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Functionalized cellulose nanocrystals as nanocarriers for sustained fragrance release

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ABSTRACT: The desire to extend the release time of highly volatile scents has led to the development of different types of fragrance release systems. We here report a new family of nanocarrier-based pro-fragrances, which have a high affinity to cotton textiles and release their payload under everyday life conditions. The new release systems were created by decorating cellulose nanocrystals (CNCs) with β-damascone, using a short linker that serves to bind the fragrance molecules to the CNC surface and permits their slow release via a retro 1,4-Michael-type reaction. The fragrance release was investigated in aqueous suspensions by gas chromatography coupled to mass spectrometry as a function of pH; the data show that the new pro-fragrances are stable under acidic conditions, but release the β-damascone at basic pH. The chemical resemblance of the CNCs and cotton was thought to be beneficial in terms of adhesion of the nanocarriers on textile substrates, which is an important requirement for the use of pro-fragrances in laundry applications. Thus, the deposition of the new pro-fragrances onto cotton and subsequent fragrance release were probed by dynamic headspace analysis in combination with gas chromatography. The data show that β-damascone is indeed slowly released, and that the quantity of fragrance released after 3 days is up to 80 times higher than in the case of reference experiments, where the tissue was treated with the neat fragrance under identical conditions.

KEYWORDS: cellulose nanocrystals, nanocarriers, fragrances, small molecule release, pro-fragrances

INTRODUCTION

Fragrances are used to impart characteristic scents such as freshness and cleanness to a seemingly ever growing range of consumer products.[1-2] In many applications, for example cleaning, laundry, and personal care products, a characteristic scent over a long time is desirable, but due to the highly volatile nature of olfactory compounds, long-lasting effects are often difficult to achieve.[3] Therefore, a range of delivery systems has been developed, which captures volatile molecules using either a physical or chemical barrier that permits slow release of the cargo.[4-7] Physical barriers are exploited in encapsulation or incorporation methods, where the release is triggered by degradation of the barrier or by the diffusion of the payload molecules through the shell.[8-9] In this context, polymers have been widely studied as scaffolds or as nanocarriers for the delivery of different kinds of fragrance molecules.[10-14] Chemical barrier systems used for small molecule delivery rely on labile bonds that dissociate upon exposure to – ideally mild – external stimuli such as heat, light, or a change in pH.[4-5] The covalent connection of fragrance molecules with substrates thus results in so-called pro-fragrances, i.e., non-volatile, odorless precursors from which the active olfactory agents can be released.[4-5] The chemical and physical conditions of formulations used in various consumer applications as well as the conditions and rates under which fragrance release should occur can vary over wide ranges, and therefore many different substrates and labile bonds have been explored in the context of polymer-based pro-fragrance design.[15-22] Based on the hypothesis that monosaccharide-based pro-fragrances adsorb well on cotton textiles, which is an important requirement for their use in laundry products,[20] we recently reported the investigation of new galactose-based pro-fragrances that were prepared by reacting δ-damascone in a 1,4-addition with mercaptoacetic acid and coupling this product with 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose, which was optionally deprotected to a more hydrophilic variant.[23] Fragrance release occurred *via* a retro 1,4-Michael-type reaction over the course of days, at rates that were pH dependent, and at a level that represents an increase by over two orders of magnitude in comparison to the neat fragrance. Typically, the precursor is stable at acidic pH and releases the fragrance under neutral or alkaline conditions.[22-24] In many cases, the application of personal care products is followed by rinsing with water, which dilutes the product and leads to neutralization of the pH. Therefore thioether pro-fragrances should be particularly suitable for acidic product formulations, because hydrolysis during the storage of the product is expected to be slow and the release of the fragrance preferentially occurs after application. Moreover, we observed rather different release kinetics for the hydrophobic and hydrophilic galactopyranose-based pro-fragrances studied, demonstrating that small changes in the molecular structure can have a significant impact.[23] The difficulty to predict the influence of polarity on the deposition and fragrance release was also documented in a recent study in which the structure of small-molecule thioether-based pro-fragrances was varied.[24] We here report on the investigation of a nanocarrier, which resembles chemically the monosaccharide substrate employed in our earlier studies,[23] as a pro-fragrance substrate. Besides their possible interactions with cotton through hydrogen bonding, (poly)saccharides offer many other attractive features, including their abundance and renewable nature, low cost, and nontoxic nature. We thus turned our attention to the use of cellulose nanocrystals (CNCs),[25-26] which have been suggested as a substrate for drug delivery[27] and described as excipient[28] but to the best of our knowledge, have not yet been used as a pro-drug or a pro-fragrance. CNCs are isolated from cellulosic biomass by the removal of non-cellulosic components and the chemical degradation of amorphous domains by hydrolysis using strong inorganic acids such as sulfuric,[29] hydrochloric[30] or phosphoric acid.[31] The dimensions of these rod-like crystals can vary from 10 – 30 nm x 100 – 3000 nm, depending on the source from which they are extracted and the extraction conditions.[32] CNCs have several highly attractive characteristics, including extraordinary strength and stiffness, biodegradability and a large surface area.[33] CNCs can be produced at low cost, and several companies have recently started to produce CNCs on a commercial scale.[34] CNCs have been widely used as reinforcing filler for polymers where they strengthen the material and optionally contribute to stimuli responsive mechanical characteristics.[35-37] CNCs appear to be benign,[38-39] which is relevant in the context of skin contact and possible inhalation, offer a large specific surface area, and an abundance of surface hydroxyl groups lends itself to a wide range of surface modification reactions.[33,40] These characteristics make CNCs a promising delivery platform for drugs or small molecules.[41] Based on the assumption that the amphiphilic nature of CNCs might lead to CNC-based pro-fragrances that adsorb well onto cotton and display an adequate release rate, we developed a new delivery system based on CNC nanocarriers and β-damascone, which is reported here. β-Damascone was employed as representative of the large family of enones, which are widely used in perfumery industries due to their fresh and pleasant smell.[2,42-44] We employed a short thioether linker that served to bind the fragrance molecules to the CNCs and permits their release under neutral or basic conditions *via* the previously explored retro 1,4-Michael-type reaction.[22-24] The release of β-damascone from the CNC-based pro-fragrances was studied in aqueous suspension as a function of pH, and after deposition of the nanoparticles onto different substrates under conditions that emulate the use in laundry applications as well as in an all-purpose surface cleaner.

