PONTIFÍCIA UNIVERSIDADE CATÓLICA
DO RIO DE JANEIRO

Luis Miguel Gutiérrez Beleño

Development of oil-in-water emulsions stabilized by sustainable materials for controlled release of N, N'-Diethyl-3-methylbenzamide (DEET)

Doctoral Thesis

Thesis presented to the Graduate Program in Chemistry at PUC-Rio in partial fulfillment of the requirements for the degree of PhD in Chemistry.

Advisor: Prof. Aurora Pérez Gramatges

Co-advisor: Prof. Karen J. Edler

Rio de Janeiro, August 2022

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Abstract

Gutiérrez Beleño, Luis Miguel; Pérez Gramatges, Aurora (advisor:); Edler J, Karen (co-advisor). **Development of oil-in-water emulsions stabilized by sustainable materials for controlled release of N, N'-Diethyl-3-methylbenzamide (DEET)**. Rio de Janeiro, 2022. 154p. Thesis of PhD - Department of of Chemistry Pontifícia Universidade Católica do Rio de Janeiro.

Mosquitoes are the main vectors of transmission of diseases such as zika, dengue and chikungunya, causing more than 700,000 cases per year. The use of repellents, mainly topical formulations, is one of the best protective measures to reduce and/or prevent the transmission of many insect-borne diseases. In this work, we formulated DEET/oil-in-water emulsions for the controlled release of repellents as a strategy to develop repellent formulations with a protection time greater than 6 h. We investigated the effects of the HLB value on the stability and viscosity of the emulsions, using a blend of surfactants (Span 80 and Tween 80) to formulate emulsions in an HLB range between 4-14. We also evaluated droplet size, rheological behavior, mixing method and emulsion stability index. The developed DEET-oil-in-water emulsions containing N, N-diethyl-3-methylbenzamide (DEET) were stabilized using two positively and negative charged nanomaterials, silica nanoparticles (SiNP) and cationic cellulose nanofibrils (CCNF), and a blend of food-grade nonionic surfactants to prevent precipitation by electrostatic aggregation. The results indicate that at HLB 10, this mixture can stabilize the emulsions regardless of the type of oil (mineral or vegetable), and the emulsions present Newtonian behavior regardless of the mixing method. The formulations were stable for more than four months at room temperature, showing to be strongly resistant to destabilization by centrifugal and thermal stress when using nanomaterials in combination with surfactants. The CCNF and SiNP kept the droplet size distribution stable due to the increase in the viscosity of the continuous phase. The combined action of these materials in stabilizing the DEET-containing oil phase significantly decreased the rate of active compound release compared to non-emulsified DEET, producing a sustained release of DEET within the first 6 h.

Keywords

Slow release. DEET. Cationic cellulose. Emulsions. Silica nanoparticles

Resumo

Gutiérrez Beleño, Luis Miguel; Pérez Gramatges, Aurora (advisor:); Edler J, Karen (co-advisor). **Desenvolvimento de emulsões óleo em água estabilizadas por materiais sustentáveis para liberação controlada de N, N'-Dietil-3-metilbenzamida (DEET))**. Rio de Janeiro, 2022. 154 p. Teses de doutorado - Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro.

Os mosquitos são os principais vetores de transmissão de doenças como zika, dengue e chikungunya, causando mais de 700 mil casos por ano. O uso de repelentes, principalmente formulações tópicas, é uma das melhores medidas de proteção para reduzir e/ou prevenir a transmissão de muitas doenças transmitidas por insetos. Neste trabalho, formulamos emulsões de DEET/óleo em água para liberação controlada de repelentes como estratégia para desenvolver formulações repelentes com tempo de proteção superior a 6 h. Investigamos os efeitos do valor de HLB na estabilidade e viscosidade das emulsões, usando uma mistura de surfactantes (Span 80 e Tween 80) para formular emulsões em uma faixa de HLB entre 4-14. Também avaliamos o tamanho das gotas, comportamento reológico, método de mistura e índice de estabilidade da emulsão. As emulsões de DEET-óleo-em-água desenvolvidas contendo N, N-dietil-3-metilbenzamida (DEET) foram estabilizadas usando dois nanomateriais carregados negative e positivamente, nanopartículas de sílica (SiNP) e nanofibrilas de celulose catiônica (CCNF), e uma mistura de surfactantes não iônicos de grau para evitar a precipitação por agregação eletrostática. Os resultados indicam que no HLB 10, esta mistura pode estabilizar as emulsões independentemente do tipo de óleo (mineral ou vegetal), e as emulsões apresentam comportamento newtoniano independentemente do método de mistura. As formulações foram estáveis por mais de quatro meses à temperatura ambiente, mostrando-se fortemente resistentes à desestabilização por estresse centrífugo e térmico quando se utiliza nanomateriais em combinação com surfactantes. O CCNF e o SiNP mantiveram a distribuição granulométrica estável devido ao aumento da viscosidade da fase contínua. A ação combinada desses materiais na estabilização da fase oleosa contendo DEET diminuiu significativamente a taxa de liberação do composto ativo em comparação com o DEET não emulsificado, produzindo uma liberação sustentada de DEET nas primeiras 6 h.

Palavras-chave

Liberação lenta. DEET. Celulose catiônica. Emulsões. Nanopartículas de sílica

Summary

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Glossary of terms

CCNF -	Cationic cellulose nanofiber
DEET -	N,N'-Diethyl-3-methylbenzamide
DLS-	Dynamic light scattering
HLB-	Hydrophobic lyophilic balance
LVE	Legion viscoeslatic
SiNP -	Silica nanoparticle
SiNPNH2 -	Silica nanoparticle modified with 3-aminopropyl trimethoxy silane
Span 80-	Sorbitan monooleate, sorbitan oleate
Tween 80 -	sorbitan monooleate polyethoxylated

1. Introduction

Emulsions are highly important owing to their widespread applications in industries such as pharmaceutical, agricultural, cosmetic, food, oil, and gas. Emulsions are mixtures of two immiscible liquids; therefore, they are thermodynamically unstable, and consequently, can undergo phase separation by different physical–chemical processes, such as gravitational separation, flocculation, coalescence, and Ostwald ripening. However, emulsions can be stabilized by surfactants, biopolymers solid particles, and their combinations [1].

Emulsifiers improve the stability of emulsions by adsorbing at the interface, thereby facilitating the formation of drops with smaller sizes, generating repulsive forces between drops, and modifying the viscosity of the continuous phase. In contrast, solid particles stabilize emulsions mainly by two mechanisms (i) adsorption at the liquid–liquid interface, forming a solid layer that prevents the coalescence of nearby drops and (ii) increasing the viscosity. All above methods generate emulsions with better performance for use in different applications, particularly in the development of cosmetics [2].

Mosquitoes are vectors of many diseases such as malaria, chikungunya, the Zika virus, and yellow fever. One method to avoid mosquito bites is the application of repellents, which has become a useful practice that can reduce and prevent the transmission of many diseases transmitted by insects. Mosquito repellents are volatile chemical compounds that, when applied to human skin, repel the insects, avoiding contact and biting.

The currently available repellents in the market have a durability of up to 2 h. Moreover, although studies on sodium chloride (NaCl)/pH-responsive emulsions have been conducted, to our best knowledge, N, N'-Diethyl-3-methylbenzamide (DEET) release by stimulus such as pH and NaCl is not reported in the literature. In this study, we developed formulations of mosquito repellents by encapsulating the active compound in highly stable emulsions, using non-toxic sustainable materials in combination with biodegradable surfactants as stabilizers, being the combination also non-irritating to the skin. These emulsions released the DEET in a controlled manner, under the action of stimuli such as pH and NaCl.

1.1. Emulsions

Emulsions are mixtures of two immiscible liquids, in which one liquid is dispersed in the form of drops (called the dispersed structure) in the other liquid called the continuous phase. They are thermodynamically unstable and tend to separate phase under the effects of the differences in their densities, gravity, and intermolecular repulsions, which can be kinetically controlled by using stabilizers such as surfactants, particles and polymers [3,4].

Emulsions are formed when oil and water are mixed in certain quantities. The emulsification process involves the dispersion of one of the liquids within the other using mechanical equipment such as Ultra-Turrax homogenizer, ultrasound sonicator, membranes, and microfluidizer devices. The chosen method depends on the desired droplet size. Homogenization methods can be of low, medium, and high energy, depending on the final application of the emulsion. Generally, smaller droplet sizes are achieved by high-energy application, whereas larger droplet sizes are obtained using low-energy methods [5].

Another important component in the formation of emulsions is the emulsifier, which are usually amphiphilic molecules that adsorb at the liquid–liquid interface. The adsorption of emulsifiers plays an important role in that it decreases the interfacial tension, because the stabilization of the newly formed drops avoids spontaneous coalescence. Typical emulsifiers are surfactants, which can be classified as ionic, non-ionic, and zwitterionic, [6], as shown in Figure 1.

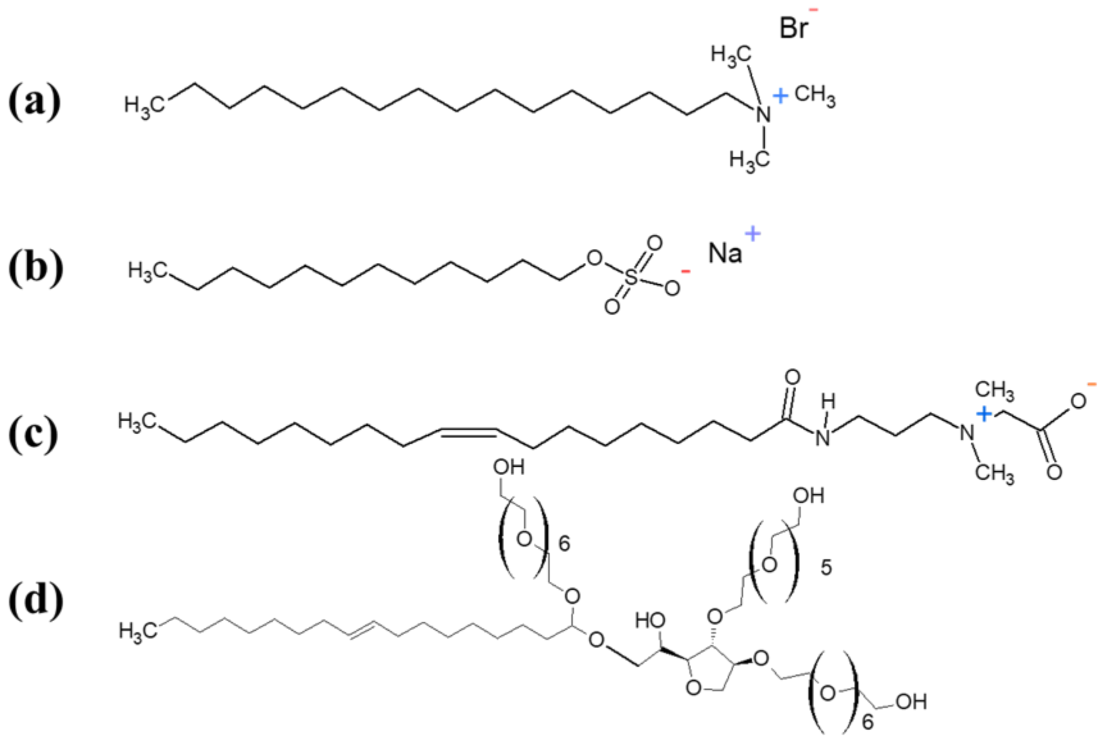


Figure 1. Chemical structure of different types of surfactants: (a) cationic (Hexadecyltrimethylammonium bromide), (b) anionic (Sodium dodecyl sulfate (SDS)), (c) zwitterionic (cocamidopropyl betaine) and (d) non-ionic (polyethoxylated sorbitan, Span 80).

Emulsions are classified according to the type of the continuous phase. Specifically, an emulsion in which water is the continuous phase is an oil-in-water (o/w) emulsion, and that in which oil is the continuous phase is a water-in-oil (w/o) emulsion. These types of emulsions are known as simple emulsions, as shown in Figure 2a and 2b.

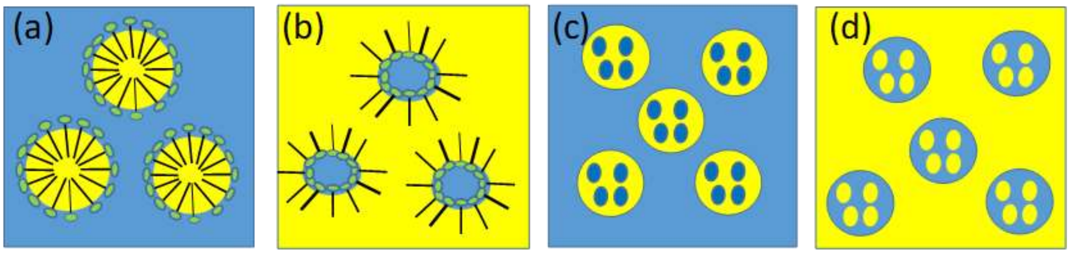


Figure 2. Types of emulsions: a) oil-in-water (w/o), b) water-in-oil (o/w), c) water-in-oil-in-water (w/o/w), d) oil-in-water-in-oil (o/w/o).

