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Article

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Efficient Encapsulation and Controlled Release of *N*,*N*-Diethyl-3-methylbenzamide (DEET) from Oil-in-Water Emulsions Stabilized by Cationic Nanocellulose and Silica Nanoparticles

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Oil-in-water emulsions containing *N*,*N*-diethyl-3-methylbenzamide (DEET) were developed aiming to extend the sustained release of the active compound, by using two oppositely charged nanomaterials, namely silica nanoparticles (SiNP) and cationic cellulose nanofibrils (CCNF), as stabilizers, and a mixture of food-grade nonionic surfactants to avoid precipitation by electrostatic aggregation. The formulations were stable for more than four months at room temperature, and strongly resistant to destabilization upon centrifugal and thermal stress. The results were correlated with the effect of SiNP on strengthening the network formation of CCNF in the aqueous phase by electrostatic interactions, which increased the viscosity of the external phase and, hence, emulsion stability. There was a significant size reduction of the internal oil phase containing DEET in the presence of CCNF, which was attributed to the increased viscosity in the external aqueous phase, as well as to interfacial stabilization. The combined action of CCNF and unmodified SiNP in the stabilization of the DEET-containing oil phase significantly decreased the release rate of the active compound, compared to non-emulsified DEET. Moreover, in the emulsions containing the CCNF/SiNP mixture there was a more sustained release for the period of 6 h, demonstrating the potential of these formulations for extended protection.

Keywords: DEET, emulsions, slow-release, silica nanoparticles, cationic cellulose, stability

Introduction

The use of repellents, mainly topical formulations, is one of the best protective measures to reduce and/or prevent transmission of many insect-borne diseases. *N*,*N*-Diethyl-3-methylbenzamide (DEET) is a broadly employed repellent for a large number of insects (e.g., mosquitos, flies, ticks).[1] DEET is fairly insoluble in water and soluble in polar and nonpolar organic solvents, such as glycerin, ethanol, and isopropyl alcohol. Due to the solubility problem of DEET, its topical formulations usually have alcoholic bases, but these kind of formulations increase skin permeation and also systemic absorption of DEET, which leads to some toxic effects.[2] Thus, novel formulations containing DEET are based on oil-in-water (O/W) emulsions (e.g., topical creams, lotions), which do not leave the skin feeling oily and are focused on ease of application and cosmetic acceptance.[2] However, these DEET formulations have short periods of protection (a few hours), requiring frequent application due to environmental effects such as excessive sweating, humidity and insect activity.[1] To achieve extended repellency, the active compound is usually formulated at high concentrations, up to 100% in some commercial products, or in controlled-release systems that allow sustained delivery of DEET for longer times.[3] The latter strategy has been gaining more relevance in the last few years, since it can contribute to decrease the potentially toxic effects of DEET by allowing a more efficient use of the active compound.[4]

Development of formulations containing extended release systems have been based on encapsulation of DEET in vehicles that range from classical emulsions[5-7] to solid lipid nanoparticles,[8] and polymer microcapsules,[9,10]

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[†] In memory of our colleague Janet Scott, 1964-2022

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micelles,[11] and nanospheres.[12] Early work[6] showed that using cyclodextrins in the aqueous phase of formulations can decrease the release rate of DEET by a combined mechanism of complexation and emulsion stabilization. Barradas *et al.*[11] used concentrated solutions of the copolymer Pluronic®F127 to form thermoreversible gels that allowed sustained DEET release for up to 7 h. Another approach used to encapsulate DEET was reported by Gomes *et al.*[12] using poly(*n*-butyl methacrylate-*co*-methyl methacrylate) nanospheres via direct miniemulsion polymerization and sodium lauryl sulfate as suitable surfactant. The release rate of the encapsulated DEET provided repellency for over 9 h, and the mechanism of release could be tuned by adjusting the copolymer glass-transition temperature. Recently, Kadam *et al.*[10] reported the encapsulation of DEET in oil-rich microcapsules through interfacial polycondensation using stearic acid functionalized cellulose nanofiber. The cellulose nanofibrils (CNF) acted both as Pickering emulsifier and strengthened the barrier properties of the microcapsules, resulting in significant reduction of the DEET release rate.

As literature shows, there are different mechanisms that allow an efficient retention and release of the active compound; thus, the combination of several strategies can be advantageous for developing novel formulations aiming extended DEET release. In O/W emulsions, the main mechanisms that control the release of the active compounds are the diffusion from the internal oil phase, as well as the coalescence of the oil droplets, leading to phase separation.[3] Therefore, to obtain formulations with long-lasting repellency the aim should be retarding the migration of DEET through the aqueous bulk phase by, for instance, increasing the stability of the emulsions. Alternatively, a second strategy would be controlling the migration of DEET through the aqueous bulk phase. A recent paper from our group[7] showed that silica nanoparticles (SiNP) functionalized with ammonium quaternary groups and anionic polymers were able to stabilize oil-in-water emulsions containing DEET and retard its release. The emulsion stability was achieved due to the strong interfacial adsorption of the solid nanoparticles mediated by bridging of the polymeric chains with a high degree of polymerization. Although this approach proved the advantage of using oppositely charged species for stabilization of O/W emulsions with slow DEET release, the functionalization of the nanoparticles required several synthesis steps that might be difficult to scale for pharmaceutical applications. In addition, the use of other nanomaterials with higher aspect ratio than SiNP, such as the cellulose nanofibrils, can lead to increased viscosity of the aqueous phase and contribute to stabilization of the emulsions.

In the present work, we report the preparation of O/W emulsions containing DEET stabilized using two oppositely charged nanomaterials, namely SiNP and cationic cellulose nanofibrils (CCNF), mediated by a mixture of food-grade nonionic surfactants, to avoid precipitation by electrostatic aggregation. The combined action of these components, which are non-toxic and easily available, aimed to retain the active compound for longer times in the formulation based on the strong stabilization of droplets containing DEET by decreasing diffusion due to the polymer network formation in bulk.

