszajca, M.; Łasocha, W.; Macyk, W. Singlet Oxygen Generation in the Presence of Titanium Dioxide Materials Used as Sunscreens in Suntan Lotions. J. Photochem. Photobiol. A Chem. 2010, 213, 158–163.
- (19) Lapidot, N.; Gans, O.; Biagini, F.; Sosonkin, L.; Rottman, C. Advanced Sunscreens: UV Absorbers Encapsulated in Sol-Gel Glass Microcapsules. *J. Sol-Gel Sci. Technol.* **2003**, *26*, 67–72.
- (20) Cui, H.; Zayat, M.; Parejo, P. G.; Levy, D. Highly Efficient Inorganic Transparent UV-Protective Thin-Film Coating by Low Temperature Sol-Gel Procedure for Application on Heat-Sensitive Substrates. *Adv. Mater.* **2008**, *20*, 65–68.
- (21) Kim, S. S.; Kim, V.; Kim, Y. B. Preparation and Characterization of Polysilsesquioxane Particles Containing UV-Absorbing Groups. *Macromol. Res.* **2012**, *20*, 437–446.
- (22) Wissing, S. A.; Muller, R. H. Solid Lipid Nanoparticles as Carrier for Sunscreens: In Vitro Release and in Vivo Skin Penetration. *J. Control. Release* **2002**, *81*, 225–233.
- (23) Perugini, P.; Simeoni, S.; Scalia, S.; Genta, I.; Modena, T.; Conti, B.; Pavanetto, F. Effect of Nanoparticle Encapsulation on the Photostability of the Sunscreen Agent, 2-Ethylhexyl-p-Methoxycinnamate. *Int. J. Pharm.* **2002**, *246*, 37–45.
- (24) Oliveira, C. A. De; Peres, D. D. A.; Graziola, F.; Chacra, N. A. B.; Araújo, G. L. B. De; Flórido, A. C.; Mota, J.; Rosado, C.; Velasco, M. V. R.; Rodrigues, L. M.; Fernandes, A. S.; Baby, A. R. Cutaneous Biocompatible Rutin-Loaded Gelatin-Based Nanoparticles Increase the SPF of the Association of UVA and UVB Filters. *Eur. J. Pharm. Sci.* **2016**, *81*, 1–9.
- (25) Scalia, S.; Casolari, A.; Iaconinoto, A.; Simeoni, S. Comparative Studies of the Influence of Cyclodextrins on the Stability of the Sunscreen Agent, 2-Ethylhexyl-p-Methoxycinnamate. J. Pharm. Biomed. Anal. 2002, 30, 1181–1189.
- (26) Hayden, D. R.; Imhof, A.; Velikov, K. P. Biobased Nanoparticles for Broadband UV Protection with Photostabilized UV Filters. *ACS Appl. Mater. Interfaces* **2016**, *8*, 32655–32660.
- (27) Vílchez-Maldonado, S.; Calderó, G.; Esquena, J.; Molina, R. UV Protective Textiles by the Deposition of Functional Ethylcellulose Nanoparticles. *Cellulose* **2014**, *21*, 2133–2145.
- (28) Bizmark, N.; Ioannidis, M. a. Effects of Ionic Strength on the Colloidal Stability and Interfacial Assembly of Hydrophobic Ethyl Cellulose Nanoparticles. *Langmuir* **2015**, *31*, 9282–9289.
- (29) Bizmark, N.; Ioannidis, M. a.; Henneke, D. E. Irreversible Adsorption-Driven Assembly of Nanoparticles at Fluid Interfaces Revealed by a Dynamic Surface Tension Probe. *Langmuir* 2014, 30, 710–717.
- (30) Zhong, Q.; Jin, M. Zein Nanoparticles Produced by Liquid-Liquid Dispersion. *Food Hydrocoll.* 2009, 23, 2380–2387.
- (31) Zhang, C.; Pansare, V. J.; Prud, R. K.; Priestley, R. D. Flash Nanoprecipitation of Polystyrene Nanoparticles. *Soft Matter* **2012**, *8*, 86–93.
- (32) Rossi, L.; Seijen ten Hoorn, J. W. M.; Melnikov, S. M.; Velikov, K. P. Colloidal Phytosterols: Synthesis, Characterization and Bioaccessibility. *Soft Matter* **2010**, *6*, 928–936.

- (33) Lokhande, A. B.; Mishra, S.; Kulkarni, R. D.; Naik, J. B. Preparation and Characterization of Repaglinide Loaded Ethylcellulose Nanoparticles by Solvent Diffusion Technique Using High Pressure Homogenizer. *J. Pharm. Res.* **2013**, *7*, 421–426.
- (34) Schäfer-Korting, M.; Mehnert, W.; Korting, H. C. Lipid Nanoparticles for Improved Topical Application of Drugs for Skin Diseases. *Adv. Drug Deliv. Rev.* **2007**, *59*, 427–443.
- (35) Othman, R.; Vladisavljević, G. T.; Nagy, Z. K.; Holdich, R. G. Encapsulation and Controlled Release of Rapamycin from Polycaprolactone Nanoparticles Prepared by Membrane Micromixing Combined with Antisolvent Precipitation. *Langmuir* **2016**, *32*, 10685–10693.
- (36) Marinova, K. G.; Alargova, R. G.; Denkov, N. D.; Velev, O. D.; Petsev, D. N.; Ivanov, I. B.; Borwankar, R. P. Charging of Oil–Water Interfaces Due to Spontaneous Adsorption of Hydroxyl Ions. *Langmuir* **1996**, *12*, 2045–2051.
- (37) Delgado, A. V.; Dekker, M. Interfacial Electrokinetics and Electrophoresis. In *Surfactant Science Series*; New York, 2002; Vol. 106.
- (38) Damiani, E.; Baschong, W.; Greci, L. UV-Filter Combinations under UV-A Exposure: Concomitant Quantification of over-All Spectral Stability and Molecular Integrity. J. Photochem. Photobiol. B Biol. 2007, 87, 95–104.
- (39) Oresajo, C.; Yatskayer, M.; Galdi, A.; Foltis, P.; Pillai, S. Complementary Effects of Antioxidants and Sunscreens in Reducing UV-Induced Skin Damage as Demonstrated by Skin Biomarker Expression. J. Cosmet. Laser Ther. 2010, 12, 157–162.
- (40) Chaudhuri, R. K.; Lascu, Z.; Puccetti, G.; Deshpande, A. a; Paknikar, S. K. Design of a Photostabilizer Having Built-in Antioxidant Functionality and Its Utility in Obtaining Broad-Spectrum Sunscreen Formulations. *Photochem. Photobiol.* **2006**, *82*, 823–828.
- Afonso, S.; Horita, K.; Sousa E Silva, J. P.; Almeida, I. F.; Amaral, M. H.; Lobão, P. A.; Costa, P. C.; Miranda, M. S.; Esteves Da Silva, J. C. G.; Sousa Lobo, J. M. Photodegradation of Avobenzone: Stabilization Effect of Antioxidants. *J. Photochem. Photobiol. B Biol.* 2014, 140, 36–40.
- (42) Niculae, G.; Lacatusu, I.; Bors, A.; Stan, R. Photostability Enhancement by Encapsulation of α-Tocopherol into Lipid-Based Nanoparticles Loaded with a UV Filter. *Comptes Rendus Chim.* 2014, 17, 1028–1033.
- (43) Sabliov, C. M.; Fronczek, C.; Astete, C. E.; Khachaturyan, M.; Khachatryan, L.; Leonardi, C. Effects of Temperature and UV Light on Degradation of α-Tocopherol in Free and Dissolved Form. JAOCS, J. Am. Oil Chem. Soc. 2009, 86, 895–902.
- Séite, S.; Moyal, D.; Richard, S.; De Rigal, J.; Lévêque, J. L.; Hourseau, C.; Fourtanier, A. Mexoryl[®]
 SX: A Broad Absorption UVA Filter Protects Human Skin from the Effects of Repeated Suberythemal Doses of UVA. *J. Photochem. Photobiol. B Biol.* **1998**, *44*, 69–76.

Appendix

Preparation of ECNPs and Investigation of the Particle Size Range

In **Figure 3.1** we found that ECNPs could be prepared with sizes ranging from 50 nm to 165 nm. Attempts to prepare larger particles than 165 nm resulted in bimodal, very polydisperse distributions of particles as shown in **Figure S3.1** below.



Figure S3.1. Size distribution as determined by DLS for when 0.025 g mL⁻¹ EC in ethanol is used for the antisolvent precipitation. Note that the DLS measurement shows a very polydisperse bimodal distribution of particles.

The yield of ECNPs for the series in which increasing concentrations of EC in ethanol solution were used for the antisolvent precipitation was quantified (100 × particles formed mass/mass of EC originally added). Yield values (**Figure S3.2**) were determined by taking 10 mL of the dispersion and drying in a glass vial at 80 °C overnight. The mass of the dried particles was then measured, multiplied by 5 (to correspond to the total 50 mL dispersion), and then this value was divided by the initial mass of EC added. This was done two-fold. We



Figure S3.2. The yield for the series is plotted as a function of the concentration of EC in ethanol. The two in the series concerning the lowest two concentrations of EC in ethanol (1.6×10^{-3} g mL⁻¹ and 2×10^{-3} g mL⁻¹) are not measured because the particle sizes for the lowest three concentrations were all similar.

see that the yield decreases for the larger concentrations of EC in ethanol. Thus, higher concentrations of EC in ethanol allow the preparation of larger ECNPs but the yield is sacrificed.

The complete raw data set for **Figure 3.1***i*, **Figure S3.1** (conc. EC in ethanol 2.50×10⁻² g mL⁻¹) and **Figure S3.2** can be found in **Table S3.1** below. Both the mean and modal average are reported for the particle size for completeness but the graph in **Figure 3.1***i* only uses the values for the mean average particle size.

Conc. of EC in ethanol (g mL ⁻¹)	Particle size modal average (nm)	Particle size mean average (nm)	Particle size error (modal average,	Particle size error (mean average,	PDI	PDI diameter (nm)	Yield average (%)	Yield error (%)
1.60 ×10 ⁻³	35	50	0.197	0.280	0.230	23.7	-	-
2.00 ×10 ⁻³	37	58	0.639	0.540	0.280	30.9	-	-
2.75 ×10 ⁻³	39	53	0.630	0.784	0.204	23.9	94.5	5.5
5.50 ×10 ⁻³	64	71	0.277	0.389	0.158	27.4	82.0	4.0
1.00 ×10 ⁻²	87	100	0.239	0.373	0.157	39.3	77.0	0.5
1.50 ×10 ⁻²	113	118	0.921	1.164	0.135	42.4	53.6	9.6
2.00 ×10 ⁻²	148	146	0.176	0.224	0.126	52.8	35.5	0.5
2.25 ×10 ⁻²	177	160	0.265	0.482	0.136	60.7	23.0	1.0
2.50 ×10 ⁻²	206	165	5.231	1.064	0.243	83.1	14.7	7.8

Table S3.1. The raw data for Figure 3.1i.

Loading of UV filters into ECNPs (71 nm) and Investigation of the Maximum Particle Loadings

Table S3.2 shows the compositions of a series of 7 ECNP dispersions which were prepared by dissolving constant amounts of EC and increasing amounts of UV filter into ethanol before undergoing the antisolvent precipitation.

Table S3.2. Compositions of the amounts of EC and UV filter initially dissolved in 50 mL ethanol for the three series of seven dispersions (one series of 7 dispersions for each UV filter).

Dispersion	Mass EC (g)	of Mas avo	ss of UV filter oxybenzone/ benzone/octinoxate (g)	UV filter added expressed as a weight percentage (wt%)
1	0.275	0.00)7	2.4
2	0.275	0.01	14	4.8
3	0.275	0.02	21	7.1
4	0.275	0.02	28	9.0
5	0.275	0.05	55	16.0
6	0.275	0.13	38	33.4
7	0.275	0.27	75	50



Figure S3.3. Size distributions as determined by DLS for the ECNP dispersions which contained the largest loadings of UV filter. (a) ECNPs with incorporated octinoxate (54.5wt%). Mean average particle size 90 nm. (b) ECNPs with incorporated avobenzone (7.8wt%). Mean average particle size 70 nm. (c) ECNPs with incorporated oxybenzone (13.8wt%). Mean average particle size 70 nm.

Interestingly, despite the larger amounts of precipitating material, we found that the resultant particle sizes were constant for all dispersions (70 nm) except the ECNPs dispersion containing the highest loading of octinoxate (90 nm). In **Figure S3.3** below we see the DLS measurement for this dispersion which contains the highest loading of octinoxate (**Figure S3.3a**) compared with the DLS measurements which contain the highest loadings of avobenzone and oxybenzone (these spectra are identical to the spectra of non-loaded ECNPs prepared from the same concentration of EC in ethanol). The DLS measurements of all other ECNP dispersions in the series were also identical to **Figure S3.1b** and **Figure S3.1c** below, with an average particle size of 70 nm.

When attempting to prepare ECNPs with large amounts of encapsulated UV filter we would dissolve large amounts of the UV filter in ethanol along with the ethyl cellulose before undergoing the antisolvent precipitation.

When large amounts of UV filter avobenzone were dissolved in ethanol (before undergoing the antisolvent precipitation) we found that the ECNPs would become saturated and the excess UV filter would form a secondary set of larger droplets (**Figure S3.4**). We hypothesize that the secondary set of larger particles are likely stable droplets of pure UV filter (or possibly droplets of UV filter stabilized with some EC at the interface) because performing the antisolvent precipitation with only UV filter avobenzone (and no EC) resulted in *only* similarly-sized larger particles (**Figure S3.5**).



Figure S3.4. Size distribution as determined by DLS for the dispersion in which EC (0.275 g) and avobenzone (0.275 g) were dissolved in ethanol (50 mL) before undergoing the antisolvent precipitation. Note the bimodal distribution in which larger micron-sized particles are formed as well as the expected ECNPs.



Figure S3.5. Size distribution as determined by DLS for the dispersion in which *only* avobenzone (0.275 g) was dissolved in ethanol (50 mL) before undergoing the antisolvent precipitation. Note the formation of larger, self-stabilized avobenzone particles.

A similar story is seen when using large amounts of octinoxate instead of avobenzone. **Figure S3.6** shows the DLS measurement for when large amounts of octinoxate was dissolved in ethanol along with ethyl cellulose and larger particles are observed. We therefore hypothesize again that the secondary peaks in the DLS measurement in **Figure S3.6** are likely due to stable particles of either pure octinoxate or octinoxate droplets partially stabilized with EC because performing the antisolvent precipitation with only UV filter octinoxate (and no EC) resulted in *only* similarly-sized larger particles (**Figure S3.7**). Lastly, we also investigated preparing particles with larger loadings of oxybenzone. Moreover, we wanted to investigate whether the particle size was mostly determined by the total mass of precipitating material or mostly determined by the total mass of precipitating ethyl cellulose. Therefore, here we used half the usual concentration of EC (half of 0.0055 g mL⁻¹, thus 0.0028 g mL⁻¹) along with an equal concentration (0.0028 g mL⁻¹) of UV filter. The total concentration of precipitating material is therefore 0.0055 g mL⁻¹, however, half of this is ethyl cellulose and half of this is UV filter oxybenzone. Therefore, we dissolved a total mass of 0.275 g in a 1:1 ratio (0.138 g of EC and 0.138 g of UV filter) in ethanol before doing the antisolvent precipitation procedure.



Figure S3.6. Size distribution as determined by DLS for the dispersion in which EC (0.275 g) and octinoxate (0.55 g) were dissolved together in ethanol (50 mL) before undergoing the antisolvent precipitation. Not the multimodal distribution in which larger (micron sized and greater) particles are formed as well as the ECNPs.



Figure S3.7. Size distribution as determined by DLS for the dispersion in which only octinoxate (0.55 g) was dissolved in ethanol (50 mL) before undergoing the antisolvent precipitation. Note the formation of large micron sized self-stabilized octinoxate particles.

We found this resulted in a bimodal particle distribution (**Figure S3.8**), similar to what we saw previously with attempting higher loadings of avobenzone and octinoxate. Again, we hypothesize that the peaks in the DLS measurement were due to ECNPs (presumably with encapsulated UV filter too) as well as larger stable droplets of pure oxybenzone/droplets of pure oxybenzone stabilized with some EC at the interface - because **Figure S3.9** shows that similarly-sized large particles are formed when performing the antisolvent precipitation with *only* oxybenzone (and no EC). Interestingly, in **Figure S3.8** we also found that the modal average particle size of the ECNPs was 40 nm – lower than when precipitating 0.0055 g mL⁻¹ of *only* ethyl cellulose. (N.B. we compare the modal average values here instead of mean because the mean average is disrupted by the fact the measurement is bimodal) According to **Table S3.1**, performing the antisolvent precipitation with 0.0028 g mL⁻¹ of EC would predict a modal average particle size of around 40 nm. We therefore hypothesize that the concentration of ethyl cellulose is the principal factor in the resultant ECNP particle size.



Figure S3.8. Size distribution as determined by DLS for the dispersion in which EC (0.138 g) and oxybenzone (0.138 g) were dissolved in ethanol before undergoing the antisolvent precipitation. Note the existence of micron sized particles along with the ECNPs.



Figure S3.9. Size distribution as determined by DLS for the dispersion in which only oxybenzone (0.275 g) was dissolved in ethanol (50 mL) before undergoing the antisolvent precipitation. Not the formation of micron sized self-stabilized particles of pure oxybenzone.

The complete raw data set for Figure 3.4 can be found in Table S3.3 below.

Table S3.3. All the data used for **Figure 3.3**. The measurements were performed in triplicate therefore the values for the loaded UV filter below are an average of three measured values. This is the source of the standard error.

UV filter added (wt%)	Loaded oxybenzone (wt%)	Standard error (wt%)	Loaded octinoxate (wt%)	Standard error (wt%)	Loaded avobenzon e (wt%)	Standard error (wt%)
2.4	3.1	0.02	1.4	0.33	0.9	0.05
4.8	5.2	0.09	4.0	0.54	3.3	0.09
7.0	6.2	0.19	5.9	0.18	5.8	0.11
9.1	8.0	0.41	7.9	0.43	7.0	0.50
16.6	13.5	3.90	15.8	0.25	7.8	0.55
33.3	13.8	3.18	32.8	0.64	7.0	0.45
50	11.8	1.20	54.5	2.50	-	-

Loading of UV filters into larger ECNPs and Investigation of the Maximum Particle Loadings

We investigated the maximum loading values of the three UV filters oxybenzone, avobenzone, and octinoxate into larger ECNPs to give further mechanistic insight. If the UV filter were to be exclusively present on the surface of the ECNPs, we would expect that larger particles would result in a smaller weight percentage of loaded UV filter because the surface area to volume ratio is lower for larger particles. In fact, we find that the maximum loading of oxybenzone and avobenzone in the ECNPs is considerably greater (27.8 wt% and 28.4 wt% respectively) when the ECNPs are larger (**Figure S3.10**), and the loading of octinoxate remains similar (47.2 wt%). This is evidence that the UV filters are distributed throughout the particles and are not exclusively at the surface.



Figure S3.10. Amount of UV filter loaded into the larger ECNPs (~100 nm, see exact sizes for all dispersions in **Table S3.5**) as a function of the amount of UV filter dissolved in the solvent phase in the synthesis. Actual values are reported in **Table S3.4**.

The complete raw data set for **Figure S3.10** can be found in **Table S3.4** below. The measurements were performed in duplicate to obtain the error values.

UV filter added (wt%)	Loaded oxybenzone (wt%)	Standard error (wt%)	Loaded octinoxate (wt%)	Standard error (wt%)	Loaded avobenzon e (wt%)	Standard error (wt%)
4.8	2.9	0.5	2.2	0.9	2.6	0.8
9.1	8.8	1	7.2	1.3	8.1	0.5
16.6	15.4	1.1	13.8	1.2	10.4	1.1
33.3	27.8	1.8	30.1	1.5	28.4	1.9
50	27.4	2.2	47.2	0.9	9.5	1.0

Table S3.4. All the Data for Figure S3.10.

The particle size data for the dispersions of larger ECNPs with loaded UV filters can be found in **Table S3.5** below.

UV (wt%)	filter	added	Particle size mean average (nm)	PDI width (nm)
Oxybenz	one			
4.8			106	40
9.1			99	41
16.6			101	42
33.3			104	46
50			108	50
Avobenz	one			
4.8			105	39
9.1			101	44
16.6			104	41
33.3			119	49
50			115	68
Octinoxa	te			
4.8			106	39
9.1			105	43
16.6			110	47
33.3			109	44
50			177	111

Table S3.5. The Particle Size Data and PDI for the Dispersions in Figure S3.10.

Co-Encapsulation of UV Filters with an Antioxidant Photostabilizer

Below we show the absorption profile for ECNPs with encapsulated α -tocopherol (EC: 0.275 g, α -tocopherol 0.028 g). Note the absorbance of the α -tocopherol at ~ λ = 280-300 nm.



Figure S3.11. Absorption spectrum of ECNPs with α -tocopherol incorporated.

Chapter 4. Fully Biobased UV-Absorbing Nanoparticles from Ethyl Cellulose and Zein for Environmentally Friendly Photoprotection

Effective photoprotection is a vital consumer issue. However, there are many concerns regarding the adverse environmental and health impacts associated with current organic and inorganic UV filters. Here, we prepare fully-biobased UV-absorbing nanoparticles from ethyl cellulose (ECNPs) and zein (ZNPs) with encapsulated biobased photoprotectants obtainable from plants and foods (quercetin, retinol, and p-coumaric acid), which have the potential to satisfy both environmental and health issues in photoprotection. We show the ability for ECNPs and ZNPs to be easily tuned compositionally to obtain uniform, broadband UV spectrum absorbance profiles, and prepare transparent UV-absorbing coatings from the ECNPs. We find maximum loadings for retinol, quercetin, and p-coumaric acid into the ECNPs are 31 wt%, 14 wt%, and 13 wt% respectively. The ECNP size remains constant (except for the largest loading of retinol, 31 wt%) and the absolute zeta potential increases upon increasing loading of quercetin and retinol, whereas increasing loading of p-coumaric acid results in increasing particle size and lower absolute zeta potential. We find that quercetin and retinol are effectively retained inside the ECNPs with 64-70% after 72 hours. These results have significant implications for the development of novel photoprotection technologies and functional nanoparticles.

4.1 Introduction

Excessive exposure to UV radiation from sunlight can lead to the degradation of foods and packaging materials, as well as multiple adverse health effects such as sunburn, accelerated skin aging, and the vast majority of skin cancers^{1,2}. Protection against UV radiation via the use of sunscreens is therefore vital for many consumer products and crucial for human health.

Many current cosmetic sunscreens provide protection across the entire UV spectrum ($\lambda = 290-380$ nm) via the use of multiple synthetic organic and inorganic UV filters. Despite the effectiveness of synthetic organic and inorganic UV filters in protecting against UV radiation, their use in large quantities has been reported to have significant adverse environmental and health effects. For example, large concentrations of synthetic organic UV filters in coastal recreational areas have been strongly linked with accelerated damage to coral reefs^{3,4} and marine phytoplankton⁵ by promoting viral infections – which has significant implications for the local ecosystem. Inorganic UV filters such as TiO₂ nanoparticles have also been reported as potentially toxic to marine life because they remain photocatalytically active in the environment^{6,7}. As for health concerns, inorganic and synthetic organic UV filters have been identified as phototoxic^{8,9}, potent skin allergens¹⁰, and have been reported to penetrate the skin and act as endocrine disruptors in the bloodstream^{11,12}.

One method to address the health concerns of inorganic and synthetic organic UV filters is to minimize skin contact with the UV filters. Skin contact can be minimized for inorganic UV filters via coating the particles (i.e. with silica/alumina) and for synthetic organic UV filters via encapsulation into nanoparticles from materials such as silica¹³, gelatin¹⁴, lipids¹⁵, poly-lactide¹⁶, and ethyl cellulose (EC)⁹. The coating of inorganic UV filters and encapsulation of synthetic organic UV filters can result in significant reduction of direct skin contact^{12,17}, skin penetration¹², and improvements in the phototoxicity^{9,13}. Furthermore, encapsulation of synthetic organic UV filters into nanoparticles also reduces the need for unnecessary chemicals (i.e. surfactants used in formulation), can increase photostability¹⁶, and potentially allows higher loadings in formulations - the amount of UV filter in the formulation is no longer limited by its solubility¹⁸. Despite the advantages of nanoencapsulation, the adverse health effects of inorganic and synthetic organic UV filters cannot be completely eliminated and their environmental impact remains a concern. Thus, the replacement of these UV filters with eco-friendly, natural, biobased photoprotectants which are potentially safer¹⁹ – and encapsulation into biocompatible eco-friendly nanoparticles has the potential to satisfy both environmental and health concerns whilst providing effective photoprotection.

There are many known biobased photoprotectants, such as flavonoids (i.e. quercetin and rutin), lignin (i.e. p-coumaric acid), and carotenoids (i.e. β -carotene, lutein, lycopene, retinol). Quercetin and rutin are the most extensively studied biobased photoprotectants, and both have even been individually incorporated into solid-lipid nanoparticles (SLNs) for sunscreen applications^{14,19,20}. Despite this, there is still a need to develop UV-absorbing nanoparticles with an entirely natural composition which can: (i) efficiently encapsulate multiple biobased photoprotectants, (ii) provide broadband and uniform UV absorbance, (iii) prepare effective UV-protective coatings, and (iv) be potentially used for multiple solvent systems e.g. oil and emulsion (SLNs may dissolve in oil continuous formulations), for effective eco-friendly photoprotection. Here, we develop fully-biobased UV-absorbing nanoparticles via the encapsulation of multiple biobased photoprotectants together into biobased ethyl cellulose nanoparticles (ECNPs) and biobased zein nanoparticles (ZNPs) using an upscalable technique. As a protein, zein may not be suitable for skincare applications but is potentially very interesting for edible photoprotection applications such as food coatings, and is moreover an interesting material because it absorbs UV light itself primarily due to the presence of xanthophylls and β -carotene²¹. We show that the fully-biobased UV-absorbing ECNPs and ZNPs with encapsulated quercetin, retinol, and p-coumaric acid can provide effective uniform broadband UV spectrum protection. We then focus on the UV-absorbing ECNPs and prepare transparent, flexible coatings with tunable thicknesses and study the photodegradation of these coatings. Additionally, we study the retention of the biobased photoprotectants inside the ECNPs and study the incorporation of the biobased photoprotectants into the ECNPs.