There also exist multiple emulsions, in which an emulsion is dispersed in other emulsions, and they can be water-in-oil-in-water and oil-in-water-in-oil emulsions as shown in Fig. 2c and 2d. Multiple emulsions are used strategically to disperse liquids of different polarities and have wide-ranging applications in the pharmaceutical and cosmetic industries [7] [8] .

1.1.1. Emulsion stabilization mechanisms

Emulsions are thermodynamically unstable systems owing to the difference between the interfacial tension of the two immiscible phases, as a consequence of which the free energy of the system is not minimized. An emulsion is kinetically stabilized by adjusting certain conditions, such as the concentration of the surfactant and the necessary energy applied to adsorb emulsifiers at the liquid–liquid interface. The most basic method to stabilize emulsions is using surfactants, which stabilize both w/o and o/w emulsions.

The main objective the use of a surfactant is to reduce the interfacial tension between the immiscible phases, thus minimizing the free energy of the system and subsequently stabilizing the oil–water interface to resist coalescence. Surfactants can stabilize emulsions by two mechanisms [9]. One mechanism is electrostatic stabilization, which occurs when an ionic emulsifier is used, generating an electrostatic repulsive force between the electrical layers on the surfaces of o/w emulsion droplets. The second is steric hindrance, in which surfactants through their carbon chains prevent two drops from combining, resulting in the coalescence of the emulsion. In this mechanism, mainly non-ionic and polymeric surfactants are used, owing to their large molecular sizes [10]. The main emulsion stabilization mechanisms are shown in Figure 3a and 3b.

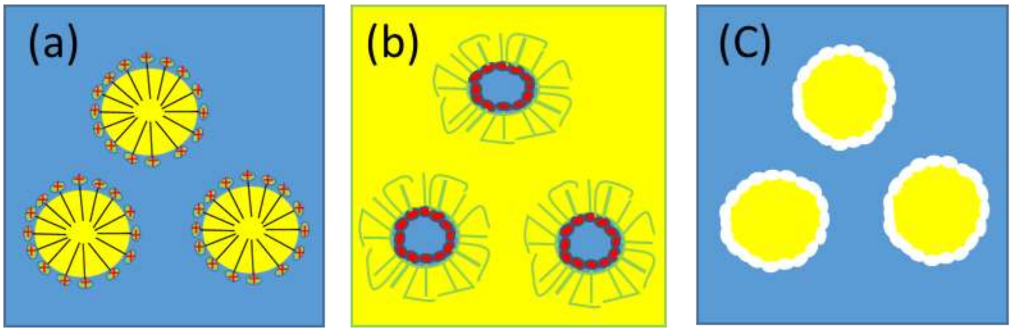


Figure 3. Stabilization mechanisms of emulsions: (a) electrostatic, (b) steric and (c) solid particles.

Emulsions can also be stabilized using nanoparticles (NPs) (known as stabilization by solid particles), and such emulsions are called ***Pickering*** emulsion as shown in Fig 3c. This type of emulsion has gained prominence in the recent decades owing to their versatility for different industrial applications such as energy, cosmetics, and food. Use of NPs improves the stability of emulsions owing to their high adsorption energy at the liquid–liquid interface, as expressed in equation 1 [8], where *E* is the energy of adsorption at the oil-water interface, $\gamma_{o/w}$ represents the surface tension at the o/w interface, *r* represents the radius of a single spherical particle, and $\theta_{o/w}$ is the contact angle of the liquid on the nanoparticle surface.

$$\Delta E = \pi \times r^2 \times \gamma_{o/w} (1 \pm \cos \theta_{o/w})^2 \qquad \textbf{Eq .1}$$

An important parameter when using NPs is the contact angle (θ) because it determines the type of emulsion formed. Solid particles that are preferentially wetted by the oil phase with a contact angle > 90° act as emulsifying agents for w/o emulsions. In contrast, particles preferably wetted by the water phase with a contact angle < 90° produce o/w emulsions [11]. Another important mechanism to stabilize emulsions is the Gibbs–Marangoni effect, which occurs when a liquid–liquid interface is stretched, thereby producing differences in the emulsifier concentration in different areas of the droplet surfaces. This creates a flow of the surfactant towards the region of lower concentration, avoiding the weakening of the interfacial film and stabilizing the interface [12].

1.1.2. Mechanism of destabilization of emulsions

The destabilization of emulsions is conditioned by their applications deliver the active principle at the right time. The destabilization of emulsions is a process that is occasionally undesirable. It depends on the objective of the application, and therefore, determining the mechanisms that govern destabilization is important to achieve the best performance. The main mechanisms for breaking emulsions are drainage and gravity-driven separations, such as creaming and sedimentation, flocculation, coalescence, and Ostwald ripening and coarsening [13].

The formation of creams and sedimentation are essentially governed by the difference in the densities of the two liquid phases. These processes are caused by the agglomeration of the drops in emulsions and the subsequent formation of larger aggregates. These events precede coalescence and Ostwald ripening. Main mechanisms that can lead to emulsion destabilization are shown in Figure 4.

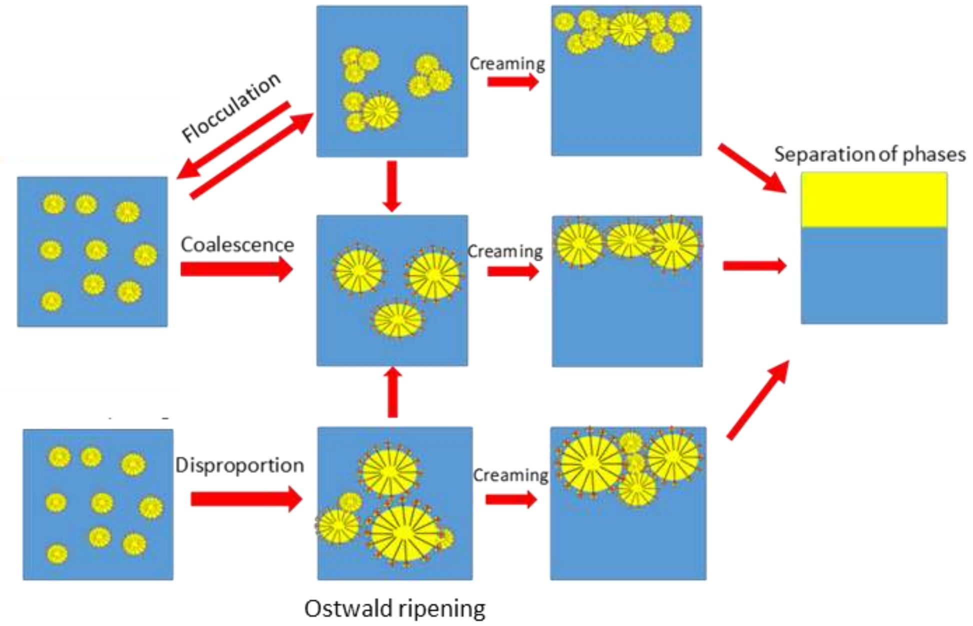


Figure 4. Diagram of the main destabilization mechanisms of emulsions. (Adapted from F. Ravera et al., 2021 [1]).

Coalescence is the fusion of two droplets into one, which is related to the thinning and rupture of the liquid emulsifier film between the drops. Therefore, it is strongly influenced by the interfacial properties of the adsorbed layers. Ostwald ripening consists of partial dissolution of the dispersed liquid phase favoured by the capillary pressure, which causes mass transfer of small to large droplets, narrowing the droplet size distribution but increasing the droplet size, which leads to destabilization of the emulsion. This process is mainly driven when the dispersed liquid has sufficient freedom of movement owing to the presence of a low-viscosity continuous phase.

The above destabilization processes are interconnected and can influence each other during aging of emulsions. They can be accelerated by the application of external triggers, such as changes in the pH [14], addition of salts and demulsifying agents, and environmental factors (e.g., changes in the temperature).

These destabilization strategies using triggers are highly interesting because they allow enhancing the use of the active principle, keeping it trapped when it is not needed and releasing it in a controlled manner during stimulation. This improves the efficiency of the corresponding emulsion [15].

1.1.3. Triggers

Sensitive or smart emulsions have gained research interest owing to their advantages as agents for sustained and controlled release. For efficient development of responsive emulsions, it is necessary to know how each trigger works and where each one will act. This is because the application of a trigger depends on the materials that act as stabilizers and co-stabilizers.

pH-responsive emulsions are generally developed with surfactants or materials that possess proton donor or acceptor groups in their structures, such as amino (RNH_2) and acid (RCOOH) groups. The mode of action of a trigger for such emulsions is essentially through binding to the above-mentioned active sites, causing desorption of the stabilizers (surfactants or particles) from the liquid–liquid interface [16], as shown in Figure 5. Changes in the pH cause neutralization of the charge of the stabilizers, affecting the electrical double layer, thereby favouring the closeness of drops, subsequently their flocculation and coalescence, and finally, the separation of the phases of the emulsion [17]. Changes in pH (acid or basic) can also be used as triggers, mainly acting by increasing the ionic strength of the aqueous phase, which causes the encapsulated active principle to undergo protonation or deprotonation (depending on their chemical nature), and then to migrate via diffusion from the dispersed droplets. In addition, inorganic salts can be used as triggers to alter the double electrical layer of the stabilizers, shielding the surface charge, causing flocculation of drops, and subsequently breaking the emulsions [18]. These stimuli are used topromote the release of emulsified active principles in a controlled and sustained manner, increasing the efficiency of the formulated products.

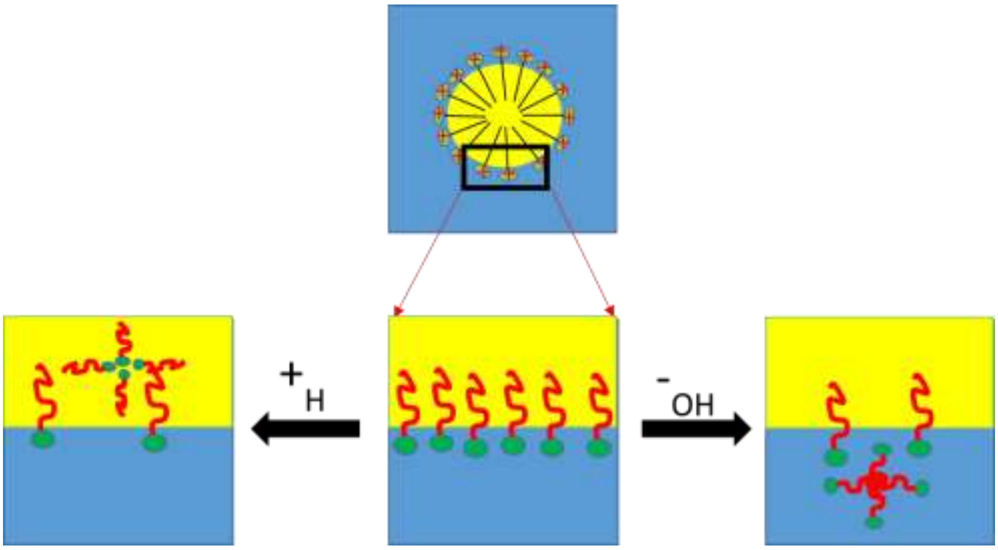


Figure 5. Schematic representation of the location of an acid-based surfactant at the oil/water interface, upon pH changes.

1.1.4. Cosmetic emulsion formulations

Cosmetic emulsions are formulated differently from those found in other industries, because of the necessity of pleasant sensory sensations, preservation of physicochemical characteristics, and fulfilling the functions for which they are developed. Examples of these functions are cleaning, moisturizing, hydrating, and nourishing at the skin level. Parameter conservation in an emulsion for use in cosmetics is aimed at reducing permeability in the skin, increasing the sensory effects, controlling and gradually releasing the active principle, improving the rheological profile, and finally, harmlessness to the skin [19].