Experimental

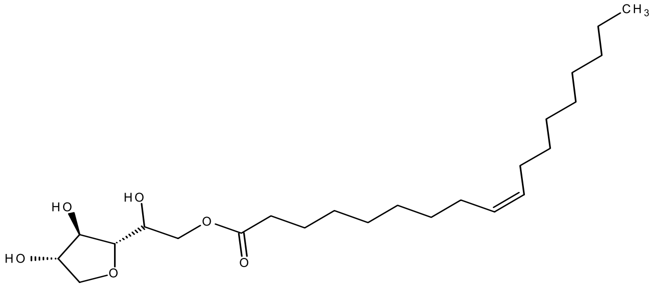
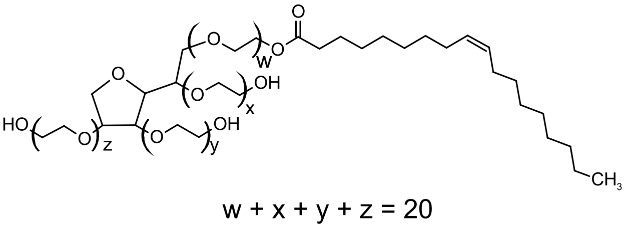
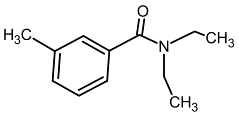
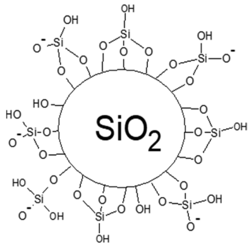
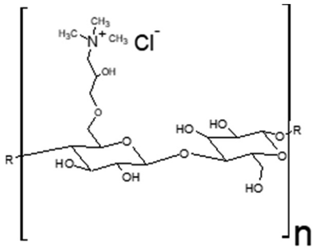
Materials

The repellent active principle used in this work was *N*,*N*-diethyl-3-methylbenzamide (DEET, 97%). The commercial food-grade nonionic surfactants used were Span®80 (sorbitan oleate) and Tween®80 (polyoxyethylene sorbitan monooleate). Silica nanoparticles (99.8% SiO_2) were non-porous and have a nominal diameter of 12 nm and Brunauer-Emmett-Teller (BET) surface area of 175-225 $m^2 g^{-1}$ (as informed by the supplier). A lightweight mineral oil (0.84 g mL^{-1} at 25 °C, BioReagent) composed of a mixture of liquid saturated hydrocarbons was chosen as oil phase, for being a common ingredient in lotions, cold creams, ointments, and cosmetics.[13] All the above reagents and materials were purchased from Sigma-Aldrich (São Paulo, Brazil), and their chemical structures are depicted in Table 1.

Alpha-cellulose (product number C8002) and the cationizing reagent glycidyl trimethylammonium chloride (GTMAC, ≥ 90%) were purchased from Sigma-Aldrich (UK). Cationic cellulose nanofibrils (CCNF, Table 1) were synthesized from the reaction of cellulose with GTMAC using the procedure described elsewhere,[14] and purified and characterized following the protocols reported by Courtenay *et al.*[15] Cellulose reacted with GTMAC in basic medium resulting in cationization of the nanofibrils due to the presence of quaternary ammonium groups. Cellulose cationization was confirmed by conductometric titration with $AgNO_3$ and the degree of substitution was 21.8% (see details of procedure in Supplementary Information (SI) section, Figure S1). The final CCNF stock dispersion was 2.2 wt.%.

To evaluate the effect of salt and pH in the emulsion stability and DEET release experiments, sodium chloride (NaCl, ACS reagent, ≥ 99.0%), hydrochloric acid, sodium hydroxide, and methanol (anhydrous, 99.8%) from Sigma-Aldrich (São Paulo, Brazil) were used. Ultrapure water

Table 1. Chemical structure of the components of the oil-in-water emulsions developed in this work

Sorbitan oleate (Span®80)		Polyoxyethylene sorbitan monooleate (Tween®80)
		
N,*N*-Diethyl-3-methylbenzamide (DEET)	Silica nanoparticles (SiNP)	Cationic cellulose nanofibrils (CCNF)
		

(conductivity of 18 MΩ cm, MilliQ® system) was used in all the experiments.

Characterization of nanomaterials by dynamic light scattering

The ζ-potential and hydrodynamic diameter (d_h) values of SiNP and CCNF were obtained using a Horiba SZ-100 dynamic light scattering (DLS) instrument from Reno, USA. Three samples of each nanomaterial were first dispersed in water using a FB-505 ultrasonic processor (Fisher, Newtown, USA, 200 W cm^{-2}) equipped with a 0.63 cm probe, in continuous mode for 2 min and an amplitude of 30%. The dispersions were diluted several times, and then the electrophoretic mobility was measured six times (at 25 °C) and converted to ζ-potential using the Smoluchowski approximation, which yields the hydrodynamic particle size.[16] Uncertainties in these values are reported as the standard deviations of six measurements. The pH of the samples was adjusted with small amounts of $HCl_{(aq)}$ or $NaOH_{(aq)}$.

Preparation of O/W emulsions containing DEET

The O/W emulsions were prepared at an oil:water ratio of 30:70 (v:v), as is common for repellent formulations.[3] The aqueous phase was prepared in the following order, to avoid extensive precipitation by electrostatic aggregation between the nanomaterials: first, SiNP were dispersed by 1-min sonication into a Tween®80 aqueous solution, and then CCNF stock dispersion was added to achieve final concentrations of the mixture in the emulsion of 0.01 and 0.3 wt.% for the SiNP and CCNF, respectively. After mixing, the SiNP-CCNF suspension, composing the aqueous phase of the O/W emulsion, was sonicated for 2 min. The sonication steps were performed using an FB-505 ultrasonic processor (Fisher, Newtown, USA, 200 W cm^{-2}) equipped with a 0.63 cm probe operating in continuous mode (amplitude of 30%). The oil phase was prepared by mixing first the mineral oil with DEET and Span®80 using an Ultra Turrax (IKA T25, Staufen, Germany) high-speed homogenizer at 13 000 rpm, then slowly adding dropwise this mixture to the aqueous phase, and homogenizing for two more minutes (procedure adapted from Li *et al.*[17]). The DEET total concentration in the formulations was 10 wt.%, and surfactants used as emulsifiers were at a 1:1 ratio (Tween®80:Span®80, wt.%:wt.%) and 0.5 wt.% total concentration.

Characterization of O/W emulsions containing DEET

Rheological behavior

The rheological behavior of the O/W emulsions was obtained using a rotational stress controlled rheometer (Discovery HR-3, TA Instruments, New Castle, UK) equipped with a plate-plate geometry (plate diameter of 40 mm). The temperature was fixed at 25 °C through a Peltier system for all the experiments. Flow curves were obtained by measuring the viscosity as a function of the increasing shear rate ($\dot{\gamma}$) in steady-state intervals within an operating range of $0.01 \leq \dot{\gamma} \leq 100$ s^{-1}. Analogous rheological measurements were performed for the aqueous phase of the emulsions, and aqueous CCNF dispersions prepared at different wt.% suspended in 0.5 wt.% Tween®80 aqueous solutions (in the presence and absence of SiNP).