EXPERIMENTAL SECTION

All commercially available reagents and solvents were used without further purification, unless otherwise stated. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance III 300 MHz spectrometer at room temperature (RT) and chemical shifts δ are reported in ppm relative to tetramethylsilane (TMS), using the internal solvent peak as a standard. FT-IR spectra were recorded on a Perkin Elmer Spectrum

65 spectrometer in ATR mode; peak positions are expressed in cm^{-1}. High resolution mass spectra (HR-MS) were recorded on a Bruker Esquire HTC mass spectrometer.

2-((4-Oxo-4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-yl)tio)acetic acid (1). (*E*)-1-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-2-en-1-one (β-damascone, 1.0 g, 5.2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.08 mL, 0.1 mmol) were dissolved in methanol (10 mL) before mercaptoacetic acid (0.45 mL, 6.2 mmol) was added and the solution was stirred at RT for 24 h. The solvent was then removed under reduced pressure and the solid residue was re-dissolved in ethyl acetate (10 mL), washed consecutively with 0.5 M HCl (15 mL), water (15 mL) and brine (15 mL). Purification by flash chromatography using hexane and ethyl acetate (1:3) as eluent resulted the title product as a pale yellow viscous oil (1.25 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.48 (m, 1H), 3.41-3.29 (m, 2H), 2.97-2.69 (m, 2H), 1.94 (m, 1H), 1.64 (m, 1H), 1.57 (s, 3H), 1.42 (m, 2H), 1.37 (d, 6.8 Hz, 1H), 1.05 (s, 6H) ppm. ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 208.77 (s), 175.99 (s), 142.54 (s), 129.92 (s), 52.68 (t), 38.89 (t), 35.65 (d), 33.31 (s), 33.05 (t), 31.23 (t), 28.72 (q), 28.71 (s), 21.26 (q), 20.80 (q), 18.78 (t) ppm. IR (neat): 2965, 2926, 2870, 1707, 1689, 1361, 1293, 1149, 953, 686 cm^{-1}. HS-MS (ESI-MS pos.): calcd. for $C_{15}H_{24}O_3S$, [M + Na]+ 307.1338, found 307.1343.

2-((4-Oxo-4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-yl)thio)acetyl chloride (2). 2-((4-Oxo-4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-yl)thio)acetic acid (1.0 g, 3.5 mmol) was dissolved in dichloromethane (10 mL) and the solution was cooled to 0 °C. Freshly distilled thionyl chloride (0.28 mL, 3.8 mmol) was added and the mixture was allowed to warm up to RT and stirred for 2 h. After the reaction was complete the solvent and the excess of thionyl chloride were evaporated under reduced pressure to afford the title compound as a dark brown liquid (1.06 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.92-3.74 (dd, 39.3, 15.9 Hz, 1H), 3.46 (m, 1H), 2.97-2.71 (m, 2H), 1.95 (m, 2H), 1.65 (m, 2H), 1.58 (s, 3H), 1.43 (m, 2H), 1.38 (d, 6.9 Hz, 2H), 1.06 (d, 2.9 Hz, 6H) ppm. ^{13}C NMR: (75 MHz,

$CDCl_3$) δ = 208.32 (s), 170.87 (s), 142.44 (s), 130.03 (s), 52.97 (t), 45.02 (t), 38.88 (t), 36.15 (d), 33.35 (s), 31.23 (t), 28.75 (q), 21.50 (q), 20.82 (q), 18.77 (t) ppm. IR (neat): 2965, 2922, 2873, 1796, 1708, 1449, 1348, 1003, 944, 695, 630 cm^{-1}.

Isolation of cellulose nanocrystals. Cellulose nanocrystals (CNCs) where prepared from Whatman No. 1 filter paper according to established protocols.[45-46] In brief, Whatman filter paper No. 1 (5 g) was cut into small pieces and soaked in 50 mL of deionized water (50 mL) for 10 min. Water (200 mL) was added and the mixture was blended for 5 min in an Intertronic brand kitchen blender until it had formed a homogenous pulp. This pulp was then transferred into a 1 L beaker and cooled in an ice bath to 5 °C. Concentrated sulfuric acid (140 mL) was slowly added under vigorous stirring so that the temperature was kept below 30 °C. After complete addition, the slurry was heated under stirring for 4 h at 50 °C. The CNCs were separated by centrifugation at 7500 rpm for 12 min and subsequently washed with deionized water until the supernatant reached a pH of 3. Finally the CNCs were placed into dialysis tubes and dialyzed against water for 4 days, changing the water every day twice, before the dispersion was frozen in liquid nitrogen, and lyophilized in a VirTis BenchTop 2K XL lyophilizer for 4 days to yield a white fluffy material.