These findings are significant for the development of safer and more environmentally friendly methods of photoprotection, in applications such as cosmetic sunscreen formulations, packaging materials and food coatings. Furthermore, these findings also have important implications for the more general fields of UV protective coatings and functional nanoparticles.

4.2 Results and Discussion

4.2.1. Preparation of Fully-Biobased UV-Absorbing Nanoparticles from EC and Zein

We investigated the incorporation of three biobased photoprotectants quercetin, retinol, and p-coumaric acid (chemical structures in **Chart 4.1**) into ECNPs and ZNPs. The biobased photoprotectants were specifically chosen because of their solubility properties (hydrophobic) and because together they absorb across the entire UV spectrum (290-380 nm) – broadband UV spectrum protection is of paramount importance for sunscreen applications. The three photoprotectants were incorporated into ECNPs and ZNPs (<100 nm) via a simple, upscalable, surfactant-free "antisolvent precipitation" technique^{9,22}. The antisolvent precipitation procedure relies on the co-precipitation of the hydrophobic photoprotectants together with the hydrophobic EC or zein.

Chart 4.1. Molecular structures of the biobased photoprotectants: quercetin, retinol, and p-coumaric acid.



Quercetin

Retinol

p-Coumaric acid

By using this procedure, we prepared fully-biobased ECNPs and ZNPs with encapsulated quercetin, retinol, and p-coumaric acid. Both the prepared ECNPs and ZNPs with encapsulated biobased photoprotectants were roughly spherical and had an average size of 70-76 nm, as seen by dynamic light scattering (DLS) measurements and SEM imaging (**Figure 4.1a**, **Figure 4.1d** and DLS measurements **Figure S4.1**). We found that we could tune the amount of each biobased photoprotectant encapsulated simply by varying the ratio of each

photoprotectant initially dissolved in the ethanol or ethanol/water solution before undergoing the antisolvent precipitation. Therefore, we always added the same total amount of the three biobased photoprotectants (10 wt% of the amount of EC/zein). We always kept the total loading below 10 wt% because ECNPs are known to encapsulate organic UV filters efficiently to this weight percentage²³, and we wanted to stay under this value so that all photoprotectants were efficiently encapsulated and therefore the absorbance profiles from the ECNPs were reproducible (Figure S4.3 shows unpredictability of the ECNP absorbance profile upon loadings greater than 10 wt%). We found that we could achieve a uniform absorbance profile across the entire UV spectrum for both ECNPs and ZNPs with encapsulated quercetin, p-coumaric acid and retinol in a ratio of 7:1.5:1.5 (Figure 4.1b and Figure 4.1e), whereas a 1:1:1 ratio resulted in greater absorbance in the UVB region of the spectrum (Figure S4.2b). Interestingly, the absorbance of zein itself contributed slightly to the overall absorbance of the ZNPs (cf. Figure 4.1b and Figure 4.1e), in which the absorbance profile remained approximately uniform but slightly greater in the UVB region and also with some absorbance at wavelengths greater than 425 nm. The absorbance of the ZNPs at higher wavelengths resulted in a distinctive yellow colour of the ZNPs²⁴ compared to the ECNPs²⁵ (Figure 4.1c left vial vs. Figure 4.1f left vial). The encapsulation of quercetin, which was necessary to achieve absorbance in the UVA region of the spectrum, also gave the dispersions a yellow colour due to its absorbance in the blue region of the visible light spectrum¹⁹. Therefore, the resultant ECNP and ZNP dispersions both had a yellow colour (Figure 4.1c right vial and Figure 4.1f right vial), unlike UV-absorbing ECNPs prepared with synthetic UV filters encapsulated which can provide uniform absorbance across the entire UV spectrum without a yellow colour because synthetic UVA filters have been developed which do not absorb into the visible spectrum⁹. We found that the ECNPs had a zeta potential of -25 mV (at the pH of milliQ water, pH=5-6), the ZNPs had a zeta potential of +30 mV (at pH=3.5) and both were colloidally stable at these respective pH values.

4.2.2. Preparation of Fully-Biobased, Transparent, UV-Absorbing Coatings from the UV-Absorbing ECNPs

We then focused on the ECNPs because of their superior colour and ease of handling with regards to their stability at close-to neutral pH – zein has an isoelectric point at pH 6.8²². The ability for these UV-absorbing nanoparticles to form coatings is essential for photoprotection applications such as cosmetic sunscreens and packaging materials.

We prepared uniform and transparent coatings from the fully-biobased UV-absorbing ECNPs (Figure 4.2(a-b)), with uniform absorbance profiles across the entire UV spectrum, via spin coating. We could completely tune the coating absorbance simply via the spin coating of



Figure 4.1. (a, d) SEM images of (a) ECNPs and (d) ZNPs with encapsulated biobased photoprotectants: quercetin (Q), retinol (R), and p-coumaric acid (C). Scale bar 200 nm. (b, e) Absorbance measurements of the (b) ECNPs and (e) ZNPs with encapsulated UV filters showing a broadband absorbance profile across the UV spectrum. Both dispersions were measured by spectrophotometry at equal concentration (5 x 10^{-2} g L⁻¹). (c, f) Photo of the (c) ECNP and (f) ZNP dispersions without (left) and with (right) encapsulated photoprotectants.

additional layers on top of the original (**Figure 4.2c**), and we found that a 4-layer coating had a thickness of 373 ± 17 nm (**Figure S4.5**). Despite the noticeable yellow colour of the ECNP dispersions, the coating appeared very transparent and a yellow tinge is only visible upon close inspection of the coating at the edges (**Figure 4.2a**), due to the well-known "coffee-ring" drying effect where higher concentrations of particles end up at the edges in coatings prepared by spin coating²⁶. The 373 ± 17 nm fully-biobased coating therefore shows effective and uniform absorbance across the entire UV spectrum although, interestingly, does demonstrate a lower absorbance (average 0.44 A.U. between λ = 290-380 nm) than a similarly thick (~312 nm) coating of similarly-sized ECNPs with a similar loading of synthetic UV filters of ~10 wt% (average coating absorbance 0.81 A.U. between λ = 290-380 nm)⁹.

We also investigated the photostability of the coating – photostability is essential if coatings are to provide effective photoprotection. Moreover, the photostability is known to vary greatly between commercialised UV filters^{2,27,28}. In order to investigate the photostability of the fully-biobased UV-absorbing ECNPs, the coating was irradiated by artificial sunlight for 4 hours and the coating absorbance was measured at hourly intervals (**Figure 4.2d**). The coating was subjected to a total flux of 432 kJ m⁻² UV irradiation (between λ =300-400 nm) over the 4 hours which translates to 2 hours and 24 minutes of summer sunlight in Nice (France) at noon²⁹. We found that the morphology of the ECNPs on the coating surface remained identical after this dose of UV irradiation (**Figure 54.6**). However, the coating absorbance degraded by approximately 50% after the total of 4 hours of irradiation. The



Figure 4.2. (a) Photo of a transparent and uniform coating of the ECNPs on a glass cover slip. A blue dashed line indicates the outside edge of the glass cover slip. (b) SEM image of the coating surface, scale bar 1 μ m. (c) Absorbance measurements of the ECNP coating showing the absorbance of each successive spin coated layer. Wavelengths lower than λ = 300 nm are not shown as they are absorbed by the glass coverslip. (d) Absorbance measurements showing the degradation of the absorbance of the 4-layer coating of ECNPs when irritated by artificial sunlight for 4h.

absorbance loss can be attributed to the degradation of the plant-based photoprotectants encapsulated inside the ECNPs, which are known to lose absorbance upon UV irradiation^{27,30,31}. This coating degradation is about two times as large as what is reported for ECNPs with encapsulated synthetic organic UV filters⁹. Interestingly, the coating absorbance begins to degrade very quickly, especially in the UVB region – but this degradation then plateaus after about 2 hours of irradiation. This is likely due to the photoisomerisation of retinol into a mixture of isomers²⁷ as well as the cis-trans isomerisation of p-coumaric acid³⁰, which both result in absorbance degradation in the UVB region. Overall, the coating maintains effective uniform absorbance across the entire UV spectrum upon irradiation by artificial

sunlight and, therefore, these fully-biobased UV-absorbing ECNPs can prepare effective UV protective coatings with photodegradation values comparable with synthetic UV filters.

4.2.3. Investigation into the Encapsulation of Biobased Photoprotectants into ECNPs

We then investigated the encapsulation of the individual biobased photoprotectants into the ECNPs. Studying the encapsulation of each of these photoprotectants into the ECNPs is crucial in giving us a better understanding of how to prepare effective fully-biobased UV-absorbing NPs. Here, we studied two phenomena: i) the maximum loadings of the biobased photoprotectants into the ECNPs, and ii) the effect of increasing loadings on the size and stability of the ECNPs. It is important that the ECNPs can efficiently encapsulate large amounts of the biobased photoprotectants whilst remaining colloidally stable and without significant changes to the morphology of the particles – preferably the ECNPs will remain small (<100 nm) to minimize scattering of visible light when applied as a coating and therefore remain attractive for cosmetic applications.

We found that the biobased photoprotectants could all be efficiently encapsulated into the ECNPs (Figure 4.3a), but the extent of loading varied between photoprotectants. For example, 14 wt% quercetin and 13 wt% p-coumaric acid could be encapsulated into the ECNPs, whereas up to 31 wt% for retinol (Figure 4.3a). Addition of larger amounts of quercetin to the synthesis simply resulted in similar particle loading for the ECNPs (12-14 wt%, Figure 4.3a), indicating that the ECNPs become saturated with the maximum amount of quercetin that can be incorporated at 12-14 wt% and the excess quercetin used in the synthesis simply precipitates out. This hypothesis is supported by the experimental observation that more precipitate is observed after the antisolvent precipitation when larger amounts of quercetin are added to the synthesis. Addition of larger amounts of p-coumaric acid than 17 wt% led to the formation of a separate set of micron-sized particles of excess p-coumaric acid, as seen by DLS (Figure 54.7). This is why Figure 4.3 does not show data points for larger additions of p-coumaric acid.

Addition of larger amounts of retinol than 50 wt% similarly resulted in the formation of a separate set of much larger particles of excess retinol (**Figure S4.8**). Interestingly, these maximum loading values of retinol, quercetin, and p-coumaric acid into ECNPs are comparable to the synthetic UV filters octinoxate, oxybenzone, and avobenzone, which show maximum loadings of 47 wt%, 14 wt%, and 8 wt% respectively into similarly-sized ECNPs (70-90 nm)²³. We have previously hypothesised that the discrepancy between loadings between compounds may be a result of the varying solubility of the compound in EC²³, which is analogous to the high loadings of SLNs with lyophilic drugs³² and polycarbonate NPs with hydrophobic drugs³³.

The size and stability of the ECNPs as a function of increasing loadings of quercetin and retinol (except for the largest loading of retinol) demonstrated similar behaviour in which the average particle size remained constant (70-76 nm) and absolute zeta potential would increase, indicating increased dispersion stability. This behaviour is similar to the behaviour displayed by the synthetic UV filters oxybenzone, octinoxate, and avobenzone upon encapsulation into ECNPs, where dispersion stability also increased as a function of loading



Figure 4.3. (a) Amount of photoprotectant loaded into the ECNPs (Loaded Photoprotectant) as a function of the amount of photoprotectant added to the synthesis (specifically: the amount dissolved in the ethanol phase before undergoing the antisolvent precipitation). **(b)** The average ECNP size measurements as determined by DLS as a function of the photoprotectant loading. **(c)** The zeta potential measurements of the ECNP dispersions as a function of the photoprotectant loading. The raw data for **Figure 4.3(a-c)** is shown in **Table S4.1**.

and average particle size remained constant (for loadings below 50 wt%)²³. However, increasing loadings of p-coumaric acid undesirably resulted in a large average particle size increase (70-140 nm) and decreasing absolute zeta potential – indicating decreased dispersion stability. Loading the ECNPs with quercetin and retinol is therefore not limited by

decreasing dispersion stability, unlike for p-coumaric acid.

Quercetin and retinol are therefore very attractive for the preparation of effective fully-biobased UV-absorbing NPs from EC because of i) high maximum loading values of quercetin and (particularly) retinol when compared with synthetic UV filters into similarly-sized ECNPs²³, and ii) because higher loadings of quercetin and retinol are not limited by decreasing dispersion stability.

4.2.4. Retention of Biobased Photoprotectants inside ECNPs

We also investigated the retention of the three biobased photoprotectants inside the ECNPs. Physical encapsulation of UV filters into nanoparticles is known to be prone to leaching, since the molecules are not covalently bound to the nanoparticle^{13,34}. Although covalent encapsulation of organic UV filters is possible and has been shown to dramatically increase retention¹³, covalent encapsulation is unrealistic for commercial use because these modified molecules need to be legislatively reapproved. For example, in the U.S. only 3 new UV filters (zinc oxide, avobenzone, and ecamsule) have been approved since 1978³⁵.

We found that the biobased photoprotectants quercetin and retinol were effectively retained inside the ECNPs with 64-70% after 72 hours (**Table 4.1**, **Figure S4.11**), where retention values were similar to synthetic UV filters physically encapsulated into nanoparticles developed for sunscreen applications^{13,36,37}. The retention of p-coumaric acid could not be measured accurately via our spectrophotometric method because the molecule is prone to cis-trans isomerisation which changes the absorbance³⁰. For these retention experiments the absorbance of 10 mL of the UV absorbing nanoparticles in dialysis tubing was measured at time intervals when sitting in a very large body of water (300 mL). It must be noted that these results represent the retention in the particles when in large bodies of water, as opposed to smaller volumes of water, like in formulations. The retention in the ECNPs in formulations. These biobased UV-absorbing nanoparticles therefore demonstrate effective retention values, with the additional advantage that if these biobased photoprotectants are released into the environment they are not reported to have such detrimental environmental impacts as their synthetic counterparts to i.e. coral reefs.

Table 4.1. Percentage of Biobased Photoprotectants Retained in the ECNPs as a Function ofTime

	Retention (%)				
Sample	72h				
Quercetin	86	71	70		
Retinol	85	67	64		

4.3 Conclusions

In conclusion, we prepared UV-absorbing nanoparticles with an entirely biobased composition via the encapsulation of biobased photoprotectants into nanoparticles from ethyl cellulose and zein. The composition of the ECNPs and ZNPs could be easily tuned in order to exhibit uniform, broadband UV spectrum absorbance profiles. We then prepared fully-biobased, transparent coatings with broadband and tunable absorbance by spin coating aqueous dispersions of the UV-absorbing ECNPs. We found that all the biobased

photoprotectants could be encapsulated efficiently into the ECNPs to maximum loadings of 31 wt%, 14 wt% and 13 wt% for retinol, quercetin and p-coumaric acid respectively, which is comparable to synthetic organic UV filters encapsulated into similarly-sized ECNPs. We found that increasing loadings of quercetin and retinol inside the ECNPs resulted in more favourable behaviour than increasing loadings of p-coumaric acid, where the ECNP size remained <100 nm (except for the largest loading of retinol, 31 wt%, where the size increased to 102 nm) and the absolute zeta potential (thus dispersion stability) increased. We also found that the biobased photoprotectants were effectively retained inside the ECNPs with 64-70% after 72 hours, which is comparable to synthetic organic UV filters encapsulated into nanoparticles for sunscreen applications.

Photoprotection technology is pushing towards biobased alternatives for inorganic and synthetic organic UV filters because of their association with many adverse health and environmental effects. Here we develop effective fully-biobased UV-absorbing nanoparticles, representing significant progress towards potentially satisfying these issues, but further studies on the toxicity/environmental impact of plant-based photoprotectants are required.

4.4 Materials and Methods

Materials

Ethyl cellulose (100 cP, lot number MKBT0521V), zein (lot number SLBL9380V), quercetin (\geq 95%, water solubility 60 mg/L at 16°C (DrugBank Database)), retinol (\geq 95%, water solubility 0.67 mg/L at 25°C (DrugBank database)), and p-coumaric acid (98%, water solubility 1.02 mg/L at 24°C (DrugBank database)), were all purchased from Sigma Aldrich. Ethanol (100%) was purchased from Interchema and pure water was used from a Millipore system.

ECNPs/ZNPs with Biobased Photoprotectants Encapsulated

ECNPs and ZNPs with quercetin, retinol and p-coumaric acid were prepared via an antisolvent precipitation technique. Briefly, 0.275 g of EC (for ECNPs) or 0.35 g zein (for ZNPs) and 7 wt% quercetin (thus 0.020 g for ECNPs and 0.025g for ZNPs), 1.5 wt% p-coumaric acid and 1.5 wt% retinol (0.004 g for ECNPs and 0.005 g for ZNPs) were dissolved together in 50 mL ethanol (for ECNPs) or 50 mL of an ethanol/water mixture (80 (v/v)% EtOH) (for ZNPs). This solution was then poured into water (150 mL) under fast magnetic stirring, resulting in the spontaneous formation of ECNPs/ZNPs with encapsulated UV filters. Rotary evaporation removed ethanol and some water to give a 50 mL aqueous dispersion of ECNPs or ZNPs with encapsulated UV filters. The ECNP/ZNP dispersions were filtered through filter paper to remove any large aggregates formed during the antisolvent precipitation. The particles were characterised by Scanning Electron Microscopy (SEM, FEI XL30FEG, samples were sputter coated with platinum), spectrophotometry (HP 8452a), Dynamic Light Scattering (DLS) and zeta potential measurements (Malvern Zetasizer, particle size distributions obtained using a CONTIN fitting and zeta potential measurements performed in presence of 10 mM NaCl background salt one day after preparation of the particles). DLS and zeta potential measurements were performed in triplicate.

Preparation of UV-Protective Coatings and Photodegradation Studies

Coatings were prepared by spin coating (SCS P6700) a concentrated dispersion (30 g L⁻¹) of the fullybiobased UV-absorbing ECNPs onto plasma-cleaned, circular glass microscope cover slips at 1800 rpm for 1 minute. For photodegradation studies, the coating was subjected to irradiation by a 75 W Xenon lamp at a distance of 20 cm (a flux of 3 mW cm⁻² between 300-400 nm). The absorbance was measured hourly for four hours. Coating thickness was measured by SEM imaging (**Figure S4.5**).

Determination of the Maximum Loadings of Photoprotectants into ECNPs

Loadings of photoprotectant inside ECNPs were determined by a spectroscopic method. Briefly, the absorbance of a known concentration of aqueous ECNPs containing encapsulated photoprotectant at the peak of the spectrum was compared with a calibration curve prepared from a series of known concentrations of photoprotectant dissolved in ethanol. Here we make the assumption that the contribution of particle scattering to absorbance is negligible, which is supported by **Figure S4.9**. Particle dispersions were prepared in duplicate to give the error bars in **Figure 4.3a**.

Retention of Photoprotectants inside ECNPs

The retention of the photoprotectants inside the ECNPs was measured as follows: 10 mL of ECNPs with one biobased photoprotectant encapsulated (at 3 wt% for retinol and 4 wt % for quercetin, dispersion concentrations both 5 g L⁻¹) in dialysis membrane tubing (pore size 40 kDa) was placed into 300 mL of water at room temperature under gentle magnetic stirring. Retention was determined by measuring the absorbance of the contents of the dialysis tubing after 24h, 36h, and 72h, in separate experiments to avoid inaccuracy through measurement.

4.5 Acknowledgements

This project was performed in collaboration with Heleen V. M. Kibbelaar. This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs. We thank Chris Schneijdenberg and Dave van den Heuvel for technical assistance.

4.6 References

- (1) Stavros, V. G. Photochemistry: A Bright Future for Sunscreens. *Nat. Chem.* **2014**, *6*, 955–956.
- (2) Bens, G. Chapter 25. In *Sunlight, Vitamin D and Skin Cancer*; Orleans, 2014; pp 429–463.
- (3) Danovaro, R.; Bongiorni, L.; Corinaldesi, C.; Giovannelli, D.; Damiani, E.; Astolfi, P.; Greci, L.; Pusceddu, A. Sunscreens Cause Coral Bleaching by Promoting Viral Infections. *Environ. Health Perspect.* **2008**, *116*, 441–447.
- (4) Downs, C. A.; Kramarsky-Winter, E.; Segal, R.; Fauth, J.; Knutson, S.; Bronstein, O.; Ciner, F. R.; Jeger, R.; Lichtenfeld, Y.; Woodley, C. M.; Pennington, P.; Cadenas, K.; Kushmaro, A.; Loya, Y. Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. *Arch. Environ. Contam. Toxicol.* **2016**, *70*, 265–288.
- (5) Sanchez-Quiles, D.; Tovar-Sanchez, A. Sunscreens as a Source of Hydrogen Peroxide Production in Coastal Waters. *Environ. Sci. Technol.* **2014**, *48*, 9037–9042.
- (6) Hund-Rinke, K.; Simon, M. Ecotoxic Effect of Photocatalytic Active Nanoparticles (TiO2) on Algae and Daphnids. *Environ. Sci. Pollut. Res.* **2006**, *13*, 225–232.
- (7) Kümmerer, K.; Menz, J.; Schubert, T.; Thielemans, W. Biodegradability of Organic Nanoparticles in the Aqueous Environment. *Chemosphere* **2011**, *82*, 1387–1392.
- (8) Hanson, K. M.; Gratton, E.; Bardeen, C. J. Sunscreen Enhancement of UV-Induced Reactive Oxygen Species in the Skin. *Free Radic. Biol. Med.* **2006**, *41*, 1205–1212.
- (9) Hayden, D. R.; Imhof, A.; Velikov, K. P. Biobased Nanoparticles for Broadband UV Protection

with Photostabilized UV Filters. ACS Appl. Mater. Interfaces 2016, 8.

- (10) Maier, T.; Korting, H. C. Sunscreens Which and What For? *Skin Pharmacol. Physiol.* **2005**, *18*, 253–262.
- (11) Krause, M.; Klit, A.; Blomberg Jensen, M.; Søeborg, T.; Frederiksen, H.; Schlumpf, M.; Lichtensteiger, W.; Skakkebaek, N. E.; Drzewiecki, K. T. Sunscreens: Are They Beneficial for Health? An Overview of Endocrine Disrupting Properties of UV-Filters. *Int. J. Androl.* 2012, 35, 424–436.
- (12) Deng, Y.; Ediriwickrema, A.; Yang, F.; Lewis, J.; Girardi, M.; Saltzman, W. M. A Sunblock Based on Bioadhesive Nanoparticles. *Nat. Mater.* **2015**, *14*, 1278–1285.
- (13) Tolbert, S. H.; McFadden, P. D.; Loy, D. A. New Hybrid Organic/Inorganic Polysilsesquioxane– Silica Particles as Sunscreens. *ACS Appl. Mater. Interfaces* **2016**, *8*, 3160–3174.
- (14) Oliveira, C. A. De; Peres, D. D. A.; Graziola, F.; Chacra, N. A. B.; Araújo, G. L. B. De; Flórido, A. C.; Mota, J.; Rosado, C.; Velasco, M. V. R.; Rodrigues, L. M.; Fernandes, A. S.; Baby, A. R. Cutaneous Biocompatible Rutin-Loaded Gelatin-Based Nanoparticles Increase the SPF of the Association of UVA and UVB Filters. *Eur. J. Pharm. Sci.* **2016**, *81*, 1–9.
- (15) Latif, R.; Refai, H.; Tawakkol, S. Photostabilization of Sunscreen Oil through Preparation of a Free-Flowing Powder Photostabilization of Sunscreen Oil through Preparation of a Free-Flowing Powder. J. Microencapsul. 2011, 28, 159–165.
- (16) Perugini, P.; Simeoni, S.; Scalia, S.; Genta, I.; Modena, T.; Conti, B.; Pavanetto, F. Effect of Nanoparticle Encapsulation on the Photostability of the Sunscreen Agent, 2-Ethylhexyl-p-Methoxycinnamate. *Int. J. Pharm.* 2002, 246, 37–45.
- (17) Wissing, S. A.; Muller, R. H. Solid Lipid Nanoparticles as Carrier for Sunscreens: In Vitro Release and in Vivo Skin Penetration. *J. Control. Release* **2002**, *81*, 225–233.
- (18) Herzog, B.; Hüglin, D.; Borsos, E.; Stehlin, A.; Luther, H. New UV Absorbers for Cosmetic Sunscreens A Breakthrough for the Photoprotection of Human Skin. *Chimia (Aarau).* **2004**, *58*, 554–559.
- (19) Cefali, L. C.; Ataide, J. A.; Moriel, P.; Foglio, M. A.; Mazzola, P. G. Plant Compounds as Active Photo Protectants in Sunscreens. *Int. J. Cosmet. Sci.* **2016**, *38*, 346–353.
- (20) Tan, Q.; Liu, W.; Guo, C.; Zhai, G. Preparation and Evaluation of Quercetin-Loaded Lecithin-Chitosan Nanoparticles for Topical Delivery. *Int. J. Nanomedicine* **2011**, *6*, 1621–1630.
- (21) Sessa, D. J.; Eller, F. J.; Palmquist, D. E.; Lawton, J. W. Improved Methods for Decolorizing Corn Zein. *Ind. Crops Prod.* **2003**, *18*, 55–65.
- (22) Bizmark, N.; Ioannidis, M. a. Effects of Ionic Strength on the Colloidal Stability and Interfacial Assembly of Hydrophobic Ethyl Cellulose Nanoparticles. *Langmuir* **2015**, *31*, 9282–9289.
- (23) Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Size and Optically Tunable Ethyl Cellulose Nanoparticles as Carriers for Organic UV Filters. *ChemNanoMat* **2018**, *4*, 301–308.
- (24) Patel, A. R.; Velikov, K. P. Zein as a Source of Functional Colloidal Nano- and Microstructures. *Curr. Opin. Colloid Interface Sci.* **2014**, *19*, 450–458.
- (25) Bizmark, N.; Ioannidis, M. a.; Henneke, D. E. Irreversible Adsorption-Driven Assembly of

Nanoparticles at Fluid Interfaces Revealed by a Dynamic Surface Tension Probe. *Langmuir* **2014**, *30*, 710–717.