Emulsified repellents, when applied to the skin, should have a shear thinning behaviour (that is to say that its viscosity decreases as it is spreaded), leaving a smooth sensory feel. Viscosity of a fluid is a measure of its resistance to deformation at a given rate. Therefore, the viscosity of these emulsions should be adjusted by the addition of colloidal materials, such as cellulose and silica (SiO_2) NPs, mediated using surfactants. When using colloidal materials for the co-stabilization of emulsions, their contact angle must be considered, because high contact angles (very hydrophobic) can accelerate the coalescence of the emulsions [20].

Different studies have shown that the addition of colloidal particles improves the sensory properties of cosmetic emulsions, and the success or failure of a cosmetic is determined by how it is perceived by the senses of the customers [21].

The use of colloidal particles considerably improves the performance of cosmetic emulsions. In addition, the conditions of such formulations can be adjusted according to the use for which they are developed, i.e., they are easily adaptable to increase or decrease the skin permeability, while maintaining fixed rates of release.

In these emulsions, a mosquito repellent can be incorporated which can be released in a controlled manner by different release mechanisms.

1.2. Controlled release

In the development of emulsions loaded with active ingredients, knowing the different mechanisms involved in the delivery of the drugs is highly important [22]. The delivery mechanism is dependent on the external stimuli and the stabilizing materials of the dispersion. Figure 6 shows some controlled delivery mechanisms [2].

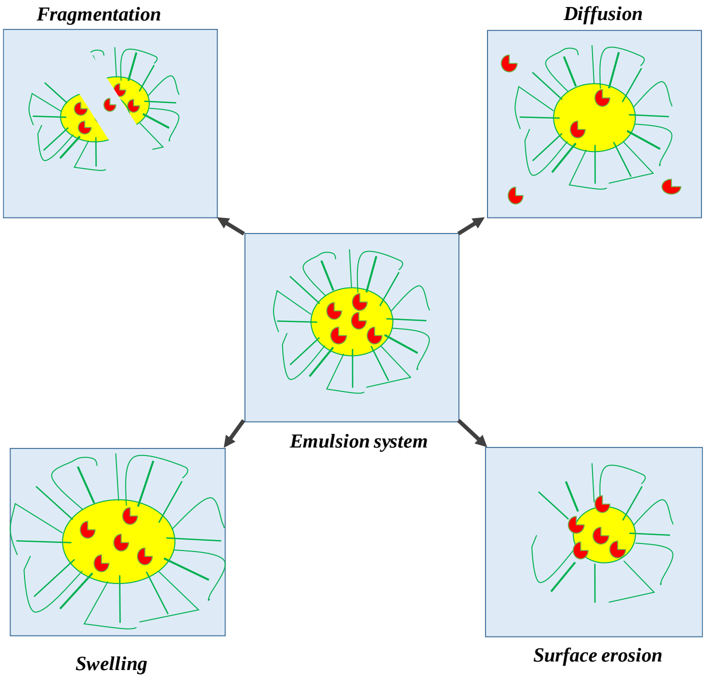


Figure 6. Controlled release mechanisms of active compounds in emulsions. (Adapted from S. Boostani, S. M. Jafari, 2021 [2]).

1.2.1. Diffusion

Diffusion is one of the dominant mechanisms in emulsions for the delivery of active principles. It is caused by the random movement of the bioactive molecules in emulsions, which is guided by a concentration gradient, under which molecules move from the inside of the drops (higher concentration) to their outside (lower concentration). Diffusion is dependent on the permeability of the active principle in an emulsion as well as on the ability of the outer phase to solvate this compound to improve the solubility and make more drug accessible. Notably, in the diffusion process, emulsion drops undergo little deformation.

1.2.2. Fragmentation

Fragmentation occurs when a drop is cracked or broken by external conditions such as pH, ionic strength, environmental factors such as temperature, shear, and biological materials. These triggers react with the stabilizers of emulsions, thereby changing their intermolecular properties, fracturing drops, and releasing the active principle. The speed of release is associated with the size of the fracture, which, in turn, depende on the intensity of the trigger. The fragmentation mechanism is associated with rapid and accelerated release, because in addition to the release of the load, it destroys the drops of the emulsions, breaking the emulsions.

1.2.3. Swelling

Swelling is caused by the penetration of the continuous phase towards the dispersed phase, producing a swelling of the drops, followed by diffusion of the drug towards the external phase. This release depends on the speed at which the incipient stabilizers control the drops swell, and this mechanism or the polymeric matrix used in the formulations of the emulsions.

1.2.4. Surface Erosion

The erosion mechanism occurs when stabilizers such as surfactants, polymers, and particles are desorbed from the liquid–liquid interface, causing the drug to exit from the drops. This desorption is caused by a disturbance in the equilibrium of the stabilizers, which generally shifts towards the continuous phase. Concurrently, the release rate depends on the amount of eroded film. This mechanism also facilitates the diffusion of drugs due to the weakening of the protective layer of the drops.

1.1. Techniques

1.1.1. Rheology

Rheology is the study of the deformation behaviour and flow of a matter subjected to stresses under certain thermodynamic conditions over time. These measurements can be made in liquid and solid states. The studied properties include elasticity, viscosity, and plasticity.

Rheological characterization is important because it provides information on the performance of domestic and personal products, e.g., silkiness and general appearance. Another important parameter obtained by rheological characterization is the ease of manufacture (mixing) and application. It also offers information on the destabilization processes that manufactured products can undergo. Among the different aspects of rheology, viscosity and viscoelasticity are the primary parameters [23].

To study the rheological behavior, a fluid was placed between two parallel flat plates of area *A* and separated by a distance *h*. A force *F* was applied at the top, moving the top plate at a constant velocity V_x with respect to the bottom plate, which is kept fixed, as shown in Figure 7. This force generates a force of a similar intensity in the fluid but in the opposite direction, called the shear force. It is only produced by the cohesion forces of the fluid with the walls of the plates and between the layers of the fluid, in the case of a laminar regime. The shear force generates a velocity gradient between the plates. If there is no fluid sliding through the walls of the plates, the fluid velocity is zero on the lower plate *V* on the upper plate [24].

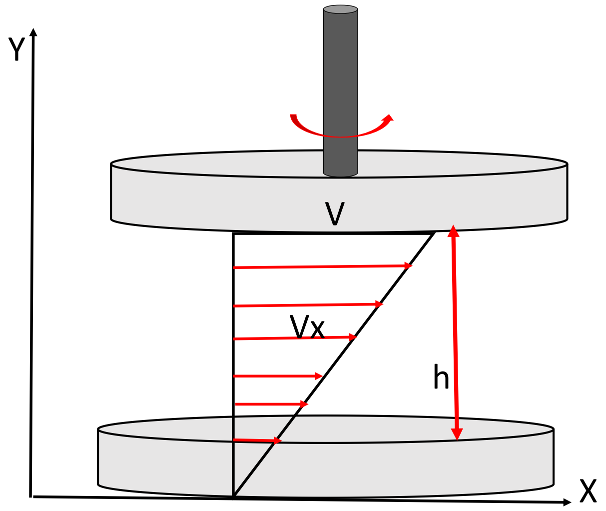


Figure 7. Schematics of shear rate and shear stress experiment for layers of fluid sliding over one another. (*h*: distance between the plates, *V*: velocity of the moving plate, V_x: velocity of the fluid).

1.1.2. Definitions

Shear rate: It is the velocity gradient measured across the diameter of a fluid-flow channel, which can be a pipe, an annulus, or another shape. It is the rate of change in the velocity at which one layer of the fluid passes over an adjacent layer [25] (Equation 3).

$$\textbf{Shear rate } (\dot{\boldsymbol{\gamma}}) = \frac{\textbf{dv}}{\textbf{dh}}\ (s^{-1}) \qquad \textbf{Eq. 3}$$

Shear stress: It is the force per unit area required to sustain a constant rate of fluid movement (Equation 4). Consider a fluid is placed between two parallel plates spaced 0.5 cm apart and a force of 3.0 dyne is applied to each centimetre square of the surface of the upper plate to keep it in motion. Thus, the shear stress in the fluid would be 3 N/m^2 at any point between the two plates.

$$\textbf{Shear stress } (\boldsymbol{\tau}) = \frac{\textbf{Force(N)}}{\textbf{Area } (m^2)} (\boldsymbol{Pa}) \qquad \textbf{Eq. 4}$$

Viscosity: It is a property of fluids and slurries reflecting their resistance to flow, and defined as the ratio of the shear stress to the shear rate. Newton's law of viscosity states that the relationship between the force of shear (shear force × area) and the local gradient of velocity is linear, where the proportionality constant is the fluid viscosity. Thus, all fluids that follow this behaviour are called Newtonian fluids [23]. It can be defined by Equation 5, where τ_X is the shear stress in the x direction (in N/m^2), and $\dot{\boldsymbol{\gamma}}$ is the velocity gradient or the shear rate.

$$\textbf{Viscosity } (\boldsymbol{\eta}) = \frac{\tau_X}{\dot{\gamma}} (\boldsymbol{Pa\, s^{-1}}) \qquad \textbf{Eq. 5}$$

Applying a shear stress to a fluid involves momentum transfer, with the shear stress being equal to the moment transfer rate (momentum flow) to the upper fluid layer. This momentum is transferred downward through the fluid layers and accompanied with a reduction in the kinetic energy and velocity, which is due to the energy loss caused by friction. The coefficient of proportionality between shear velocity and shear stress is viscosity, also known as dynamic viscosity (η). Shear viscosity describes the internal friction between the layers of a fluid, and a higher viscosity implies a slower system movement due to energy loss. Equation 6 expresses the dynamic viscosity. In Newtonian fluids, the viscosity varies with the temperature or pressure of the flow, whereas it is independent of the rate of deformation. Specifically, the viscosity tends to increase as the pressure increases and the temperature decreases [26].

$$\boldsymbol{\sigma} = \boldsymbol{\eta} \times \boldsymbol{\gamma} \qquad \textbf{Eq. 6}$$

Fluids can be classified according to the relationship between their strain rate and shear stress. This class covers all non-polymeric and homogeneous gases and liquids, e.g., water, milk at physiology conditions, sucrose solutions, and vegetable oils. Newtonian fluids are fluids that show a linear relationship between the shear rate and the shear stress, i.e., their viscosity is invariant. Non-Newtonian fluids are fluids that present a non-linear relationship between the shear rate and the shear stress, i.e., the viscosity varies as function of the applied shear stress or shear rate. Figure 8 shows the change in viscosity with shear rate.

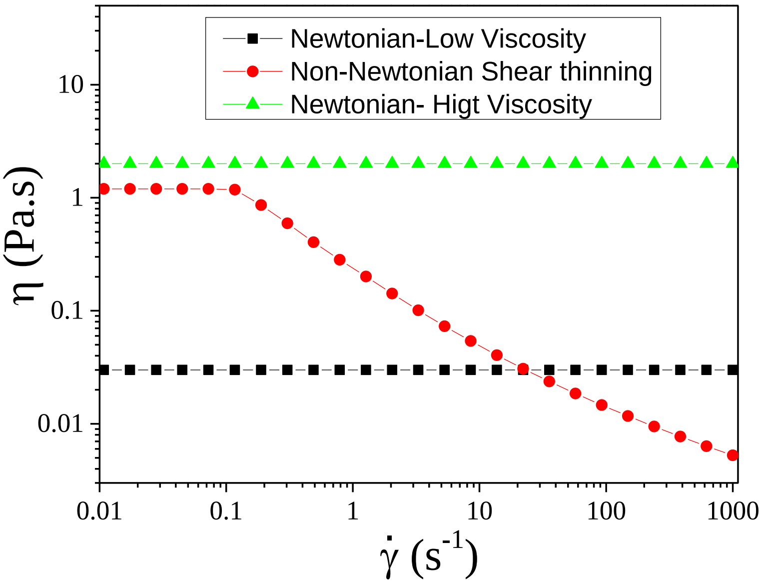


Figure 8. Flow curves of shear stress and viscosity across a range of shear rates. (Adapted from https://fluidan.com/rheology-basics/).

Non-Newtonian fluids can be further classified into viscoelastic, time-dependent, and time-independent ones [27]. The slope in non-Newtonian liquids is greater than in Newtonian liquids. Figure 9 shows the change in shear stress with shear rate.