Stability, emulsion morphology and phase separation

Physical stability of the emulsions was monitored by time lapse images (using a photographic camera with a 16-megapixel, 1/2.6" sensor having an aspect ratio of 16:9, aperture of f/2.2, 31 mm focal length, and 1.12 µm pixel size). Microscopic characterization to determine emulsion morphology (droplet size and distribution) was performed using a Brunel Microscope SP60P coupled to an EOS 1300D Canon camera (Chippenham, UK). An aliquot of 20 µL of the emulsion was diluted into 1000 µL of the aqueous phase, and dispensed on a glass slide prior to imaging. Experiments were carried out at room temperature (ca. 25 °C).

Droplet size and distribution were obtained using a Mastersizer 3000 Hydro with a small volume dispersion unit, from Malvern Instrument (UK). The lens used can detect diameters ranging from 0.5 µm up to several mm. Emulsions were diluted in the equipment's accessory to avoid erroneous results due to the aggregation of the drops. The refraction index used was 1.33 and 1.47 for the water and mineral oil, respectively. The reported particle size was obtained form an average of five measurements. Representative sizes were evaluated based on the D90, referred to as the particle size (diameter) below which the 90 vol% of the droplet population is present.

To determine the extent of phase separation and compare the effect of nanomaterials in the emulsion stabilization, the samples were submitted to mechanical (centrifugation) and thermal (heating) stresses. Aliquots of emulsion were centrifuged at 3889 RCF for 30 min using a Thermo Scientific Heraeus Megafuge (Hampton, United States), or kept at 45 °C in a laboratory oven (Memmert oven UN 30, Schwabach, Germany) for 72 h, and then monitored by visual observation. In addition, since the mechanism of emulsion stabilization was based on electrostatic interactions, the effect of changes in solution pH and of salt addition was evaluated. In an initial volume of 5 mL of emulsion, the pH was adjusted from the initial pH of 6.0 to pH values of 3 or 11 with 0.1 M HCl or 0.1 M NaOH, respectively, using an Analiser 650 MA pH-meter (São Paulo, Brazil). For the salt addition experiment, NaCl in powder form was added directly to the emulsion to reach a 3.5 wt.% concentration, and the emulsion was shaken manually for 2 min. Since only small changes were observed immediately after the destabilization procedure, stability of emulsions was monitored for up to 24 h. In all studies, the appearance of an aqueous bottom layer was used as a criterion for destabilization of the emulsion, calculated as an instability index (ratio of separated aqueous phase to total volume of solution).

Quantification of DEET release from O/W emulsions

The release of DEET from the emulsions was evaluated using a procedure reported previously, which consisted of immersing an Eppendorf vial containing 1 mL of emulsion, covered with a dialysis membrane (MWCO 12400 Da from Sigma-Aldrich, UK), into a 50-mL test tube, which contained 45 mL of 30:70 (v:v) methanol:water mixture.[7] The receiver medium was selected to generate a strong gradient of polarity for the diffusion of DEET from the donor (emulsion) to the receiver compartment, given the non-polar nature of the active compound.[6] The systems were placed in a Stuart Orbital Shaker SSL1 (Staffordshire, UK) at 90 rpm for up to 6 h. Aliquots were collected at 2, 4 and 6 h, and the quantification of released DEET was obtained by UV-Vis spectrophotometry (Cary 60 Agilent Technologies spectrometer, Santa Clara, USA) using UV-grade quartz cuvettes. A calibration curve at 204 nm (wavelength of the DEET absorption maximum) was prepared at a DEET concentration range of 0.264 to 8.423 mg L^{-1}, using the methanol:water mixture. The released amounts were quantified at different times by sampling 50 µL aliquots of the external release medium and diluting it 20 times with the methanol:water mixture. This procedure was also employed for the study of pH and salt effects on DEET release from the W/O emulsions.

Results and Discussion

Effect of pH on ζ-potential of aqueous dispersions of SiNP and CCNF

Both nanomaterials used in this work, namely, silica nanoparticles and cationic cellulose nanofibrils, present functional groups that determine their surface charge in aqueous solution, and therefore, the possible electrostatic interactions. Therefore, it is important to study the ζ-potential behavior at different pH values, to understand the extension and nature of interactions at the working pH, as well as possible behavior under acid/basic conditions in the formulations. The ζ-potential measurements of SiNP dispersions showed a predominance of negative surface over all the pH range studied, due to deprotonation of surface silanol (Si–OH) acid groups at pH values higher than the isoelectric point (IEP ca. 2,

Figure 1). An opposite behavior was observed for CCNF, since positive ζ-potentials were obtained, confirming the surface modification of cellulose with positively charged tetra-alkylammonium groups from grafting with GTMAC. It is interesting to note that, despite the fact that the charge on the quaternized amine group in CCNF is not pH-dependent, there is a decrease in ζ-potentials with increase of pH. This behavior can be attributed to the presence of some carboxylic groups (pK_a ca. 2.5) on the CCNF surface, originating from the residual hemicellulose in the pulp and some cellulose oxidation, which increase dissociation as the pH increases.[18] Therefore, the decrease observed in ζ-potential values would be an effect of the compensation of positive and negative charges on the surface of the CCNF.[19] In addition, the screening of charges due to the increasing ion concentration in solution, as more NaOH was added should also be considered.[20] The results obtained for the surface charge behavior of the nanomaterials in aqueous solutions as a function of pH were in agreement with the differences observed in hydrodynamic diameter (Figure S2, in SI section). In both cases, there was a tendency to increase the size of agglomerates in solution as the ζ-potential decreased, following the changes in pH, which also indicated that the DLS was measuring size of the agglomerates rather than individual particle size.

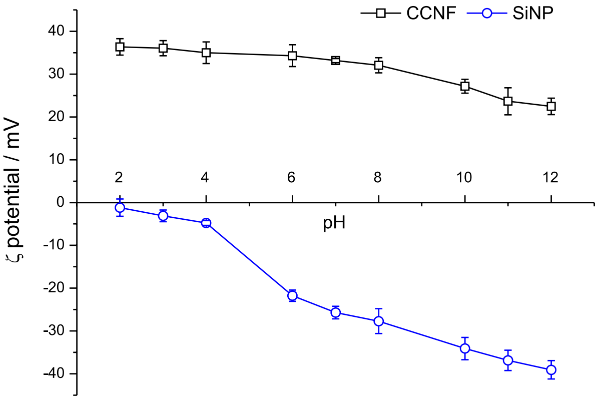


Figure 1. ζ-Potential of aqueous dispersions of cationic cellulose (CCNF) and bare silica nanoparticles (SiNP) at different pH values.