- (26) Yunker, P. J.; Still, T.; Lohr, M. A.; Yodh, A. G. Suppression of the Coffee-Ring Effect by Shape-Dependent Capillary Interactions. *Nature* **2011**, *476*, 308–311.
- Tolleson, W. H.; Cherng, S. H.; Xia, Q.; Boudreau, M.; Yin, J. J.; Wamer, W. G.; Howard, P. C.; Yu, H.; Fu, P. P. Photodecomposition and Phototoxicity of Natural Retinoids. *Int. J. Environ. Res. Public Health* 2005, *2*, 147–155.
- (28) Morabito, K.; Shapley, N. C.; Steeley, K. G.; Tripathi, a. Review of Sunscreen and the Emergence of Non-Conventional Absorbers and Their Applications in Ultraviolet Protection. *Int. J. Cosmet. Sci.* **2011**, *33*, 385–390.
- Séite, S.; Moyal, D.; Richard, S.; De Rigal, J.; Lévêque, J. L.; Hourseau, C.; Fourtanier, A. Mexoryl[®]
 SX: A Broad Absorption UVA Filter Protects Human Skin from the Effects of Repeated Suberythemal Doses of UVA. J. Photochem. Photobiol. B Biol. **1998**, 44, 69–76.
- (30) Kort, R.; Vonk, H.; Xu, X.; Hoff, W. D.; Crielaard, W.; Hellingwerf, K. J. Evidence for Trans-Cis Isomerization of the p-Coumaric Acid Chromophore as the Photochemical Basis of the Photocycle of Photoactive Yellow Protein. *FEBS Lett* **1996**, *382*, 73–78.
- (31) Dall'Acqua, S.; Miolo, G.; Innocenti, G.; Caffieri, S. The Photodegradation of Quercetin: Relation to Oxidation. *Molecules* **2012**, *17*, 8898–8907.
- (32) Schäfer-Korting, M.; Mehnert, W.; Korting, H. C. Lipid Nanoparticles for Improved Topical Application of Drugs for Skin Diseases. *Adv. Drug Deliv. Rev.* **2007**, *59*, 427–443.
- (33) Othman, R.; Vladisavljević, G. T.; Nagy, Z. K.; Holdich, R. G. Encapsulation and Controlled Release of Rapamycin from Polycaprolactone Nanoparticles Prepared by Membrane Micromixing Combined with Antisolvent Precipitation. *Langmuir* **2016**, *32*, 10685–10693.
- (34) Kumari, A.; Yadav, S. K.; Pakade, Y. B.; Singh, B.; Yadav, S. C. Development of Biodegradable Nanoparticles for Delivery of Quercetin. *Colloids Surfaces B Biointerfaces* **2010**, *80*, 184–192.
- (35) Lautenschlager, S.; Wulf, H. C.; Pittelkow, M. R. Photoprotection. *Lancet* 2007, 370, 528–537.
- (36) Kidsaneepoiboon, P.; Wanichwecharungruang, S. P.; Chooppawa, T.; Deephum, R.; Panyathanmaporn, T. Organic–inorganic Hybrid Polysilsesquioxane Nanospheres as UVA/UVB Absorber and Fragrance Carrier. J. Mater. Chem. 2011, 21, 7922–7930.
- Lacatusu, I.; Niculae, G.; Badea, N.; Stan, R.; Popa, O.; Oprea, O.; Meghea, A. Design of Soft Lipid Nanocarriers Based on Bioactive Vegetable Oils with Multiple Health Benefits. *Chem. Eng. J.* 2014, 246, 311–321.
- Rutz, J. K.; Zambiazi, R. C.; Borges, C. D.; Krumreich, F. D.; Da Luz, S. R.; Hartwig, N.; Da Rosa, C.
 G. Microencapsulation of Purple Brazilian Cherry Juice in Xanthan, Tara Gums and Xanthan-Tara Hydrogel Matrixes. *Carbohydr. Polym.* 2013, *98*, 1256–1265.

Appendix

Preparation of Fully-Biobased ECNPs and ZNPs with Broadband UV Absorbance

The ECNPs and ZNPs with quercetin, p-coumaric acid, and retinol encapsulated together in a 7:1.5:1.5 ratio respectively (from **Figure 4.1**) show average particle size measurements of 72 nm (for ECNPs, PDI = 26.6 nm) and 76 nm (for ZNPs, PDI = 30.2 nm) as determined by DLS (**Figure S4.1**).

We found that we could simply tune the amount of biobased photoprotectant encapsulated into the ECNPs by the addition of varying ratios of photoprotectant (absorbance profiles shown in **Figure S4.2a**) in the ethanol solution. We found that a 1:1:1 (3.3 wt% ratio for each of retinol, p-coumaric acid and quercetin) resulted in an absorbance profile which was lacking in UVA coverage (**Figure S4.2b**). We always kept the total loading below 10 wt.% because ECNPs are known to encapsulate organic UV filters efficiently to this weight percentage²³, and we wanted to stay under this value so that the encapsulation efficiency remained high. This meant that the absorbance profiles from the ECNPs were reproducible. We found that a ratio of 7:1.5:1.5 provided uniform UV spectrum coverage.

We also investigated loading the ECNPs with greater than 10 wt% material in order to prepare thinner coatings with higher absorbance. Spin coating multiple layers can sometimes result in non-uniformity and this can be avoided by preparing thinner coatings with already high absorbance. We found that this was possible by simply dissolving more material in the initial ethanol solution, and that the ECNPs showed a much greater absorbance at the same concentration of particles (**Figure S4.3** vs. **Figure S4.2b**). However, we found that the absorbance profile was not easy to predict, probably because not all the biobased photoprotectants which were added to the synthesis ended up encapsulated. For example in **Figure S4.3** we see that a 10:3:3 ratio [Q:R:C] results in higher absorbance below 350 nm than a 15:15:4 ratio. This is likely because the ECNPs are unable to encapsulate material after a certain maximum point, which is consistent with our "Maximum Particle Loading" results in



Figure S4.1. Size distributions as determined by DLS for the (a) ECNPs and (b) ZNPs with quercetin, retinol and p-coumaric acid encapsulated in the ratio 7:1.5:1.5.



Figure S4.2. (a) Absorbance profiles of quercetin, coumaric acid, and retinol all measured at equal concentrations of photoprotectant in ethanol $(1 \times 10^{-2} \text{ g L}^{-1})$. (b) Absorbance measurements of ECNPs with the three UV filters encapsulated in varying ratios in which the total loading was kept under 10 wt.%. All measured at equal ECNP concentrations (5 x $10^{-2} \text{ g L}^{-1})$.

Figure 4.3a where we find that quercetin and p-coumaric can only be encapsulated to a maximum of between 12-14 wt.%. Also, the final ratio of the UV filters encapsulated may then be a result of factors other than just the maximum loading for each UV filter, including potential interactions between UV filter molecules with each other (and also with ethyl cellulose) when confined into a small space.

Preparation of Coatings from Full-Biobased UV-absorbing ECNPs

In **Figure 4.2** we showed the preparation of spin coatings of the ECNPs with quercetin, pcoumaric acid and retinol encapsulated in the ratio 7:1.5:1.5. We also prepared spin coatings from the ECNPs with greater amounts of encapsulated material, such as those from **Figure S4.3** (**Figure S4.4**). We found that spin coating the ECNPs with a 15:15:4 ratio of encapsulated



Figure S4.3. Absorbance profiles of ECNPs with the three biobased photoprotectants encapsulated in varying ratios. All measured at equal ECNP concentrations (5 x 10^{-2} g L⁻¹).



Figure S4.4. Absorbance measurements of the ECNP coating prepared from the ECNPs with a 15:15:4 ratio of encapsulated quercetin, p-coumaric acid, and retinol. The absorbance is shown for 4 successive spin coated layers. Wavelengths lower than λ = 290 nm are not shown as they are absorbed by the glass coverslip.

UV filters (quercetin, p-coumaric acid, and retinol) gave thinner coatings with greater absorbance than an equal concentration (30 g L⁻¹) of the ECNPs with less encapsulated material (**Figure S4.4** vs. **Figure 4.2b** in main article). The spin coatings were again prepared onto plasma-cleaned, circular glass microscope cover slips at 1800 rpm for 1 minute. This way, highly transparent and thin coatings high absorbance could be prepared.



Figure S4.5. SEM images of the cross section of a glass slide covered with a 4 layer coating of fullybiobased UV-absorbing ECNPs, (a) at low magnification and (b) at high magnification where the thin coating is visible.



Figure S4.6. SEM image showing the coating surface after UV irradiation. Scale bar $1 \, \mu m$.

In **Figure S4.5** we show a SEM image of the cross section of a glass slide with a 4 layer coating. This SEM image was used to determine the coating thickness $(373 \pm 17 \text{ nm})$ on the glass slide by taking the average of 10 measurements at different areas along the cross section.

In **Figure S4.6** we show a SEM image of the coating surface after irradiation by UV light. We can see that there is no significant change in size or shape of the ECNPs as a result of the irradiation with UV light.



Figure S4.7. Size distributions as measured by DLS for the ECNP dispersions with encapsulated p-coumaric acid in **Figure 4.3**.

Investigation into the Encapsulation of Individual Biobased Photoprotectants into ECNPs

Increasing loadings of p-coumaric acid into the ECNPs resulted in a considerable change in the average ECNP size (**Figure S4.7**). Moreover, we found that the addition of p-coumaric acid greater than 17 wt% resulted in the formation of a second set of micron-sized particles. This larger set of particles is likely pure p-coumaric acid or p-coumaric acid with some EC at the interface: the same micron-sized particles are formed when the antisolvent precipitation is performed with pure p-coumaric acid and no EC (**Figure S4.7f**). This observation has previously been reported for the loading of large amounts of synthetic organic UV filters into ECNPs of similar size (<100 nm)²³.

Retinol could be loaded into the ECNPs very efficiently, up to 31 wt%. The average particle size did not change upon increasing loadings (70-76 nm) except for the largest loading in which the size increased slightly to 102 nm (**Figure S4.8**). The increase in size probably arises because of with the greater amount of material in the particle, and this phenomenon has been seen previously for ECNPs with high loadings (47 wt%) of the UV filter octinoxate²³. We found that the addition of large amounts of retinol (66 wt%) resulted in the formation of much larger particles along with the ECNPs. Similarly as to the case with p-coumaric acid, these larger particles are likely pure retinol or retinol stabilised by some EC at the interface: large



Figure S4.8. Size distributions as measured by DLS for the ECNP dispersions with encapsulated retinol in **Figure 4.3**.
particles are also formed upon performing the antisolvent precipitation of retinol alone (Figure S4.8h).

Increasing loadings of quercetin into the ECNPs resulted in no change in the average particle size (**Figure S4.9**). We hypothesise that this is because the loadings are still relatively low.



Figure S4.9. Size distributions as measured by DLS for the ECNP dispersions with encapsulated quercetin in **Figure 4.3**.

4

The raw data for Figure 4.3 can be found in Table S4.1 below.

Table S4.1.	Raw	data	for	Figure	4.3 .
-------------	-----	------	-----	--------	--------------

Sample	Loading	error	z-average size (nm)	Error (nm)	PDI width (nm)	Zeta pot. (mV)	error
5 wt% Quercetin	3.8	1.6	76	0.1	28	-27.9	0.1
9 wt% Quercetin	7.1	0.7	75	0.1	30	-33	0.5
17 wt% Quercetin	12.0	1.4	76	1.1	32	-36.5	0.1
26 wt% Quercetin	14.0	0.8	77	0.7	31	-32	0.5
33 wt% Quercetin	13.0	2.5	77	0.5	34	-33.5	0.1
50 wt% Quercetin	8.6	2.6	82	0.9	33	-35	0.4
5 wt% Retinol	2.7	0.1	68	0.1	27	-26.0	0.5
9 wt% Retinol	7.1	0.3	69	0.1	29	-34.6	0.5
17 wt% Retinol	12.6	2.8	71	0.5	29	-38.2	1
26 wt% Retinol	15.9	0.1	72	0.5	31	-36.5	0.5
33 wt% Retinol	20.2	3.9	73	0.1	34	-37	1
50 wt% Retinol	30.9	3.5	102	0.5	46	-38.8	0.5
5 wt% p-Coumaric	1.9	0.2	93	0.5	32	-24	0.1
9 wt% p-Coumaric	6.9	0.5	120	0.1	31	-16	0.5
17 wt% p-Coumaric	12.8	0.9	131	0.5	51	-12	0.5
Empty ECNPs	0	0	71	0.4	24	-26	1.8

The loadings from **Figure 4.3** were determined by a spectroscopic method, where the absorbance of a known concentration of aqueous ECNPs containing encapsulated photoprotectant at the peak of the spectrum was compared with a calibration curve prepared from a series of known concentrations of photoprotectant dissolved in ethanol. Here we make the assumption that the contribution of particle scattering to absorbance is minimal. This assumption is supported by Figure S4.10 which shows the absorbance shown by the ECNPs alone is very small compared to the absorbance profile from the series which were used to determine the loadings, when measured at similar concentrations (actual concentrations shown in Figure S4.10). The potential inaccuracy of the final loading which arises as a result of this assumption is smaller than 0.6 wt% for the loadings of p-coumaric acid, which is the photoprotectant for which the particle scattering contributes most to the peak absorbance (because the peak maximum of p-coumaric acid is at the shortest wavelength λ =310 nm of all the photoprotectants and the absorbance due to particle scattering is greatest at lower wavelengths). The potential inaccuracy due to this assumption is much lower for the other photoprotectants (<0.1 wt%). We therefore consider the potential inaccuracy due to this assumption negligible for all the photoprotectants. We determined the loadings in this way and not by drying the particles and re-dissolving in another solvent because retinol is unstable upon direct contact with air and both retinol and p-coumaric acid are unstable as a result of elevated temperatures which would be required to dry the particles^{30,38}. This way, we could



Figure S4.10. Absorbance measurements of ECNPs with encapsulated p-coumaric acid, retinol, quercetin, and ECNPs with nothing encapsulated. These absorbance measurements were used to determine the particle loadings. The absorbance of the ECNPs due to scattering is very small compared to the absorbance profiles for the used to determine the particle loadings. The legends show the amount of photoprotectant added to the synthesis (akin to the x-axis on **Figure 4.3a**) as well as the concentration of the ECNP dispersions at which they were measured by spectrophotometry.

determine the loadings for all the UV filters using an identical technique and without damaging the photoprotectants (and therefore reducing the chance of inaccurate results).

Retention of the Biobased Photoprotectants inside the ECNPs

In **Figure S4.11** we show the absorbance measurements of 10 mL aliquots of ECNPs containing retinol and quercetin (both dispersions 5 g/L) in dialysis tubing measured after 0, 1, 2, and 3 days. Percentage retention is in **Table 4.1**.



Figure S4.11. Absorbance measurements showing the decrease in absorbance due to the release of ECNPs containing (a) 3 wt% of retinol and (b) 4 wt% quercetin from dialysis tubing into the surrounding water medium over a period of 3 days.

Chapter 5. Fully Biobased Highly Transparent Nanopaper with UV-Blocking Functionality

The development of green alternatives for petroleum-based plastics is essential for the protection of our environment and its ecosystems. Here, we demonstrate broadband UV-blocking, highly transparent composite nanopaper films from a waste source of cellulose nanofibrils with embedded tunable UV-absorbing nanoparticles (NPs) from ethyl cellulose. These functional nanopaper films are highly-transparent, selectively block UV light, and show excellent photostability, therefore with great potential as high-performance, renewable, sustainable and biodegradable materials for photoprotection applications. Moreover, the integration of functionalized NPs from ethyl cellulose into nanopaper is a platform for novel advanced sustainable materials with a myriad of functionality.

5.1 Introduction

The problem of plastic waste is one of the greatest challenges faced by the current generation. Every year, millions of tons of plastic pollutes our oceans resulting in potentially major damage to marine life, biodiversity, food security, and human health. The development of renewable, sustainable, and biodegradable alternatives for plastic materials is therefore essential.

Nanopaper has attracted attention as a low-cost, environmentally-friendly, highperformance material with strong potential to replace plastic substrates in many electronic and material applications.^{1–4} Nanopaper is prepared from the same chemical constituents as regular paper but uses very thin cellulose nanofibrils (<20 nm) instead of thicker fibers. These very thin cellulose nanofibrils can advantageously be obtained from diverse sources (multiple plants or bacteria) including waste sources, instead of thicker fibers that are typically obtained from wood pulp. Preparing a material from these thinner nanofibrils results in a material that exhibits superior mechanical and barrier properties to regular paper, and significantly, is also optically transparent.^{1,4,5} The transparent property arises because nanofibrils are much less effective in scattering visible light than larger cellulose fibrils, and can also pack together more efficiently.^{1,3} The transparent nature of nanopaper has opened up the potential for optically functionalized paper-based materials.

Paper-based materials like nanopaper are excellent substrates for functionalization by nanoparticles (NPs) because the porous structure allows for high NP loadings.⁶ Additionally, paper-based materials can be effectively functionalized by a wide *variety* of NPs, resulting in materials suitable for a wide range of applications. For example, the use of inorganic NPs (e.g. TiO₂, Au, Ag) can produce paper-based materials with excellent catalytic,⁷ antibacterial,⁸ sensing⁹ and anticounterfeit¹⁰ properties. Despite the easy functionalization of regular paper-based materials with inorganic NPs, the opaque nature of regular paper limits the potential for optical functionalization. Moreover, inorganic NPs are: (i) non-biobased which limits the renewable and sustainable nature of the resultant material, and (ii) unable to easily utilize functional organic molecules. Therefore, there is a need to develop materials from transparent nanopaper which can be functionalized with biobased organic NPs containing functional organic molecules as encapsulants – which offer a great range of functionality.

Here, we develop transparent UV-blocking nanopaper by embedding tunable UVabsorbing NPs from ethyl cellulose¹¹ into nanopaper. The embedded NPs are used as carriers for two types of UV-absorbing species: (i) organic UV filters commonly used in sunscreens (oxybenzone, octinoxate, and avobenzone), and (ii) biobased UV-absorbing molecules from plants (quercetin, retinol, and p-coumaric acid). Significantly, the nanopaper prepared with the latter set of embedded NPs is entirely biobased. Both the NPs and nanopaper preparation processes are upscalable and moreover, we adopt a more environmentally-friendly route to prepare our nanopaper by retaining the fibrillar structure of cellulose instead of dissolving it using toxic solvents (dissolution is often performed to prepare highly transparent cellulosebased materials, i.e., cellophane and nanopaper containing cellulose nanocrystals (CNCs)).^{5,12,13} Effective UV blocking cellulose films can also be prepared by incorporating inorganic ZnO^{14,15} or synthetic fibers¹⁶ into nanopaper, or even using chemically-modified UVabsorbing CNCs as fillers in a polymeric matrix¹⁷, but the resulting materials are all nonbiobased and the latter two examples involve toxic solvents. Fully-biobased UV-blocking cellulose films can be prepared by incorporating lignin-containing cellulose nanofibrils into nanopaper,^{12,18} but this technique significantly compromises film appearance and

transparency. In our method, the incorporation of biobased UV-absorbing NPs advantageously allows us to more selectively block UV light while maintaining excellent transparency and appearance of the films. We envisage that our transparent UV-blocking nanopaper has great potential for low-cost, high-performance, sustainable materials in a wide variety of photoprotection applications including food and beverage packaging, eye-protection, consumer goods packaging, photoresist templates, UV protective materials for solar panels, and UV filter window shades. Even though we focus here on the potential of transparent UV protective materials, the NPs we use are potential vehicles for a vast range of organic molecules with a variety of properties, including: dyes,¹⁹ fragrances,²⁰ antimicrobial,²¹ photochromic molecules,²² and potentially many more.

Therefore, the novel principle of functionalizing nanopaper with size and compositionally tunable ethyl cellulose NPs²³ allows for the facile fabrication of environmentally-friendly materials with a potentially almost unlimited functionality, as an alternative to plastics in a tremendous range of applications.

5.2 Results and Discussion

We first prepared simple nonfunctionalized nanopaper using cellulose nanofibrils from two sources: (i) bacteria and (ii) primary cell walls in citrus peels waste after production of pectin. The fibrils from both sources are a few tens of nanometers in width in their native state,²⁴ unlike in the case of wood pulp where extensive mechanical/mechanochemical treatment needs to be applied to obtain fibrils in the nanometer range. However, in a dispersed state, these fibrils have a tendency to aggregate due to their attractive van der Waals and hydrogenbonding interactions.²⁵ Since the cross-sectional dimension of the fibrils plays a crucial role in fabricating nanopaper, it is important to individualize the nanofibrils in the dispersed state. This was done by a combination of a TEMPO-mediated surface oxidation reaction²⁶ and a high-energy mechanical deagglomeration process.²⁵ The oxidation reaction results in the conversion of some hydroxyl groups to charged carboxyl groups at the fibril surface leading to electrostatic stabilization of the nanofibrils against agglomeration. Both cellulose sources underwent the same individualization process, which disintegrates the fibrils into much thinner elementary fibril components (nanofibrils are composed of bundles of elementary fibrils with a width ~4 nm^{1,25}). The structure of fibrils obtained after the oxidation reaction is known to be strongly dependent on the starting material,²⁷ and indeed we found that the width of cellulose nanofibrils from bacteria remained similar after the individualization process (mean width 43 nm, Figure 5.1a and discrete size distributions shown in Figure S5.5), which has been reported previously,²⁸ while the individualization process on citrus cellulose yielded much thinner nanofibrils with a mean width of 7 nm (Figure 5.1c and discrete size distributions shown in Figure S5.5), close to that of elementary fibrils.

We prepared nanopaper from both cellulose sources via a simple vacuum filtration followed by gentle heating and pressing. Remarkably, the nanopaper prepared using cellulose



Figure 5.1. (a, c) SEM and TEM images, respectively, of the oxidized bacterial and (negatively stained) citrus cellulose nanofibrils. (b, d) Photos of the nanopaper films prepared from bacterial (b) and citrus (d) cellulose sources, 1 cm away from the text. (e-h) SEM images of the surface and cross section of the nanopaper film from bacterial (e, f) and citrus (g, h) cellulose sources. Scale bars: (a, e, g) 1 μ m, (c) 100 nm, and (f, h) 2 μ m.

nanofibrils from the citrus source was significantly more transparent and less hazy than the nanopaper prepared using the nanofibrils from bacterial cellulose (Figure 5.1b vs Figure 5.1d), at a similar thickness (8 - 12 μ m as measured by SEM imaging of cross sections in Figure 5.1). We hypothesize that the higher transparency of the nanopaper prepared from citrus cellulose nanofibrils arises because these nanofibrils are much thinner than the cellulose nanofibrils from the bacterial source, despite both cellulose sources undergoing the same individualization process. These thinner nanofibrils result in more transparent nanopaper films because they scatter visible light less effectively than the thicker nanofibrils (Rayleigh's scattering theory predicts $\sigma_{set} \alpha D^4$ for long thin fibers, where σ_{set} is the scattering cross section and D is the fiber diameter³) and can also pack together more efficiently. The denser packing of nanofibrils results in fewer air gaps within the cellulose film and therefore less haze,⁴ which arises as a result of the refractive index mismatch between the cellulose and structural air gaps causing light scattering. Moreover, nanopaper prepared with thinner fibrils is reported to have better mechanical properties than nanopaper prepared with thicker fibrils, by an anomalous scaling law.²⁹ The difference in nanofibrils size between the bacterial and citrus cellulose sources is also apparent when observing the nanopaper film surface in Figure 5.1, where the individual nanofibrils are distinguishable on the surface of the bacterial cellulose nanopaper (Figure 5.1e), but indistinguishable on the surface of the citrus cellulose nanopaper (Figure 5.1g). We therefore found that this citrus waste cellulose source is very beneficial for preparing highly transparent nanopaper.

Consequently, we chose to use cellulose nanofibrils from the citrus source, instead of bacterial, to prepare transparent UV-blocking nanopaper. We prepared these films by premixing the cellulose nanofibrils with UV-absorbing ethyl cellulose NPs before proceeding with the same filtration, heating, and pressing technique used for the preparation of the pure nanopaper. We used two types of UV-absorbing ethyl cellulose NPs: (i) "sunscreen-NPs"



Figure 5.2. (a, b) Photos of nanopaper films functionalized with (a) sunscreen-NPs and (b) biobased-NPs, 1 cm away from underlying text. (c, d) SEM images of the surface of the nanopaper films (a) and (b), respectively, in which sunscreen-NPs and biobased-NPs are visible on the respective surfaces. Both scale bars are 500 nm. (e) Spectral transmission measurements for the three films. (f) Total transmission measurements for the three films.

containing encapsulated commonplace organic UV filters from cosmetic sunscreens (oxybenzone, avobenzone, and octinoxate),^{11,23} and (ii) "biobased-NPs" containing encapsulated biobased UV-absorbing molecules obtainable from plants (quercetin, retinol, and p-coumaric acid).¹⁹ Both the sunscreen-NPs and biobased-NPs were prepared by an upscalable "antisolvent precipitation" technique, and were prepared using 10 and 34 wt% UV-absorbing material respectively in the antisolvent precipitation because these recipes are known to give stable aqueous NP dispersions and provide effective uniform absorbance across the entire UV spectrum.^{11,19,23} The resultant prepared sunscreen-NPs and biobased-NPs show average particle diameters of 70 and 90 nm respectively (DLS measurements in **Figure S5.1** and SEM images in **Figure S5.2**), and demonstrate effective uniform absorbance across the entire UV spectrum λ =290-400 nm (**Figure S5.3**).^{11,23} The biobased-NPs also absorb up to λ =450 nm due to the broad absorbance profile of quercetin, resulting in a yellow appearance (**Figure S5.4**).