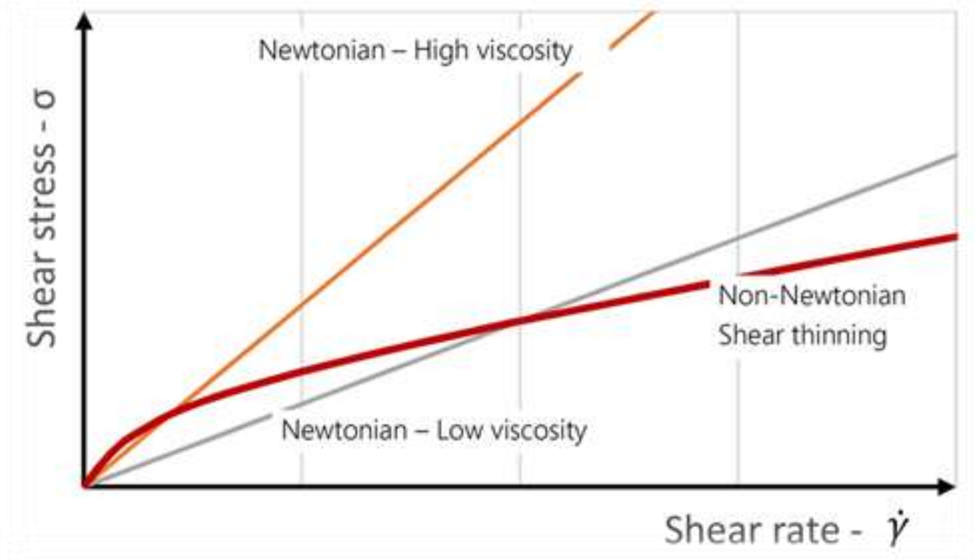


Figure 9. Behavior of shear stress versus shear rate for different types of fluids. (Adapted from https://fluidan.com/rheology-basics/).

1.1.3. Shear thinning

Emulsions need to have a rheological profile such that their products are stable in containers as well as have a good application flow on the skin surface. The profile should also enable the application evenly to the surface of the skin to achieve the most blemish-free appearance possible and allow good adhesion on the skin for satisfactory user experience. This is achieved with emulsions formulations that have a shear-thinning behavior [28].

In rheology, shear thinning is probably the simplest and most frequent rheological behaviour that can be found in emulsions and some gels. Shear thinning here concerns a steady shear rate dependence of viscosity (see red circles in Fig. 14). It is occasionally considered synonymous with pseudoplastic behaviour and is typically defined excluding time-dependent effects, such as thixotropic effects. In shear thinning, viscosity instantaneously returns to the original low-shear value when the shear stress is removed. Thixotropy is a phenomenon in which viscosity decreases when a constant shear rate is applied over a period. When the shear force is removed, viscosity returns approximately to the original value, with a time delay. Emulsions are desired to present the behaviour of shear thinning, because a low shear implies a high viscosity, which will them stable during storage. In comparison, a high shear corresponds to a low viscosity, which allows it to flow and cover the largest possible surface. [8]

When fitting models to the linear portion of flow curves, using the power law (Equation 7) quantitative information about emulsions can be obtained and compared to their stability.

$$\boldsymbol{\eta = K\dot{\gamma}m} \tag{Eq. 7}$$

In this equation, η is the viscosity, $\dot{\gamma}$ is the shear rate, K is the flow consistency index, and m is the flow behaviour index. This index provides insight regarding the storage stability and application properties of the coating [29].

1.1.4. **Amplitude sweep**

To understand the rheology of emulsions, namely, how they respond to stresses and strains, the material behavior must be probed across a wide range of amplitude and frequency. The amplitude sweep is an important assay to properly determine the LVER (Linear Viscoelastic Region) to define rheological stability within either a % strain or stress value that is a critical input parameter for subsequent frequency [23].

1.1.5. **Frequency sweep**

Frequency sweeps are methods that give us information about the behavior and internal structure of polymers as well as the stability of long-term emulsions, additionally, the frequency sweep gives us information about how interlocked the polymers are and whether or not they form a three-dimensional network in the dispersions. Before performing the frequency barrier analysis, it is necessary to do an amplitude sweep.

1.1.6. Dynamic light scattering (DLS)

DLS is an optical measurement technique for the characterization of dispersed systems, mainly particles. It is one of the most commonly used methodologies to rapidly obtain particle size distributions of colloidal particles. More frequently, DLS is used to quantify the Brownian motion of individual particles in liquids for the purpose of particle size analysis. It is important to know the sizes, shapes, concentration and aggregation, chemical states, and electric charges of particles to understand their applicability as a stabilizer of emulsions [30].

Brownian motion causes dispersed particles to diffuse into the solution. Considering that particles are in Brownian motion, knowing the measurement temperature (T) and the system viscosity (η), the average size (hydrodynamic radii R_h) of the observed molecules can be calculated using the Stokes–Einstein equation, as expressed in equation 8.

$$\boldsymbol{D = \frac{K_B T}{3\pi\eta R_h}} \qquad \textbf{Eq. 8}$$

where K_B is the Boltzmann constant and D is the diffusion coefficient of the particles in the solvent.

In the case of small particles, light scattering intensity fluctuations are fast but of low amplitude, and vice versa for large particles, as shown in Figure 10. These trends lead to a more rapid diffusion for small particles compared to that for large particles.

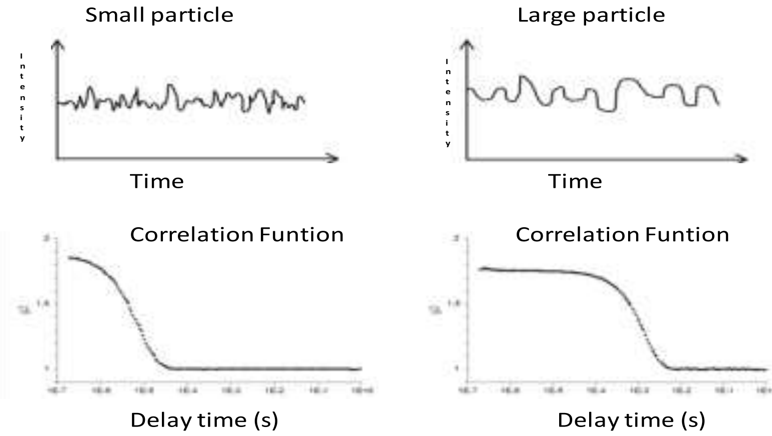


Figure 10. Schematics of dynamic light scattering (DLS) measurements, showing typical intensity fluctuations for small and large particles and their corresponding correlation functions.

If an obtained intensity curve is measure over a small time shift (τ), the curves for larger particles will have a longer delay than those for smaller particles. The similarity of the intensity versus the applied time shift results in an exponentially decaying curve. This curve is called a correlogram, which measures the degree of similarity between two signals, and calculates the intensity autocorrelation function, $G^{(2)}$ (τ) (Equation 9).

$$G^{(2)}(\tau) = < I(t)\, I(t+\tau) > t = lim\rightarrow\infty\ 1\ n \sum j\ NIjIj+n \qquad \textbf{Eq .9}$$

In correlation spectroscopy, signal analysis is performed in the time domain and the normalized autocorrelation function, G(2) (τ), as expressed in equation 9, is calculated. However, for a diluted system, equation 9 is reduced to equation 10.

$$G\,(1)\,(\tau) = \frac{<E(t)E(t+\tau)>t}{<I>} \qquad \textbf{Eq. 10}$$

To use DLS to quantify the properties of individual particles, the sample should be appropriately diluted to exclude any impact of particle concentration on the measured correlation functions and frequency spectra (amplitude, mean decay rate, and shape). To avoid these interferences, different adjustments are applied, such as the method of cumulants for correlation functions.

1.1.7. ζ-potential

ζ-potential is the electrical potential that NPs develop in a solution by the formation of a double electrical layer, called the Stern layer, and a secondary diffuse layer in which counterions are relatively sparsely packed. ζ-potential is generally analysed to measure the surface charge and stability of NPs in solution [31] (Figure 11). Both the stability and surface charge affect the adsorption of NPs at the oil–water interface of an emulsion. In general, a change in the pH or the ionic strength causes a change in the surface charge and stability of the NPs in the medium. Therefore, it is highly recommended to measure the values of the ζ-potentials for all materials used to stabilize emulsions [32].

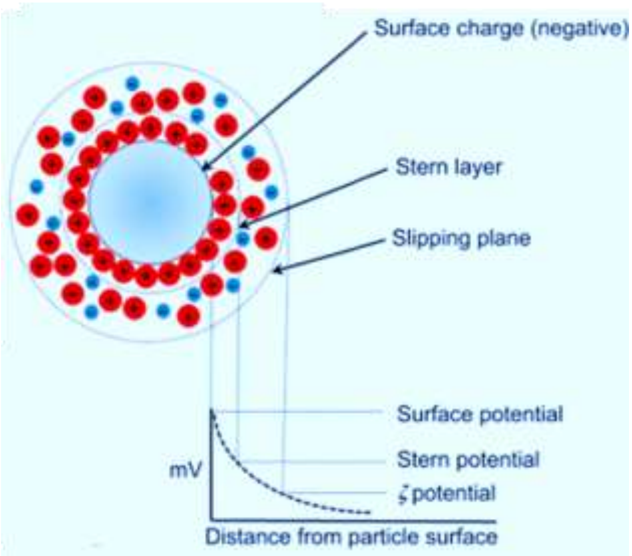


Figure. 11. Schematics of electric double layer around a particle/droplet in aqueous solutions (Adapted from K. Pate, P. Safier, in Advances in Chemical Mechanical Planarization (CMP), 2016).

The ζ-potential measured based on the movement of particles when subjected to an electric field when they are in a suspension; this movement is known as electrophoretic mobility (Ue). In contact with a polar solvent (water), most particles show a defined surface with a charge as a result of ionization, attracting counterions, which adsorb and act as a part of the particles. The adsorbed counterions move together with the particles when an electric field is applied, in contrast to the counterion layer called the diffuse layer, which does not migrate with the particles

This double layer consists of a charged surface and a neutralized surplus of opposite and equally charged ions that spread diffusely through the medium [33]. The potential that is generated between the diffuse layer and the second layer of adsorbed charges is called the ζ-potential and is highly important in all particulate matter applications. The ζ-potential of particles in a polar solvent can be calculated using the Henry equation:

$$\boldsymbol{U_e = \frac{2\varepsilon z f(\kappa a)}{3\eta}} \qquad \textbf{Eq.11}$$

Where *Ue* is the electrophoretic mobility, ε is the dielectric constant, η is the zero-shear viscosity of the medium, *f(κa)* is the Henry function, and $\boldsymbol{\kappa a}$ is a measure of the ratio of the particle radius to the Debye length. NPs with ζ-potentials between −10 and +10 mV are considered approximately neutral, and thus unstable and likely to aggregate rather than staying as stable dispersed colloids, whereas those with ζ-potentials greater than +30 mV or less than −30 mV are considered strongly cationic and anionic, respectively, and stable [32]

1.2. Literature Review

1.2.1. Responsive emulsions

Mosquitoes are vectors of numerous diseases including dengue, the Zika virus, chikungunya, and yellow fever, and are responsible for more than 500,000 deaths each yearin the world [34,35]. Among the above-mentioned diseases, only for yellow fever a vaccine is available. The Sanofi-Pasteur group has developed a vaccine against the virus that causes dengue fever; however, it is yet unavailable for mass vaccination [36]. Therefore, one method to prevent these diseases is using repellents, either by physical means using cloth and ultrasound or chemically by direct application of certain chemical compounds to the skin [37]. These products can be applied in different forms:

creams, gels, aerosols, and emulsions [38]. Emulsions present excellent media for repellent encapsulation, because they can be developed to control various parameters such as the release rate or control rheological behaviour, and sensory characteristics [21].

Currently, there is significant research interest in generating new dispersed systems (foams and emulsions) that are sensitive to stimuli, owing to their numerous applications in various fields such as drug administration, food, oil and gas, and agriculture. This widespread application is owing to the spontaneous and controlled changes in the physical and chemical properties of such systems, which can be reversible or irreversible changes, in the presence of different stimuli. Examples of stimuli are pH, temperature, electric field, ionic strength, and light, which impart unique properties to a stimulus responsive system [16] [39] [40]. Among them, emulsified systems whose properties are sensitive to stimuli have gained prominence, owing to their nano and micro sizes, which allow them to be used in various medical and industrial applications. Because of the wide variety of responsive emulsions which can be obtained, in this study, we focused on responsive emulsions stabilized by mixing non-ionic surfactants (Span 80 and Tween 80), cationic cellulose, and SiO_2 NPs.

Compared to conventional emulsions, which are stabilized by reducing the oil–water interfacial tension, Pickering emulsions are stabilized by anchoring solid particles at the oil–water interface, which prevents coalescence of the emulsion droplets. Owing to the high energy of the particle adsorption at the oil–water interface, the adsorption of particles can be considered irreversible. Pickering emulsions stabilized by surfactant and colloidal particles are known to have superior stability to conventional emulsions stabilized by surfactants or polymers. However, when de-emulsification is necessary, the ultra-stability of Pickering emulsions can be challenging. Sensitive Pickering emulsions that can be activated by triggers are highly interesting when only temporary stability is required, such as in drug and repellent deliveries. In particular, there is an interest in bio-based materials such as cellulose to be used as a Pickering emulsifier, in response to a growing awareness of the need to chemicals and materials based on renewable resources. Furthermore, cellulose has an active surface that can be modified and adjusted to different hydrophobicity or hydrophilicity according to its use. For all these reasons, cellulose is an appropriate candidate for formulations that encapsulate different active ingredients in the form of emulsions or gels.