Synthesis of β-damascone decorated CNCs (βD-CNCs, 3). β-Damascone decorated CNCs (βD-CNCs, **3**) were synthesized from lyophilized CNCs (500 mg), which were dispersed in dry dimethylformamide (DMF, 250 mL) by sonication in a Sono swiss SW3H sonication bath for 5 h. Taking into account that around 30% of all hydroxyl groups comprised in the CNCs are present on their surface[47] and available for reaction, a stoichiometric amount of compound **2** (0.8 g, 2.6 mmol) was slowly added and the mixture was heated to 80 °C and stirred at this temperature for 12 h. The reaction mixture was then cooled to RT and the CNCs were isolated by centrifugation in 50 mL falcon tubes at 7500 rpm for 12 min. The brown supernatant, containing reaction byproducts and unreacted acid chloride, was discarded and replaced with DMF (40 mL), and the mixture was centrifuged again. This procedure was repeated until the supernatant remained colorless. The CNCs were subsequently washed 3 times in the same manner with ethyl acetate and dried under high vacuum over night to yield βD-CNCs **3** as a pale orange powder (502 mg). The amount of attached β-damascone was determined by gas chromatography (GC) using an Agilent 6850 Series GC system equipped with a HP-5 capillary column (30 m, i. d. 0.32 mm, film thickness 0.25 μm) coupled with an Agilent 5975 Series MSD mass spectrometer. The fragrance was analyzed using a temperature gradient starting from 80 °C to 220 °C at 20 $°C \cdot min^{-1}$. The injection temperature and the detector temperature were both 250 °C. After heating a dispersion of βD-CNCs (10 mg) in DMF (5 mL) for 24 h at 130 °C, which releases the fragrance as the thioether is labile when heated (Supporting Fig. S8); evaluation of the data against an external calibration curve prepared by measuring ethanol solutions of the fragrance with 5 different concentrations (Supporting Fig. S9-S11) resulted in a releasable fragrance content of 16 mg per 1 g of CNCs.

FT-IR spectroscopy. Neat and fragrance-modified CNCs were dried under vacuum (1.0×10^{-2} mbar) overnight. The FT-IR spectra were taken in the range of 4000 to 600 cm^{-1} performing 8 scans for each sample. The spectra were normalized to the peak at 1167 corresponding to an antisymmetric ring vibration which remains unchanged during the modification reaction.[48]

Transmission electron microscopy. Transmission electron microscope (TEM) pictures were taken on a Philips CM 100 microscope with an accelerating voltage of 80 kV. The samples were prepared by dropping dispersions of neat and modified CNCs in DMF (CNC content around 0.1 $mg \cdot mL^{-1}$, the dispersions were prepared by sonication for 4 h) onto carbon supported copper grids (Electron Microscopy Science, Hatfield, Pennsylvania, U.S.A.) and subsequent drying in an oven at 70 °C for 4 h. The CNC and βD-CNC dimensions were determined by analyzing 10 TEM images of both types of

CNCs for more than 200 CNCs. The dimensions were measured using the image tool software measureIT® and reported as average values ± standard error.

Release of β-damascone from βD-CNCs (3) in aqueous buffer solutions. Aqueous buffer solutions of pH 4, 7 and 10 were prepared according to the protocol described previously using deionized water instead of deuterium oxide.[23] βD-CNCs (15 mg) were dispersed by short sonication (1 min) in the buffer solution (1 mL) and the resulting dispersion was covered by a mixture of ethyl acetate and hexane (1:3, 2 mL). Aliquots (2 µL) from the organic phase were taken in time intervals of 30 min, after 12 h the intervals were adapted to lager intervals, and analyzed continuously *via* GC to determine the amount of fragrance released (using the methodology as described in the βD-CNCs synthesis section). The amount of desorbed fragrance was determined *via* an external calibration curve made using five different solutions of β-damascone in ethanol, integrating the peak areas and plotting the data against the concentration.

Preparation of aqueous surfactant emulsions. An aqueous fabric surfactant emulsion was prepared according to protocols established earlier[20-24,49] by combining 16.5% Stepantex® VK90 (90% w/w methyl bis[ethyl (tallowate)] -2- hydroxyethyl ammonium methyl sulfate, 10% w/w isopropanol), 0.2% of an aqueous calcium chloride solution (10%) and 83.3% water. The resulting homogeneous emulsion had a pH of around 3.1. An all-purpose surface cleaner (APC) formulation was prepared from 5.0% Shell Neodol® 91-8 (ethoxylated C9-11 primary alcohols with an average of ca. 8 moles of ethylene oxide per mole of alcohol), 4.0% Marlon® A 375 (75% w/w n-C10-C13-alkylbenzene sodium sulphonate), 2% sodium cumolsulphonate, 0.2% Kathon® CG (a preservative containing 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) and 88.8% water.[24] All percentages are given in weight. The resulting formulation had a pH of around 5.

Deposition of βD-CNCs (3) and β-damascone reference onto ceramic tiles. As ceramic tiles are widely used flooring materials, they served as reference surface to simulate a typical APC application. The APC formulation (1 mL) was mixed with βD-CNCs (**3**, 27.5 mg), corresponding to a releasable amount of 0.4 mg of β-damascone, under intense stirring with a magnetic stir bar until the mixture appeared homogeneous to the unassisted eye (ca. 4 min). Then 9 mL of deionized water were added and the mixture was again stirred for 1 min. The mixture thus produced showed well distributed nanocarriers to the unassisted eye, but after ca. 1 min the nanocarriers started to settle down due to the absence of stabilizing ionic surfactants. A portion (0.75 mL) of this mixture was placed onto a non-painted pre-cleaned ceramic tile (10 x 5 cm). The tiles were further protected against dust and environmental influences and dried under ambient conditions for 3 d. The neat β-damascone was applied as a reference in similar manner, using 200 µL of a stock solution of β-damascone/ethanol (2 $mg \cdot ml^{-1}$) for the preparation of the formulation. All samples were prepared in duplicate.

Deposition of βD-CNCs (3) and β-damascone reference onto cotton tissue. The deposition of the CNC nanocarriers onto cotton followed protocols previously reported for pro-fragrances based on polymers and small molecules.[20-24,49] Dry βD-CNCs (100 mg) were mixed directly with the aqueous surfactant emulsion (1.8 g) and deionized water (1 mL) was added to permit better mixing. The mixtures were stirred for 10 min, transferred into 1 L beakers and diluted with deionized water (600 g). A cotton sheet (Swiss Federal Laboratories for Materials Science and Technology, cotton test cloth Nr. 221, cut into 12 x 12 cm sheets, average mass of 3.12 g, prewashed with an unscented detergent powder) was placed into the beaker and was manually stirred for 3 min, left to rest for 2 min, and wrung out to obtain a wet cotton sample with a mass of 7.0 g +/- 0.1 g. These tissues were further line dried in a dark cupboard for 3 days under ambient conditions. Unmodified β-damascone (1.6 mg) was deposited as reference in a similar manner. All samples were prepared in duplicate.