We found that the nanopaper containing the sunscreen-NPs is almost identical in appearance to the nonfunctionalized nanopaper (Figure 5.1d vs Figure 5.2a), demonstrates similar direct and total transmission values in the visible spectrum (Figure 5.2e-f) but completely blocks out UV light (<1% average between λ =290-380 nm, Figure 5.2e). The nanopaper containing the biobased-NPs is also similar in appearance to the other two films, completely blocks out UV light (Figure 5.2e), but has a slight yellow tinge (Figure 5.2b) due to the presence of quercetin in the biobased-NPs. This fully biobased nanopaper also appears slightly hazier than the other films, which is confirmed by slightly lower direct and diffuse transmission values (Figure 5.2e-f). This is likely due to the increased scattering caused by the slightly larger biobased-NPs compared to the sunscreen-NPs, especially considering that the thickness and root-mean-square (rms) surface roughness for both films are very similar to each other, where the thickness is 9.5 ± 0.4 and 9.6 ± 0.3 µm for the films functionalized with sunscreen-NPs and biobased-NPs, respectively (Figure 55.7a-b), and the rms surface roughness is 67 and 68 nm respectively (Figure 55.8). Improving the transparency of nanopaper can be achieved with more advanced nanopaper preparation methods and

75



Figure 5.3. (a, b) Photos of a glass vial containing 2 nm CdSe quantum dots which fluoresce as a result of stimulation by a λ =375 nm laser light. In (a), the UV laser light is shined through the nonfunctionalized nanopaper (from **Figure 5.1d**, shown in the inset) and the quantum dots are strongly fluorescing, whereas in (b) the UV laser is shined through the nanopaper functionalized with biobased-NPs (from **Figure 5.2b**, shown in the inset) and the fluorescence is significantly suppressed. (c) Photo of nanopaper functionalized with biobased-NPs with text printed on, being bent to demonstrate flexibility.

techniques. For example, postprocessing techniques such as polishing,¹ heat pressing,³ and irreversible collapsing by capillary action by the evaporation of water during pressing¹ are all known to result in far greater transparency. We therefore believe that the transparency/haziness of these films could be improved further by such post-processing techniques. Despite this, we find that our films are highly transparent (direct transmission 51%, 57%, and 39% and total transmission 95%, 94%, and 89% at λ =600 nm for nanopaper, nanopaper with sunscreen-NPs, and nanopaper with biobased-NPs respectively), with far greater transparency values than UV blocking films containing lignin.^{12,18} Moreover, the haze (H_T) values of these films (47%, 39%, and 56% respectively at λ =600 nm),^{5,30} as determined by:⁵

$$H_{\rm T} = 100 \times \frac{(\text{Total Transmission} - \text{Direct Transmission})}{\text{Total Transmission}}$$
(1)

This is remarkable considering we use such a simple technique of nanopaper production.

The presence of the NPs in both the resultant UV-blocking films is supported by SEM imaging of the surface (**Figure 5.2c** and **Figure 5.2d**) and cross section (**Figure S5.7c**). In order to achieve complete UV-blockage, we used a mass ratio of 44:56 [NPs:nanofibrils] for the preparation of the films. We did find that some particles were lost upon preparing the films via vacuum filtration, but this was only a small amount: ~95% of both the added sunscreen-NPs and biobased-NPs were retained in the resultant films respectively (meaning the other 5% passed through the filter), which we determined from the absorbance of the filtrate. Improving the transparency of the films (e.g. with postprocessing techniques) may result in more NPs required for complete UV blockage than what we use, because more transparent



Figure 5.4. (a-c) Photos and (d) transmission measurements of the three films from **Figure 5.2** after 5 months of sunlight exposure. (a) Nanopaper, (b) nanopaper with embedded sunscreen-NPs, and (c) fully biobased nanopaper with embedded biobased-NPs.

films will typically have greater transmission of UV light too, therefore requiring more NPs in order to fully block UV radiation. In this case, both: (i) the amount of NPs embedded in the film as well as (ii) the loading of the UV-absorbing molecules into the NPs, can simply be tuned either by varying the amount of NPs used in the nanopaper preparation or by varying the amount of UV-absorbing material in the NP preparation (a.k.a. antisolvent precipitation) process, in order to provide the desired UV protection. As a visual demonstration of the UVblocking ability of the fully-biobased UV-blocking nanopaper, we show that the fluorescence of CdSe quantum dots, which fluoresce on exposure to a λ =375 nm UV laser, is significantly suppressed when the UV laser is shined through our UV-blocking nanopaper, as compared to the nonfunctionalized nanopaper (**Figure 5.3a-b**). Moreover, we demonstrate the flexibility and ability to print onto the fully-biobased UV-blocking nanopaper in **Figure 5.3c**. We observed no obvious difference in the mechanical properties of the UV-blocking films compared to the non-functionalized film, where all films are flexible and can be repeatedly bent without damage. The UV-blocking films demonstrate effective photostability as a function of time, maintaining identical appearance (Figure 5.4a-c) and transparency (Figure 5.4d, transmission of visible light λ =400-700 nm is almost identical to Figure 5.2e) when exposed to sunlight over a period of 5 months on a windowsill. This duration and intensity of sunlight exposure can be compared, for example, to many food packaging materials in supermarkets. The UV-blocking ability of both the nanopaper with sunscreen-NPs and nanopaper with biobased-NPs remained effective but did degrade slightly, in which the films show average direct transmission values of 10% and 9%, respectively (between λ =290-380 nm), as opposed to <1% before sunlight exposure. Despite this, even after the extensive sunlight exposure, these values are still much lower than that of the non-functionalized nanopaper which transmits 36% (Figure 5.4d). Since the UV absorption is defined by the degradation of the compounds encapsulated inside the NPs,^{19,23} the photostability of both films could be vastly improved simply by loading the films with more NPs or loading the NPs with more UV-absorbing compounds, so that the films maintain full UV blockage despite degradation of the UVabsorbing compounds. Additionally, the UV-blocking films also demonstrated thermal stability very similar to typical nanopaper (Figure S5.9).

5.3 Conclusion

In conclusion, we prepared highly transparent UV-blocking nanopaper with embedded UVabsorbing NPs from ethyl cellulose, via a completely upscalable technique. We use a waste source of cellulose nanofibrils resulting in highly transparent films. We then prepare nanopaper which selectively blocks UV light without compromising film quality by embedding sunscreen-NPs. Significantly, we also prepare transparent UV-blocking nanopaper with a fully biobased composition by embedding biobased-NPs with encapsulated plant-based UVabsorbing compounds. The films blocked UV light completely (<1% direct transmission between λ =290-380 nm) while exhibiting high transparency (57%/94% and 39%/89% direct/total transmission at λ =600), low haze (39% and 56% at λ =600), and good thermal and photostability (still <10% direct transmission between λ =290-380 nm after 5 months of sunlight exposure). Crucially, these films are entirely upscalable and the embedded ethyl cellulose NPs are potential vehicles for a huge range of organic molecules, with a wide range of functionality. This system therefore is highly promising for the innovation of novel and advanced sustainable functional materials for many applications.

5.4 Materials and Methods

Materials

Bacterial cellulose (BC) nanofibrils in the form of pellicles of the strain *Acetobacter* were sourced from a commercial Nata de coco product (Cozzo food industries, Malaysia). Citrus fibers (Herbacel-AQ Plus, type N, ~75% cellulose containing water insoluble fraction) were obtained from Herbafood. Ethyl cellulose (100 cP, lot number MKBT0521V), octinoxate (98%), avobenzone (\geq 99%), oxybenzone (98%), retinol (\geq 95%), p-coumaric acid (98%), quercetin (\geq 95%), and DURAPORE membrane filters (25 mm, pore size 0.65 µm) were obtained from Sigma Aldrich. Ethanol was purchased from Interchema. Deionized water from a Millipore system was used in all experiments.

Preparation of Oxidized Nanofibrils from Bacterial and Citrus Sources

Citrus nanofibril dispersion was prepared by mixing a weighed amount of citrus fibre powder with water, first using a shear mixer (Silverson, L5M-A) for 10 mins at 3000 RPM and then passing it once

through a Z-chamber (87 µm channel diameter) in a Microfluidizer[™] (Microfluidics Corp, M-110S) operated at a maximum pressure of 1200 bar. Bacterial cellulose pellicles were immersed in a 0.1 M NaOH solution followed by thoroughly washing with water and then breaking it down using a hand blender (Braun 4185545). The resulting BC slurry was then subjected to 8 washing cycles involving vacuum filtration and re-dispersion in water, in order to remove soluble impurities. A macroscopically homogeneous dispersion was obtained after passing the suspension once through the Microfluidizer™ operated at a maximum pressure of 1200 bar. The weight fraction of the nanofibrils in the dispersion was determined gravimetrically, as the average of three samples from which water was evaporated at 45°C under a pressure of 40 mbar in a vacuum oven (Memmert Celcius). A combination of TEMPOmediated oxidation reaction and high energy mechanical deagglomeration was applied to disintegrate cellulose nanofibrils from both the sources, described as follows. The oxidation reactions on 0.2 wt% dispersions were done in TEMPO/NaClO₂/NaClO system at neutral conditions for 48 hours at 60°C.²⁶ After the reaction, the oxidized nanofibrils were purified by repeated centrifugation at 10000 g (Hettich Rotina 380R) for 30 mins, decantation and re-dispersion steps, 6 times. In order to individualize the nanofibrils, the purified dispersions were passed through the Microfluidizer™ 5 times, operated at a maximum pressure of 1200 bar. The concentration of the final dispersion was remeasured gravimetrically by the aforementioned procedure.

Preparation of Non-Functionalized Nanopaper Films

Bacterial or citrus cellulose nanofibril dispersion (1 mL at concentration 5 g $L^{-1}/0.5$ wt%) was vacuum filtrated onto a 22 mm hydrophilic polyvinylidene fluoride membrane filter (pore size 0.65 μ m, DURAPORE) cut to fit an 18 mm Buchner funnel for 10 mins until a gel-like cake formed. The filter with the gel-like cake was then removed from the funnel, placed in a petri dish, and put in an oven at 50°C for 10 mins. The nanopaper film was then peeled from the filter with tweezers and pressed for 1 minute with 10 tons of pressure using a hydraulic press (Specac) at room temperature.

Preparation of UV-Absorbing Ethyl Cellulose NPs (Sunscreen-NPs and Biobased-NPs)

Ethyl cellulose NPs with encapsulated UV absorbing molecules were prepared via an antisolvent precipitation technique.¹¹ Briefly, ethyl cellulose (0.275 g) was dissolved in ethanol (50 mL) along with i) oxybenzone (0.008 g), avobenzone (0.008 g), and octinoxate (0.008 g) for the sunscreen-NPs, or ii) quercetin (0.019 g), p-coumaric acid (0.008 g), and retinol (0.014 g) for the biobased-NPs, before being poured into water (150 mL, pH 5-6) under fast magnetic stirring resulting in the spontaneous formation of NPs. The dispersions were then evaporated to 50 mL and filtered through filter paper to remove any large aggregates. These dispersions were then concentrated to 10 g L⁻¹ (by rotary evaporation, gravimetric determination of concentration and adjustments using water) for the preparation of the UV-blocking nanopaper films.

Preparation of UV-Absorbing Nanopaper Films

Aqueous cellulose nanofibril dispersion (1 mL at concentration 5 g $L^{-1}/0.5$ wt%) from the citrus source was first mixed with a quantity (0.65 mL of concentration 10 g L^{-1} in **Figure 5.2**) of either of the aqueous NP dispersions in a small glass pot. This resultant dispersion was then vacuum filtrated, dried, and pressed as described above.

Characterization

Nanopaper films were characterized with SEM imaging (FEI XL30FEG) in which samples were sputter coated with platinum, direct transmission measurements using a HP 8452a spectrophotometer, total transmission measurements using a home-built set-up with a spectrophotometer (HR4000, Ocean Optics) equipped with a 15 cm diameter integrating sphere (barium sulfate coated, Labsphere) and a Tungsten Halogen light source (HL-2000-FHSA-LL, Ocean Optics), and photographs which were taken

with a Nikon D70 camera. NPs were characterized with SEM imaging as described above and also DLS (Malvern Zetasizer Nano ZS) in which particle size distributions were obtained using a CONTIN fitting. Nanofibrils were characterized with SEM imaging as described above and also TEM imaging (Philips TECNAI20) in which the imaged nanofibrils were negatively stained using uranyl acetate and dried on a BUTVAR-coated TEM grid. AFM measurements were used to characterize the surface roughness. Measurements were performed with a JPK Nanowizard II Atomic Force Microscope at room temperature in non-contact mode with a silicon cantilever (BRUKER model: OTESPA-R3) and the roughness of the films was determined from the height images over a 25 μ m² area and are presented as root-mean-square (rms) values. Thermogravimetric analysis (TGA) was performed with a TGA Q50 V6.7 Build 203, using approximately 2-3 mg of the respective samples, under an air atmosphere with a ramp of 15.00 °C/min to 800.00 °C. Printing of text onto the film was performed by sticking the nanopaper onto a sheet of A4 paper with tape and subsequent printing using a Xerox WorkCentre 7855.

5.5 Acknowledgments

This project was performed in collaboration with Srivatssan Mohan. This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of The Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs, as well as the Foundation for Fundamental Research on Matter (FOM), financially supported by a FOM program grant (Grant No. 143). We thank Chris Schneijdenberg and Dave van den Heuvel for technical assistance, Roland Gouzy for his assistance in processing the nanofibril dispersions, Relinde J. A. van Dijk-Moes for providing the quantum dots in **Figure 5.3**, Heleen Kibbelaar for providing the biobased-NPs, L. Christiaan Post for AFM measurements, J. Lennart Weber for TGA measurements, and Tonnishtha Dasgupta for critical reading of the manuscript. D R Hayden and S Mohan contributed equally to this work.

5.6 References

- (1) Nogi, M.; Iwamoto, S.; Nakagaito, A. N.; Yano, H. Optically Transparent Nanofiber Paper. *Adv. Mater.* **2009**, *21*, 1595–1598.
- (2) Lin, Y.; Gritsenko, D.; Liu, Q.; Lu, X.; Xu, J. Recent Advancements in Functionalized Paper-Based Electronics. *ACS Appl. Mater. Interfaces* **2016**, *8*, 20501–20515.
- (3) Hongli, Z.; Sepideh, P.; Colin, P.; Oeyvind, V.; Zhichao, R.; Liangbing, H. Transparent Nanopaper with Tailored Optical Properties. *Nanoscale* **2013**, *5*, 3787–3792.
- Hsieh, M. C.; Koga, H.; Suganuma, K.; Nogi, M. Hazy Transparent Cellulose Nanopaper. *Sci. Rep.* 2017, 7, 41590.
- (5) Xu, X.; Zhou, J.; Jiang, L.; Lubineau, G.; Ng, T.; Ooi, B. S.; Liao, H. Y.; Shen, C.; Chen, L.; Zhu, J. Y. Highly Transparent, Low-Haze, Hybrid Cellulose Nanopaper as Electrodes for Flexible Electronics. *Nanoscale* **2016**, *8*, 12294–12306.
- (6) Hui, Y.; Li, D.; Simon, G. P.; Garnier, G. Paper Surfaces Functionalized by Nanoparticles. *Adv. Colloid Interface Sci.* **2011**, *163*, 23–38.
- (7) Li, S. X.; Lin, X.; Zheng, F. Y.; Liang, W.; Zhong, Y.; Cai, J. Constituting Fully Integrated Visual Analysis System for Cu(II) on TiO2/Cellulose Paper. *Anal. Chem.* **2014**, *86*, 7079–7083.
- (8) Tankhiwale, R.; Bajpai, S. K. Graft Copolymerization onto Cellulose-Based Filter Paper and Its Further Development as Silver Nanoparticles Loaded Antibacterial Food-Packaging Material. *Colloids Surfaces B Biointerfaces* **2009**, *69*, 164–168.

- (9) Niarchos, G.; Dubourg, G.; Afroudakis, G.; Tsouti, V.; Makarona, E.; Matović, J.; Crnojević-Bengin, V.; Tsamis, C. Paper-Based Humidity Sensor Coated with ZnO Nanoparticles: The Influence of ZnO. *Procedia Eng.* **2016**, *168*, 325–328.
- (10) Zhang, Y.; Aslan, K.; Previte, M. J. R.; Geddes, C. D. Metal-Enhanced Fluorescence from Paper Substrates: Modified Spectral Properties of Dyes for Potential High-Throughput Surface Analysis and Assays and as an Anti-Counterfeiting Technology. *Dye. Pigment.* 2008, 77, 545– 549.
- (11) Hayden, D. R.; Imhof, A.; Velikov, K. P. Biobased Nanoparticles for Broadband UV Protection with Photostabilized UV Filters. *ACS Appl. Mater. Interfaces* **2016**, *8*, 32655–32660.
- (12) Sadeghifar, H.; Venditti, R.; Jur, J.; Gorga, R. E.; Pawlak, J. J. Cellulose-Lignin Biodegradable and Flexible UV Protection Film. *ACS Sustain. Chem. Eng.* **2017**, *5*, 625–631.
- (13) Yang, Q.; Fukuzumi, H.; Saito, T.; Isogai, A.; Zhang, L. Transparent Cellulose Films with High Gas Barrier Properties Fabricated from Aqueous Alkali/Urea Solutions. *Biomacromolecules* 2011, 12, 2766–2771.
- (14) Feng, X.; Zhao, Y.; Jiang, Y.; Miao, M.; Cao, S.; Fang, J. Use of Carbon Dots to Enhance UV-Blocking of Transparent Nanocellulose Films. *Carbohydr. Polym.* **2017**, *161*, 253–260.
- (15) Jiang, Y.; Song, Y.; Miao, M.; Cao, S.; Feng, X.; Fang, J.; Shi, L. Transparent Nanocellulose Hybrid Films Functionalized with ZnO Nanostructures for UV-Blocking. J. Mater. Chem. C 2015, 3, 6717–6724.
- (16) Luo, J.; Zhang, M.; Yang, B.; Liu, G.; Tan, J.; Nie, J.; Song, S. A Promising Transparent and UV-Shielding Composite Film Prepared by Aramid Nanofibers and Nanofibrillated Cellulose. *Carbohydr. Polym.* **2019**, *203*, 110–118.
- (17) Sirviö, J. A.; Visanko, M.; Heiskanen, J. P.; Liimatainen, H. UV-Absorbing Cellulose Nanocrystals as Functional Reinforcing Fillers in Polymer Nanocomposite Films. *J. Mater. Chem. A* **2016**, *4*, 6368–6375.
- (18) Wang, Q.; Du, H.; Zhang, F.; Zhang, Y.; Wu, M.; Yu, G.; Liu, C.; Li, B.; Peng, H. Flexible Cellulose Nanopaper with High Wet Tensile Strength, High Toughness and Tunable Ultraviolet Blocking Ability Fabricated from Tobacco Stalk: Via a Sustainable Method. J. Mater. Chem. A 2018, 6, 13021–13030.
- (19) Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Fully-Biobased UV-Absorbing Nanoparticles from Ethyl Cellulose and Zein for Environmentally Friendly Photoprotection. *RSC Adv.* 2018, *8*, 25104–25111.
- (20) Sansukcharearnpon, A.; Wanichwecharungruang, S.; Leepipatpaiboon, N.; Kerdcharoen, T.; Arayachukeat, S. High Loading Fragrance Encapsulation Based on a Polymer-Blend: Preparation and Release Behavior. Int. J. Pharm. 2010, 391, 267–273.
- Pan-In, P.; Banlunara, W.; Chaichanawongsaroj, N.; Wanichwecharungruang, S. Ethyl Cellulose Nanoparticles: Clarithomycin Encapsulation and Eradication of H. Pylori. *Carbohydr. Polym.* 2014, 109, 22–27.
- (22) Vílchez-Maldonado, S.; Calderó, G.; Esquena, J.; Molina, R. UV Protective Textiles by the Deposition of Functional Ethylcellulose Nanoparticles. *Cellulose* **2014**, *21*, 2133–2145.
- (23) Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Size and Optically Tunable Ethyl

Cellulose Nanoparticles as Carriers for Organic UV Filters. ChemNanoMat 2018, 4, 301–308.

- (24) Mohan, S.; Jose, J.; Kuijk, A.; Veen, S. J.; van Blaaderen, A.; Velikov, K. P. Revealing and Quantifying the Three-Dimensional Nano- and Microscale Structures in Self-Assembled Cellulose Microfibrils in Dispersions. *ACS Omega* **2017**, *2*, 5019–5024.
- Kuijk, A.; Koppert, R.; Versluis, P.; Dalen, G. Van; Remijn, C.; Hazekamp, J.; Nijsse, J.; Velikov, K.
 P. Dispersions of Attractive Semi Fl Exible Fiberlike Colloidal Particles from Bacterial Cellulose Micro Fi Brils † *1. Langmuir* 2013, *29*, 14356–14360.
- (26) Saito, T.; Hirota, M.; Tamura, N.; Kimura, S.; Fukuzumi, H.; Heux, L.; Isogai, A. Individualization of Nano-Sized Plant Cellulose Fibrils by Direct Surface Carboxylation Using TEMPO Catalyst under Neutral Conditions. *Biomacromolecules* **2009**, *10*, 1992–1996.
- (27) Siró, I.; Plackett, D. Microfibrillated Cellulose and New Nanocomposite Materials: A Review. *Cellulose* **2010**, *17*, 459–494.
- (28) Saito, T.; Nishiyama, Y.; Putaux, J. L.; Vignon, M.; Isogai, A. Homogeneous Suspensions of Individualized Microfibrils from TEMPO-Catalyzed Oxidation of Native Cellulose. *Biomacromolecules* **2006**, *7*, 1687–1691.
- (29) Zhu, H.; Zhu, S.; Jia, Z.; Parvinian, S.; Li, Y.; Vaaland, O.; Hu, L.; Li, T. Anomalous Scaling Law of Strength and Toughness of Cellulose Nanopaper. *Proc. Natl. Acad. Sci.* **2015**, *112*, 8971–8976.
- (30) Fang, Z.; Zhu, H.; Yuan, Y.; Ha, D.; Zhu, S.; Preston, C.; Chen, Q.; Li, Y.; Han, X.; Lee, S.; Chen, G.;
 Li, T.; Munday, J.; Huang, J.; Hu, L. Novel Nanostructured Paper with Ultrahigh Transparency and Ultrahigh Haze for Solar Cells. *Nano Lett.* **2014**, *14*, 765–773.
- (31) Bizmark, N.; Ioannidis, M. a. Effects of Ionic Strength on the Colloidal Stability and Interfacial Assembly of Hydrophobic Ethyl Cellulose Nanoparticles. *Langmuir* **2015**, *31*, 9282–9289.
- Jin, H.; Zhou, W.; Cao, J.; Stoyanov, S. D.; Blijdenstein, T. B. J.; de Groot, P. W. N.; Arnaudov, L. N.; Pelan, E. G. Super Stable Foams Stabilized by Colloidal Ethyl Cellulose Particles. *Soft Matter* 2012, *8*, 2194.
- (33) Sehaqui, H.; Liu, A.; Zhou, Q.; Berglund, L. A. Fast Preparation Procedure for Large, Flat Cellulose and Cellulose/Inorganic Nanopaper Structures. *Biomacromolecules* **2010**, *11*, 2195–2198.
- (34) Hsieh, M. C.; Kim, C.; Nogi, M.; Suganuma, K. Electrically Conductive Lines on Cellulose Nanopaper for Flexible Electrical Devices. *Nanoscale* **2013**, *5*, 9289–9295.
- (35) Guo, J.; Uddin, K. M. A.; Mihhels, K.; Fang, W.; Laaksonen, P.; Zhu, J. Y.; Rojas, O. J. Contribution of Residual Proteins to the Thermomechanical Performance of Cellulosic Nanofibrils Isolated from Green Macroalgae. ACS Sustain. Chem. Eng. 2017, 5, 6978–6985.

Appendix

Ethyl Cellulose Nanoparticles (NPs) Characterization

Both the sunscreen-NPs and biobased-NPs were characterized with Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM) imaging, and absorbance measurements. The DLS measurements show an average particle diameter of 70 nm for the sunscreen NPs and 90 nm for the biobased-NPs (**Figure S5.1**).



Figure S5.1. Size distributions as determined by DLS showing the sunscreen-NPs with an average size of 70 nm and the biobased-NPs with an average size of 90 nm.

The SEM imaging shows that both sets of NPs are spherical with sizes consistent with the DLS measurements (Figure S5.2).



Figure S5.2. SEM images of (a) sunscreen-NPs and (b) biobased-NPs. Scale bars 200 nm.

The absorbance measurements show that both the sunscreen-NPs and the biobased-NPs demonstrate absorbance over the entire UV spectrum, and the biobased-NPs also absorb up to 450 nm (**Figure S5.3**).



Figure S5.3. Absorption spectra for (a) sunscreen-NPs and (b) biobased-NPs, both measured at a concentration of 1×10^{-1} g L⁻¹.

The photograph of both NP dispersions in **Figure S5.4** shows that the biobased-NPs are yellow compared to the colourless sunscreen-NPs (slightly blue-tinged due to the Tyndall effect). It is also worth noting that the fully-biobased particles have been investigated for their environmental durability in wet conditions in previous work, where the UV absorbing molecules are effectively retained within the NPs but some migration can occur.¹⁹ Despite this, EC is insoluble in water and these NPs are known to be stable also at low pH values and across a range of ionic strengths.^{31,32}



Figure S5.4. Photo showing dispersions of sunscreen-NPs (left vial) and biobased-NPs (right vial).

Nanofibril characterization

The size distributions for the cellulose nanofibrils from a bacterial source and the cellulose nanofibrils from a citrus source are shown in **Figure S5.5**. The cellulose nanofibrils from the bacterial and citrus sources have mean average widths of 43 nm and 7 nm respectively.



Figure S5.5. Discrete size distributions for the average width of the cellulose nanofibrils from (a) the bacterial source and (b) the citrus fruits source. Information was obtained from the TEM and SEM images in **Figure 5.1**.