Recently, Shi *et al.* prepared o/w emulsions via electrostatic interactions between cationic and anionic cellulose. The oppositely charged cellulose nanofibrils enhanced the stability of the emulsions when soybean oil was used [41]. Silva *et al*. [42] used cationic cellulose to stabilize an oleic acid–water interface, based on an electrostatic complexation at the oil–water interface. They prepared two classes of cationic cellulose with different degrees of modification (obtained by the addition of the cationization reagent glycidyltrimethylammonium chloride - GTMAC), and used them as stabilizers for Pickering emulsions. They found that the electrostatic attraction between the positively charged trimethylammonium groups and the negatively charged deprotonated oleic acid efficiently stabilized the liquid–liquid interface in the Pickering o/w emulsions. Silva *et al*. also revealed that cationic nanofibrillated cellulose (cNFCs) dispersed in the aqueous phase increased the viscosity and decelerated the coalescence of the oil drops. Therefore, stabilization of the cNFC Pickering emulsions had a synergistic effect involving the electrostatic complexation at the liquid–liquid interface and the network formation in the aqueous phase that increased viscosity. Concurrently, they lead to the possibility that using oleic acid can develop pH-responsive emulsions.

Zhu *et al.* [43] stabilized emulsions using carboxymethylcellulose and cationic chitosan, presenting high stability in different conditions such as pH, salts, temperature, and long-term scaffolding. They utilized this stability to encapsulate curcumin developing a Pickering emulsion delivery system with improved stability and sustainability.

Chen *et al*. [44] developed emulsions which respond to stimuli, by using different stabilizers (namely, N-isopropyl acrylamide and N, N-methylenebisacrylamide), each one responding to a distinct stimulus. They found that the synthesized microcapsules could respond to temperature and pH changes, and be able to selectively and independently release Nile Red (NR) and oil-soluble fluorescent green (OG) from the shell and the core without any mutual interference. Most importantly, the release of the NR and the OG could be controlled by pre-programming the order of the responses to the stimuli, in such a way that if the temperature rises, Nile Red was released and, in contrast, if the pH changes, OG was released.

Li *et al*. [45] aimed at finding green and recyclable emulsifiers and developing pH-sensitive Pickering emulsions. Their strategy consisted of synthesizing cellulose nanocrystals (CNCs) modified by benzyl-polyethyleneimine (called Ben-PEI-CNC), via periodate oxidation of CNCs and amination reduction. These emulsions were sensitive to pH owing to the existence of hydrophilic amino groups and hydrophobic benzyl groups. Li *et al*. developed cyclic emulsions, i.e., they could be stabilized and destabilized simply by adjusting the pH, and in addition allowed oil recovery and recycling of the emulsifiers.

Le *et al*. [46] stabilized emulsions using hydrophobically modified cellulose, to investigate and understand their ability to remain stable under changes in the pH and the in ionic strength (presence of NaCl). It was found that these formulations could respond by flocculation at low pH and ionic forces, showing their promise in the design of delivery vehicles.

Bao *et al.* [47] optimized the efficiency of cellulose for stabilizing emulsions and used a strategy of mixing an ionic surfactant with cellulose to obtain emulsions with better yields. In this approach, they also explored the effects of pH and the presence of NaCl, finding that the stability of the emulsions was dependent on the concentration of NaCl, first increasing and subsequently decreasing. This trend could be elucidated by the electrostatic shielding and aggregation caused by NaCl. The addition of NaCl induced electrostatic shielding of the CNC negative charges by counter-ions (Na^+). This study found that the Pickering emulsions stabilized by cetyltrimethylammonium bromide/CNC complexes may have the potential for large-scale applications in food, cosmetics, and medicines. However, the toxicity of CTAB can be a serious limitation due to potential health risks.

Chen *et al.* [48] used SiO_2-modified alginate to stabilize emulsions. They obtained stable emulsions in a pH range of 2.0–6.2, owing to the interactions of the polymer chains leading to the formation of a three-dimensional network. The charges and interactions of the NPs improved with the increase in the pH of the emulsions from 6.2 to 8.0, which enhanced the stability of the emulsions. However, the stability of the emulsions decreased when the pH exceeded 9.0. These emulsions released the model drug, λ-cyhalothrin, at pH values above 8, opening the field of study to obtain new formulations for drug release using similar strategies.

Slavova *et al*. [49] stabilized emulsions to encapsulate essential oils (limonene and citronellol) using SiO_2 with surfactants (carboxylate–potassium oleate) and polymers (Carbopol), with ultrasound as the homogenizing method. These capsules showed stability in a pH range between 3 and 10; however, they released their loads at pH greater than 11. According to Slavova *et al*., the developed methodology can find applications in any area where reverse encapsulation of oily substances is necessary.

Owing to the use of solid particles, instead of traditional surfactants, as emulsifiers, Pickering emulsions have unique advantages such as environmental friendliness, recyclability, and responsiveness to stimuli. Lu *et al*. [50] used sustainable amphoteric lignin (AML) for in situ hydrophobization of SiO_2 NPs by electrostatic adsorption, followed by preparation of pH-sensitive Pickering o/w emulsions. When the pH was above 4, only a small amount of AML was adsorbed on the SiO_2 surface owing to its higher hydrophilicity and strong electrostatic repulsion between nanoparticles. In contrast, the Pickering emulsions synergistically stabilized with AML and SiO_2 responded to pH owing to their amphoteric character. However, in this study there were not performed stability tests for emulsions, not evaluating the stability of these emulsions during storage.

Fan e*t al.* [51] concluded that alkyl-SiO_2 NPs can increase the viscosity of emulsions, therefore improving the stability by modifying the rheological properties. Another significant finding of their study was that when the carbon chain of the modifier was octadecyl, the increase in the viscosity was higher than with short methyl chains. Although the above study is applicable for the oil industry, these results could also be used in cosmetics.

These findings help in the discussion of the results obtained in the study presented in this thesis. Our strategy included the use of non-ionic surfactants to prevent the precipitation of the complexes formed by CCNF and silica nanoparticles (included as emulsion stabilizers), and the repellent in the oil phase. The study presented in this thesis also addressed the gap that exists in the literature regarding the role played by SiO_2 NPs and their ability to be multifunctional in the co-stabilization of emulsions and the involved mechanisms. Example mechanisms are adsorption at the oil–water interface and enhancement of viscosity increse in the presence of rheological modifiers, such as cationic cellulose.

Another key point that was addressed in the present study was theunderstanding of the destabilization mechanisms of emulsions to develop formulations that can respond to triggers such as pH and NaCl. Liu *et al*. [52] prepared pH-sensitive o/w emulsions using SiO_2 NPs and traces of surfactants (carboxyl betaine). The emulsions were stable to coalescence at pH ≤ 5, where the phases separated completely at pH > 8.5. K. This study explained the stabilization–destabilization mechanisms. These mechanisms were strongly dependent on the electrostatic interactions of the materials involved in the formulation of the emulsions. Liu *et al*. also demonstrated that these pH-sensitive emulsions can oscilate between stable and unstable states many times by alternating the pH of the aqueous phase. However, the mean droplet size in the low-pH re-stabilized emulsions gradually increased after four cycles, owing to the accumulation of NaCl which partially screens the electrostatic interaction between the positively charged of carboxyl betaine surfactants and the negatively charged particles. Although the contributions of this group are highly relevant, tests showing release of a drug as a proof of concept were lacking.

Emulsions are inherently capable of serving as vehicles for the administration of active agents, and generating sustainable, high-performance formulations is gaining interest and importance in various industries, such as personal care, pharmaceuticals, and oil. Drakontis *et al.* developed a cosmetics lip gloss using emulsions stabilized by biosurfactants and SiO_2. They studied the sizes of the NPs and their effects on the rheology. [53] Their results provide information on the design of formulations stabilized by SiO_2, particularly lip and makeup formulations. We extended the rheological study to formulations of emulsions stabilized by non-ionic surfactants, cationic cellulose, and SiO_2 NPs for the controlled release of repellents.

1.2.2. Formulation of emulsions containing N,N'-diethyl-m-toluamide

Insect repellents are used to repel mosquitoes, ticks, flies, and other biting insects. Repellents are not meant to kill insects but to repel them to prevent bites and the spread of disease. Because some insects act as vectors for some diseases, the use of insect repellents is essential when other forms of protection are unavailable. As mosquitoes are propagators of diseases, developing more effective repellent formulations that help combat the spread of diseases is necessary. In this context, we reviewed the literature to find the existing repellent formulations, based on which we improved the performance of the formulations and developed emulsions with sustainable materials.

N,N'-diethyl-m-toluamide (DEET) is the most common active ingredient in insect repellents. It was developed in the 1940s, and works by interfering with the ability of insects to detect lactic acid in hosts. Because it is a broad-spectrum repellent, DEET is used for the development of repellents. 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. 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. 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

Lorenzo *et al.* [54] prepared poly(L-lactic acid) (PLLA)/DEET mixtures in various compositions. DEET was used as a plasticizer for PLLA and the obtained material presented good repellent activity. In addition, the results reported on the miscibility of the components of the mixtures allowed plotting the phase diagram of the PLLA/DEET mixtures, which had previously been reported in the literature only for mixtures in which DEET is the main component.

Kadam *et al.* [55] encapsulated DEET by polycondensation with cellulose, adjusting the release profiles to fit various kinetic models. Daza *et al*. [56] developed emulsions containing DEET, which were stabilized by SiO_2, modified SiO_2, and polymers. The most stable emulsions were obtained with NPs coated with polyacrylic acid, owing to the improvement in the synergy of the strong interfacial adsorption of the NPs by the long polymer chains and the weak polyelectrolytic character of the polymers. The NPs could prevent the diffusion of DEET. However, tests on the rheological characterization, oil type effect, and responses to stimuli of these formulations were not conducted.

The studies discussed above show that most investigations in this area only deal with emulsification and the release of repellent by temperature as a trigger. On the other side, some research address emulsions that respond to NaCl and pH although without DEET encapsulation. Understanding the activation mechanisms of emulsions providing controlled release and the behaviour of colloidal materials can help develop emulsions with improved performance. In this context, the study presented in this thesis addressed the characterization of o/w emulsions containing DEET and their repellent release mechanisms when subjected to stimuli. Concurrently, we evaluated the interfacial properties of colloidal materials and their interactions under external stimuli as well as their roles in stabilizing the emulsions.

Advances in nanotechnology have led to the emergence of new NPs for various applications, including the well-known cosmetics preparation. In these cases, the main focus is on cationic cellulose and SiO_2-based NPs, because of their major importance in the development of products for application to the skin and high application prospects in current cosmetic formulations. Based on the literature, nanomaterials such as cellulose and SiO_2 play important roles in improving the stability of emulsions. In the study presented in this thesis we attempted to explore various gaps that, to our knowledge, have not been addressed by other investigations.

1.3. Statement of Research Problem

Worldwide each year, more than 300 million cases of diseases transmitted by mosquitoes are reported [34]. One available approach to prevent these diseases is using repellents, among which DEET has gained importance because it is a wide-spectrum repellent. However, the use of DEET in concentrations exceeding 10% is not recommended because it can be harmful to children and pregnant women. An alternative to applying this repellent to vulnerable people is using DEET emulsification; most DEET formulations have a protection time of up to 4 h, probably owing to its rapid absorption by the skin. Therefore, research on generating repellent formulations with a longer protection time without the need for concentrations exceeding 10% to enable use by pregnant women and babies is highly interesting.

Concurrently, many traditional repellent formulations use components that are non-biodegradable. The presented study addressed the stabilization of emulsions using biodegradable surfactants (Tween 80/Span 80) and sustainable materials such as cellulose that stabilize emulsions by several mechanisms. These materials were selected because, to our knowledge, they have been scarcely investigated for the formulation of slow-release repellent emulsions. Furthermore, the stabilization of emulsions by biodegradable and renewable materials has the advantages of lower toxicity, economic benefits associated with easy access, and abundance of cellulose and SiO_2 NPs.

1.3.1. General Objective

To develop oil-in-water emulsions stabilized by cationic cellulose, silica nanoparticle and surfactants for controlled release of DEET .