It should be noted that both nanomaterials showed relatively high ζ-potentials (> |20| mV) at pH 6, which is the typical pH of skin,[21] therefore, the ideal condition for topical repellent formulations. The O/W emulsions in this work were prepared at pH 6, which was the resultant pH after incorporation of all the components. The positive surface charge of CCNF, opposite to that of SiNP at the working pH, can promote the attractive interactions leading to electrostatic complexation and strengthening of the network formation in bulk, as well as to enhance interfacial adsorption. Since strong complexation was expected between the charged nanomaterials used as stabilizers at this condition, a mixture of nonionic surfactants was used to regulate the interactions and avoid electrostatic precipitation. Moreover, the high ζ-potential values obtained at the formulation pH ensure good dispersion of the nanomaterials in the aqueous external phase of the emulsion during the preparation of the formulations.

Optimization of CCNF concentration as rheological modifier

The viscosity of the external phase of the O/W emulsions plays an important role in decreasing the diffusion of DEET from the oil droplets, delaying its release from the formulation. A high viscosity of the aqueous phase increases the stability of the emulsion by reducing the drainage and coalescence of the droplets. Since CCNF aqueous dispersions can significantly affect the viscosity and the rheological properties of the aqueous phase, a study was carried out to optimize the CCNF concentration in the emulsion formulation. Aqueous dispersions containing different concentrations of CCNF were first prepared in surfactant solutions (0.5 wt.% Tween®80) to resemble the external phase of emulsions (Span®80 was not included due to its low solubility in water).[22] The results of viscosity at different shear rates showed that the presence of the CCNF increased the viscosity of the aqueous phase, and that at 0.3 wt.% the suspensions started showing a more pronounced shear thinning behavior, i.e., a decrease in viscosity with shear rate (Figure 2). At the highest CCNF concentration (1.0 wt.%), the viscosity was 10^4 times the water viscosity (0.9×10^{-3} Pa s) at low shear rates, due to the network formation of entangled fibrils.[23] In general, the viscosities measured in this work were similar than the typical values reported in the literature for CCNF dispersions.[18,24] Based on these results, a CCNF concentration of 0.3 wt.% was selected

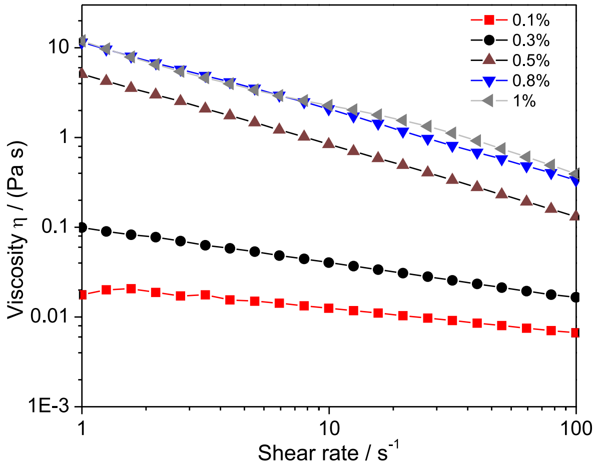


Figure 2. Flow curves for CCNF suspensions at different concentration (wt.%), suspended in a 0.5 wt.% Tween 80 aqueous solution.

for the preparation of the emulsion containing DEET, since higher concentrations might lead to gelation,[25] which can affect the emulsion preparation protocol and the release of the active compound (DEET).

Secondly, the possible effect of the silica nanoparticles on the viscosity of the aqueous phase at the chosen CCNF concentration was evaluated. It is hypothesized that SiNP can strengthen the network formation of CCNF in aqueous solutions due to the attractive electrostatic. A SiNP concentration of 0.01 wt.% was used to achieve a synergy in the emulsion stabilization, while avoiding extensive aggregation at the working pH (ca. 6), where CCNF and SiNP have opposite surface charges (as shown in ζ-potential experiments). As can be seen in Figure 3, the presence of SiNP, even at this low concentration, increased the viscosity of the aqueous solution containing 0.3 wt.% CCNF and Tween®80, maintaining the shear thinning behavior. The latter is a desired characteristic for topical formulations, since a decrease in viscosity can be obtained by skin rubbing, facilitating the spreading of the product and, thus, enhancing the delivery of the active compounds. Based on the results obtained in the rheological characterization, CCNF and SiNP concentrations of 0.3 and 0.01 wt.%, respectively, were chosen for the formulation of the emulsions containing DEET, aiming to obtain a synergy in the interactions in bulk and at the droplet interface towards emulsion stabilization, without compromising the intended release of DEET from the emulsion droplets.

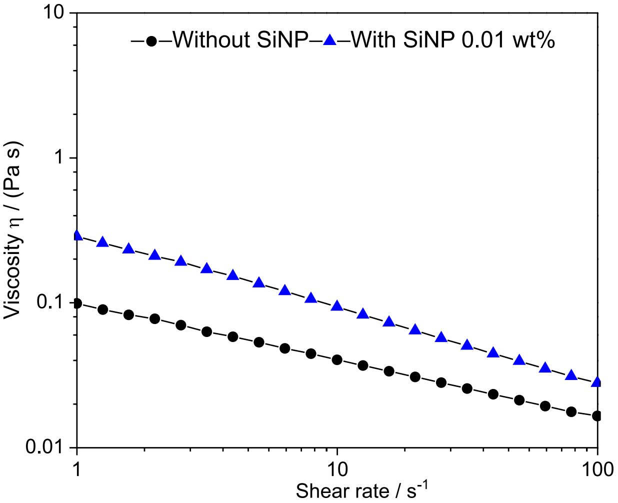


Figure 3. Effect of silica nanoparticles on rheological behavior of aqueous dispersions of 0.3 wt.% CCNF in 0.5 wt.% Tween®80 solutions.

Formulation and characterization of CCNF/SiNP stabilized O/W emulsions containing DEET

The first step in the preparation of the O/W emulsions was the solubilization of the active compound (DEET) in the mineral oil containing Span®80, followed by high-shear mixing into the aqueous phase containing Tween®80 and CCNF, SiNP, or both nanomaterials. The use of DEET in repellent formulations is recommended in concentrations from 7 to 10% for short repellent action (up to 2 h) and 20-30% for longer periods (up to 6 h).[26] In this work, a DEET concentration of 10 wt.% was used to facilitate the evaluation of the extended release action, and to highlight the potential of the formulations for more efficient use of the active compound. For comparison purposes, O/W emulsions were also prepared using only the mixture of surfactants (Tween80®/Span80®), SiNP or CCNF.