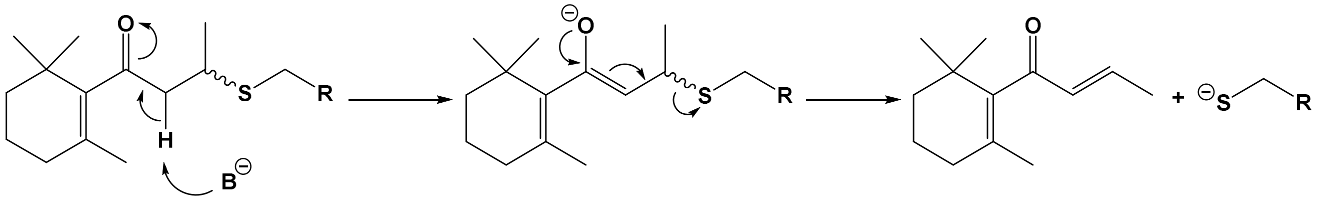
To determine the deposition efficiency, mixtures of βD-CNCs and β-damascone were directly deposited onto cotton tissues, which were washed with an unperfumed softener formulation as above-quoted. A stock solution of βD-CNCs was created by mixing βD-CNCs (250 mg) with dimethylsulfoxide (DMSO) (500 µL) under vigorous stirring for 10 min until a homogenous distribution of nanocarriers was achieved. Subsequently, 200 µL of the stock solution (containing a releasable amount of 1.6 mg of β-damascone) was placed onto the freshly washed tissue and line dried in a dark cupboard for 3 days. All samples were prepared in duplicate.

Dynamic headspace analysis of β-damascone released from ceramic tiles. Dynamic headspace analysis was performed using previously reported protocols.[23-24] The ceramic tiles were placed in homemade headspace cells (volume = 360 mL) and exposed to a constant air flow (200 mL·min^{-1}) which passed first through active charcoal and then through a saturated sodium chloride (NaCl) solution to guarantee a constant humidity of 75%. The experiment was first equilibrated for 15 min with a dummy cartridge, before a fresh cartridge was inserted and the measurement was started. The data collection lasted for 15 min (corresponding to a volume of 3 L of air) and was repeated every 45 min over an 8 h period. All samples were measured in duplicate and the sample cartridges were thermally desorbed on a Perkin Elmer TurboMatrix ATD 350 desorber coupled to an Agilent Technologies 7890A gas chromatograph equipped with a HP-1 capillary column (30 m, i. d. 0.32 mm, film thickness 0.25 µm) and a flame ionization detector. The volatiles were analyzed using a two-step temperature gradient starting from 60 °C to 130 °C at 15 °C·min^{-1} and then heating to 220 °C at 40 °C·min^{-1}. The injection temperature and the detector temperature were both at 250 °C. The amount of desorbed fragrance was determined *via* an external calibration curve using five different solutions of β-damascone in ethanol. The peak areas of the calibration solutions were then plotted against the concentration. All samples were measured in duplicate.

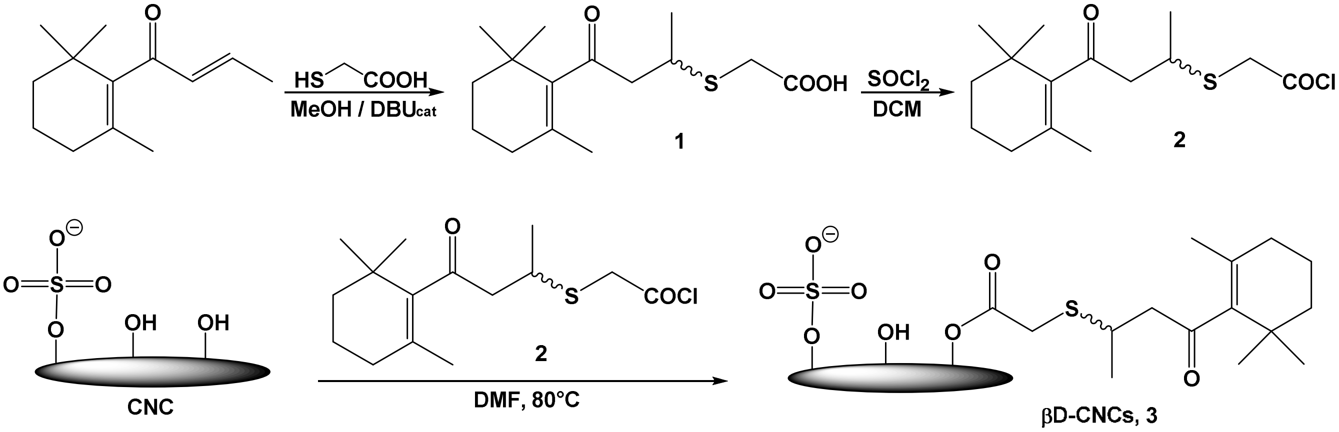
Dynamic headspace analysis of β-damascone released from cotton tissue. Dynamic headspace analysis was performed using previously reported protocols.[23-24,49] The line dried cotton sheets onto which the βD-CNCs or the neat β-damascone reference had been deposited were placed into temperature-controlled (25 °C) headspace sampling cells (volume = 160 mL) and analyzed using the same experimental installation as described *vide supra* for the tile release. The amount of desorbed fragrance was determined *via* an external calibration curve using five different solutions of β-damascone in ethanol. The peak areas of the calibration solutions were then plotted against the concentration.