1.3.2. Specific objectives

- Characterize the nanomaterials by dynamic light scattering (DLS) (size hydrodynamics and zeta (ζ)- potential).
- Understand the influence of modified cellulose in altering the rheological behaviour of the emulsions.
- Study the destabilization mechanisms of the emulsions in the presence of triggers (NaCl and pH).
- Evaluate the types of oils (mineral and vegetable) used in the formulation of the emulsions.
- Characterize the rheological behaviour of the emulsions.
- Determine the release kinetics of the repellent encapsulated in the emulsions.

1.3.3. Hypotheses

The following hypotheses were tested in this research:

1. Cellulose improves the stabilization of the repellent emulsions by increasing the viscosity.
2. Cellulose acts as a rheological modifier of the emulsions owing to the electrostatic interactions with the SiO_2 NPs.
3. The increased viscosity retards the release of DEET from the emulsions.

1.3.4. Significance of Research

This study was aimed at improving the quality of life and health of people who may be affected by mosquito-borne diseases. The growing demand for repellents with longer protection times compared to traditional ones motivated us to search for repellent formulations that meet the requirements of the World Health Organization. The development of these controlled release emulsion formulations will introduce a route of stabilizing emulsions using sustainable and biodegradable materials for the pharmaceutical and food industries. This is inferred considering that the knowledge generated with these repellent formulations can be extrapolated to the food and pharmaceutical industries.

1.3.5. Expected Outcomes and Contributions of Research

The results of the research presented in this thesis will provide insights into the applications of the materials used in the formulation of controlled release repellent emulsions. They will also present various industries with knowledge about the formulation of stabilized emulsions using sustainable, inexpensive, and easily accessible materials. In addition, they will provide an understanding of the variables that affect or improve the stability of emulsions, supported by rheological measurements.

Based on this study, an article entitled ***Efficient encapsulation and controlled release of N,N-diethyl-3-methylbenzamide (DEET) from oil-in-water emulsions stabilized by cationic nanocellulose and silica nanoparticles*** was published. In this paper, we presented information on the preparation of repellent formulations from emulsions stabilized by sustainable materials.

2. Materials and methods

The repellent active principle, DEET (97%), was purchased from Sigma Aldrich (Brazil). Surfactants Span®80 and Tween®80, mineral oil (BioReagent), NaCl (≥99%), hydrochloric acid (HCl) (≥35%), sodium hydroxide (NaOH) (≥50%), and methanol (≥99.8%) were also purchased from Sigma Aldrich (UK). NaOH and HCl were adjusted to the required concentrations with deionised (DI) water, whereas ultrapure water (MilliQ) was used for all experiments (conductivity of 18 MΩ cm).

2.1. Modifications of materials

The α-cellulose and the cationizing reagent, glycidyltrimethylammonium chloride, (GTMAC, ≥90%) were also purchased from Sigma-Aldrich (U.K). Cationic cellulose was obtained from the reaction of cellulose with GTMAC by a procedure described elsewhere [57,58]. Following the semi-dry procedure described in J. C. Courtenay et al., [58,59], 8 wt% NaOH (relative to the corrected film mass) dissolved in 20 mL DI water was added to α-cellulose contained in polyethylene bags. Accurately weighed GTMAC (1.2–1.6 g) in molar ratios of 0.5–3.0, relative to anhydroglucose units (AGUs) of the weighed cellulose, was added dropwise.

The sample was kneaded to achieve homogenisation, prior to reaction at 65 °C (water bath) for 75 min. Subsequently, the mixture was cooled to 25 °C, and the reaction was quenched by addition of ethanol. To purify the resultant cationic cellulose nanofibril dispersion, the suspension was centrifuged (5 min at 3600 RCF), and the pellet was collected and redispersed in DI water; the above process was repeated ten times. These GTMAC-modified α-cellulose are referred as ‘cationic cellulose nanofibrils’ or ‘CCNFs’ in this thesis.

SiO_2 NPs (12 nm, 98.8%) (hereafter referred as SiNPs) were purchased from Sigma-Aldrich (Brazil) and modified with 3-(aminopropyl) trimethoxysilane (APTMS, 97%) purchased from

Sigma-Aldrich (Brazil). SiNPs were modified following the procedure reported in [56]. Previous to the functionalization procedure, SiNPs were dried in an oven at 150 °C for 24 h to remove the physisorbed water. A mass of 2 g dried and activated SiNPs was mixed with 50 mL anhydrous toluene and 3 mL APTMS. This mixture was heated and maintained under reflux (150 °C) for 24 h. The amino-functionalized SiNPs (SiNPNH2) obtained by this procedure were purified by centrifugation and sequential washing with toluene, ethanol, and water thrice. Subsequently, they were dried at room temperatu (25 °C) under vacuum to obtain a dry powder [60].

2.1.1. Degree of substitution of CCNFs

The degree of substitution (DS) of the CCNFs was determined by conductometric titration of chloride ions with $AgNO_3$ (0.003 M). A sample of 22 mg CCNFs was dispersed in 50 mL ultrapure water with magnetic stirring for 10 min. The DS was calculated using the following equation 2:

$$\mathbf{DS} = \frac{(\mathbf{162.15 \times C \times V})}{\mathbf{w - (151.63 \times C \times V)}} \times \mathbf{100} \qquad \textbf{Eq. 2}$$

where C is the molar concentration of $AgNO_3$ solution, V is the volume of $AgNO_3$ solution (in mL), and w is the weight of the dried cationic cellulose sample (in g). Molar mass of cellulose was 151.63 g mol^{-1} and molar mass of substituent group was 162.15 g mol^{-1}. The reported value (DS = 21.8%) is the result of triplicate determinations. Figure 7 shows the results of the conductometric titration conducted to determine the DS of the cationic cellulose.

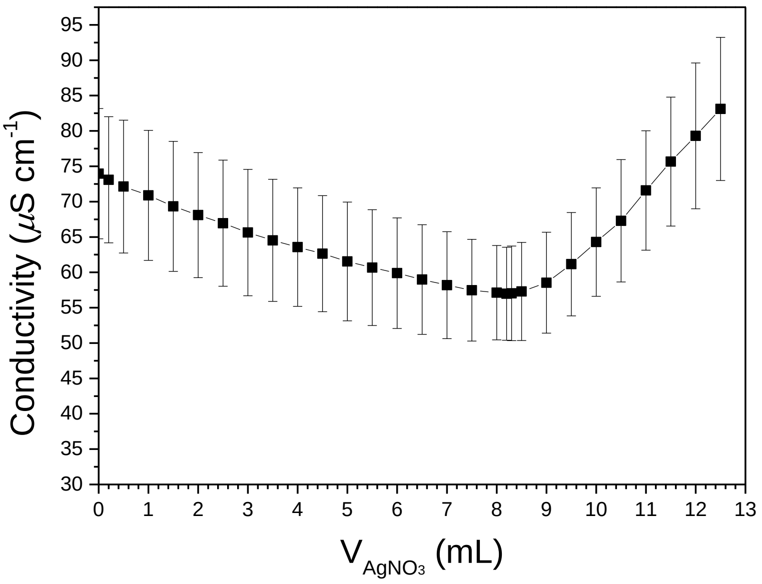


Figure 12. Conductometric titration of cationic cellulose with silver nitrate.

2.2. Materials characterization

2.2.1. Measurement of ζ-potential of nanomaterials by dynamic light scattering

ζ-potential is the electric potential that NPs develop in a solution by the formation of a electrical double layer. It is a measure of the surface charge and stability of NPs. Both these properties affect the profiles of NPs to stabilize emulsions. The ζ-potential values of SiNPs and the CCNFs were obtained by measurement of electrophoretic mobility using a dynamic light scattering (DLS) equipment (Horiba SZ-100). Three samples of each system were diluted in water until a concentration of 20 mg L^{-1} was obtained. Subsequently, they were dispersed using a FB-505 ultrasonic processor (Fisher, 200 W·cm^{-2}), equipped with a 0.63 cm probe, in continuous mode for 2 min and at an amplitude of 30%. These samples were measured six times at 25 °C. Electrophoretic mobility was converted to the ζ-potential using the Smoluchowski approximation, which yields the hydrodynamic particle size [32]. The reported results are the averages of the six measurements, and the uncertainties in these values are the standard deviations. The pH values of the samples were adjusted by adding small amounts of HCl or NaOH [61].

2.2.2. Emulsion formation

Preparation of o/w emulsions containing DEET

Emulsions were prepared in 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 of the nanomaterials. First, SiNPs were dispersed by 1-min sonication into an aqueous Tween®80 solution, and subsequently, the CCNF stock dispersion was added to achieve final concentrations of the mixture in the emulsion: 0.01 wt% and 0.3 wt% for SiNPs and CCNFs, respectively. After mixing, the SiNP–CCNF suspension, composing the aqueous phase of the o/w emulsion, was sonicated for 2 min using an FB-505 ultrasonic processor (Fisher, 200 W·cm^{-2}) equipped with a 0.63 cm probe operated in the continuous mode (amplitude 30%). The oil phase was prepared by first mixing mineral oil with DEET and Span®80 using an Ultra-Turrax (IKA T25) high-speed homogenizer at 13 000 rpm. Subsequently, this mixture was gradually added dropwise to the aqueous phase, and homogenization was performed for 2 min (procedure adapted from [13]). The mixing scheme of the emulsions. The DEET total concentration in the formulations was 10 wt%, and the surfactants used as the emulsifiers were taken in a 1:1 ratio (Tween®80:Span®80, wt:wt) and 0.5 wt% total concentration are shown in Figure 8.

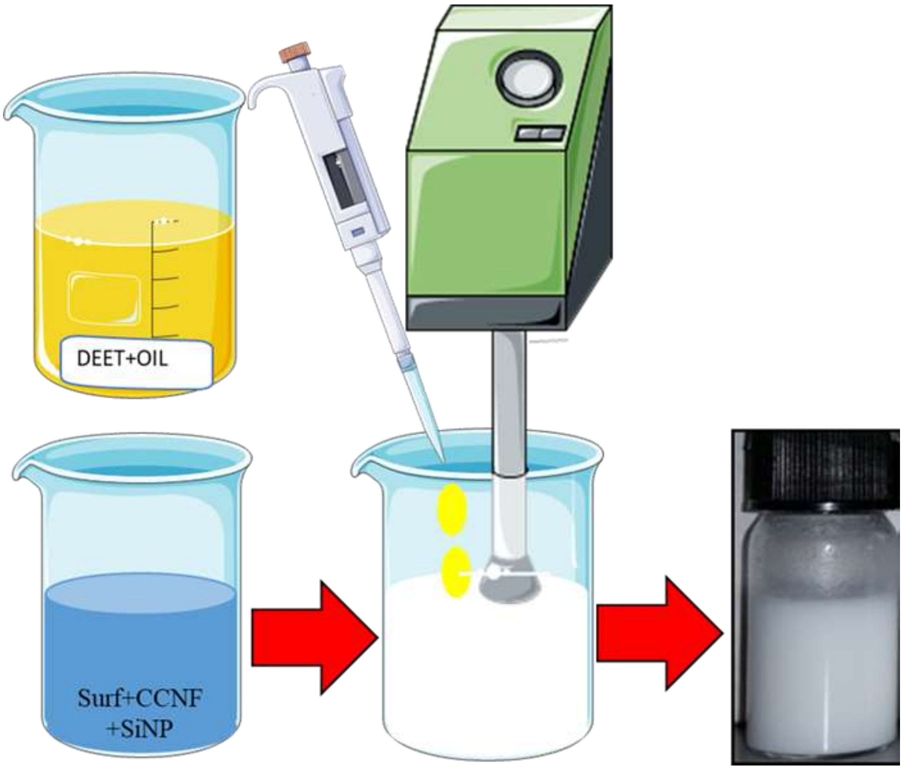


Figure 13. Scheme of the procedure for formulation of o/w emulsions containing DEET.

2.3. Characterization of emulsions

2.3.1. Rheological Behaviour

The rheological behaviour of the emulsions was obtained using a revamped Discovery HR rheometer (TA Instruments) at 25 °C with a plate−plate geometry (40 mm) with a gap of 1000 μm. The viscosity was monitored by increasing the shear from 0.01 to 1000 s^{-1}. All experiments were conducted under controlled temperature (25 °C).

2.3.2. Optical Microscopy

The morphology of the drops of the internal phase of each emulsion was determined by optical microscopy using a Brunel Microscope SP60P model coupled to an EOS 1300 D Cannon camera (the EOS Utility program was used for editing the images). A 20 μL aliquot of an emulsion was diluted in 1000 µL aqueous phase and subsequently dispersed on a watch glass to obtain better images (Figure 9). All experiments were conducted at room temperature (25 °C).