It was not possible to obtain an emulsified phase when using the nanomaterials (SiNP, CCNF or a combination) in the absence of surfactants, at the concentrations and conditions used in this work (Figure 4A). In the case of SiNP, the very low concentration used in the formulation was not enough to stabilize the oil-water mixture. A recent study reported by Silva *et al.*[23] reported the stabilization of O/W Pickering emulsions by cationic nanocelluloses at 0.5 and 1 wt.%. The stabilization mechanism was attributed to the electrostatic attraction between the positively charged trimethylammonium groups on the surface of the nanofibrils and the negatively charged deprotonated oleic acid groups of the almond oil used as oil phase. However, these interactions cannot be considered for the light mineral oil used in the present work (and in common cosmetic and pharmaceutical formulations), containing a mixture of saturated hydrocarbons, since the partial polarity (log K_{ow} = 2.02)[27] of DEET molecule tends to reduce its partitioning in the mineral oil phase of the emulsions.

The combination of nonionic surfactants allowed the formation of the O/W emulsion, but after 24 h a creaming phenomenon was observed, characterized by the concentration of large dispersed oil droplets in the upper phase, along with a separated aqueous phase at the bottom of the flask (Figure 4B). A similar result was obtained for the combination of surfactants and SiNP, exhibiting a phase separation after 24 h, although the micrographs of the creamed phase showed smaller droplet diameters and a less polydisperse distribution (Figures 4Cb and 4Cc and Figure S3 in SI section), compared to the surfactant-only stabilization (Figure 4Ca). Although the presence of surface silanol groups in the SiNP can interact with the ethoxylated chains of Tween®80, and at some extent with the Span®80 headgroups, being able to strengthen the interfacial film and decreasing the coalescence,[28] these interactions were not sufficient to maintain the emulsion stability over time.

The inclusion of CCNF in the formulations brought a significant improvement in the emulsion stability, both in the absence and in the presence of SiNP (Figure 4B). These

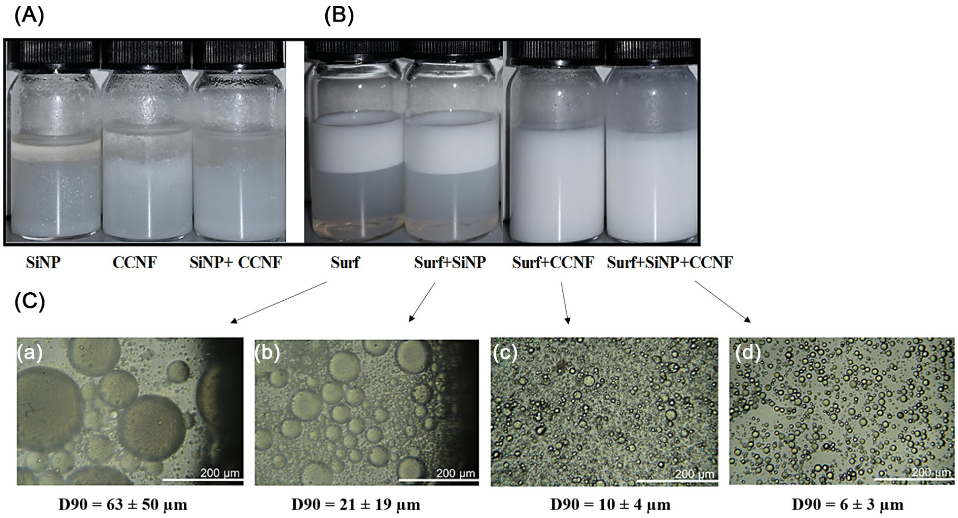


Figure 4. Photographs of O/W emulsions containing DEET and stabilized with (A) nanomaterials only and (B) mixtures of nonionic surfactants and nanomaterials (0.5 wt.% surfactants, 0.3 wt.% CCNF, 0.01 wt.% SiNP). (C) Images obtained by optical microscopy of formulations that showed creaming ((a) Surf, (b) Surf + SiNP) or stable emulsion formation ((c) Surf + CCNF, (d) Surf + SiNP + CCNF). Pictures and micrographs were taken 24 h after preparing the emulsions.

emulsions were stable for more than four months after being prepared in the laboratory, not showing any indication of creaming or phase separation. The emulsion stability initially assessed by visual observation was confirmed by the smaller droplet size and more homogeneous droplet distribution (Figures 4Cc and 4Cd and Figure S3 in SI section). These microscopic properties are characteristics of stable emulsions, decreasing the coarsening and coalescence rates. There was a drastic reduction in the diameter of the internal oil phase containing DEET compared to the results without CCNF, which supports the hypothesis of various mechanisms acting in synergy at both the interfacial film and in bulk between surfactants and CCNF, as well as the need for the multicomponent system for the emulsion stabilization. This is in agreement with results reported for Pickering emulsions with CCNF and coconut oil, where an increase in emulsion stability and a decrease in droplet size was observed after adding nonionic surfactant.[29] For the emulsion containing SiNP and CCNF, a very narrow droplet distribution was observed after 24 h of preparation (Figure S3d in SI section), which confirmed the synergy between the two nanomaterials in the stabilization of the oil phase containing DEET, required for delaying release of the active compound.

Effect of CCNF/SiNP interactions on rheological behavior of emulsions

The presence of SiNP, at the low concentration used in this work, did not significantly affect the macroscopic stability of the O/W emulsion, compared to the formulation containing only surfactants and CCNF. In this case, despite the electrostatic attractions occurring between the opposite surface charges at the working pH, the effect of the cationic nanocellulose governs the viscosity of the aqueous phase and, hence, the stability of the DEET emulsion.[10] To explore further this effect, rheological measurements were performed using the stable emulsions obtained with CCNF, in the absence and the presence of SiNP (Figure 5). Both emulsions presented a shear-thinning behavior, and in the presence of SiNP there was a further increase in viscosity (ca. 3 times), in agreement with the results obtained for the aqueous phases (Figure 3).