(Citrus) Films Characterization

When too few sunscreen-NPs or biobased-NPs are used to prepare the films, the films do not completely block UV radiation (Figure S5.6).



Figure S5.6. Transmission measurements for the nanopaper films prepared with (a) sunscreen-NPs, and (b) biobased-NPs, showing that when too few NPs are used there is transmission of UV radiation. Concentration of particles used for nanopaper is always 10 g L^{-1} for both the sunscreen-NPs and biobased-NPs.

The film thicknesses for the nanopaper films functionalized with the sunscreen-NPs and the biobased-NPs (shown in **Figure 5.2** in the main article) were 9.6 \pm 0.3 μ m and 9.5 \pm 0.4 μ m respectively, determined by measuring the thickness at ten points across the SEM images of the cross sections of both films in **Figure S5.7a-b**. Additionally, from SEM imaging of the cross section of another area of the nanopaper film with embedded sunscreen-NPs we found that the NPs are not just distributed on the surface of the films as seen in **Figure 5.2**, but also



Figure S5.7. (a-b) SEM images of the cross sections of the functionalized nanopaper films from **Figure 5.2** in the main article, in which (a) is an image of the nanopaper film functionalized with sunscreen-NPs and (b) is an image of the nanopaper film functionalized with biobased-NPs. Both scale bars 10 μ m. (c) SEM image of the cross section of a nanopaper film functionalized with sunscreen-NPs from **Figure S5.2** showing that sunscreen-NPs are visible between the layers. Scale bar 500 nm.

between the layers (Figure S5.7c).

The surface roughness values of the prepared films were determined by Atomic Force Microscopy (AFM) from height profiles over a 25 μ m² area, shown in **Figure S5.8**. The surface roughness values are presented as rootmean-square (rms) values. The root mean square (rms) roughness values were measured as 42 nm, 26 nm, 67 nm, and 68 nm for: i) pure bacterial cellulose nanopaper (from **Figure 5.1b**) ii) pure citrus cellulose nanopaper (from **Figure 5.1b**) ii) pure citrus cellulose nanopaper (from **Figure 5.2a**), and iv) citrus cellulose nanopaper functionalized with biobased-NPs respectively (from **Figure 5.2b**). These values are all typical for nanopaper³³ which is much smaller than typical values for regular paper (rms values ~5-6 microns³⁴).



Figure S5.8. AFM height profiles for: (a) pure bacterial cellulose nanopaper (b) pure citrus cellulose nanopaper, (c) citrus cellulose nanopaper functionalized with sunscreen-NPs, and (d) citrus cellulose nanopaper functionalized with biobased-NPs.

The thermal stability of the nanopaper films was investigated using Thermal Gravimetric Analysis (TGA). We found that the nanopaper films were stable until 180-220°C, where they decomposed significantly up to 450°C (**Figure S5.9**). This decomposition can be attributed to the decomposition of cellulose which has been previously observed in the TGA of nanopaper.³⁵ Interestingly, we find second degradation peaks at ~400°C for two of the films, which we cannot explain. Although interesting, the films have already heavily degraded by this temperature, therefore we emphasize that the important information from this data is that all the films are stable until 180-220°C.



Figure S5.9. TGA profiles for the pure nanopaper (from **Figure 5.1d**), nanopaper with sunscreen-NPs (from **Figure 5.2a**), and nanopaper with biobased-NPs (from **Figure 5.2b**), indicating their thermal stability under an air atmosphere. (a) Weight loss as a function of temperature. (b) Temperature first derivative weight loss profiles (dW/dT) of the samples.

UV-Blocking Nanopaper from Bacterial Cellulose

We found that nanopaper from bacterial cellulose could also be prepared and functionalized in the same way as with citrus cellulose (**Figure S5.10**). Fewer NPs were required in order to achieve full UV blockage here – 0.1 mL sunscreen-NPs at 10 g L^{-1} – because the transparency was much lower anyway).



Figure S5.10. (a-b) Photographs of a nanopaper film using cellulose nanofibrils from a bacterial source (1 mL at 5 g L⁻¹) functionalized with sunscreen-NPs (0.1 mL at 10 g L⁻¹). (c) SEM image of the surface of the nanopaper film clearly showing the large nanofibrils and the particles embedded. Scale bar 500 nm. (d) Direct and (e) total transmission measurements of both the pure bacterial cellulose nanopaper film (the film shown in **Figure 5.1b**) and the bacterial cellulose nanopaper film functionalized with the sunscreen-NPs.

Chapter 6. Photochromic Nanoparticles from Ethyl Cellulose for Smart Materials

The development of photochromic nanoparticles (NPs) is important for the development of novel smart materials in many applications. Here, we develop photochromic NPs from ethyl cellulose (ECNPs) by encapsulating an organic photochrome from the spirooxazine family by an upscalable technique. The photochromic ECNPs exist in as a dispersion in water and have an average diameter of 78 nm. We then prepare transparent thin films on glass substrates and transparent nanopaper from the ECNPs and observe the photochromic activity. These results have great implications for the development of photochromic nanoparticles and smart materials.

6.1 Introduction

Photochromism is the reversible transformation of a chemical species between two forms with different absorption spectra, with the use of a light stimulus.¹ The word photochromism derives from Greek and originally referred to a general light-induced ("*photo*") colour change of compounds ("*chromism*"), but has since been replaced by the more specific above definition.² The property of photochromism in compounds can be exploited for many applications, perhaps most-commonly known for darkening lenses for glasses, but also in data storage,³ energy storage,⁴ and textiles.⁵

Many compounds exhibit photochromism, including both organic and inorganic. Typical families of photochromic organic compounds include spiropyrans, spirooxazines, dithienylethenes, stilbenes, and azobenzenes.² Some inorganic metal halides also exhibit photochromism such as AgCl, which is the active ingredient in many darkening lenses. The origin of the colour change is usually a result of either a photoisomerization, which is the case for organic molecules, or a photoreduction of metallic ions, which is the case for inorganic compounds. Organic photochromes offer a greater variety of properties than inorganic photochromes and are more tunable. For example, new organic photochromes are being developed commercially with novel colour or solubility properties by synthetic modifications of the molecular structures of existing photochromes.^{6,7} Despite this, there is currently not such a wide variety of photochromic compounds and therefore the properties and potential applications are limited. These limitations have driven research towards the incorporation of organic photochromes into NPs.⁸

The incorporation of photochromic molecules into NPs can combine the unique properties of the photochrome with effective properties of NPs. For example, the incorporation of these predominantly water-insoluble photochromic molecules into water-dispersible NPs allows the use of these photochromes in aqueous media. The ability to use these photochromes in aqueous environments is interesting for biological imaging, ^{9,10} colloidal imaging, as well as the use of these photochromes in the preparation of many materials and coatings which require a water solvent. Additionally, incorporation of photochromes into NPs can result in suppressed degradation,¹¹ as well as enhanced light emission.¹² Photochromes have been incorporated into micron-sized particles for use in water-based coatings,¹³ but such large particles on the micron instead of nano-scale scatter light very effectively and therefore cannot be used for transparent materials. Despite the potential of photochromic NPs, the field is still very young. The only significant commercial application of photochromic NPs which can be used for the development of novel materials with photochromic NPs which can be used for the development of novel materials with photochromic properties.

Herein, we develop photochromic NPs from ethyl cellulose (ECNPs) with an encapsulated photochrome from the spirooxazine family and demonstrate their use in the preparation of novel colour-changing materials. ECNPs are known effective carriers for multiple organic compounds,^{14–16} are size-tunable,¹⁷ and can be prepared using an upscalable process. Moreover, ECNPs can produce thin films on substrates^{14,16} and effectively functionalize textiles⁵ and paper-based materials.¹⁵ We therefore prepare transparent films of photochromic ECNPs on glass substrates and investigate the photochromic activity. We then prepare transparent photochromic paper-based materials by combining the photochromic ECNPs with cellulose nanofibrils. These paper-based materials are interesting

particularly for the development of sensor applications. For example, such a transparent material which responds to UV light could be used in food packaging and give information about the extent of UV light that the packaged food is exposed to, which relates directly to food spoilage. Moreover, we investigate the potential to stimulate only particular sections of the nanopaper material by UV light, which has implications for the development of erasable messages and untraceable documents.

6.2 Results and Discussion

We first identified a suitable organic photochromic molecule from the spirooxazine family to encapsulate into the ECNPs (**Scheme 6.1**). We chose this photochrome because it shares the solubility properties of EC (soluble in ethanol, insoluble in water) which are required for the encapsulation using an upscalable antisolvent precipitation method. Moreover, this photochrome changes molecular conformation to a species which is not charged, unlike other photochromes.⁸ It is important that the molecule does not become charged because this will likely change the solubility properties to water soluble, and the solubility properties for the antisolvent precipitation rely on the molecule remaining water-insoluble.

Scheme 6.1. Reaction scheme for the spirooxazine molecule (full name: 1,3-Dihydro-1,3,3-tri-methylspiro[2H-indole-2,3'- [3H]naphth[2,1-b][1,4]oxazine]) conformation change upon UV irradiation.



We encapsulated the photochrome using the established antisolvent precipitation method, which has previously been used to effectively encapsulate UV filters into ECNPs.^{14,15} Here, the photochrome was dissolved in ethanol together with the EC, before undergoing the antisolvent precipitation process to form an aqueous dispersion of ECNPs with encapsulated photochrome. We found that the ECNPs had an average diameter of 78 nm (as measured by Dynamic Light Scattering, DLS), which is expected for the amount of EC used in the antisolvent precipitation process.¹⁷ The resultant photochromic ECNPs have a zeta potential of -28 mV, which is similar to pure ECNPs (-26 mV)¹⁷ and desirably shows that the incorporation of the photochrome does not destabilize the ECNPs, which can happen with UV filters.¹⁶

We found that an aqueous dispersion of the photochromic ECNPs changed colour from slightly turbid to a distinct blue colour upon irradiation by 1 minute under a UV lamp



Figure 6.1. (a) Photographs of an aqueous dispersion of ECNPs (concentration 5 g/L) before (left) and directly after (right) UV irradiation. (b) Absorbance spectrum for the ECNPs, measured at a concentration of 0.25 g/L.

(Figure 6.1a). This colour change confirms that the conformation change of the photochrome is not prevented by confinement into the ECNPs. The presence of the photochrome in the ECNPs was further confirmed by the absorbance spectrum (Figure 6.1b), which also showed that the photochrome (in its relaxed state) absorbs from λ =380 nm. The colour change of the photochromic ECNP dispersion is reversible, where the colour change from blue back to colourless quickly occurs in a matter of seconds (Figure S6.1).

We then investigated the ECNPs for their ability to make coatings. The ability to form coatings is important for the application of these ECNPs to form thin films and functionalize various substrates. We found that the photochromic ECNPs could form uniform transparent coatings on glass slides simply by spin coating (**Figure 6.2a**). The presence of ECNPs on the surface of the glass slide was confirmed using SEM imaging, where the ECNP size is consistent with the DLS measurements (**Figure 6.2b**). We found that the transparent coatings of photochromic ECNPs demonstrated the colour change from colourless to blue upon irradiation by UV light, similar to the photochromic ECNP dispersion. Interestingly, we found that the colour change for the coatings still hadn't completely reversed from blue back to colourless after 2 minutes (**Figure S6.2**). This slower colour change is likely due to the photochrome now being in the solid state, which reduces the speed of the molecular conformation change, as has been previously observed.¹⁸

Finally, we investigated the ability for these photochromic nanoparticles to make freestanding transparent paper-based materials. It is known that functional UV-absorbing ECNPs can effectively functionalize transparent paper-based materials (a.k.a. nanopaper), simply by filtering a mixture of an aqueous dispersion of ECNPs with cellulose nanofibrils.¹⁵ We found that the photochromic ECNPs could also effectively be combined with cellulose nanofibrils to prepare transparent nanopaper (**Figure 6.3**). The functional nanopaper exhibited photochromic behavior, where the colour of the material changed from transparent to blue upon irradiation by UV light. Similar to the spin coatings, we also found that the colour change



Figure 6.2. (a) Photographs of a glass slide coated with the photochromic ECNPs, before (left) and directly after (right) UV irradiation. (b) SEM image of the surface of the glass slide showing the photochromic ECNPs.

from blue back to colourless took much longer (>2 minutes) than when the ECNPs were in the liquid state (Figure S6.3).

Another remarkable observation is that particular sections of the nanopaper could be stimulated to change colour by the use of a UV laser (Figure 6.3c), and also with the use of a template (Figure 6.3d). This property allows the nanopaper to be written onto using laser light and without physical contact, which is interesting for the development of erasable messages and untraceable documents. Additionally, we found that the intensity of the UV light was directly dependent on brightness of the blue colour exhibited by the nanopaper. In Figure 6.3b, the nanopaper is stimulated with a UV lamp for 1 minute and the blue colour is fainter than in Figure 6.3c, where the nanopaper is stimulated with a high-intensity UV laser. In Figure 6.3d, the nanopaper is placed underneath a template before being stimulated by a UV lamp for a minute (in the same way as for Figure 6.3b), and the nanopaper is even fainter than the nanopaper in Figure 6.3b because the template blocks some UV light.



Figure 6.3. Photographs of nanopaper containing photochromic ECNPs. (a) Photochromic nanopaper. (a) The nanopaper after irradiation by a UV lamp for 1 minute. (b) The nanopaper is irradiated by a UV laser for 10 seconds. (c) The nanopaper after irradiation by a UV lamp through a template, demonstrating that text can be temporarily "printed" on using UV light.

6.3 Conclusions

In conclusion, we developed an aqueous dispersion of photochromic NPs from ethyl cellulose (average diameter 78 nm) by encapsulating a photochromic molecule from the spirooxazine family via an upscalable technique. We then prepared transparent thin films from these NPs by spin coating and transparent paper-based materials by combining the NPs with cellulose nanofibrils. The thin films and paper-based materials both demonstrated effective photochromic activity, where the speed of the colour change of the photochromic NPs in the solid state was significantly slower than compared with the aqueous dispersion. Moreover, we found that the use of templates resulted in the ability to "write" on the paper-based

materials using UV light. We also found that the higher intensity UV irradiation resulted in greater photochromic activity. We believe that these results have great implications for the development of photochromic NPs for smart materials, such as transparent food-packaging which can give an insight into the extent of food-spoilage, as well as erasable messages and untraceable documents.

6.4 Materials and Methods

Materials

Cellulose fibers (Herbacel-AQ Plus, type N, ~75% cellulose containing water insoluble fraction) were obtained from Herbafood. Ethyl cellulose (100 cP, lot number MKBT0521V), the photochrome (1,3-Dihydro-1,3,3-tri- methylspiro[2H-indole-2,3'-[3H]naphth[2,1-b][1,4]oxazine], >98%) and DURAPORE membrane filters (25 mm, pore size 0.65 μ m) were obtained from Sigma Aldrich. Ethanol was purchased from Interchema. Deionized water from a Millipore system was used in all experiments.

Preparation of Photochromic ECNPs

ECNPs with encapsulated photochrome were prepared via an antisolvent precipitation technique.¹⁴ Briefly, ethyl cellulose (0.275 g) was dissolved in ethanol (50 mL) along with the photochrome (0.028 g) before being poured into water (150 mL, pH 5-6) under fast magnetic stirring resulting in the spontaneous formation of NPs. The dispersions were then evaporated to 50 mL and filtered through filter paper to remove any large aggregates. These dispersions were then concentrated to 42 g L⁻¹ (by rotary evaporation, gravimetric determination of concentration and adjustments using water) for the spin coating and to 10 g L⁻¹ for the preparation of the nanopaper.

Spin Coating of Photochromic ECNPs

A concentrated dispersion of the photochromic ECNPs (42 g L⁻¹, 300 μ L) was spin-coated onto plasma-cleaned glass microscope cover slips (No. 1, 22mm) at 1500 rpm for one minute.

Preparation of Oxidized Cellulose Nanofibrils

The oxidized cellulose nanofibril dispersion was prepared by mixing a weighed amount of cellulose fibre powder with water, first using a shear mixer (Silverson, L5M-A) for 10 mins at 3000 RPM and then passing it once through a Z-chamber (87 µm channel diameter) in a Microfluidizer™ (Microfluidics Corp, M-110S) operated at a maximum pressure of 1200 bar. The weight fraction of the nanofibrils in the dispersion was determined gravimetrically, as the average of three samples from which water was evaporated at 45°C under a pressure of 40 mbar in a vacuum oven (Memmert Celcius). A combination of TEMPO-mediated oxidation reaction and high energy mechanical deagglomeration was applied to disintegrate the cellulose nanofibrils, described as follows. The oxidation reactions on 0.2 wt% dispersions were done in TEMPO/NaClO₂/NaClO system at neutral conditions for 48 hours at 60°C.¹⁹ After the reaction, the oxidized nanofibrils were purified by repeated centrifugation at 10000 g (Hettich Rotina 380R) for 30 mins, decantation and re-dispersion steps, 6 times. In order to individualize the nanofibrils, the purified dispersions were passed through the Microfluidizer™ 5 times, operated at a maximum pressure of 1200 bar. The concentration of the final dispersion was remeasured gravimetrically by the aforementioned procedure.

Preparation of Photochromic Nanopaper

An aqueous dispersion of oxidized cellulose nanofibrils (1 mL at concentration 5 g $L^{-1}/0.5$ wt%) was mixed with the photochromic ECNPs (0.65 mL, 10 g L^{-1}) and vacuum filtrated onto a 22 mm hydrophilic polyvinylidene fluoride membrane filter (pore size 0.65 μ m, DURAPORE) cut to fit an 18 mm Buchner

funnel for 10 mins until a gel-like cake formed. The filter with the gel-like cake was then removed from the funnel, placed in a petri dish, and put in an oven at 50°C for 10 mins. The nanopaper film was then peeled from the filter with tweezers and pressed for 1 minute with 10 tons of pressure using a hydraulic press (Specac) at room temperature.

Characterization

Photochromic ECNPs were characterized by: Spectrophotometry (HP 8452a), SEM (FEI XL30FEG) and DLS (Zetasizer Nano ZS, Malvern). Coatings were prepared with a spin coater (SCS P6700). Photographs were taken with a Nikon D70 camera. The aqueous dispersion of photochromic ECNPs and spin coated glass substrates were irradiated with a 6 Watt UVGL-58 Handheld UV lamp at λ =365 nm at a distance of 10 cm. The nanopaper was irradiated with the same UV lamp and also a 0.07 Watt λ =375 nm LED UV Pen Light (Varta), both at a distance of 10 cm.

6.5 Acknowledgements

This project was performed in collaboration with Maria Spanou. The part of this project involving nanopaper was performed in collaboration with Srivatssan Mohan. This research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of The Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs, as well as the Foundation for Fundamental Research on Matter (FOM), financially supported by a FOM program grant (Grant No. 143). We thank Chris Schneijdenberg and Dave van den Heuvel for technical assistance and Roland Gouzy for his assistance in processing the nanofibril dispersions.

6.6 References

(1) Irie, M. Photochromism: Memories and Switches-Introduction. *Chem. Rev.* **2002**, *100*, 1683–1684.

(2) Wang, L.; Li, Q. Photochromism into Nanosystems: Towards Lighting up the Future Nanoworld. *Chem. Soc. Rev.* **2018**, *47*, 1044–1097.

(3) Feringa, B. L.; Jager, W. F.; de Lange, B. Organic Materials for Reversible Optical Data Storage. *Tetrahedron* **1993**, *49*, 8267–8310.

(4) Kucharski, T. J.; Tian, Y.; Akbulatov, S.; Boulatov, R. Chemical Solutions for the Closed-Cycle Storage of Solar Energy. *Energy Environ. Sci.* **2011**, *4*, 4449–4472.

(5) Feczkó, T.; Samu, K.; Wenzel, K.; Neral, B.; Voncina, B. Textiles Screen-Printed with Photochromic Ethyl Cellulose-Spirooxazine Composite Nanoparticles. *Color. Technol.* **2013**, *129*, 18–23.

(6) Tian, H.; Yang, S. Recent Progresses on Diarylethene Based Photochromic Switches. *Chem. Soc. Rev.* **2004**, *33*, 85–97.

(7) Lukyanov, B. S.; Lukyanova, M. B. SPIROPYRANS: SYNTHESIS, PROPERTIES, AND APPLICATION. *Chem. Heterocycl. Compd.* **2005**, *41*, 1–31.

(8) Harbron, E. J. Photochromic Nanoparticles. *Photochemistry* **2011**, *39*, 211–227.

(9) Xiong, Y.; Rivera-Fuentes, P.; Sezgin, E.; Vargas Jentzsch, A.; Eggeling, C.; Anderson, H. L. Photoswitchable Spiropyran Dyads for Biological Imaging. *Org. Lett.* **2016**, *18*, 3666–3669.

(10) Fernández-Suárez, M.; Ting, A. Y. Fluorescent Probes for Super-Resolution Imaging in Living Cells. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 929–943.

(11) Zhu, M. Q.; Zhu, L.; Han, J. J.; Wu, W.; Hurst, J. K.; Li, A. D. Q. Spiropyran-Based Photochromic Polymer Nanoparticles with Optically Switchable Luminescence. *J. Am. Chem. Soc.* **2006**, *128*, 4303–4309.

(12) Lim, S. J.; An, B. K.; Sang, D. J.; Chung, M. A.; Soo, Y. P. Photoswitchable Organic Nanoparticles and a Polymer Film Employing Multifunctional Molecules with Enhanced Fluorescence Emission and Bistable Photochromism. *Angew. Chemie - Int. Ed.* **2004**, *43*, 6346–6350.

(13) Periyasamy, A. P.; Vikova, M.; Vik, M. A Review of Photochromism in Textiles and Its Measurement. *Text. Prog.* **2017**, *49*, 53–136.

(14) Hayden, D. R.; Imhof, A.; Velikov, K. P. Biobased Nanoparticles for Broadband UV Protection with Photostabilized UV Filters. *ACS Appl. Mater. Interfaces* **2016**, *8*, 32655–32660.

(15) Hayden, D. R.; Mohan, S.; Imhof, A.; Velikov, K. P. Fully Biobased Highly Transparent Nanopaper with UV-Blocking Functionality. *ACS Appl. Polym. Mater.* **2019**, *1*, 641–646.

(16) Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Fully-Biobased UV-Absorbing Nanoparticles from Ethyl Cellulose and Zein for Environmentally Friendly Photoprotection. *RSC Adv.* **2018**, *8*, 25104–25111.

(17) Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Size and Optically Tunable Ethyl Cellulose Nanoparticles as Carriers for Organic UV Filters. *ChemNanoMat* **2018**, *4*, 301–308.

(18) Di Nunzio, M. R.; Gentili, P. L.; Romani, A.; Favaro, G. Photochromism and Thermochromism of Some Spirooxazines and Naphthopyrans in the Solid State and in Polymeric Film. *J. Phys. Chem. C* **2010**, *114*, 6123–6131.

(19) Saito, T.; Hirota, M.; Tamura, N.; Kimura, S.; Fukuzumi, H.; Heux, L.; Isogai, A. Individualization of Nano-Sized Plant Cellulose Fibrils by Direct Surface Carboxylation Using TEMPO Catalyst under Neutral Conditions. *Biomacromolecules* **2009**, *10*, 1992–1996.



Figure S6.1. Photographs of aqueous dispersions of photochromic ECNPs before and after irradiation with a UV lamp for 1 minute. Six photographs are taken at increasing time periods after irradiation to monitor the speed of the reversing colour change from blue back to colourless.



Figure S6.2. Photographs of spin coatings of photochromic ECNPs before and after irradiation with a UV lamp for 1 minute. Six photographs are taken at increasing time periods after irradiation to monitor the speed of the reversing colour change from blue back to colourless.



Figure S6.3. Photographs of nanopaper containing photochromic ECNPs before and after irradiation with a UV lamp for 1 minute. Six photographs are taken at increasing time periods after irradiation to monitor the speed of the reversing colour change from blue back to colourless.
Part 2

UV-Absorbing Composite "Matchsticks" for Functional Materials

Chapter 7. Seeded-Growth of Silica Rods from Titania Particles and Self-Assembly

Seeded-growth of silica rods from titania particles is a facile method to synthesize novel, complex, matchstick-shaped particle structures which are particularly interesting for optical applications. However, the synthesis and assembly of these matchsticks is challenging and not well explored. Here, we explore the tunability of these matchsticks and their assembly into arrays with liquid crystalline order via sedimentation and supraparticles via spherical confinement. These results are valuable for the development of novel particle structures and particle assembly.

7.1 Introduction

Photonic crystals are materials which can manipulate light because of periodic order, or more specifically, the materials contain a periodically varying index of refraction. Many examples of photonic crystals exist in nature, including the colouring in peacock feathers and some butterfly wings, as well as the reflective 'eyeshine' seen in many nocturnal animals.¹ Photonic crystals find use wherever light needs to be manipulated, therefore have many potential applications, particularly in optics,^{2–4} reflective coatings,¹ and structural colouration.⁵

One potential novel bottom-up approach towards photonic crystals is through rodshaped silica particles. The use of colloidal particles to form photonic crystals is attractive because this gives the chemist control of the nano/micron-sized building blocks and a bottomup approach to their assembly. Rod-shaped silica particles are particularly interesting because they can be made with an anisotropic shape,⁶ hybrid and anisotropic composition,^{7–9} are sizetunable at the length scale appropriate for optical applications (the wavelength of visible light),^{6,7,10} and can assemble into 2D and 3D liquid crystal phases.¹¹

The synthesis of rod-shaped silica particles with titania on one end has recently been demonstrated in literature, where the particles have a "matchstick" like structure with a silica tail and titania head.⁷ Titania is a high refractive index material, which makes these hybrid matchsticks particularly interesting for photonic crystals. However, the synthesis and assembly of these novel structures is not well studied. There is a therefore a need to explore the synthesis and tunability of these hybrid matchsticks, and investigate their assembly into 2D and 3D ordered structures.