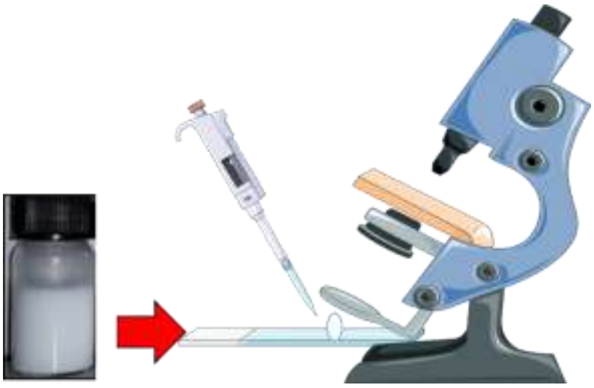


Figure 14. Scheme for optical microscopy measurements of emulsions.

2.3.3. Laser Diffraction for Particle Size Analysis

Particle size and distribution were obtained using a Mastersizer 3000 Hydro model with a small volume dispersion unit (Malvern Instruments, UK). The used lens can detect diameters ranging from 0.5 µm up to several millimetres. The emulsions were diluted in the accessory of the equipment, to avoid erroneous results from the aggregation of drops. Refraction indexes of 1.33 and 1.47 were used for water and mineral oil, respectively. The volumetric diameter (D4,3) was taken as the average of five measurements, and the reported values are D90, where D90 implies that 90% drops are below the volumetric diameter.

2.3.4. Stability, emulsion morphology, and phase separation

Physical stability of the emulsions was monitored by time lapse imaging (using a photographic camera with a 16-megapixel, 1/2.6” sensor having aspect ratio 16:9, aperture f/2.2, focal length 31 mm, and pixel size 1.12 µm). To determine the extent of phase separation and compare the effects of the nanomaterials in the emulsion stabilization, the samples were subjected to mechanical (centrifugation) and thermal (heating) stresses. Aliquots of the emulsions were centrifuged at 2566 RCF for 10 min using a Thermo Scientific Heraeus Megafuge equipment or kept at 45 °C in a laboratory oven (Memmert oven UN 30) for 72 h, and subsequently visually monitored. In addition, because the mechanism of emulsion stabilization was based on electrostatic interactions, the effect of changing the solution pH and adding a salt were evaluated. The pH of 5 mL of the initial volume of an emulsion was adjusted from the initial pH value of 6.0 to 3 or 11 with 0.1 M HCl or 0.1 M NaOH, respectively, using a 650 MA pH meter. For the salt addition experiments, NaCl solid was added directly to an emulsion to reach 3.5 wt% concentration, following which the emulsion was shaken manually for 2 min. Because only small changes were observed immediately after the destabilization procedure, the stability of each emulsion was monitored for up to 24 h. In all studies, the appearance of an aqueous bottom layer was used as a criterion for the destabilization of an emulsion and calculated as an instability index (ratio of the separated aqueous phase to the total volume of the solution).

2.3.5. Release and quantification of DEET from o/w emulsions

The release of DEET from the emulsions was evaluated using a procedure reported previously. Specifically, an Eppendorf vial containing 1 mL emulsion was covered with a dialysis membrane (MWCO 12400 Da from Sigma-Aldrich, UK) and immersed into a 50-mL test tube containing 45 mL 30:70 (v:v) methanol:water mixture. [56] A receiver medium was selected to generate a strong polarity gradient for the diffusion of DEET from the donor (emulsion) to the receiver compartments, given the non-polar nature of the active compound. The systems were placed in a Stuart Orbital Shaker SSL1 at 90 rpm for up to 6 h. Aliquots were collected at 2, 4, and 6 h. However, when vegetable oil was used to formulate the emulsion, the release method changed. Specifically, 0.3 mL repellent formulation was taken on a tape paper, and in an oven at 25 °C, five samples were placed to analyse the residual DEET on the papers. DEET was extracted from each paper at 2, 4 and 6 h, using a 30:70 methanol:water solution. The two methods described for evaluating the release of DEET are shown in Figure 10.

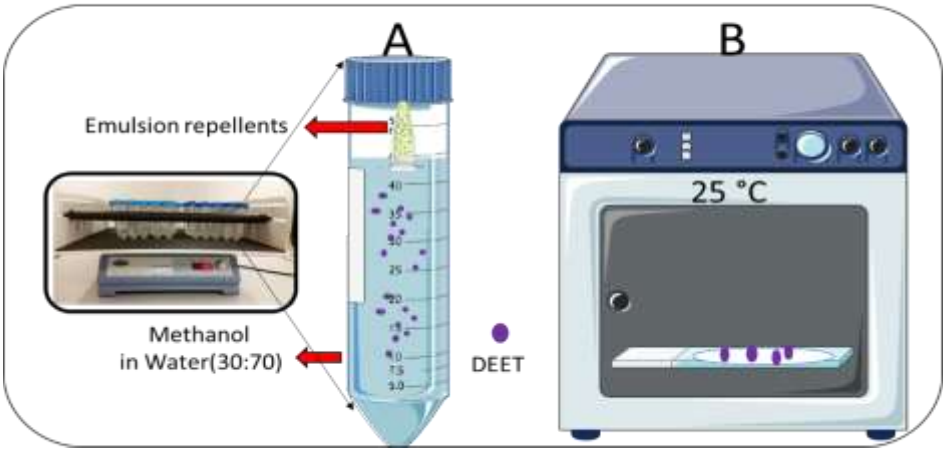


Figure 15. Scheme showing the two approaches for studying the release of DEET: (A) using 30:70 (v:v) methanol:water mixture as receptor medium, (B) using temperature controlled release (at 25 °C) and without a receptor medium.

The cumulative release of DEET was quantified by UV–visible spectrophotometry (Cary 60 Agilent Technologies spectrometer) using UV-grade quartz cuvettes. First, it was obtained the absorption spectrum of DEET to determine the wavelength of the DEET absorption maximum (Figure 11).

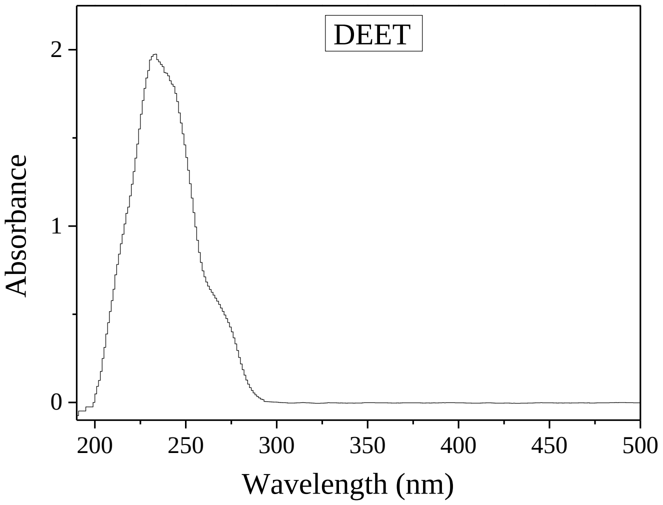


Figure 16. Absorption spectrum of DEET.

Next, a analytical curve was constructed by measuring the absorbance at the maximum wavelength (λ_{max} = 204 nm), over a concentration range of 0.264–8.423 mg L^{-1}, using the methanol:water 30:70 (vol:vol) mixture as solvent (Figure 12). The amounts of DEET released at different times were quantified by sampling 50 μL aliquots of the external receiver medium, and diluting it 20 times with the methanol:water 30:70 (vol:vol) mixture. This procedure was also employed for studying the pH and salt effects on the DEET release from the w/o emulsions.

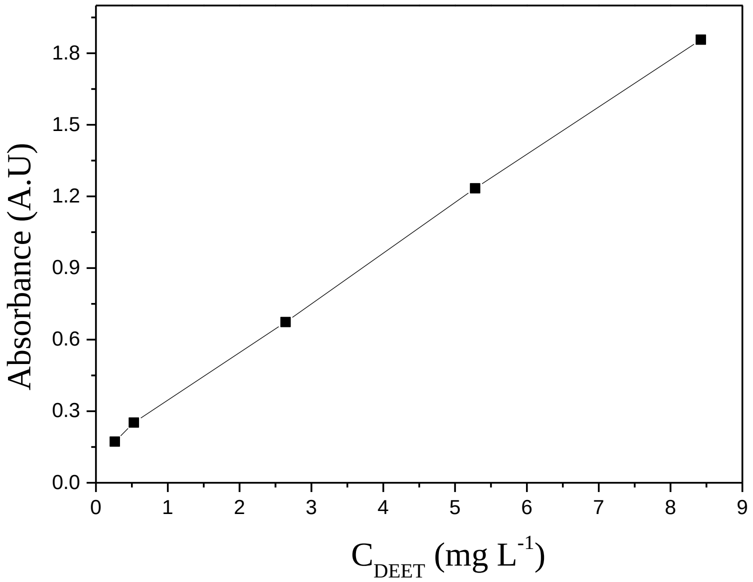


Figure 17. Calibration curve of DEET in 30:70 (v:v) methanol:water mixture (λ = 204 nm, linear regression: y = 0.206x + 0.132, R^2 = 0.999).

3. Results and discussion

For the development of repellents in an emulsion form, choosing an oil that offers additional properties to those for repelling insects is necessary; these properties range from the reduction in lactic acid, which attracts insects, to improvement in sensory properties [62]. Therefore, the study presented in this thesis began by determining the formulation variables: selection of the type of oil, hydrophilic–lipophilic balance (HLB), and concentration of surfactants. These were subsequently used for the development of repellents in emulsion forms.

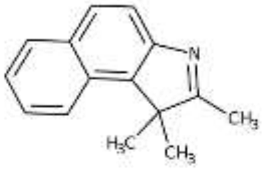
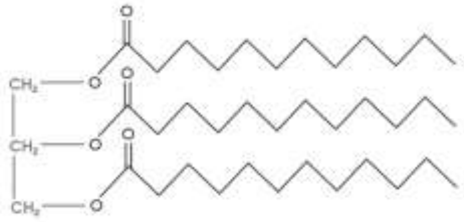
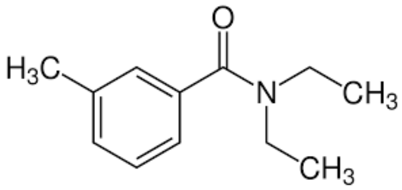
3.1. Influence of composition variables

3.1.1. Oils of study

Emulsions are mainly classified into two classes: w/o and o/w. In skin care emulsions, emollients are the major ingredient after water, being used at a level between 3 and 40% (w/w). Therefore, in o/w emulsions, the type of oil plays an important role owing to the rheological behaviour and sensory characteristics they endow to the emulsions. To develop a repellent in the form of emulsions, the solubility of DEET were evaluated in four types of oils: Vaseline, mineral oil, olive oil, and soybean oil. These oils were chosen based on their structural differences and chemical compositions.

The chosen oils have different polarities, which depend mainly on the chemical groups that compose them, the size of the carbon tail, and their origin, e.g., animal, vegetable, or mineral. In this context, selecting a type of oil that allows the development of repellent formulations without causing adverse effects on the skin, regardless of its solubilization of the different formulation components, is important. In Table 1, the structures of the oils used to determine the solubility of DEET are presented. Therefore, the objective of this study was to develop formulations with improved physical–chemical stability and releasing the repellent in a controlled and sustained manner.

Table 1. Chemical structure of the oils.

Oil name	Chemical structure
Petrolatum or Vaseline	
Mineral oil	
Vegetable oil (Soybean and olive oil)	
N, N'-Diethyl-3-methylbenzamide (DEET)	

The solubilization tests showed that DEET was soluble in the vegetable oils, olive oil has a higher percentage of the lauric chain linked to the glycerol group compared to soybean oil. This is attributed to the vegetable oils containing glycerol or carboxylic acid groups in their structures, which improve the interactions with the amide groups of DEET, generating a mixture of the two compounds. In contrast, vaseline and mineral oil do not possess atoms or functional groups that help intermolecular interactions; consequently, the compounds repel each other, generating two phases and there is no solubilization. Figure 18 shows images of the DEET solubilization tests conducted in the different oils, DEET concentration 10 v/v% was used.