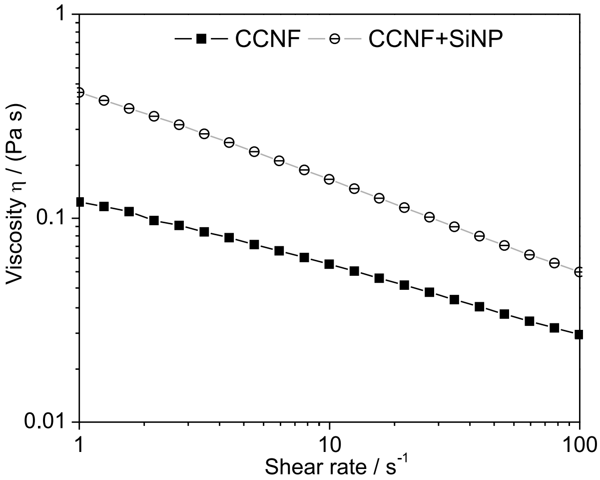


Figure 5. Effect of silica nanoparticles on rheological behavior of O/W emulsions containing DEET (10 wt.%) and stabilized by 0.3 wt.% cationic cellulose (CCNF) and nonionic surfactants.

The further increase in viscosity of emulsions observed in the presence of SiNP is an indication of the strong association by electrostatic interactions with CCNF in the aqueous phase, even at low concentrations of these nanoparticles. In a study by Gong *et al.*[25] it was found that the addition of colloidal SiNP enhanced the viscosity of dilute hydroxyethyl cellulose solution, leading to formation of gels by a mechanism of physical adsorption of nanoparticles on the polymer. Although to the best of our knowledge the combination of CCNF and unmodified SiNP used in this work has not been explored yet for stabilization of emulsions, there are reports on similar systems that support this hypothesis. Huan *et al.*[30] recently published a study using cellulose nanofibrils (CNF), which have high negative surface charge, and cationic nanochitin (NCh) to produce Pickering emulsions with sunflower oil. The authors found that the interfacial adsorption of CNF/NCh complexes formed by the oppositely charged species can be tuned using the mass/charge ratio of the CNF to NCh, going from a dominant dispersed system in the aqueous phase to a strongly associated system, both providing stable Pickering multiphase systems.

Effect of external factors on the stability of O/W emulsions

Physical destabilization by mechanical and thermal stress

The use of the nanomaterials with the nonionic surfactant mixture led to very stable oil-in-water emulsions, able to retain DEET in the internal phase. Therefore, to investigate the impact of combining SiNP with CCNF on the emulsion stability, tests of accelerated destabilization were needed. For that, the emulsions were submitted to centrifugation and heating, and the stability was assessed by the extent of the separated phase. All the emulsions showed a behavior characterized by the release of free water, but no oil after centrifugation and heating. This is in agreement with the results obtained by Aaen *et al.*,[31] who found that emulsions stabilized by cellulose nanofibrils formed a separated water phase after centrifugation due to the relatively stable network formed in the continuous water phase, entrapping the oil droplets as well as adsorbing at the O/W interface. The results from these accelerated destabilizations tests also revealed that the emulsion containing SiNP had greater stability than the emulsion with CCNF and surfactant mixture, since there was less phase separation after centrifugation and heating at 45 °C (Figure 6a). The difference in stability between the two systems was best observed upon centrifugation, since the emulsion containing SiNP had less separated water phase (by ca. 20%) than the emulsion containing only CCNF and surfactants. This behavior can be related to the stabilizing effect of the adsorbed electrostatic complexes formed between CCNF and SiNP at the oil-water interface, hindering droplet coalescence. Lu *et al.*[32] reported the stabilization of soybean O/W Pickering emulsions using an amphoteric lignin (modified with quaternary ammonium groups) and SiNP, which adsorbed synergistically at the oil-water interface via electrostatic interactions. The high stability of the emulsions was attributed to the coating of hydrophilic SiNP with the polymer, and the formation an interfacial gel-like structure with a certain rigidity, which prevented the coalescence of the droplets effectively.

The effect of increasing temperature on the emulsion stability was milder than that of centrifugation, without significant differences between the two types of emulsions. The increase in temperature increases the Brownian movement of the emulsion droplets, but the presence of CCNF in both systems provided a high viscosity of the aqueous phase (Figure 3), slowing down the migration speed and collisions of the droplets, which contributed to the stability of the emulsion.

Emulsion destabilization by pH and salt

In addition to the physical destabilization tests, the emulsions were submitted to changes in chemical conditions to assess the impact on the interactions between

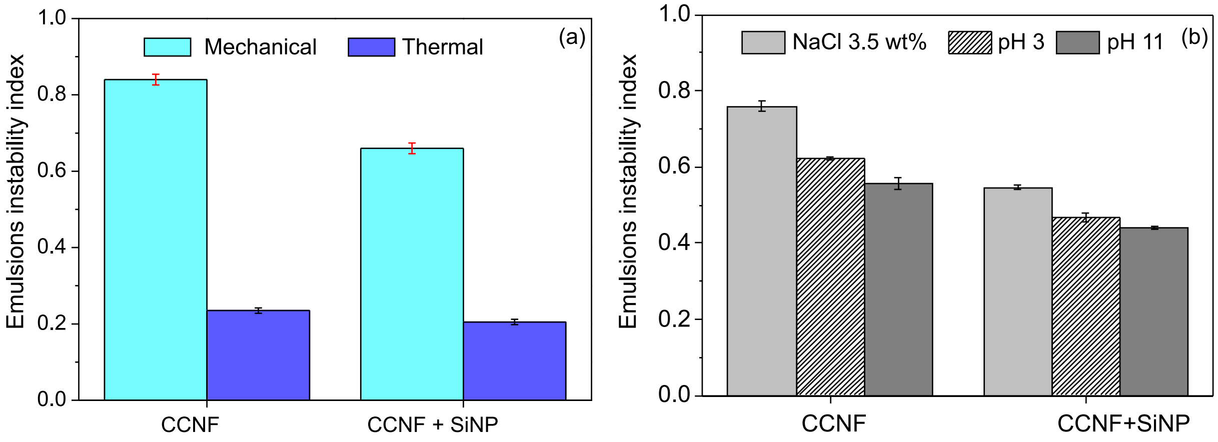


Figure 6. Destabilization of O/W emulsions containing DEET by (a) mechanical (centrifugation) or thermal (T = 45 °C) stress, and by (b) addition of salt (NaCl) or varying pH (stability was assessed after 24 h of preparing the emulsions). Emulsion instability index represents extent of aqueous phase separation.

the stabilizers, and thus on emulsion destabilization mechanisms. The stability of the emulsions after 24 h was evaluated in the presence of NaCl and extreme pH values (3 and 11), since these factors can greatly influence the extent of electrostatic interactions responsible for emulsion stabilization and DEET retention. The results obtained in the presence of NaCl were similar than those found upon centrifugation: the emulsion containing SiNP presented less separated aqueous layer in comparison with the emulsion stabilized only by CCNF (Figure 6b). The presence of salt screened the charge on the cationic cellulose adsorbed at the oil-water interface, reducing the electrostatic repulsion between droplets and promoting aggregation and coalescence (see ζ-potential values in SI section, Table S3). It has been reported that the CCNF layer around the oil droplets in O/W emulsions becomes thinner in the presence of NaCl, as observed from small angle neutron scattering (SANS) measurements.[19] This effect might have been counteracted, to some extent, by the presence of SiNP at the droplet interface (ca. 30% increase in emulsion stability).