RESULTS AND DISCUSSION

Design and synthesis of β-damascone decorated CNCs (βD-CNCs, 3). Conjugated enones such as damascones can undergo an efficient, but reversible 1,4-Michael reaction with thiols, and the retro 1,4-Michael-type reaction of the resulting thioethers represents a broadly useful release mechanism (Scheme 1) that is well understood and already commercially exploited in small-molecule based pro-fragrances.[50-51] Using this well-established framework as a testbed we selected β-damascone [(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one] as typical representative of the rose ketone family and elected to attach a thiol-ene adduct of this motif to the hydroxyl groups present on the surface of the CNCs (Scheme 2). It has been shown in previous studies that small molecules or macromolecules can be grafted successfully onto the CNC surface using different chemistries, including isocyanate chemistry,[46,52] etherification,[53-54] and ester formation.[55] We opted to attach the payload by reacting acid chlorides with the (primary) hydroxyl groups of the CNCs, as the moderate temperature and the *in-situ* produced hydrochloric acid appeared to be beneficial of attaching retro-Michael based pro-fragrances. To minimize the amount of added weight, we elected to use mercaptoacetic acid as the shortest commercially available linker that supports the targeted reactions.[24] We thus first reacted mercaptoacetic acid with β-damascone to produce intermediate **1** *via* a 1,4-Michael reaction (Scheme 2). The NMR spectrum clearly shows the disappearance of the double bond (peaks at 7.27 and 6.11 ppm) and the appearance of the linker protons (peaks at 2.72 – 2.99 ppm) indicating a complete reaction (Supporting Fig. S1). The thiol-ene adduct **1** was subsequently treated with thionyl chloride to prepare the corresponding acid chloride **2**. The transformation into the acid chloride was quantitative, as indicated by the absence of signals related to the starting material in the ^{1}H NMR spectrum after the reaction (Supporting Fig. S3).



Scheme 1 Reaction mechanism of the base-induced retro 1,4-Michael-type reaction of thiol-ene adducts of β-damascone.



Scheme 2 Synthesis of β-damascone decorated cellulose nanocrystals (βD-CNCs, **3**).

The CNCs used in this study were isolated from cotton according to well established protocols by hydrolysis of pulp prepared from cotton-based filter paper with sulfuric acid.[45] Transmission electron microscopy (TEM) images show well-defined and well isolated rod-like particles of typical appearance (Fig. 1).[56] Evaluation of the TEM images revealed that the CNCs had an average length of 213 ± 54 nm, an average width of 23 ± 6 nm, and an aspect ratio of ca. 10, which are typical values for CNCs isolated from cotton.[25, 38-39,45] The hydrolysis of cellulose pulp with sulfuric acid leads to the formation of a small number of sulfate ester groups (as a side reaction), which help to stabilize dispersions of the CNCs in water and other solvents. Conductometric titration, which was performed as described in detail elsewhere,[31] resulted in a surface charge density of about 35 mmol·kg^{-1}, which falls also in the typical range for CNCs isolated from cotton under the conditions used here. [38-39] These neat CNCs were decorated with the thiol-ene adduct of β-damascone by esterification with acid chloride **2**. Thus, neat CNCs were dispersed in dry DMF, before a stoichiometric amount (relative to all available OH groups on the CNCs' surface) of **2** was added and the mixture was stirred at 80 °C for 16 h. The resulting βD-CNCs (**3**) were separated from the reaction mixture by centrifugation and subsequently dried. Gratifyingly, TEM images of βD-CNCs that were re-dispersed in DMF reveal no statistically significant difference in size or shape (235 ± 61 nm / 25 ± 7 nm) after the modification step (Fig. 1). The induced functionalization of the CNCs' surface renders the nanoparticles more hydrophobic, which affects the dispersibility in polar solvents and results in an increase in aggregates and agglomerations. In line with previous reports on other modified CNC systems,[57-59] the typically low degree of surface functionalization only allows for a few reliable characterization techniques. The success of the esterification reaction was qualitatively confirmed by infrared (IR) spectroscopy (Fig. 2). The IR spectra clearly show the appearance of a characteristic ester band at 1727 cm^{-1}, which indicates the successful linkage of the intermediate **2** with the CNCs surface hydroxyl groups. Despite the fact that the corresponding bands for acid and ester bonds are sometimes overlapping, they could be clearly distinguished in the present case (Supporting Fig. S7). Furthermore, a carbonyl band at 1640 cm^{-1}, which corresponds to the ketone of the attached fragrance, was observed. Finally, the band observed in the region between 3000 and 3500 cm^{-1} has been reported to be characteristic of cellulose.[58-60] The amount of attached β-damascone was quantitatively determined by gas chromatography (GC) after thermally releasing the fragrance by heating a dispersion of βD-CNCs in DMF. The value of 1.6% w/w is lower than the decoration level that has been reported for isocyanate functionalized CNCs,[46,52] but we note that the latter chemistry requires conditions under which the thiol-ene adduct suffers from cleavage.

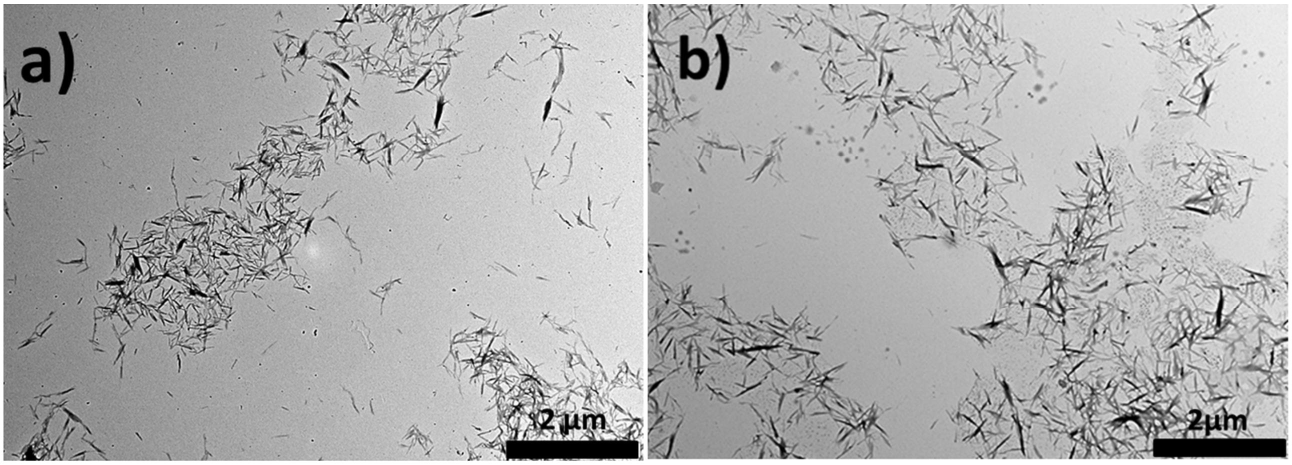


Fig. 1 Transmission electron microscopy (TEM) images of a) neat CNCs and b) βD-CNCs deposited from 0.1 wt% suspensions in DMF.