In this work, we grow silica rods from titania particles to form matchstick-shaped hybrid particles. We then assemble these matchsticks via sedimentation and spherical confinement. We explore the formation of nematic and smectic liquid crystal phases when assembling the matchsticks into arrays via sedimentation. The ability to vary the liquid crystal phase simply by varying the particle concentration is interesting for fundamental studies. The formation of smectic liquid crystal phases is particularly desirable because this results in 1D periodic long-ranged ordering of the high refractive index titania "heads", which is interesting for photonic crystals. We then investigate the assembly of these matchsticks into spherical structures consisting solely of clusters of matchstick particles, termed supraparticles. Supraparticles can themselves be building blocks for further hierarchical structures.^{12,13}

We believe these results have great implications for the development of novel complex hybrid particle structures with potential applications in photonic crystals, as well as being fundamentally of great interest.

7.2 Results and Discussion

7.2.1 Growth of Silica Rods from Titania Particles

We began by synthesizing titania particles with an average size 432 nm (polydispersity δ = 4%, where δ = (standard deviation)/(mean diameter)) (**Figure S7.1**). These particles were synthesized by an established technique in literature, involving the hydrolysis and condensation of titania precursor titanium tetraisopropoxide.¹⁴ Next, we grew silica rods from titania particles. The growth of silica rods from colloidal titania particles in this size range is already an established synthesis in literature, which results in anisotropic matchstick-shaped particles with a titania "head" and silica "tail".⁷ The experimental conditions to grow silica



Figure 7.1. (a, b) SEM images of matchsticks with titania "heads" and silica "tails". (c-e) TEM images of the resulting matchsticks when the reaction time is shortened to: (c) 15 minutes, (d) 1 hour, (e) 2 hours.

rods from titania particles exploits the well-established emulsion-droplet based growth of anisotropic silica structures.⁷ In this synthesis, a water-in-oil emulsion is formed where the water droplets are stabilized by sodium citrate and poly(vinyl pyrrolidone) (PVP), in a continuous phase of 1-pentanol which also contains dispersed titania particles. The water droplets interact with the hydrophilic surface of the titania particles, and silica rods then grow from the ammonia-catalysed condensation of TEOS inside the emulsion droplets.

We prepared matchsticks using this technique and the resulting particles have lengths ranging between 4-5 μ m, widths 300-400 nm, and aspect ratios ((rod length)/(rod diameter)) 8-13 (**Figure 7.1a, b**). We could also prepare matchsticks with lower aspect ratios by using larger titania particles (786 nm, δ = 5%, **Figure S7.2**). We found that it was essential to age the titania particles in water for at least one day before the growth of silica rods from the titania particles, and not doing this resulted in dispersions of pure silica rods and titania particles with no silica rods grown from the surface, similar to another study.⁷ Interestingly, we also observed that some silica rods grew from multiple titania particles, which can be explained by the favourable free energy of the water droplet attachment at concave regions, as has been previously observed.¹⁵ We also found that we could tune the length, and therefore aspect ratio, of the matchsticks simply by varying the reaction time (**Figure 7.1c-e**).



Figure 7.2. Confocal images in reflection mode of the bottom layer of matchsticks, which have been sedimented in a capillary to form liquid crystal phases. The bright dots are indicative of the high refractive index titania. (a, b) The sedimentation column was filled with 500 μ L of a solution of matchsticks in ethanol at a concentration of 0.14 g/L and a nematic phase was formed. (c, d) The sedimentation column was filled with 500 μ L of a solution of 1.06 g/L (volume fraction 0.4) and regions of smectic phases formed.

7.2.2 Self-Assembly of Matchsticks into Liquid Crystalline Phases via Sedimentation

We then left dispersions of these matchsticks (500 μ L, concentration 0.14 g/L and 1.06 g/L) to sediment in ethanol for 3 days, in hand-made sedimentation columns prepared from the end of a glass pipette glued to a glass cover slip.

Interestingly, we found that the bottommost layers of the sedimented matchsticks formed nematic and smectic liquid crystal phases. We found that the type of liquid crystal phase (nematic or smectic) was dependent on the concentration of sedimenting matchsticks (**Figure 7.2**). We believe that we were able to form both nematic and smectic liquid crystal phases because of the high aspect ratio (9-13) of these matchsticks. This hypothesis is consistent with the phase diagram for spherocylinders which shows that the nematic and smectic phases form over a wider range of packing fractions for higher aspect ratios.¹⁶



Figure 7.3. (a) SEM image of the clusters of rods formed for the high aspect ratio matchsticks from **Figures 7.1 and 7.2**. (b, c) Spherical supraparticles formed from lower aspect ratio matchsticks (**Figure S7.2**).

7.2.3 Self-Assembly of Matchsticks into Supraparticles via Spherical Confinement

Finally, we assembled the matchsticks into supraparticles via spherical confinement. Here, the matchsticks were assembled into supraparticles simply by emulsifying an aqueous dispersion of the matchsticks into a water-in-oil emulsion, followed by slow evaporation of the solvent in the suspended water droplets.¹⁷

We first found that supraparticles from the high-aspect ratio rods did not form spherical supraparticles, instead forming supraparticles composed of small clusters of nearly parallel matchsticks (**Figure 7.3a**). We believe that the small size of the emulsion droplets relative to the large matchstick length facilitated the formation of small clusters of parallel matchsticks instead of spherical supraparticles. We then found that lower aspect ratio matchsticks (**Figure 57.2**) did form spherical supraparticles (**Figure 7.3b, c**), which is consistent with our hypothesis that a large emulsion droplet size relative to the particle size helps facilitate the formation of spherical supraparticles. We did not find that the matchsticks inside the spherical supraparticles were particularly ordered. Despite this, we believe that optimizing conditions and increasing supraparticles size may result in greater order. For example, more controlled emulsification procedures, such as microfluidics, would allow the formation of well-defined and controllable droplet sizes.^{18–20} Moreover, slower speeds of the self-assembly (thus longer drying times) has been reported to improve ordering.²¹ The formation of such supraparticles is very interesting for 3D photonic crystalline materials.

7.3 Conclusions

In conclusion, we prepared matchstick-shaped hybrid particles with titania "heads" and silica "tails". We explored the size tunability of these particles, ability to control the length and aspect ratio, and ability to grow from multiple sizes of titania (average diameter 432 nm and 786 nm). We then assembled these structures into arrays via sedimentation and supraparticles via spherical confinement. We found nematic and smectic liquid crystal phases from the assembly via sedimentation. The matchsticks inside the supraparticles did not show any order. However, we believe that optimizing the conditions of supraparticles formation could result in ordered supraparticles. These results are valuable for the development of novel particle structures and particle assembly.

7.4 Materials and Methods

Materials

Sodium citrate dihydrate (99%), 1-pentanol (99%), tetraethylorthosilicate (TEOS, 98%), ammonium hydroxide (NH4OH, 25 w/w%), Polyvinylpyrrolidone-40 (PVP, Weight Average Molecular Weight MW = 40 kg/mol), PVP-360 (MW = 360 kg/mol), (3- aminopropyl)-triethoxysilane (APTES, 98%), methanol (99%), acetonitrile (99%), titanium(IV) isopropoxide (TTIP, 97%), dodecylamine (>99%), and Span 80 were purchased from Sigma Aldrich. Ethanol (100%) was purchased from Interchema and pure water was used from a Millipore system. n-Hexadecane (for synthesis) was obtained from Merck.

Preparation of Titania Particles

Titania particles were prepared by a modified method from literature.¹⁴ Here, acetonitrile (49 mL), H_2O (0.18 mL), dodecylamine (350 µL), methanol (101 mL) were added to a 250 mL round bottomed flask and stirred vigorously. TTIP (1.0 mL) was then added. The reaction is left to proceed for 8 hours. After 8 hours, the titania particles were isolated via centrifugation, washed with ethanol, and then finally isolated via centrifugation and subsequent drying in an oven at 50 °C. The following day, the dried particles were then redispersed in water to make a 2.5 wt% solution. For the lower aspect ratio matchsticks in **Figure S7.2** which were used for the supraparticles in **Figure 7.3**, the reaction was left to proceed for 24h instead of 8h resulting in larger titania seed particles.

Growth of Silica Rods from Titania Particles

Silica rods were grown via a modified procedure described by Datskos et al.⁷, in which our modification is that we prepare the matchsticks using a defined concentration of titania in water. The silica rod growth is a modification from the silica rod growth procedure originally described by Kuijk et al.⁶ Firstly, a PVP/1-pentanol stock solution was made with concentration 100 g/L. Then 9.11 g of this stock solution was added to a 15 mL centrifuge tube. Next, 500 μ L of titania particles (2.5 wt% in water) were dispersed in the PVP/1-pentanol solution via sonication. Ethanol (1.5 mL) and aqueous sodium citrate (0.18 M, 100 μ L) were then added and the mixture was vigorously shaken by hand. Ammonia (200 μ L, 25%) was then added and the mixture shaken again. TEOS (100 μ L) was then added followed again by vigorous shaking. The mixtures were then left to stand for 12 hours. The particles were washed by two rounds of centrifuging at 500g for 15 minutes, discarding the supernatant, and redispersing in ethanol. To obtain shorter matchsticks, a higher concentration of titania particles in water (5 wt%) was used.

Assembly of Matchsticks via Sedimentation

The sedimentation columns were prepared by hand from a glass Pasteur Pipette (WU Mainz, short form, 150 mm), glass cover slip (Deckglaser, No. 1.5H, 22 × 22 mm), conventional glass slide (ThermoFisher), and UV curing glue (NOA 68). Briefly, the end of a glass Pasteur Pipette (where the rubber bulb attaches) was cut off and the resulting sharp end was then sanded. One end of this glass cylinder was then glued to a glass microscope cover slip using UV curing glue. Next, this pipette end with connected cover slip was inserted through a glass microscope slide which contains a complimentarily-sized hole drilled in the centre, which is used so that the cell can be held in a conventional microscope stage. The two were then glued together resulting in a home-made sedimentation column. The dispersion of matchsticks in ethanol (500 μ L, concentration 0.14 or 1.06 g/L) was then pipetted into the end of glass pipette and the top sealed with Parafilm to prevent solvent evaporation. The matchsticks in ethanol were then left to sediment for 3 days in this sedimentation column.

Assembly of Matchsticks via Spherical Confinement

Supraparticles were made using a water-in-oil (W/O) emulsion method, in which charge stabilized colloids are compressed inside aqueous droplets dispersed in a nonvolatile organic phase.¹⁷ The matchstick particles obtained from the synthesis were centrifuged and redispersed in deionized water (10 mL, 2 g/L). 0.5 mL of this dispersion was then pipetted into hexadecane (5 mL) containing nonionic surfactant Span 80 (1 wt%) in a small glass vial (20 mL). This solution was then shaken vigorously to form the emulsion. Parafilm was then used to seal the top of the glass vial, which was then punctured with a few holes (to allow slow evaporation of the aqueous dispersed phase). The vial was then left to shake gently (IKA KS 260 basic, 250 rpm) for 2 weeks. The shaking was then stopped to allow the formed supraparticles to sediment. After 1 hour, sediment was visible on the bottom of the vial so the supernatant was carefully removed using a glass pipette. The supraparticles were then washed twice with cyclohexane (cyclohexane added, solution vortexed, left to sediment, solvent removed), eventually leaving a relatively concentrated supraparticle dispersion in <0.5 mL cyclohexane.

Characterization

Imaging of the particle morphology was performed with TEM, SEM and confocal microscopy. TEM was performed with an FEI TECNAI 20 electron microscope, in which samples were prepared by pipetting a drop of the particle dispersion in ethanol onto a Formvar/Carbon Films 200 Mesh Copper (100) grid. SEM was performed with an FEI XL30SFEG in which samples were prepared by pipetting a drop of the particle dispersion onto a silicon wafer on top of an aluminium stub and subsequently sputter-coated with a platinum layer of approximately 4 nm. Confocal microscopy was performed with a Leica SP8 confocal microscope (100x oil immersion lens).

7.5 Acknowledgements

This research was performed in collaboration with Chris L. Kennedy and Fabian Hagemans. The research is supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs, as well as by the Netherlands Center for Multiscale Catalytic Energy Conversion (MCEC), an NWO Gravitation programme funded by the Ministry of Education, Culture and Science of the government of the Netherlands. We thank Chris Schneijdenberg for technical assistance, and Ramakrishna Kotni for useful discussions.

7.6 References

- (1) Parker, A. R.; Townley, H. E. Biomimetics of Photonic Nanostructures. *Nat. Nanotechnol.* **2007**, *2*, 347–353.
- (2) Soukoulis, C. M. Photonic Crystals and Light Localization in the 21st Century, 1st ed.; 2001.
- (3) Xia, Y.; Gates, B.; Li, Z. Y. Self-Assembly Approaches to Three-Dimensional Photonic Crystals. *Adv. Mater.* **2001**, *13*, 409–413.
- (4) Birner, A.; Wehrspohn, R. B.; Gosele, U. M.; Busch, K. Silicon-Based Photonic Crystals. *Adv. Mater.* **2001**, *13*, 377–388.
- (5) Lee, H. S.; Shim, T. S.; Hwang, H.; Yang, S. M.; Kim, S. H. Colloidal Photonic Crystals toward Structural Color Palettes for Security Materials. *Chem. Mater.* **2013**, *25*, 2684–2690.
- (6) Kuijk, A.; van Blaaderen, A.; Imhof, A. Synthesis of Monodisperse, Rodlike Silica Colloids with Tunable Aspect Ratio. *J. Am. Chem. Soc.* **2011**, *133*, 2346–2349.

- (7) Datskos, P.; Cullen, D. A.; Sharma, J. Step-by-Step Growth of Complex Oxide Microstructures. *Angew. Chemie - Int. Ed.* **2015**, *54*, 9011–9015.
- (8) Kim, J.; Hwang, H. J.; Oh, J. S.; Sacanna, S.; Yi, G. Monodisperse Magnetic Silica Hexapods. J. Am. Chem. Soc. 2018, 140, 9230–9235.
- (9) Morgan, A. R.; Dawson, A. B.; Mckenzie, H. S.; Skelhon, T. S.; Beanland, R.; Franks, H. P. W.; Bon, S. A. F. Chemotaxis of Catalytic Silica–Manganese Oxide "Matchstick" Particles. *Mater. Horizons* **2014**, *1*, 65–68.
- (10) Sharma, J. Finite-Sized One-Dimensional Silica Microstructures (Rods): Synthesis, Assembly, and Applications. *ChemNanoMat* **2017**, *3*, 214–222.
- (11) Kuijk, A.; Byelov, D. V.; Petukhov, A. V.; van Blaaderen, A.; Imhof, A. Phase Behavior of Colloidal Silica Rods. *Faraday Discuss.* **2012**, *159*, 181–199.
- (12) Wintzheimer, S.; Granath, T.; Oppmann, M.; Kister, T.; Thai, T.; Kraus, T.; Vogel, N.; Mandel, K. Supraparticles: Functionality from Uniform Structural Motifs. *ACS Nano* **2018**, *12*, 5093–5120.
- (13) Wang, T.; Lamontagne, D.; Lynch, J.; Zhuang, J.; Cao, Y. C. Colloidal Superparticles from Nanoparticle Assembly. *Chem. Soc. Rev.* **2013**, *42*, 2804–2823.
- (14) Tanaka, S.; Nogami, D.; Tsuda, N.; Miyake, Y. Synthesis of Highly-Monodisperse Spherical Titania Particles with Diameters in the Submicron Range. J. Colloid Interface Sci. 2009, 334, 188–194.
- (15) Li, C.; Zhang, S.; Zhang, B.; Liu, J.; Zhang, W.; Solovev, A. A.; Tang, R.; Bao, F.; Yu, J.; Zhang, Q.; Lifshitz, Y.; He, L.; Zhang, X. Local-Curvature-Controlled Non-Epitaxial Growth of Hierarchical Nanostructures. *Angew. Chemie - Int. Ed.* **2018**, *3200003*, 3772–3776.
- (16) Bolhuis, P.; Frenkel, D. Tracing the Phase Boundaries of Hard Spherocylinders. J. Chem. Phys. **2002**, *106*, 666–687.
- (17) Nijs, B. De; Dussi, S.; Smallenburg, F.; Meeldijk, J. D.; Groenendijk, D. J.; Filion, L.; Imhof, A.; van Blaaderen, A.; Dijkstra, M. Entropy-Driven Formation of Large Icosahedral Colloidal Clusters by Spherical Confinement. *Nat. Mater.* **2015**, *14*, 56–60.
- (18) Kim, S. H.; Lee, S. Y.; Yi, G. R.; Pine, D. J.; Yang, S. M. Microwave-Assisted Self-Organization of Colloidal Particles in Confining Aqueous Droplets. *J. Am. Chem. Soc.* **2006**, *128*, 10897–10904.
- (19) Montanarella, F.; Urbonas, D.; Chadwick, L.; Moerman, P. G.; Baesjou, P. J.; Mahrt, R. F.; Van Blaaderen, A.; Stöferle, T.; Vanmaekelbergh, D. Lasing Supraparticles Self-Assembled from Nanocrystals. ACS Nano 2018, 12, 12788–12794.
- (20) Besseling, T. H.; Hermes, M.; Kuijk, A.; De Nijs, B.; Deng, T. S.; Dijkstra, M.; Imhof, A.; van Blaaderen, A. Determination of the Positions and Orientations of Concentrated Rod-like Colloids from 3D Microscopy Data. *J. Phys. Condens. Matter* **2015**, *27*, 1-13.
- (21) Wang, J.; Mbah, C. F.; Przybilla, T.; Apeleo Zubiri, B.; Spiecker, E.; Engel, M.; Vogel, N. Magic Number Colloidal Clusters as Minimum Free Energy Structures. *Nat. Commun.* **2018**, *9*, 1-10.

Appendix

Image of titania particles



Figure S7.1. TEM image of titania particles. Scale bar 1 micron.

Smaller titania rods



Figure S7.2. (a) TEM image (scale bar 2 microns) of titania particles with average size 786 nm, δ = 5%. (b-c) SEM images of lower aspect ratio matchsticks, prepared using an equal concentration (by weight %) of slightly larger seed particles. Silica rod growth from the matchsticks proceeded in an identical way as for the smaller titania particles.

Chapter 8. Seeded-Growth of Silica Rods from Silica-Coated Particles

Seeded growth of silica rods from colloidal particles has emerged as a facile method to develop novel complex particle structures with hybrid compositions and asymmetrical shapes. However, this seeded-growth technique is so far limited to colloidal particles of only a few materials. Here, we first develop a general synthesis for the seeded-growth of silica rods from silica particles. We then demonstrate the growth of silica rods from silica-coated particles with three different cores which highlight the generality of this synthesis: fluorescently labeled organo-silica (fluoresceine), metallic (Ag) and organic (PS latex). We also demonstrate the assembly of these particles into supraparticles. This general synthesis method can be extended to the growth of silica rods from any colloidal particle which can be coated with silica, such as crystalline titania.

8.1. Introduction

Fine control of the composition and shape of building blocks at the nano and micron scale is a route to novel functional materials with unique properties. Particularly interesting colloidal building blocks are colloidal silica rod-like structures. Silica rods with high uniformity can already be made via a one-pot wet-chemical synthesis method,¹ are low-cost, inert, biocompatible, highly tunable and already show great potential for many novel materials such as advanced coatings (superhydrophobic² and non-fouling³), thin films for solar cells,⁴ porous composites,^{5,6} and advanced optical materials because of their ability to form liquid crystal^{7–} ⁹ and plastic crystal phases.^{10–12}

Increasing the breadth of these potential applications as well as the complexity of potential novel materials can be achieved by increasing the complexity of the building blocks themselves. Two ways to increase the complexity of silica rods are by introducing: i) heterogeneity in composition (e.g. composites/hybrid structures), and ii) anisotropy in composition (e.g. creating asymmetry). Introducing heterogeneity in the composition of silica rods adds functionality which broadens the potential properties of the material, whilst asymmetry in these structures is essential for some potential applications in active matter, ^{13–} ¹⁶ directed assembly,⁹ and advanced optical materials.

Currently, the most effective experimental conditions to prepare silica rods exploit the emulsion-droplet based growth of anisotropic silica structures, which was developed in our group.^{17,18} In this method, silica rods grow from the ammonia-catalysed condensation of tetraethylorthosilicate (TEOS) in water emulsion droplets stabilized by sodium citrate and poly(vinyl pyrrolidone) (PVP) in a 1-pentanol continuous phase. This method reproducibly results in highly uniform silica rods with tunable aspect ratio. Moreover, with this method the silica rods can even be shaped by modifying conditions during growth,^{3,19} or via posttreatment to create even more complex particles.^{20,21} Subsequent research then found that when certain colloidal particles (e.g. TiO₂, FeO_x, and MnO_x) are also present in the synthesis, the silica rods can grow from the water droplets attached to (or Pickering stabilized by) the colloidal "seed" particles, resulting in anisotropic composite silica rods.^{14,15,19,22,23} This is known as "seeded-growth" and the resulting particles as "matchsticks". Alternatively, functional polymers can be introduced by an inverse seeded-growth method, where silica rods are used as seeds in a polymerization reaction.²⁴ Despite the seeded-growth synthesis technique being very effective for growing anisotropic composite silica rods with certain functionalities, the synthesis is so far limited to only a few colloidal particles e.g. FeO_x , TiO_2 , and MnO_x. This is probably because the colloidal particle 'seeds' must adhere to certain requirements, e.g. be dispersible in 1-pentanol and interact in a specific way with the water emulsion droplets in order to facilitate growth of a silica rod. In practice, this means that seed particles and growth conditions must be optimized for each desired material. In fact, the use of this method along with colloidal silica and silica-coated particles has shown to result in isotropic shapes with multiple silica rods grown from the seed.^{19,25} It is difficult with some colloidal particles to facilitate silica rod growth at all, where instead pure silica rods grow independently of the dispersed colloidal seeds. Therefore, there is a need for a general synthesis of silica rod growth from colloidal particles. This general synthesis will allow facile experimental preparation of advanced anisotropic composite silica rods with a wide range of functionalities.

Herein we demonstrate a general synthesis for growing silica rods from silica seeds

with potentially any functionality. We optimize experimental conditions of this seeded growth approach of growing silica rods from seed particles in order to grow silica rods from silica seeds. Then, we use the principle that almost all colloidal particles can be coated with silica.²⁶ Subsequently, as a proof-of-principle for the generality of this method, we prepare composite matchstick particles from core/shell silica particles with (i) a fluorescent silica core, (ii) metallic Ag core, and (iii) organic polystyrene (PS) core, using the same experimental conditions for matchstick growth. These three cores were specifically chosen in order to demonstrate the generality of the synthesis, highlighting that these matchsticks can potentially be prepared with a huge diversity of functionality/composition. The first anisotropic composite matchstick particles we prepare with a fluorescent core in the head are particularly interesting for applications which can utilize the fluorescent anisotropic nature of these particles, including confocal fluorescence microscopy in which single particle orientations and positions can be determined even in concentrated systems,²⁷ cell imaging, and the investigation of flow behavior. The second anisotropic composite matchstick particle we prepare introduces a catalytically active and electrically conductive material (Ag) into the rod head. The former property makes these potentially self-propelling particles with applications in active matter,^{13–16} while the latter introduces the possibility of fine and anisotropic manipulation of the rods via dielectrophoretic trapping.²⁸ The seeded growth method presented here is able to incorporate the silver head, while reliably maintaining a shape resembling a high aspect ratio rod. Current methods to incorporate metals into rod heads require Pickering stabilisation of water droplets, which results in polydisperse mixtures of particles (size range of smallest dimension generally between 100 – 1000 nm) with nonuniform shapes.¹⁵ The third anisotropic composite matchstick particles we prepare with organic PS in the heads are potentially very versatile because organic polymers – and therefore these particles – can be made with a huge range of functionality.

We then also demonstrate the assembly of the matchsticks with PS heads into supraparticles. Supraparticles are clusters of smaller particles which themselves have a defined structure.^{29,30} The formation of supraparticles commonly utilizes the technique involving the slow drying of particle-containing emulsion droplets in a nonvolatile continuous phase, and recently our group extended this technique to the formation of supraparticles also from rod-like nanoparticles.^{27,31} These hybrid matchsticks with PS heads therefore have the potential to form advanced hierarchical structures, because supraparticles can be used as building blocks themselves for hierarchically ordered structures which combine original functionalities at different length scales.^{29,30} These supraparticles assembled from the matchsticks with PS heads are particularly interesting for advanced supraparticles with tunable porosity, because the organic PS can be burned away.³² Such structures are potentially interesting for catalysis and sensing applications.

We believe these results have great implications for the development of novel complex composite particles, for applications towards novel advanced materials as well as being fundamentally of great interest.



Figure 8.1. (a-c) SEM images of matchsticks grown from silica particles.

8.2. Results and Discussion

8.2.1. Seeded Growth of Silica Rods from Silica Particles

The seeded growth of silica rods from silica particles has previously been shown to result in isotropic star-shaped particles in which multiple rods are grown from the silica seed particles.¹⁹ Using this synthesis as our starting point, we then optimized the conditions of silica rod growth in order to grow singular silica rods from pure silica particles (average size 325 nm, polydispersity δ = 6% where δ = (standard deviation)/(mean diameter), Figure S8.1). We reduced the number of rods grown from the silica seed particles by using more water in the emulsion. The effect of increased water content of the emulsion has recently been shown with FeO_x structures to better facilitate the growth of one rod onto one seed.¹⁴ However, the increasing amount of water is also known to reduce the rod aspect ratio (Figure S8.2).¹ We therefore compensated for this rod shortening by increasing the amount of ethanol present, which is known to increase rod length up to a certain point, beyond which no rods are formed at all – only spheres.¹⁷ The amount of each reagent in the recipe is a delicate balancing act, which is all the more reason why a general synthesis is necessary. By using more water and more ethanol in our recipe, we were successfully able to facilitate the growth of a singular silica rod from the silica seed particles (Figure 8.1a). We did interestingly find many instances of silica rod growth from multiple seed particles (Figure 8.1b) and this can be explained by the favourable free energy of the water droplet attachment at concave regions, as has been previously observed.³³ Despite this observation, the synthesis results in a highly uniform set of matchsticks (Figure 8.1c).