In the case of the experiments at different pH, the emulsions also showed destabilization, although the effect was smaller than with NaCl. According to ζ-potential results, the CCNF has a high positive charge (> +30 mV) at pH 3, and slightly lower at pH 11 (Figure 1 and Table S3 in SI section). The results showed that the extent of aqueous phase separation was larger at pH 3, since there would be less adsorption at the oil-water interface, and stronger repulsion among the adsorbed particles, decreasing the stabilization of droplets. Besides the effect on interfacial adsorption, pH can also affect the strength of the entangled network in the aqueous phase, responsible for the increase in viscosity.[33] At higher pH, there is an increase in the likelihood of aggregation of the nanofibrils, causing a less structured water phase and less repulsion between oil droplets, which can both contribute to reduced emulsion stability.

In general, the emulsions containing SiNP presented smaller instability indexes at both pH values than those containing only CCNF and surfactants, which was explained by the compensating effect of the silica surface charge (opposite ζ-potential behavior with pH) with that of CCNF, buffering the interactions at the droplet interface.

Release of DEET from emulsion systems: effects of salt and pH

The release of DEET from the emulsions prepared using a combination of surfactants, CCNF and SiNP was evaluated and the results were compared with a release experiment using a non-emulsified sample of DEET. Quantification of DEET released was done by UV-Vis spectrophotometry, as described in the Experimental and Supplementary Information section (Figure S4 and Table S1). A period of 6 h was chosen for comparing the release profiles (results for longer time are shown in SI section, Table S2).

The delay in release of DEET from the emulsions is a key aspect for extended repellent effect, since high initial evaporation rates reduce the concentration of DEET available at longer times, which may be one of the reasons for the short efficacy times associated with this repellent in several formulations, particularly for low dose applications.[34] As can be seen in the results in Figure 7a, there was a drastic reduction in the initial release rate of DEET from the emulsions prepared in this work, in comparison to the non-emulsified sample. In the latter, there was a very fast release in the first 2 h, and after 6 h almost all the repellent has migrated into the recipient

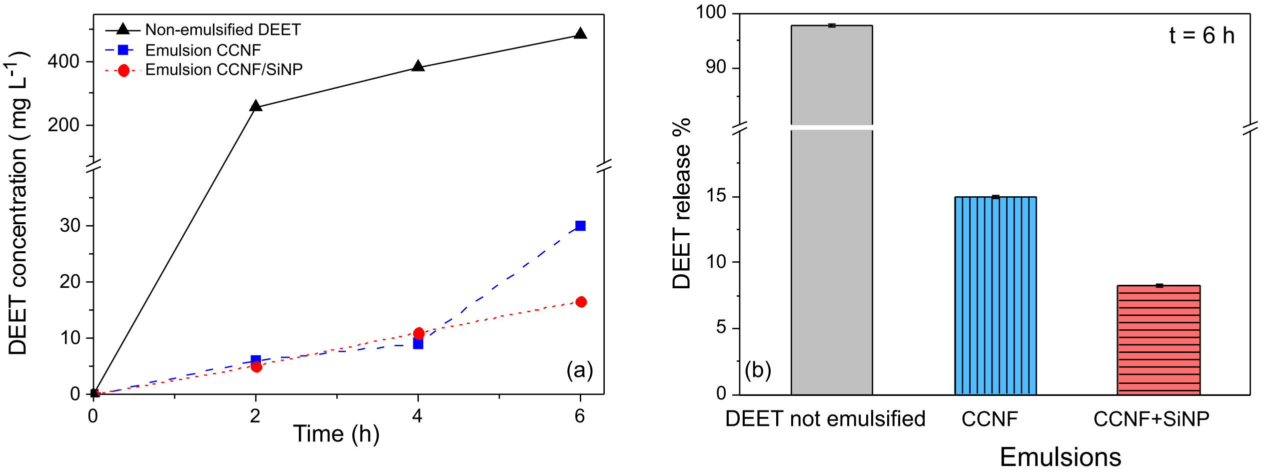


Figure 7. DEET release as a function of time from a non-emulsified sample (pure DEET) and from O/W emulsions stabilized by surfactants and CCNF (0.3 wt.%), in the absence and in the presence of SiNP (0.01 wt.%). (a) Release profiles. (b) Total amount of DEET released after 6 h (as percentage of initial amount). Standard deviation values < 6%, see values in SI section, Table S2.

phase (Figure 7b). In contrast, the two emulsions containing CCNF showed significantly slower release of DEET, exhibiting a sharp decrease in the burst release during the 6 h period (7-15% of cumulative release). In a similar study using polyurethane microcapsules (MIC) reinforced with modified cellulose nanofibers (mCNF) to encapsulate DEET, Kadam *et al.*[10] reported that the presence of mCNF led to a significant reduction in the release rate of DEET (13-15% in 6 h).

In the present work, the presence of SiNP acted as an additional component in synergy with the CCNF to obtain a linear release rate during the time of the experiment (Figure 8a). In the system containing only CCNF and surfactants, a slow release of DEET can be observed for up to 4 h, then it rapidly increased, while in the formulation containing the CCNF/SiNP combination there was a sustained release for the period of 6 h. In general, the sustained release of a microencapsulated species is controlled by its capability to permeate the capsule walls.[34] Therefore, we can assume that the increased stability of this emulsion due to complexation at the interface, as observed in the tests of accelerated destabilization, plays a key role in the retention of the active compound and extending its action. Noticeably, the differences observed in the release profiles were maintained when preparing the emulsions at pH 3 and 11, with an increase in DEET after 4 h in the absence of SiNP, and a more controlled, sustained release in the presence of the nanoparticles (Figures 8c and 8d). These results confirmed the role of electrostatic interactions between CCNF and SiNP on the stabilization of the emulsions containing DEET. A faster DEET release was obtained with salt (Figure 8b), in agreement with the higher extent of emulsion destabilization under these conditions, as discussed in the previous section. The presence of salt had a similar effect in both systems, since the principal effect is on the interfibrillar interactions related to CCNF in solution and at the interface.[19] In this case, the increase observed in DEET release with addition of NaCl can be used advantageously in formulations for controlled delivery