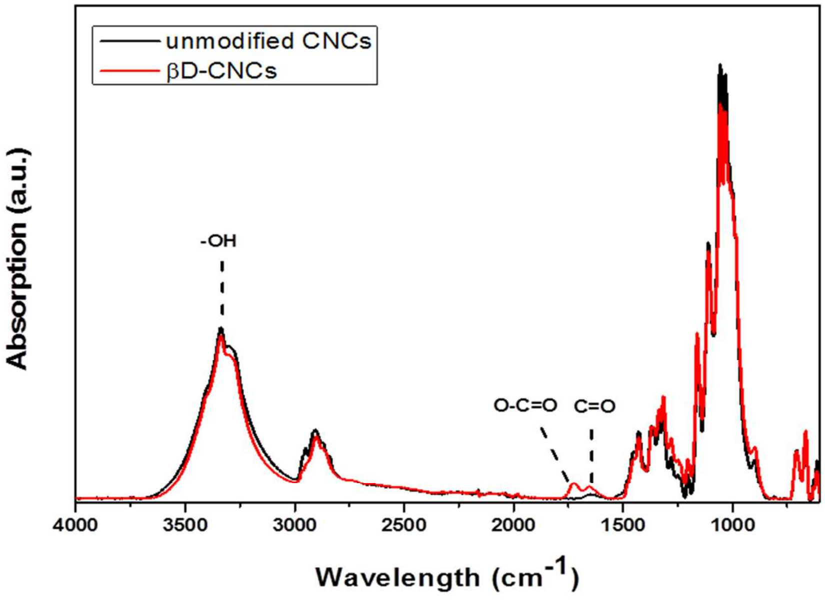


Fig. 2 Infrared spectra of unmodified CNCs and β-damascone modified CNCs (βD-CNCs), showing the appearance of a carbonyl peak at 1727 cm^{-1}, which is attributed to the ester linkage formed upon reaction of CNCs with acid chloride **2** and a carbonyl peak at 1640 cm^{-1}, which corresponds to the ketone of the attached β-damascone (Supporting Fig. S7).

Release of β-damascone from βD-CNCs in aqueous buffer dispersions at different pH. To explore the conditions and kinetics of the release of β-damascone from the nanocarrieres, we prepared dispersions of βD-CNCs in aqueous buffers at pH 4, 7 and 10 and the fragrance release over time was monitored by gas chromatography coupled with mass spectrometry (GC-MS). While other factors (in particular temperature) can also influence the rate of the retro-Michael reaction, their systematic investigation is beyond the scope of this study. The experiments were thus conducted at room temperature (20° - 25°C) in a biphasic system comprised of an aqueous buffer phase (1 mL) and an organic phase (mixture of ethyl acetate and hexane, 1:3, 2 mL) that was utilized to capture the released β-damascone. The experiments were conducted in closed systems, which may have had an influence on the equilibrium between dissociation and formation reactions.

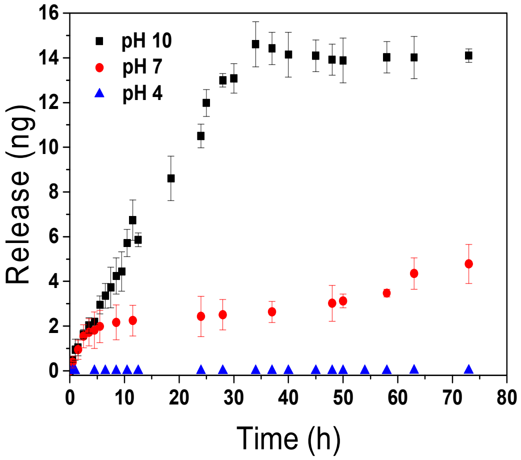


Fig. 3 Amount of β-damascone released from βD-CNCs in aqueous buffer mixtures at pH 4, 7 and 10 as function of time. Data were acquired by GC-MS spectroscopy using a biphasic system comprising an aqueous buffer phase in which βD-CNCs were dispersed at a concentration of 15 mg·mL^{-1} and a 1:3 mixture of ethyl acetate and hexane.

Fig. 3 shows the absolute amounts of β-damascone released from 15 mg βD-CNCs at different pH values. At pH 4 no release was detectable within three days, which is consistent with earlier studies that showed high stability of thioether based pro-fragrances at acidic pH.[22-23,51] At pH 7, a small amount of the fragrance was rapidly released, before after ca. 5 h the release rate dropped, and slow release was observed over several days. At pH 10 the release was accelerated due to the catalytic effect of the basic environment (Scheme 1). The release reached a plateau after 30 h indicating that all the releasable fragrance had been liberated. While it is known from previous studies that nanoparticle carriers can slow down the release of fragrance molecules in comparison with monomers, a comparison of the above data with the release kinetics observed previously for monosaccharide-based pro-fragrances using the same release chemistry suggests similar release rates.[23]