Notably, we also found that the size of the seed particle was important in facilitating singular silica rod growth. We found that using much larger seed particles sizes of >1.5 μ m diameter facilitated the growth of many rods from one seed particle, resulting in rough particles with short spikes (**Figure S8.3**). We hypothesize that this is because the size of the emulsion water droplets from which the silica rods grow are ~200-500 nm,^{17,19} therefore



Figure 8.2. (a-c) Confocal microscopy images of dried matchsticks with a single (a,c) and double (b) fluorescent head. The fluorescence signal is overlayed in red on the grayscale reflection images. The only appreciable fluorescence signal was found in the matchstick heads. Scale bars for both (a) and (b) 500 nm.

multiple emulsion droplets are able to form without coalescing on the surface of larger seed particles. In a similar way, urchin-like silica particles have been prepared²⁵.

8.2.2. Seeded-Growth of Silica Rods from Silica Particles with a Fluorescent (FITC) Core.

We then grew silica rods from silica particles with a fluorescent silica core containing fluorescein isothiocyanate isomer I (FITC). We chose a fluorescent core because this increases the utility of these particles for imaging applications. For example, this allows 3D tracking of one end of the particle from confocal image volumes, yielding information about the matchstick orientation and dynamics.

The silica particles with a fluorescent core (size 441 nm, δ = 3%) were prepared using established techniques, by a modified Stöber method, utilizing seeded-growth and the incorporation of silane coupling agents that were first reacted to a fluorescent dye (**Figure S8.4**).³⁴ The silica rods were then grown from these silica particles with a fluorescent core in the same way as with the pure silica particles. The resulting matchsticks displayed fluorescence exclusively in the head of the matchstick (**Figure 8.2**), while our group also developed the methodology to fluorescently label the silica rods.¹

8.2.3. Seeded-Growth of Silica Rods from Silica Particles with a Metallic (Ag) core

Next, we grew silica rods from silica particles with a core consisting of metallic silver (Ag). We chose Ag for the metallic core because silica rod matchsticks have not been prepared from large Ag colloidal particles, and colloidal Ag can be prepared with high yields. Moreover, Ag has (i) a high conductivity and (ii) high catalytic activity. The former means anisotropic hybrid rods with a Ag head would be responsive in an electric field, and therefore have potential applications in directed assembly. The latter means that these hybrid rods are interesting for the fields of active matter and catalysis.



Figure 8.3. (a-c) TEM images of matchsticks with a head containing an Ag core.

The silica particles with a Ag core were synthesized by an established method involving the reduction of silver ions to metallic silver by ascorbic acid, which is known to result in polydisperse dispersions of Ag with diameter around 500 nm,³⁵ followed by subsequent washing and coating of the Ag particles with silica by an established method²⁶ (Figure 8.3). The resultant Ag particles were polydisperse (Figure S8.5) with an average diameter of 560 nm (from dynamic light scattering). Next, these particles were coated with silica (Figure S8.6). We then grew silica rods from these silica particles with a Ag core in the same way as with the pure silica particles. TEM images of the resulting matchsticks display an obvious darker region in the head of the matchstick, which is due to the presence of the Ag (Figure 8.3). Interestingly, despite the high polydispersity of the seed particles, matchsticks were successfully prepared from the seed particles of all sizes and the length and diameter of the silica rods between seeds of all sizes is relatively uniform (Figure S8.7). This suggests that the relative width of the rod diameter distribution is still governed by a similar emulsion droplet size distribution as found in ordinary rod growth, while the mean rod diameter is increased by the wetting of the initial droplets on the large seeds.

8.2.4. Seeded-Growth of Silica Rods from Core-Shell Silica Particles with an Organic (Polystyrene) Core and Assembly into Supraparticles

Finally, we grew silica rods from silica particles with an organic core of polystyrene (PS). We chose an organic core in order to fully demonstrate the generality of the synthesis. We chose PS as our organic core because monodisperse PS colloidal particles can easily be prepared. Moreover, polymers can have a huge potential range of functionality, which means developing a synthesis for anisotropic composite rods with a polymer head could have many applications. Additionally, polymers can be removed via heating³² which means that the porosity of these particles could be tuned.

We chose to use cationically stabilized PS to further show the generality of the synthesis – silica itself is anionically stabilized and therefore we are demonstrating that we can also prepare matchsticks with colloids stabilized cationically, too. The silica particles with a PS core were prepared by an established surfactant-free emulsion polymerization method,³⁶



Figure 8.4. (a-c) SEM images of matchsticks with a head containing PS. (d, e) SEM images of supraparticles assembled from the matchsticks with PS heads.

followed by subsequent washing and coating of the PS particles with silica by an established method.²⁶ The size of the PS particles from TEM imaging is 372 nm, $\delta = 1\%$, (**Figure S8.8**) and then this was coated with a 32 nm layer of silica (**Figure S8.9**). We then grew silica rods from these silica particles with a PS core in the same way as with the pure silica particles. The resulting matchsticks were uniform and similarly contained matchsticks with multiple seeds (**Figure 8.4a-c, Figure S8.10**).

We then assembled the matchsticks with PS heads into supraparticles. We thus demonstrate that these matchstick particles have potential to form advanced hierarchical structures, which can lead to advanced materials in many applications. We found that the matchsticks with PS heads could be assembled into supraparticles simply by emulsifying the aqueous matchstick dispersion into a water-in-oil emulsion, followed by slow evaporation of the solvent in the suspended water droplets.³⁷ The spherical compression of the matchstick dispersion inside the shrinking droplets formed supraparticles which were composed solely

of the matchstick particles (**Figure 8.4d**, **e**) and generally ranged in size from 3 to 15 microns (**Figure S8.11**). These supraparticles assembled from matchstick rods with PS heads are particularly interesting for the development of advanced supraparticles with tunable porosity, because PS can be easily be burned away from the cores of silica particles without damage to the silica.³² Tunable porosity in supraparticles is particularly interesting because supraparticles are currently being studied for applications in catalysis – in which porosity is a key factor – as well as fundamental research which requires the formation of novel self-assembled structures.

8.3. Conclusions

In conclusion, we developed a general synthesis for the seeded-growth of silica rods from silica-coated particles. We then demonstrated the potential of this synthesis to prepare anisotropic composite matchstick particles with a range of functionality by growing silica rods from other colloidal particles with a silica shell. We show the generality of this synthesis method by growing silica rods from three general types of colloidal particles as cores: i) fluorescent (FITC), ii) metallic (Ag), and iii) organic (polystyrene, PS). We then demonstrate the assembly of the matchsticks with a PS head into supraparticles, which are particularly interesting for the development of advanced supraparticles with tunable porosity. We believe that this general synthesis method can be extended for the preparation of matchsticks with a great range of functionality, for the development of advanced materials and of interest to study how the exact shape of anisotropic particles affect their self-assembly.

8.4. Materials and Methods

Materials

Sodium citrate dihydrate (99%), 1-pentanol (99%), tetraethylorthosilicate (TEOS, 98%), ammonium hydroxide (NH₄OH, 25 w/w%), Polyvinylpyrrolidone-40 (PVP, Weight Average Molecular Weight M_w = 40 kg/mol), PVP-360 (MW = 360 kg/mol), (3- aminopropyl)-triethoxysilane (APTES, 98%), Fluorescein isothiocyanate isomer I (FITC, >90%), AgNO3 (>99%), L-ascorbic acid (>99%) and Span 80 were purchased from Sigma Aldrich. Ethanol (100%) was purchased from Interchema and pure water was used from a Millipore system. Gum arabic was obtained from Fluka. n-Hexadecane (for synthesis) was obtained from Merck.

Preparation of Silica Particles

Silica particles (325 nm, δ = 6%) were prepared via a modified Stöber method. Briefly, NH₄OH (4.0 mL), water (3.0 mL), ethanol (22.0 mL) and TEOS (1.1 mL) were added to a 50 mL round bottomed flask. The mixture was stirred for 24 hours before the particles were isolated via centrifugation and dried before then being redispersed in water to make a 2.5 wt% dispersion. Polydispersity values were obtained from 25 particles in the TEM image in **Figure S8.1**.

Preparation of Silica Particles with a Fluorescent Core

The silica particles with a fluorescent core (441 nm, δ = 3%) were synthesized by first coupling the FITC dye to APTES, followed by the addition of ammonia and TEOS using a syringe pump until the desired particle size was reached, as described by Vlug.^{34,38} Polydispersity values were obtained from 50 particles in the TEM image in **Figure S8.4**.

Preparation of Silica Particles with an Ag Core

The silica particles with an Ag core were synthesized by first synthesizing Ag colloidal particles and subsequent coating with silica. The Ag particles were synthesized as follows: First, gum arabic (0.35 g), ascorbic acid (5.86 g) were dissolved in water (50 mL). Then a separate solution of gum arabic (0.35 g), AgNO3 (1.41 g), and water (50 mL) was prepared. The second solution was quickly poured into the first solution in a 250 mL round bottomed flask and left to react for 12 hours. The Ag particles were then isolated by centrifugation. Next, these Ag particles were coated with silica as described by Graf et al.²⁶ Briefly, 5.0 mL of a 4.72 g/L solution of Ag particles in water was added to 8.7 mL of a 9.5 g/L PVP (40 kg/mol) solution in water in a 50 mL centrifuge tube and the tube was slowly rotated for 24 hours. Then, the PVP-coated Ag particles were centrifuged, the supernatant discarded, and redispersed in a solution of NH₄OH in ethanol (4.2 vol%, 8.63 mL). Then TEOS (87.2 μ L, as a 10% solution in ethanol) was added to coat the particles with a thin layer of silica. Next, double the amount of the TEOS solution (10% in ethanol) was continuously added stepwise every 60 minutes (174.4 μ L, 348.8 μ L, 697.6 μ L and so on) for 6 additions. The resultant silica coated Ag particles were then isolated via centrifugation.

Preparation of Silica Particles with a PS Core

Cationic polystyrene (PS) particles (372 nm, $\delta = 1\%$) were prepared by a surfactant-free emulsion polymerization as described by Goodwin et al.³⁶ These particles could be readily dispersed in ethanol. The PS particles were then coated with silica as described by Graf et al.²⁶ The PS particles were sedimented, the supernatant discarded, and a 4.75 mL solution of particles ethanol (0.41 g/L) was then added to 4.75 mL of a 7 g/L PVP (360 kg/mol) in ethanol. Then, ammonia (0.25 mL) was added along with 500 µL TEOS (10% in water) in order to coat the particles with a thin layer of silica. Next, double the amount of the TEOS solution (10% in ethanol) was continuously added stepwise every 60 minutes (1.0 mL, 2.0 mL, 4.0 mL and so on) for 6 additions. The resultant silica coated PS particles (436 nm, $\delta = 1\%$) were then isolated via centrifugation.

Growth of Silica Rods from Silica Particles

Silica rods were grown via a modified procedure described by Datskos et al.¹⁹, which is a modification from the silica rod growth procedure originally described by Kuijk et al.¹⁷ Firstly, a PVP/1-pentanol stock solution was made with concentration 100 g/L. Then 9.11 g of this stock solution was added to a 15 mL centrifuge tube. Next, 300 μ L of the seed particles (2.5 wt% in water) were dispersed in the PVP/1-pentanol solution via sonication. We found that the seed particles needed to have aged in water for at least 24 hours before use in the reaction to ensure growth of silica rods from the particles. Ethanol (1.5 mL) and aqueous sodium citrate (0.18 M, 100 μ L) were then added and the mixture was vigorously shaken by hand. Ammonia (200 μ L, 25%) was then added and the mixture shaken again. TEOS (100 μ L) was then added followed again by vigorous shaking. The mixtures were then left to stand for 12 hours. In the case of the Ag cores, the mixture was slowly rotated for the 12-hour reaction period to prevent sedimentation. After the 12-hour reaction, the mixtures were centrifuged at 800g for 45 minutes, the supernatant discarded, and the particles redispersed in ethanol. The particles were then washed by two rounds of centrifuging at 500g for 15 minutes, discarding the supernatant, and redispersing in ethanol. The Ag matchsticks were further isolated simply by letting the particles sediment for an hour and removing the supernatant.

Supraparticles from Matchsticks with PS Heads

Supraparticles were made using a water-in-oil (W/O) emulsion method, in which charge stabilized colloids are compressed inside aqueous droplets dispersed in a nonvolatile organic phase.³⁷ The matchstick particles with PS heads obtained from the synthesis were centrifuged and redispersed in deionized water (10 mL). 0.5 mL of this dispersion was then pipetted into hexadecane (5 mL)

containing nonionic surfactant Span 80 (1 wt%) in a small glass vial (20 mL). Parafilm was then used to seal the top of the glass vial, which was then punctured with a few holes (to allow slow evaporation of the aqueous dispersed phase). The vial was then left to shake gently (IKA KS 260 basic, 250 rpm) for 2 weeks. The shaking was then stopped to allow the formed supraparticles to sediment. After 1 hour, sediment was visible on the bottom of the vial so the supernatant was carefully removed using a glass pipette. The supraparticles were then washed twice with cyclohexane (cyclohexane added, solution vortexed, left to sediment, solvent removed), eventually leaving a relatively concentrated supraparticle dispersion in <0.5 mL cyclohexane.

Characterization

Imaging of the particle morphology was performed with TEM, SEM and confocal microscopy. TEM was performed with an FEI TECNAI 20 electron microscope, in which samples were prepared by pipetting a drop of the particle dispersion in ethanol onto a Formvar/Carbon Films 200 Mesh Copper (100) grid. SEM was performed with an FEI XL30SFEG, FEI Phenom in which samples were prepared by pipetting a drop of the particle dispersion onto an aluminium stub (**Figure 8.1**) or silicon wafer on top of an aluminium stub and subsequently sputter-coated with a platinum layer of approximately 4 nm. Confocal microscopy was performed with a Leica SP8 confocal microscope (100x oil immersion lens) with excitation by a 488 nm laser line. The reflection signal was recorded at 488 nm and the fluorescence in the range 500-600 nm. To prepare the samples, the rods with fluorescent heads were dried from ethanol on a #1.5 coverslip before adding a drop of refractive index matching liquid (glycerol/water mixture, 85:15 by mass) on top.

8.5. Acknowledgements

This project was performed in collaboration with Chris L. Kennedy. This research was supported by the Dutch Technology Foundation STW (Grant No. 13567), which is part of the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs, as well as by the Netherlands Center for Multiscale Catalytic Energy Conversion (MCEC), an NWO Gravitation programme funded by the Ministry of Education, Culture and Science of the government of the Netherlands. We thank Chris Schneijdenberg for technical assistance, Wessel Vlug for providing the silica particles with fluorescent cores, Fabian Hagemans and Ramakrishna Kotni for useful discussions.

8.6. References

(1) Kuijk, A.; van Blaaderen, A.; Imhof, A. Synthesis of Monodisperse, Rodlike Silica Colloids with Tunable Aspect Ratio. *J. Am. Chem. Soc.* **2011**, *133*, 2346–2349.

(2) Yi, D.; Xu, C.; Tang, R.; Zhang, X.; Caruso, F.; Wang, Y. Synthesis of Discrete Alkyl-Silica Hybrid Nanowires and Their Assembly into Nanostructured Superhydrophobic Membranes. **2016**, *3001*, 8515–8520.

(3) Sharma, J. Finite-Sized One-Dimensional Silica Microstructures (Rods): Synthesis, Assembly, and Applications. *ChemNanoMat* **2017**, *3*, 214–222.

(4) Byranvand, M. M.; Taghavinia, N.; Kharat, A. N.; Dabirian, A. Micron-Scale Rod-like Scattering Particles for Light Trapping in Nanostructured Thin Film Solar Cells. *RSC Adv.* **2015**, *5*, 86050–86055.

(5) Li, W.; Lu, K.; Walz, J. Y.; Anderson, M. Effects of Rod-like Particles on the Microstructure and Strength of Porous Silica Nanoparticle Composites. *J. Am. Ceram. Soc.* **2013**, *96*, 398–406.

(6) Li, W.; Chen, B.; Walz, J. Y. Positioning Growth of Scalable Silica Nanorods on the Interior and Exterior Surfaces of Porous Composites. *J. Mater. Chem. A* **2015**, *3*, 2019–2024.

(7) Kuijk, A.; Byelov, D. V.; Petukhov, A. V.; van Blaaderen, A.; Imhof, A. Phase Behavior of Colloidal Silica Rods. *Faraday Discuss.* **2012**, *159*, 181–199.

(8) Yan, J.; Chaudhary, K.; Chul Bae, S.; Lewis, J. a; Granick, S. Colloidal Ribbons and Rings from Janus Magnetic Rods. *Nat. Commun.* **2013**, *4*, 1516.

(9) Gao, Y.; Romano, F.; Dullens, R. P. A.; Doye, J. K.; Aarts, D. G. A. L. Directed Self-Assembly into Low-Density Colloidal Liquid Crystal Phases. *Phys. Rev. Mater.* **2018**, *2*, 015601.

(10) Bakker, H. E.; Dussi, S.; Droste, B. L.; Besseling, T. H.; Kennedy, C. L.; Wiegant, E. I.; Liu, B.; Imhof, A.; Dijkstra, M.; van Blaaderen, A. Phase Diagram of Binary Colloidal Rod-Sphere Mixtures from a 3D Real-Space Analysis of Sedimentation-Diffusion Equilibria. *Soft Matter* **2016**, *12*, 9238–9245.

(11) Liu, B.; Besseling, T. H.; van Blaaderen, A.; Imhof, A. Confinement Induced Plastic Crystal-to-Crystal Transitions in Rodlike Particles with Long-Ranged Repulsion. *Phys. Rev. Lett.* **2015**, *115*, 1–5.

(12) Liu, B.; Besseling, T. H.; Hermes, M.; Rs, A. F. D. O.; Imhof, A.; van Blaaderen, A. Switching Plastic Crystals of Colloidal Rods with Electric Fields. *Nat. Commun.* **2014**, *5*, 1–8.

(13) Morgan, A. R.; Dawson, A. B.; Mckenzie, H. S.; Skelhon, T. S.; Beanland, R.; Franks, H. P. W.; Bon, S. a. F. Chemotaxis of Catalytic Silica–manganese Oxide "Matchstick" Particles. *Mater. Horizons* **2014**, *1*, 65–68.

(14) Kim, J.; Hwang, H. J.; Oh, J. S.; Sacanna, S.; Yi, G. Monodisperse Magnetic Silica Hexapods. J. Am. Chem. Soc. **2018**, 140, 9230–9235.

(15) Gao, Y.; Dullens, R. P. A.; Aarts, D. G. A. L. Bulk Synthesis of Silver-Head Colloidal Rodlike Micromotors. *Soft Matter* **2018**, *14*, 7119–7125.

(16) Sen, A.; Ibele, M.; Hong, Y.; Velegol, D. Chemo and Phototactic Nano/Microbots. *Faraday Discuss.* **2009**, *143*, 15–27.

(17) Kuijk, A.; van Blaaderen, A.; Imhof, A. Synthesis of Monodisperse, Rodlike Silica Colloids with Tunable Aspect Ratio. *J. Am. Chem. Soc.* **2011**, *133*, 2346–2349.

(18) Kuijk, A.; Imhof, A.; Verkuijlen, M. H. W.; Besseling, T. H.; Van Eck, E. R. H.; van Blaaderen, A. Colloidal Silica Rods: Material Properties and Fluorescent Labeling. *Part. Part. Syst. Charact.* **2014**, *31*, 706–713.

(19) Datskos, P.; Cullen, D. A.; Sharma, J. Step-by-Step Growth of Complex Oxide Microstructures. *Angew. Chemie - Int. Ed.* **2015**, *54*, 9011–9015.

(20) Hagemans, F.; Wee, E. B. Van Der; van Blaaderen, A.; Imhof, A. Synthesis of Cone-Shaped Colloids from Rod-Like Silica Colloids with a Gradient in the Etching Rate. *Langmuir* **2016**, *32*, 3970–3976.

(21) Hagemans, F.; Pujala, R. K.; Hotie, D. S.; Thies-Weesie, D. M. E.; De Winter, D. A. M.; Meeldijk, J. D.; van Blaaderen, A.; Imhof, A. Shaping Silica Rods by Tuning Hydrolysis and Condensation of Silica Precursors. *Chem. Mater.* **2019**, *31*, 521–531.

(22) Morgan, A. R.; Dawson, A. B.; Mckenzie, H. S.; Skelhon, T. S.; Beanland, R.; Franks, H. P. W.; Bon, S. A. F. Chemotaxis of Catalytic Silica–manganese Oxide "Matchstick" Particles. *Mater. Horizons* **2014**, *1*, 65–68.

(23) Zhao, B.; Zhou, H.; Liu, C.; Long, Y.; Yang, G. Fabrication and Directed Assembly of Magnetic Janus Rods. *New J. Chem.* **2016**, *40*, 6541–6545.

(24) Peng, B.; Soligno, G.; Kamp, M.; van Nijs, B.; de Graaf, J.; Dijkstra, M.; van Roij, R.; van Blaaderen, A.; Imhof, A. Site-Specific Growth of Polymer on Silica Rods. *Soft Matter* **2014**, *10*, 9644–9650.

(25) Zhao, B.; Li, D.; Long, Y.; Yang, G.; Tung, C.; Song, K. Modification of Colloidal Particles by Unidirectional Silica Deposition for Urchin-like Morphologies. *RSC Adv.* **2016**, *6*, 32956–32959.

(26) Graf, C.; Vossen, D. L. J.; Imhof, A.; van Blaaderen, A. A General Method to Coat Colloidal Particles with Silica. *Langmuir* **2003**, *19*, 6693–6700.

(27) Besseling, T. H.; Hermes, M.; Kuijk, A.; De Nijs, B.; Deng, T. S.; Dijkstra, M.; Imhof, A.; van Blaaderen, A. Determination of the Positions and Orientations of Concentrated Rod-like Colloids from 3D Microscopy Data. *J. Phys. Condens. Matter* **2015**, *27*, *194109*.

(28) Bezryadin, A.; Dekker, C.; Schmid, G. Electrostatic Trapping of Single Conducting Nanoparticles between Nanoelectrodes. *Appl. Phys. Lett.* **1997**, *71*, 1273–1275.

(29) Wintzheimer, S.; Granath, T.; Oppmann, M.; Kister, T.; Thai, T.; Kraus, T.; Vogel, N.; Mandel, K. Supraparticles: Functionality from Uniform Structural Motifs. *ACS Nano* **2018**, *12*, 5093–5120.

(30) Wang, T.; Lamontagne, D.; Lynch, J.; Zhuang, J.; Cao, Y. C. Colloidal Superparticles from Nanoparticle Assembly. *Chem. Soc. Rev.* **2013**, *42*, 2804–2823.

(31) van Blaaderen, A.; Dijkstra, M.; van Roij, R.; Imhof, A.; Kamp, M.; Kwaadgras, B. W.; Vissers, T.; Liu, B. Manipulating the Self Assembly of Colloids in Electric Fields. *Eur. Phys. J. Spec. Top.* **2013**, *222*, 2895–2909.

(32) Deng, T.; Marlow, F. Synthesis of Monodisperse Polystyrene @ Vinyl-SiO2 Core – Shell Particles and Hollow SiO2 Spheres. *Chem. Mater.* **2012**, *24*, 536–542.

(33) Li, C.; Zhang, S.; Zhang, B.; Liu, J.; Zhang, W.; Solovev, A. A.; Tang, R.; Bao, F.; Yu, J.; Zhang, Q.; et al. Local-Curvature-Controlled Non-Epitaxial Growth of Hierarchical Nanostructures. *Angew. Chemie - Int. Ed.* **2018**, *57*, 3772–3776.

(34) van Blaaderen, A.; Vrij, A. Synthesis and Characterization of Colloidal Dispersions of Fluorescent, Monodisperse Silica Spheres. *Langmuir* **1992**, *8*, 2921–2931.

(35) Velikov, K. P.; Zegers, G. E.; van Blaaderen, A. Synthesis and Characterization of Large Colloidal Silver Particles. *Langmuir* **2003**, *19*, 1384–1389.

(36) Goodwin, J. W.; Ottewill, R. H.; Pelton, R. Studies on the Preparation and Characterization of Monodisperse Polystyrene Latices V.: The Preparation of Cationic Latices. *Colloid Polym. Sci.* **1979**, *257*, 61–69.

(37) Nijs, B. De; Dussi, S.; Smallenburg, F.; Meeldijk, J. D.; Groenendijk, D. J.; Filion, L.; Imhof, A.; van Blaaderen, A.; Dijkstra, M. Entropy-Driven Formation of Large Icosahedral Colloidal Clusters by Spherical Confinement. *Nat. Mater.* **2015**, *14*, 56–60.

(38) Vlug, W. Balls, Beams and Blocks: In Situ Observation of Colloidal Particles in Confinement and under Electron Irradiation, 2018.

Appendix

Silica particles

Silica particles (**Figure S8.1**) were prepared by a modified Stöber method, as described in the Experimental Section. Particle size average as determined from 25 particles in **Figure S8.1**: 325 nm, $\delta = 6\%$.





Rods from Silica Particles

When we first increased the amount of water used in the silica rod growth reaction, we found that this facilitated the growth of fewer spikes per seed particle, but also shortened the length of the rods (**Figure S8.2**).



Figure S8.2. TEM image of short and fat silica rods grown from silica particles.

When using much larger seed particles with an average size of 1.5 μ m, we found that multiple rods would grow from the particles (**Figure S8.3**).

Fluorescent silica particles

Silica particles with a fluorescent core had an average size of 441 nm, δ = 3%, as measured from 50 particles in **Figure S8.4**.



Figure S8.4. TEM images of silica particles containing a fluorescent core.

Ag particles and SiO2@Ag particles

The Ag particles and Ag particles coated in silica which were used as seeds for the preparation of the structures shown in **Figure 8.3** are shown in **Figure S8.5 and Figure S8.6**, respectively. We do not quote an average value for the size of the silica layer because the sample is so polydisperse. This means that the silica layer for each particle will likely be very different, and light scattering techniques will also not give an accurate value for particle sizes. Despite this, we can see for the individual particles in **Figure S8.6** that the thickness is approximately between a few nanometers and 30 nm.