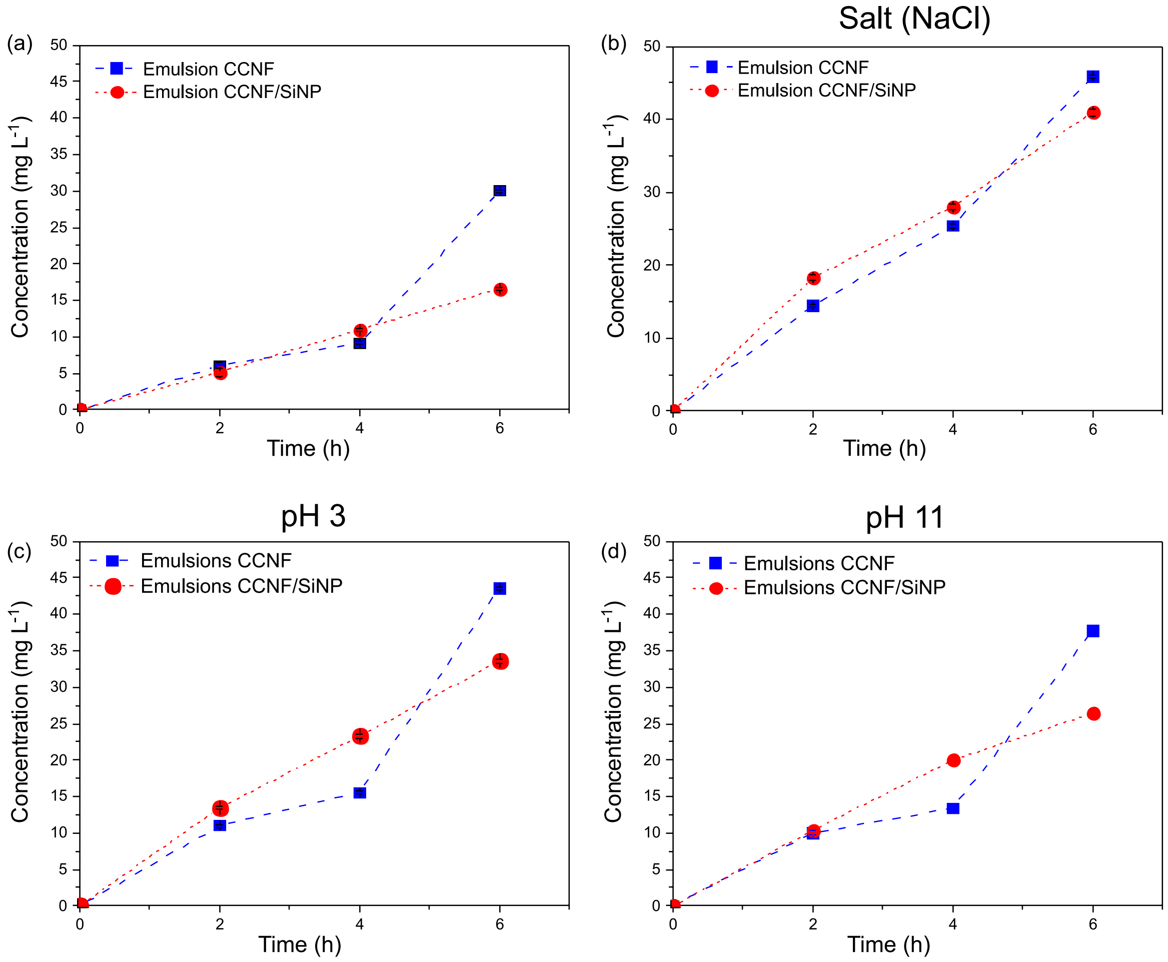


Figure 8. (a) Cumulative DEET release as a function of time from O/W emulsions stabilized by surfactants and CCNF (0.3 wt.%), in the absence and in the presence of SiNP (0.01 wt.%). (b-d) Effect of salt and pH on DEET release from emulsions. Emulsion pH was 6, unless otherwise stated. Standard deviation values < 6%, see values in SI section, Table S2.

of this active compound, with the objective of achieving extended repellent action for longer time. For example, similar levels of salt can be found in fluids commonly in contact with the skin (e.g., sweat and seawater), which will promote an additional DEET release from the emulsions prepared in this work with CCNF and SiNP.

Conclusions

Oil-in-water emulsions containing 10 wt.% DEET were obtained with long-term stability (up to 4 months from preparation, at room temperature) by combining stabilization mechanisms associated with the improvement in rheological properties of the emulsion by addition of CCNF, as well as the resistance to coalescence by enhancing interactions with SiNP in bulk and at the droplet interface. Both nanomaterials showed relatively high ζ-potentials at the working pH, which ensured good dispersion of the nanomaterials in the aqueous external phase of the emulsion during the preparation of the formulations. The positive surface charge of CCNF, opposite to that of SiNP, promoted attractive interactions that allowed strengthening of the network formation in bulk, as demonstrated by the further increase in viscosity observed even at low concentrations of these nanoparticles (0.01 wt.%).

Accelerated stability tests showed that the emulsions containing the CCNF/SiNP combination were more stable than in the absence of silica, even upon addition of salt and at large pH variations. The emulsion stability was confirmed by the smaller droplet size and a more homogeneous droplet distribution, which supported the hypothesis of various mechanisms acting in synergy between the two nanomaterials in the stabilization of the dispersed oil phase containing DEET, which was the requirement for delaying release of the active compound.

The capacity to retain DEET in the internal phase of the O/W emulsions was assessed by release studies into a model media, and the results showed a drastic reduction in the initial release rate, in comparison to the non-emulsified sample. While in the latter most of the active compound migrated into the recipient phase after 6 h, there was a significantly slower release of DEET from the two emulsions containing CCNF, exhibiting a sharp decrease in the burst release during the 6 h period (7-15% of cumulative release). In addition, the emulsions containing SiNP showed a more sustained release during the time of the experiment, which confirmed the role of electrostatic interactions between CCNF and SiNP on the stabilization of the emulsions containing DEET. This delay in the release from the emulsions is a key aspect for achieving extended repellency, increasing the concentration of DEET available at longer times, which can be used advantageously for the development of formulations with long-lasting efficacy even at low dose applications.

Supplementary Information

Supplementary information (calculation of substitution degree, Figures S1-S4 and Tables S1-S3) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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