Dynamic headspace analysis of β-damascone released from ceramic tiles. The β-damascone release from the new nanocarriers under ambient conditions was next studied using unpainted ceramic tiles as a substrate, which permitted the deposition of known amounts of the βD-CNCs, and emulates a substrate that is relevant for the application in cleaning products. The detergent had a pH of ca. 5, which represents an environment under which the pro-fragrance is likely to be very stable (*vide supra*), but drying under ambient conditions is expected to raise the pH, so that the retro 1,4-Michael-type reaction is triggered; note that the release therefore starts already during drying. In order to compare the fragrance release from the βD-CNCs with the evaporation that is observed by depositing the same amount of the neat fragrance and a drying period (where much of the neat fragrance is expected to evaporate already upon drying), βD-CNCs and β-damascone were separately mixed with an all-purpose surface cleaner (APC) formulation under vigorous stirring at a concentration of 2.75 mg·mL^{-1} and 0.04 mg·mL^{-1}, respectively (these concentrations were chosen to keep the absolute amount of fragrance the same), and known amounts of the mixtures were placed separately onto the tiles. The tiles were dried under ambient conditions for three days and the β-damascone release was analyzed using dynamic head space analysis at a flow rate of 200 mL·min^{-1} and a constant humidity of 75% according to well-established protocols.[24] The volatiles were collected on Tenax® cartridges and subsequently thermally desorbed and analyzed by GC (Supporting Fig. S13). Comparison with a calibration curve (Supporting Fig. S10) permitted the presentation of release profiles that show the concentration of β-damascone released as a function of time (Fig. 4).

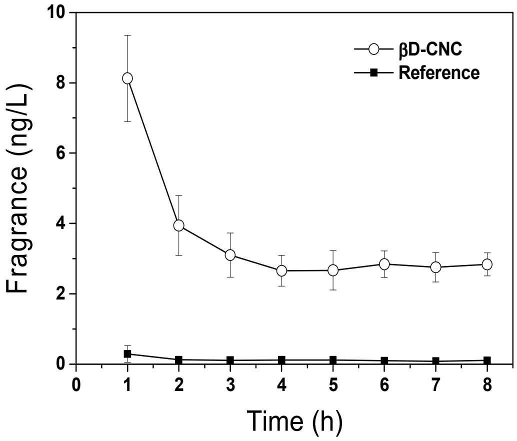


Fig. 4 Concentration of β-damascone released from βD-CNCs and the neat β-damascone reference in air passed over ceramic tiles onto which an all-purpose surface cleaner (APC) formulation containing the (pro-)fragrances at concentrations of 2.57 mg·mL^{-1} and 0.04 mg·mL^{-1}, respectively, had been deposited. Data were collected after drying the samples under ambient conditions for 3 days, using dynamic headspace analysis in combination with GC. Data points represent averages of two samples.

The data in Fig. 4 show several interesting features. First of all, the tiles treated with βD-CNCs release much more β-damascone than the ones treated with an equimolar amount of the neat fragrance reference. In the case of the βD-CNCs, the headspace concentration decreases from initially ca. 8 ng·L^{-1} to ca. 3 ng·L^{-1} within a few hours, after which the release is stable for many hours (note that the experimental setup prevented us from recording data for more than 8 h, but the steady release rate shown in Fig. 4 suggests that the fragrance is far from being exhausted). This effect is consistent with a rapidly decreasing fragrance concentration on/in the porous tiles, which can retain a significant amount of β-damascone that was released during drying at ambient (it should be recalled that at the beginning of the experiment the samples had already been dried for 3 days), and evaporation is greatly accelerated upon applying an airstream. In the steady state, the βD-CNCs release up to 20 times more fragrance than the neat reference. This significant difference clearly confirms that βD-CNCs can serve as pro-fragrance and the result is a first validation of the general approach pursued here. It is noted, however, that the release level is limited by the relatively low level of functionalization achieved (*vide supra*).

Dynamic headspace analysis of β-damascone released from softener washed cotton sheets. One of our main motivations of studying cellulose-based nanocarriers was the structural similarity of CNCs and cotton. Both consist of the same building block, which we assumed would cause the CNC pro-fragrance to adsorb well on cotton textiles. This affinity might result in an effective deposition during the softening step. Therefore, the performance of these nanocarriers in a simplified softener release test was investigated. The softener emulsion was prepared using a commercially available cationic surfactant emulsion, which had a pH of ca. 3.1. As it has been shown in the past for polymeric and small molecule systems,[22-24,49] and in the kinetic experiment shown in Fig. 3, an acidic environment is favorable for storing thiol-ene adducts, because the retro 1,4-Michael-type reaction (Scheme 1) is suppressed in the acidic environment. Another well-known positive effect of these softener emulsions is the presence of surfactants, which have the ability to facilitate the deposition of non-polar compounds onto the cotton surface and help to keep the nanoparticles stable in suspension. To study the effects of deposition and release, cotton sheets were washed with an aqueous softener mixtures containing βD-CNCs and β-damascone (which served as the reference) at concentrations of 170.0 $\mu g \cdot mL^{-1}$ and 2.7 $\mu g \cdot mL^{-1}$, respectively (again, these concentrations correspond to the same amount of the fragrance), and drying the samples for 3 days under ambient conditions. This process simulates the normal washing process of clothes. The dried cotton clothes were then tested by dynamic headspace analysis to determine the amount of fragrance released; the results are summarized in Fig. 5 (an example of the chromatograms recorded is shown in Supporting Fig. S14).

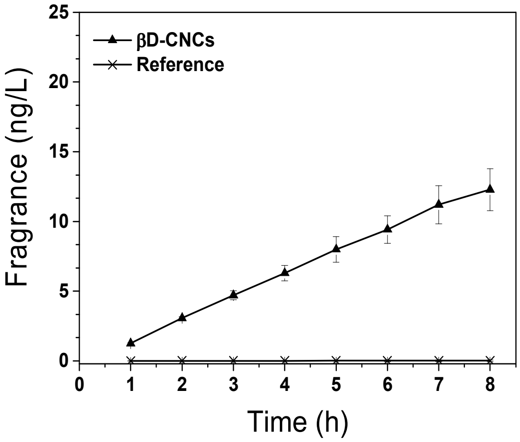


Fig. 5 Concentration of β-damascone released from βD-CNCs and the neat β-damascone reference in air passed over cotton tissues that had been washed with a fabric softener formulation containing the (pro-)fragrances at concentrations of 170.0 μg·mL^{-1} and 2.7 μg·mL^{-1} respectively. Data were collected after drying the samples under ambient conditions for 3 days, using dynamic headspace analysis in combination with GC. Data points represent averages of two samples.