Figure S8.5. TEM image of Ag particles.



Figure S8.6. TEM images of silica coated Ag particles. These particles were used as seeds for the rods grown in **Figure 8.3**.



Figure S8.7. Diameters of single-headed particles. (a) Size distributions of the silver seed particles and the rods grown from them. The rod and Ag mean values are 650 nm (10%) and 700 nm (23%) respectively. (b) Relation between the diameter of the silver particle and that of the rod grown from the same particle. The fit line has gradient 0.26 and intercept 0.47 μ m.

The diameter polydispersity of the rods grown from Ag ($\delta = 10\%$) was remarkably similar to that of the unseeded synthesis (**Figure S8.7**),¹ despite the high polydispersity of the seeds ($\delta = 22\%$). The positive correlation between the two diameters (**Figure S8.7b**), shows that the rod diameter is increased approximately 26 nm for every 100 nm increase in the seed size, suggesting a high contact angle of the droplet on the seed particle.

PS Particles and SiO2@PS Particles

The PS particles and PS particles coated in silica which were used as seeds for the preparation of the structures shown in **Figure 8.4** are shown in **Figure S8.8** and **Figure S8.9** respectively.



Figure S8.8. TEM images of PS particles. Particle size as determined from 25 particles in the images: 372 nm, $\delta = 1\%$.



Figure S8.9. TEM images of silica coated PS particles. Particle size average as determined from 25 particles in the image: 436 nm, δ = 1%.

TEM images of matchsticks with PS heads

TEM images of the matchsticks shown in **Figure 8.4** are shown in **Figure S8.10** below, where the PS cores inside the matchstick heads are more obvious.



Figure S8.10. TEM images of matchsticks with PS heads.

Supraparticles Assembled from Matchsticks with PS Heads

Further SEM imaging of the assembled supraparticles (shown in **Figure 8.4**) is shown in **Figure 88.11**. The supraparticles ranged in size from ~3-15 microns, and we also observed some 'broken' supraparticles in the sample.



Figure S8.11. SEM images of supraparticles assembled from matchsticks with heads of PS.

Summary for the Broad Public

The work presented in this thesis is most succinctly described by the title: "UV-Absorbing Colloids for Advanced Photoprotection and Functional Materials". To elaborate on this, we develop colloidal particles which can absorb UV light, for applications in photoprotection and for the development of new materials.

Colloidal particles are small, with sizes between 1-1000 nanometers. To get an idea of the scale, 1 nanometer is about the same size as a medium-sized molecule and 1000 nanometers is about the same size as bacteria/blood platelets. The width of human hair is about 100 micrometers, meaning the same width as 100 bacteria placed end-to-end. A single bacterium is then approximately the same width as 1000 molecules placed end to end. In this thesis, we develop particles which are bigger than molecules but smaller than bacteria. We develop such particles because colloidal particles are a potential solution to many of the challenges faced in current photoprotection, as well as a route towards novel materials.

Photoprotection is the protection of organisms and materials from sunlight, where the most common example is cosmetic sunscreens. The main challenges in photoprotection include effectiveness, stability, safety, and environmental impact. Ideal sunscreens, for example, should (i) effectively protect against the full range of UV light in sunlight for long periods of time, (ii) contain components which are not harmful to humans or the environment, and (iii) contain components which do not degrade to form by-products that are harmful to humans or the environment. Current sunscreens do not satisfy all these requirements. Many of the UV-absorbing components in sunscreens degrade over time when exposed to sunlight. Some UV-absorbing components are harmful to coral reefs, so much so that Hawaii became the first US state in 2018 to pass a bill banning the use of sunscreens containing these components. Many UV-absorbing components also produce harmful by-products called reactive oxygen species (ROS) upon exposure to sunlight, which are carcinogenic and promote skin ageing. However, all these challenges can potentially be addressed by the use of colloidal particles.

Sunscreens can address many of these challenges by using colloidal particles as carriers for the UV-absorbing components. The encapsulation of UV-absorbing components into colloidal particles can improve effectiveness by stabilizing the components from degradation. Moreover, encapsulation can improve the safety by minimizing direct skin contact with the components, and co-encapsulation of photostabilizers into the colloidal particles along with the UV-absorbing components can improve safety by neutralizing the harmful ROS by-products generated by the UV-absorbing components.

UV-absorbing colloidal particles have a big role to play for the development of sunscreens, but also for many materials in photoprotection e.g. food packaging and eye protection. These transparent materials also face many of the same challenges as sunscreens.

In **Part 1** of this thesis, we develop UV-absorbing nanoparticles (colloidal particles in the size range 1-100 nm) from biobased ethyl cellulose for advanced photoprotection and for advanced materials for photoprotection. We show how the biobased UV-absorbing nanoparticles can satisfy many of the challenges faced by sunscreens and we go on to use these nanoparticles for the preparation of transparent photoprotective materials which have great potential for sustainable food packaging.

In **Chapter 2**, we develop UV-absorbing nanoparticles from biobased ethyl cellulose with commonplace UV-absorbing components from sunscreens encapsulated, along with a

photostabilizing antioxidant. We show that these NPs can provide broadband UV spectrum protection and that these NPs can form transparent coatings. Significantly, we also show co-encapsulation of an antioxidant can neutralize the carcinogenic ROS.

In **Chapter 3**, we further study this system from Chapter 2. We investigate the size tunability of the nanoparticles and study the incorporation of the UV-absorbing components into the nanoparticles.

In **Chapter 4**, we develop fully biobased UV-absorbing nanoparticles from ethyl cellulose with UV-absorbing plant extracts encapsulated. We show that these nanoparticles can provide broadband UV spectrum protection, form coatings, and study the incorporation of the plant extracts.

In **Chapter 5**, we develop innovative transparent UV-blocking paper-based materials (a.k.a. "nanopaper"), by combining the NPs developed in the previous chapters with cellulose nanofibrils. We investigate the optical properties, degradation, and mechanical properties of these materials, which have great potential as sustainable solutions for food-packaging.

In **Chapter 6**, we develop photochromic (colour-changing) NPs from ethyl cellulose with unique photochromic compounds encapsulated. We show that these NPs can similarly be combined with cellulose nanofibrils to produce innovative colour-changing paper-based materials, which have great potential for food-packaging, as well as erasable documents.

In **Part 2** of this thesis we develop novel colloidal particle structures which contain a well-known UV-absorbing compound, titanium dioxide. Titanium dioxide is a material with many unique properties. Therefore, the development of novel colloidal particle structures from unique materials like titanium dioxide and the assembly of these particles is a route towards a new generation of materials with advanced/tailorable properties. However, the synthesis of novel particle structures on the colloidal size range and their assembly is unsurprisingly very challenging. We therefore investigate the synthesis and assembly of novel particle structures containing titanium dioxide. Mastering the synthesis allows the design of the exact building blocks we want, whilst mastering the assembly allows the building of structures using the building blocks from the bottom-up. Mastering of both will give complete control over new materials and their properties.

In **Chapter 7**, we synthesize matchstick-shaped particles with a titania "head" and a silica tail, and assemble these into arrays and 3D structures.

In **Chapter 8**, we develop a general synthesis for matchstick-shaped particles which can incorporate any material, not just titanium dioxide.

The results in this thesis involve the use of nanotechnology in order to make a step towards more advanced photoprotection and innovative functional materials. The results intentionally have a strong flavour of sustainability, which is widely regarded as one of the greatest challenges facing our generation. We hope that our results will inspire further research.

Samenvatting voor het Brede Publiek

Het werk gepresenteerd in dit proefschrift kan kort en bondig beschreven worden met de titel: "UV-Absorberende Colloïden voor Geavanceerde Zonbescherming en Functionele Materialen". Verder uitgebreid, wij ontwikkelen colloïdale deeltjes die UV-licht kunnen absorberen voor gebruik in zonwerende toepassingen en het ontwikkelen van nieuwe materialen.

Colloïdale deeltjes zijn klein, tussen de 1-1000 nanometer groot. Om een beter idee te krijgen over die lengteschaal, 1 nanometer is ongeveer net zo groot als een typisch molecuul en 1000 nanometer is ongeveer net zo groot als bacteriën/bloedplaatjes. De breedte van een menselijke haar is ongeveer 100 micrometer, dezelfde breedte als 100 bacteriën naast elkaar dus. Een enkele bacterie is daarom ongeveer dezelfde breedte als 1000 moleculen naast elkaar. In dit proefschrift, ontwikkelen wij deeltjes die groter zijn dan moleculen maar kleiner dan bacteriën. Wij ontwikkelen dergelijke deeltjes want colloïdale deeltjes zijn een potentiele oplossing voor veel van de uitdagingen in huidige zonwerende technologie, en bovendien zijn ze een route naar nieuwe materialen.

Zonbescherming is het beschermen van organismen en materialen tegen zonlicht. Het meest bekende voorbeeld is cosmetische zonnebrand. De hoofd uitdagingen in de veld van zonbescherming zijn effectiviteit, stabiliteit, veiligheid, en milieu-impact. Ideale zonnebrand, zou bijvoorbeeld: (i) effectief moeten beschermen tegen het volledig gebied van UV licht voor lange periodes van tijd, (ii) componenten moeten bevatten die niet beschadigend zijn voor mens en milieu en (iii) componenten moeten bevatten die niet degraderen en niet resulteren in de formatie van schadelijke nevenproducten die schadelijk zijn voor mens en milieu. Huidige zonnebrand voldoet niet aan al deze eisen. Veel van de UV-absorberende componenten in zonnebrand degraderen met de tijd door blootstelling aan zonlicht. Door blootstelling aan zonlicht, produceren sommige UV-absorberende componenten ook schadelijke nevenproducten, die kankerverwekkend zijn en resulteren in veroudering van de huid. Die nevenproducten heten reactieve zuurstofcomponenten (ROS). Al deze uitdagingen kunnen opgelost worden met het gebruik van colloïdale deeltjes.

Het inkapselen van de UV-absorberende componenten in colloïdale deeltjes kan veel van deze uitdagingen oplossen. Het kan bijvoorbeeld de effectiviteit van de componenten vergroten door de stabiliteit te verbeteren. Bovendien, het inkapselen verbetert ook de veiligheid door direct contact van de UV-absorberende componenten met de huid te verminderen. Het inkapselen van stabiliserende antioxidanten samen met de UVabsorberende componenten verbetert ook veiligheid door schadelijke ROS nevenproducten te neutraliseren.

UV-absorberende colloïdale deeltjes zijn belangrijk voor het ontwikkelen van zonnebrand, maar ook voor veel UV-absorberende materialen op het gebied van zonbescherming, zoals voedselverpakking en oogbescherming. Deze transparante materialen hebben veel van dezelfde uitdagingen als zonnebrand.

In **Deel 1** van dit proefschrift beschrijven we ontwikkelingen in UV-absorberende nanodeeltjes (colloïdale deeltjes van tussen de 1 nm en 100 nm groot) van biologisch ethyl cellulose voor geavanceerde zonbescherming en functionele materialen in zonbescherming. We laten zien dat de biologische UV-absorberende nanodeeltjes veel van de uitdagingen in huidige zonweringtechnologie op kunnen lossen. Daarna laten we zien dat we nieuwe innovatieve transparante zonwerende materialen kunnen maken met deze nanodeeltjes, die veel potentie hebben voor duurzame voedselverpakking.

In **Hoofdstuk 2**, beschrijven we de ontwikkeling van UV-absorberende nanodeeltjes waarin biologisch ethyl cellulose met UV-absorberende componenten uit normale zonnebrand samen met een stabiliserende antioxidant is ingekapseld. We laten zien dat deze nanodeeltjes over het volledig gebied van UV kunnen beschermen en dat tot transparante coatings verwerkt kunnen worden. We laten ook zien dat het inkapselen van een antioxidant samen met de UV-absorberen componenten resulteert in een vermindering van de schadelijke ROS.

In **Hoofdstuk 3**, bestuderen wij dit systeem nog verder. Wij onderzoeken het inkapselen van de UV-absorberende componenten binnen de nanodeeltjes en voor welke groottes nanodeeltjes deze techniek werkt.

In **Hoofdstuk 4**, ontwikkelen wij volledig biologische UV-absorberende nanodeeltjes van biopolymeren ethyl cellulose en zeïne, waarin UV-absorberende plantenextracten geïncorporeerd zijn. Wij laten zien dat deze nanodeeltjes over het volledig gebied van UV kunnen beschermen, tot transparante coatings verwerkt kunnen worden. Verder bestuderen wij het inkapselen van de UV-absorberende plantenextracten binnen de nanodeeltjes.

In **Hoofdstuk 5**, ontwikkelen wij innovatieve transparante UV-werend papier, door het combineren van de nanodeeltjes van de eerdere hoofdstukken met dunne cellulose vezels. Wij onderzoeken de optische eigenschappen, degradatie en mechanische eigenschappen van deze materialen, die veel potentie hebben als duurzame oplossingen voor plastic voedsel verpakkingen.

In **Hoofdstuk 6**, wij ontwikkelen fotochromische (kleur-veranderende) nanodeeltjes van ethyl cellulose waarin unieke fotochromische componenten zijn ingekapseld. Wij laten zien dat deze nanodeeltjes gecombineerd kunnen worden met dunne cellulose vezels om nieuwe, innovatieve, transparente, kleur-veranderende papier te maken, die veel potentie hebben in voedsel-verpakking en ook uitwisbare documenten.

In **Deel 2** van dit proefschrift beschrijven wij de ontwikkeling van nieuwe vormen van colloïdale deeltjes die de UV-absorberende component, titanium dioxide, bevatten. Titanium dioxide is een materiaal met veel unieke eigenschappen. Dus het ontwikkelen van nieuwe vormen van deze colloïdale deeltjes die titanium dioxide bevatten en het assembleren van deze deeltjes zou kunnen resulteren in een nieuwe generatie van materialen met geavanceerde eigenschappen. Echter, het synthetiseren van dergelijke nieuwe vormen van deeltjes op deze lengteschaal en het assembleren van deze deeltjes is vrij lastig. Daarom onderzoeken wij het synthetiseren en assembleren van deze synthese resulteert in het ontwerpen van de gewenste bouwblokken die wij willen en het beheersen van de assemblage maakt het mogelijk om nieuwe structuren te kunnen bouwen van de kleinste bouwblokken. Controle over de combinatie zou kunnen resulteren in volledig controle over nieuwe materialen en hun eigenschappen.

In **Hoofdstuk 7**, synthetiseren wij luciferhoutjevormige deeltjes die een titanium dioxide "hoofd" bevatten en een silica "staart". Daarna gebruiken wij deze deeltjes in zelfassemblage.

In **Hoofdstuk 8**, ontwikkelen wij een algemene synthese voor luciferhoutjevormige deeltjes die alle materialen kunnen bevatten, niet alleen titanium dioxide.

De resultaten in dit proefschrift gebruikt nanotechnologie om een stapje te maken naar geavanceerde zonbescherming en innovatieve functionele materialen. De resultaten zijn expres gericht op duurzaamheid, dat één van de grootste uitdagingen is voor onze generatie. Wij hopen dat onze resultaten verder onderzoek zullen inspireren.

Acknowledgements

When I arrived in Utrecht all the way back in 2015 I had no idea I would be as lucky as I have been. I have had the opportunity to work with so many wonderful people, in such a positive working environment. I couldn't speak more highly of the group and will always look back on this period of my life very fondly. In this section I will use this opportunity to thank these people, without whom the work in this thesis would not have been possible.

Firstly I would like to thank my supervisors Arnout, Krassimir, and Alfons. First and foremost, thank you for giving me the opportunity to do a PhD in your group. To my daily supervisors Arnout and Krassimir, your supervision has been irreplaceable. Thank you for providing all possible support towards the completion of this thesis. You have both always given prompt and quality feedback on everything I have written. Thank you for all your guidance, patiently listening, addressing my doubts and your encouragement. Arnout, your door has always been open to me and I thank you for always helping me out whenever I have a query, both scientific and non-scientific. You have given me so much of your time for which I am immensely grateful. Your kindness has always made me feel so comfortable. Krassimir, your enthusiasm is contagious. After every meeting I always feel reinvigorated and driven. You have always been very encouraging and supportive and I couldn't have asked for anything more. Thank you for being such a great mentor. Alfons, I couldn't speak more highly of you as a group leader. Working in an environment with you is a hugely rewarding experience and I have learned so much. All three of you have given me much freedom in my research. I owe you all so much.

Next I would like to thank my collaborators. Heleen, I was lucky to work with such an enthusiastic and fun person. Your enthusiasm and drive led to us having a very successful time working together. Srivatssan, thank you for being such a great collaborator. I really enjoyed working on the nanopaper project with you and your knowledge combined with your attitude led to such a great collaboration. Chris, I'm really happy we got the opportunity to work together on a project because, as expected, it has been really fun and your very deep understanding and knowledge has pushed the project really far. I expect we will get a nice publication. Maria, I've only worked with you for a short while but already you have produced so many results, keep it up! Rama, Albert, Fabian and Ravi, I have really enjoyed all of our helpfulness and openness together whilst working on silica rods.

I would like to give a special mention to the technical and supporting staff who have helped me so much. Dave, I have come to you many, many times asking for all sorts of help. Thank you for always having been so helpful whether it be help with equipment or explaining concepts to me. Peter, I thank you for your willingness to help and for sorting out so many problems I have encountered. Chris and Hans, thank you for having always made my trips to the microscopy department enjoyable and productive. I love the positivity that you both exude and the atmosphere that this creates. Chris, thank you for having helped me so much, I admire your pragmatism with so many of the challenges I have come to you with. Relinde and Elleke, thank you for being such great technical staff. Relinde, it has been very pleasant working with you. You have always been positive and have done a lot for me, for which I am very grateful. Dianne, Hester, Linda, and Marion, I thank you all for all you have done for me. Dianne, I have loved our chats and working together on the Debye booklet. Hester, I really enjoyed setting up our Christmas project together along with Chris. Thank you very much for the constant help during the final stages of my PhD. Marion, thank you for getting me started here and choosing such a great location for the group outing in Enkhuizen.

I would also like to thank the scientific staff at SCMB: Gerhard, Laura, Marjolein, René, Patrick, Hans Freddy, and Marijn. Thank you for all your suggestions and comments which have greatly improved my scientific research and presentations. Gerhard, I thank you for bringing a smile to my face every day for the past 4 years. I have really enjoyed our many, many long chats together and our pub quizzes. Laura, Marjolein, René, and Patrick, thanks for being so warm, social, and welcoming.

John Kelly, thanks for the squash games every week for the last 4 years. I've loved our chats and our shared passion for football.

I thank Rama, Albert, Chris, Fabian, Vassilis, Frankje, Rik, Rao, and Pepijn who all have shared an office with me for creating such a pleasant and productive work environment. I sincerely thank all my colleagues at SCMB and in the DAC, past and present. You have all contributed to such a great working environment and for that I am very grateful. I have made many lifelong friends amongst you. Chris, Toni, and Wiebke, our trip to India was such an amazing experience because of your company. I can't believe we saw a tiger and an elephant up close in the wild (and thanks Wiebke for screaming when you saw the tiger!). Rama, thanks for inviting us to your wedding in India. I had a wonderful experience on the waters of Alleppey, in the tea gardens of Munnar, and finally in Visakhapatnam, made unforgettable by the company of Chris, Toni, Naveed, Samaneh, Gabriele, and Massi. Naveed, thanks for always making me smile and, of course, your constant positivity. Giulia and Guido, thanks for organising such a memorable trip to Rome for us, and thank you Siddarth, Anna, Sergei, Chris, Eke and Pepijn for the great company. Anna, thank you for your enthusiasm to help whatever I may ask. Albert and Rama, thanks for being great gym buddies. Giulia, Guido, Toni, Chris, Wiebke, Pepijn and Siddharth thanks for all the memories from the countless number of times we met up outside of work. Chris, Wiebke, and Pepijn, thanks for getting me into bouldering! Pepijn, thanks for your constant enthusiasm. Thanks so much for correcting and muchimproving the Dutch summary for this thesis. Carmine, thank you for our in-depth football discussions, particularly concerning Mourinho. Siddharth, thank you for your easy approachability and your help particularly during my teaching periods. Giulia, thank you for your positivity and always making me laugh. Wiebke, thanks for your great friendship and significantly improving my writing skills. Ernest, thanks for all the help you've given me and for being so approachable. Chris, I have spent so much time with you over the last few years and I've really enjoyed it. Thanks for being such a great friend.

Finally, thank you to my family for the unconditional support. Thank you mum and dad, who scaled the breadth of England at a moment's notice to fetch my passport on the day before my interview for this PhD position. Thanks Catherine and Thom (and Darren and Kate) for being awesome siblings. Tonnishtha, thank you for the indescribable impact you've made. We live our lives in perpetual laughter. I look forward to a very happy future with you and I thank your family for all their support.

List of Publications

This thesis is based on the following publications:

- Hayden, D. R.; Imhof, A.; Velikov, K. P. Biobased Nanoparticles for Broadband UV Protection with Photostabilized UV Filters. *ACS Appl. Mater. Interfaces* **2016**, *8* (48), 32655–32660. (Chapter 2)
- Hayden, D. R.; Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Size and Optically Tunable Ethyl Cellulose Nanoparticles as Carriers for Organic UV Filters. *ChemNanoMat* 2018, 4 (3), 301–308. (Chapter 3)
- Hayden, D. R./Kibbelaar, H. V. M.; Imhof, A.; Velikov, K. P. Fully-Biobased UV-Absorbing Nanoparticles from Ethyl Cellulose and Zein for Environmentally Friendly Photoprotection. *RSC Adv.* **2018**, *8* (44), 25104–25111. (Chapter 4)

Featured in:

Utrecht University News

(https://www.uu.nl/en/news/utrecht-researchers-develop-technology-for-safer-more-sustainable-sunscreens)

 Hayden, D. R./Mohan, S.; Imhof, A.; Velikov, K. P. Fully Biobased Highly Transparent Nanopaper with UV-Blocking Functionality. ACS Appl. Polym. Mater. 2019, 1 (4), 641–646. (Chapter 5)

Featured in:

Utrecht University News

(https://www.uu.nl/en/news/new-nanopaper-is-transparent-and-uv-blocking) Regional broadcaster RTV Utrecht

(https://www.rtvutrecht.nl/nieuws/1907336/wetenschappers-ontwikkelen-vervangend-product-voor-plastic.html)

Dutch national newspaper Algemeen Dagblad

(https://www.ad.nl/utrecht/opvallende-utrechtse-vinding-sinaasappelschillen-als-wapen-tegen-plasticsoep~a5c74c1b/)

Dutch national radio station BNR Nieuwsradio

(https://www.bnr.nl/podcast/wetenschap-vandaag/10375439/nanopapier-van-sinaasappelschil-als-vervanging-van-plastic)

- Hayden, D. R.; Spanou, M.; Mohan, S.; Imhof, A.; Velikov, K. P. Photochromic Nanoparticles from Ethyl Cellulose for Smart Materials, *manuscript in preparation*. (Chapter 6)
- Hayden, D. R.; Kennedy, C. L.; Velikov, K. P.; van Blaaderen, A.; Imhof, A. Seeded-Growth of Silica Rods from Silica-Coated Particles, *to be submitted*. (Chapter 8)
Also by the Author:

Patent:

• Koning, C. E.; Buijsen, P. F. A.; Lansbergen, A. J. H.; Papegaaij, A.; Hayden, D. R. Polyfunctional Hydrophthalimide Monomer Compounds. WO2016097403 A1, 2016.

Conference Presentations

Parts of this thesis were presented at:

- Physics @ Veldhoven 2016 (poster presentation)
- Grenoble International Soft Matter Conference 2016 (poster presentation)
- Physics @ Veldhoven 2017 (poster presentation)
- Physics @ Veldhoven 2018 (poster presentation)
- Inter-departmental seminar within the Debye Institute (UU), a.k.a. 'Nanoseminar' 2018 (oral presentation)
- International Association of Colloid and Interface Scientists Conference 2018 (oral presentation)
- Chains 2018 (oral presentation)
- Physics @ Veldhoven 2019 (poster presentation)

About the Author



Douglas Richard Hayden was born in Bedford, UK on the 21st August 1993. He grew up in Flitwick, Bedfordshire, where he graduated from Redborne Upper School in 2011. That same year he started his undergraduate degree in Chemistry at the University of Warwick. During his degree he carried out a 12 month internship with DSM Coating Resins, Zwolle, the Netherlands, working on renewable building blocks for alkyd resins, alkyd emulsions, and powder coatings under supervision of Prof. Cor E. Koning, Alwin Papegaaij, and Dr. Paul Buijsen. This results of this research project were

patented and a company CTO subsequently presented the research as one of DSM's top five scientific highlights of the previous six months. Douglas was subsequently selected as a finalist for the award of "Placement of the Year" by Cogent UK Life Science Skills Awards. He graduated from the University of Warwick in 2015 with a First Class Honours Master of Chemistry (MChem) degree, in Chemistry with Industrial Training. His Master's project was under the supervision of Prof. Stefan A. F. Bon, and the resultant thesis was entitled: "Site-Specific Polymer Grafting from Silica Particles to Study Bio-Inspired Systems". Shortly afterwards, he started his PhD in the Soft Condensed Matter group at Utrecht University under the supervision of Dr. Arnout Imhof, Prof. Krassimir P. Velikov, and Prof. Alfons van Blaaderen. During his PhD, Douglas was the representative for his research group in the PhD committee for of the Debye Institute for Nanomaterials Science (2016-2019). The results during his PhD have been presented at national and international conferences and published in peer-reviewed scientific journals. Moreover, the results in this thesis have been highlighted by media outlets including Utrecht University, local broadcaster RTV Utrecht, national newspaper Algemeen Dagblad, and national news radio station BNR Nieuwsradio. Outside of science, he likes to do a lot of sport and play guitar. He used to be a competitive rower and nowadays does a lot of football, squash, and bouldering. He has grown up a big fan of Luton Town F.C., who have helped him through the latter stages of his PhD by getting promoted twice in as many years.