The data show that air passed over the tissue treated with the neat β-damascone reference contains only a very low (0.15 ng·L^{-1}) concentration of the fragrance, on account of evaporation of this volatile product during drying. The nanocarriers (βD-CNCs), by contrast, release much larger amounts of β-damascone. Interestingly, the β-damascone concentration in air passed over tissues treated with βD-CNCs steadily increases with time to a value of 12 ng·L^{-1} after 8 h, corresponding to an increase that is 80 times higher than the neat β-damascone reference. The recorded release profile suggested an even higher release rate, given more time, but this could not be investigated, due to the limitation of the experimental setup, which could not be modified to permit longer sampling times. The amount of fragrance attached to the CNC nanocarriers (1.6% w/w) was lower than in previous studies, which investigated pro-fragrances based on polymeric systems with the same release mechanism and 2.5 – 38% w/w payload.[20-21] Nevertheless, the present study proves that the new nanocarriers can release fragrance molecules very efficiently over a long timeframe.

Dynamic headspace analysis of β-damascone released after direct deposition onto cotton sheets. As the deposition efficiency is one of the key characteristics of fragrance delivery systems in softener applications, we also probed the release of β-damascone after direct deposition of βD-CNCs and the neat β-damascone reference onto cotton and comparison with the washed tissue. Thus, the βD-CNCs (250 mg) were dispersed in DMSO (500 μL) and 200 μL of this dispersion (100 mg βD-CNCs) were applied onto the freshly washed tissues. The tissues were line dried for 3 days under ambient conditions and the above described headspace analysis was performed (examples of gas chromatograms are shown in Supporting Fig. S15). The results are shown in Fig. 6.

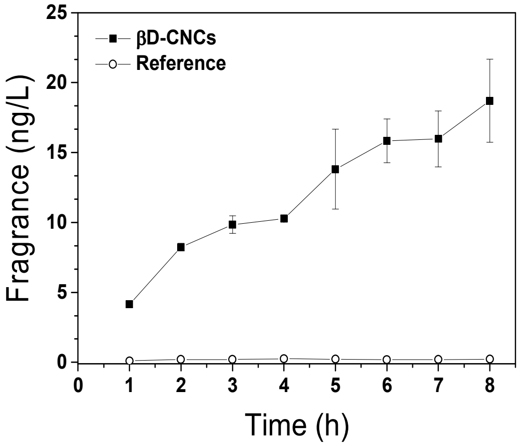


Fig. 6 Concentration of β-damascone released from βD-CNCs and the neat β-damascone reference in air passed over cotton tissues onto which DMSO (200 μL) containing 100 mg βD-CNCs or 1.6 mg β-damascone had been deposited. Data were collected after drying the samples under ambient conditions for 3 days, using dynamic headspace analysis in combination with GC. Data points represent averages of two samples.

Mirroring the situation observed for the cotton tissues washed with a softener formulation containing βD-CNCs (*vide supra*), the concentration of β-damascone in air passed over tissues treated with βD-CNCs steadily increases with time to a value of 20 ng·L^{-1} after 8 h. A comparison with Fig. 5 shows that at all time points, the values are about 60% higher than in the case of the washed tissue (12 ng·L^{-1} after 8 h). This in turn suggests a very effective deposition (60%) of the cellulose nanocarriers on the cotton fabric under the here-described conditions, as it had been described before for apolar pro-fragrances featuring a protected galactose substrate.[23]

CONCLUSIONS

Our study reports the first nanocarrier-based pro-fragrances based on cellulose nanocrystals that were decorated with β-damascone, using a short linker that permits slow fragrance release *via* a retro 1,4-Michael-type reaction. In aqueous suspension the pH-dependence and kinetics of the fragrance release mirror the situation observed for small-molecule pro-fragrances with a similar release motif. Consistent with the fact that the retro 1,4-Michael-type reaction is base catalyzed, βD-CNCs are highly stable under acidic conditions, whereas the release rate appears to increase if the pH is lowered from neutral to pH 10. The βD-CNCs were effective under conditions that emulate two realistic application scenarios as all-purpose cleaner and fabric softener, respectively. In both cases, the concentration of β-damascone released into air passed over substrates that had been treated with the pro-fragrance and dried for 3 days was much (20-80 times) larger than in the case of the neat β-damascone reference (where much of the volatile fragrance had evaporated during drying). Furthermore, the measured concentrations were sufficiently above the human olfactory threshold[43] to be easily recognized. However, in the present proof-of-concept study only a small fraction of the CNC's surface hydroxyl groups could be functionalized with fragrance moieties and a higher decoration level would be desirable for industrial applications. We note that this limitation is not intrinsic to the general idea to utilize CNCs as a carrier for pro-fragrances, but rather a constraint of the specific attachment chemistry utilized here. A higher level of functionalization is achievable with isocyanate chemistry,[15] although it is noted that the conditions must not allow the retro 1,4-Michael-type reaction to occur. Thus, the design approach may be improved by first decorating the CNCs with an appropriate anchor group (i.e., a thiol function) and forming the thiol-ene through a reaction with the particles thus functionalized. Perhaps the most intriguing finding is the fact that βD-CNCs display a very effective deposition efficiency on the cotton fabric, at least under the here-described conditions. This finding bodes well for the delivery of compounds onto textile fabrics, depending on the target application the payload might need to be increased.

ASSOCIATED CONTENT

Supporting Information. NMR, IR spectra, gas chromatograms and TGA data. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes: The authors declare no competing financial interest.

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