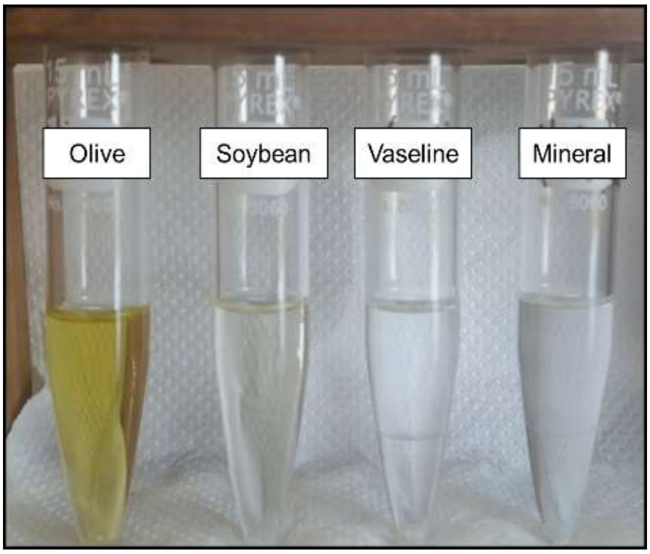


Figure 18. Solubility of DEET 10 v/v% in different oils.

Sandip *et al.* [55] used paraffin oil to prepare repellent formulations using DEET and reported an approach to encapsulate DEET, via interfacial polycondensation using modified cellulose nanofibers (CNFs) as a *Pickering* emulsifier, These authors used paraffin to create a shell to encapsulate DEET, which required to heat the paraffin up to 80 °C. In contrast, the oil used in the present work offers the advantage of not needing to be heated to formulate the emulsions. In another study, G. M. Gómez *et al.* [63] encapsulated DEET in poly(n-butyl methacrylate-co-methyl methacrylate) nanospheres via direct mini-emulsion polymerization.

Therefore, to broaden the knowledge of DEET formulations, we chose oils in which DEET was found soluble and insoluble, respectively. The latter was mineral oil, which is

FDA-approved for ophthalmic, oral, and prominent applications, listed in many different pharmacopeia, and inexpensive. Soybean and mineral oils were selected to continue with the formulation of the emulsions because they do not significantly penetrate the skin, in addition to endowing pleasant sensory properties to formulations [64] .

3.2. Determination of optimal Hydrophilic-Lipophilic Balance (HLB)

3.2.1. Determination of HLB of surfactants to stabilize emulsions using mineral oil

As mentioned above, emulsions are composed of two phases stabilized by particles or surfactants. Surfactants stabilize emulsions by reducing the interfacial tension between the oil and water phases, and, the use of a mixture of surfactants plays a key role in the development of emulsions to be applied in cosmetics, because it increases the interactions between the components of the formulations. Non-ionic surfactants are described by the hydrophilic-lipophilic balance (HLB) scale established by Griffin. Owing to all above factors, to develop stable emulsions, the HLB needs to be close to that of the oil, and because it is an empirical parameter, it is determined experimentally. Therefore, tests were conducted to calculate the optimal HLB for the production of mineral oil-based emulsions for mosquito repellent formulations.

The use of an HLB system helps rapidly identify the proportions in which the surfactants should be mixed to obtain the optimal surfactant to achieve a stable emulsion. The HLB of the mixtures was calculated by adding the multiplication of the molar fractions of the surfactants by their HLB in the pure state, that is: $HLB_{mixt} = x_{S1} \times HLB_{S1} + x_{S2} \times HLB_{S2}$. The emulsions were formulated in an HLB range of 6–14, using Tween 80 and Span 80 surfactants. All the formulations showed to be stable immediately after preparation (Figure 19).

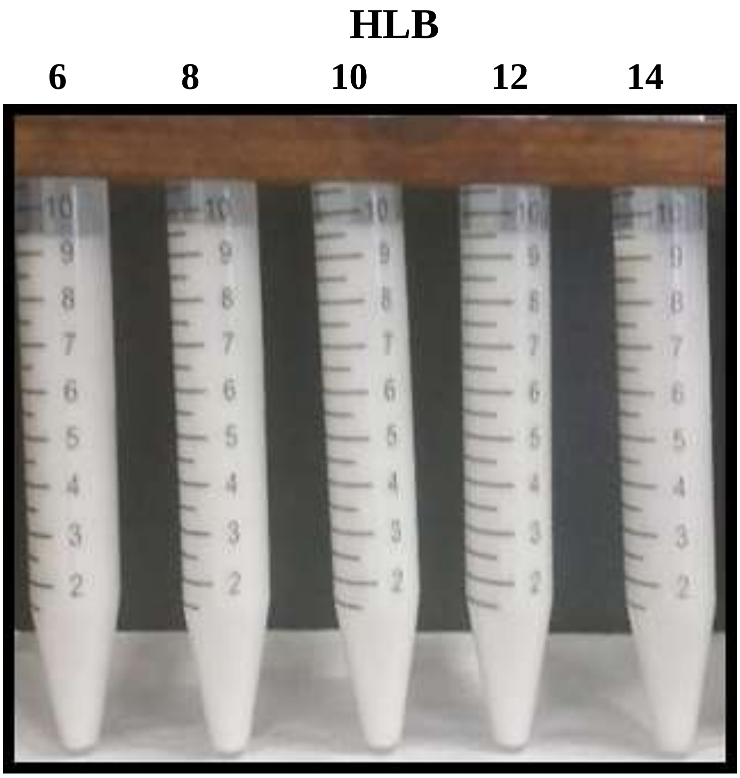


Figure 19. Initial stability of mineral oil in water emulsions using a mixture of surfactants Span®80/Tween®80 (3 wt.%) at different HLB. Pictures taken immediately after preparation.

However, there was a destabilization effect observed in some emulsions after 24 h. The emulsions formulated at HLB 12, and 14 presented drainage of ca. 15% after 24 h, while those at HLB 6, 8, and 10 presented drainage of less than 10%, in the same period as shown in Fig 20. The emulsions formulated at HLB 12 and 14 were considered unstable, which may be associated with a low adsorption of the surfactants at the oil–water interface. We hypothesize that in case of HLB 6, the Span®80 surfactant is in a higher concentration than Tween®80, generally tending to induce the formation of w/o emulsions, and therefore fails to efficiently stabilize the o/w emulsions studied here. In contrast, when we use HLB 12 and 14 formulations, the surfactant in highest concentration is Tween 80 (hydrophilic surfactant) because it has greater solubility in water than in oil., which fails to adequately stabilize the oil–water interface, causing coalescence between the oil droplets, and finally, breaking the emulsions.

We subjected the emulsions to destabilization tests using the centrifugal field to determine the most appropriate HLB to form emulsions with enhanced stability. Such tests provide information on the stability duration of a formulation in stock in the market. The conditions were 2655 RCF for 10 min at 25 °C. The results revealed that the emulsion with the highest stability was the one formulated with HLB 10, consistent with the results shown in Fig. 20. This maximum stability may be associated with the strong interactions (electrostatic interactions between hydroxyl groups) between the surfactants adsorbed on the oil–water interface, which endow properties that prevent emulsion inversion and coalescence between droplets.

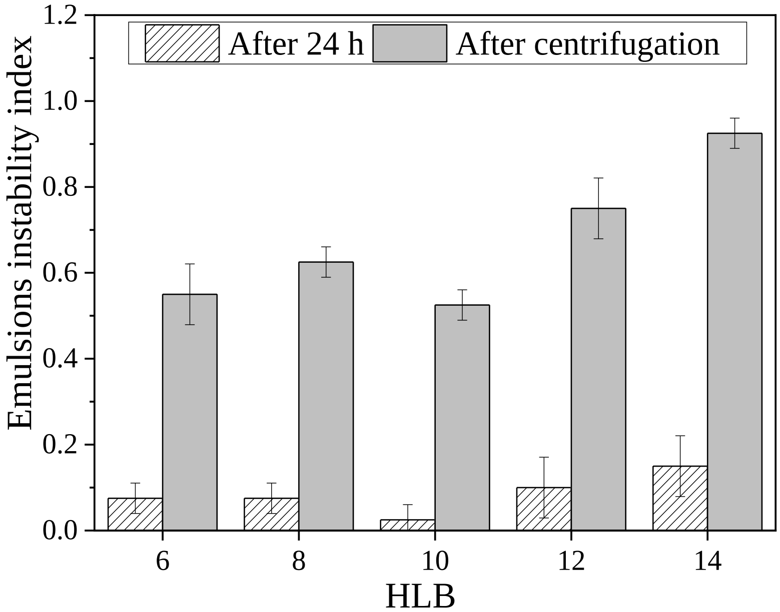


Figure 20. Initial stability of mineral oil in water emulsions using a mixture of surfactants Span®80/Tween®80 (3 wt.%) at different HLB. Pictures taken immediately after preparation.

Based on Fig. 20, the formulations with HLB 6, 8, 12, and 14 present an aqueous phase drainage greater than 55% after centrifugation. Therefore, we hypothesize that these mixtures of surfactants cannot adequately stabilize the oil–water interface when subjected to the centrifugal force. The drainage of the aqueous phase of the formulation with HLB 10 is lower than those of the other HLB cases. This may be associated with the combination of the percentages of the surfactants allowing the highest interactions with both the aqueous and oily phases, appropriately stabilizing the oil–water interface. This result is in accordance with those of Roy and Hong [4,65]. Hong *et al.* [63] studied the influence of the HLB in the formulation of emulsions using a mixture of surfactants (Span/Tween). They determined that HLB 10 presented the best performance to stabilize emulsions and stop the increase in the droplet size as well as reduced the phase separation. These results are in agreement with the HLB determined in this study, i.e., HLB 10. We hypothesize that the HLB determined herein is slightly lower because it is a mixture of mineral oil and DEET. Therefore, we chose HLB 10 to continue developing repellent formulations using mineral oil.

3.2.2. Determination of HLB surfactants to stabilize emulsions using vegetable oil

We also conducted tests to determine the optimal HLB for the development of formulations using the vegetable oils. The tests showed that when using the vegetable oils, the mixture of surfactants (Span 80/Tween 80) stabilized the emulsions in the HLB range of 4–14, which is displayed in Fig. 21. The stability of the emulsions was measured by quantifying the drained aqueous phase, and the results are also shown in Fig. 22. It can be seen that all emulsions drained less than 10% aqueous phase; therefore, we can affirm that the mixture of surfactants is effective in stabilizing the formulations when the vegetable oils are used.

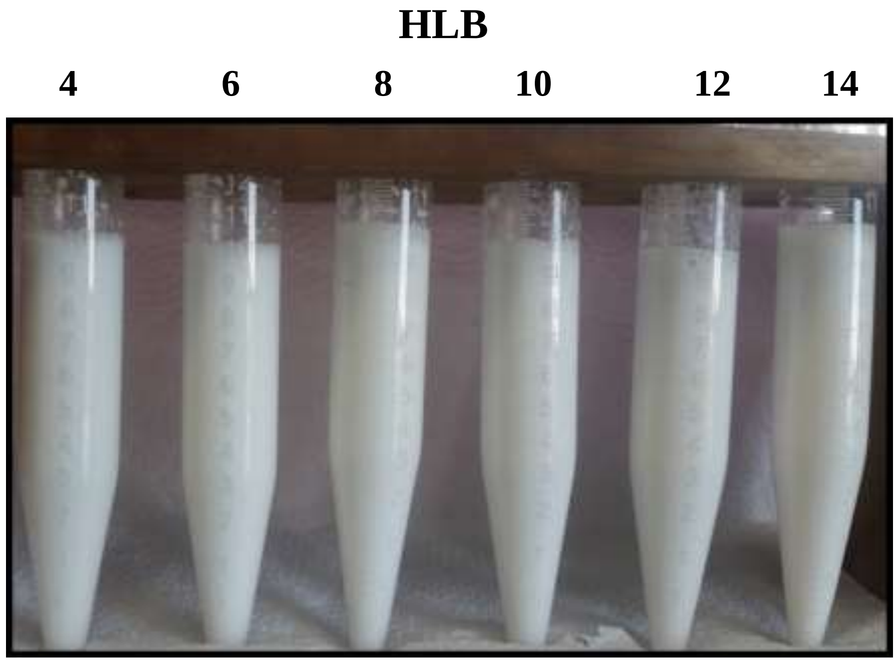


Figure 21. Initial stability of vegetable oil in water emulsions using a mixture of surfactants Span®80/Tween®80 (3 wt.%) at different HLB. Pictures taken immediately after preparation, the error bar is the product of three measurements.

As the drained values was less than 10%, the emulsions were subjected to accelerated destabilization tests to determine the HLB that best stabilizes the emulsions. As it can be seen in Fig. 22, the emulsions formulated with HLB 4, 6, 8, and 10 drained approximately 10% and 15% at HLB 12 and 14, respectively, after 24 h, whereas those with HLB 12 and 14 have drainage greater than 80% of aqueous phase after centrifugation. Although the emulsions with HLB 6, 8, and 10 drain 50%, these results are promising because the used centrifugal stress was high. Therefore, emulsions can be formulated in the HLB range of 6–10 when using vegetable oil. Thus, the range of HLB for stabilizing the emulsions varies according to the oil used.

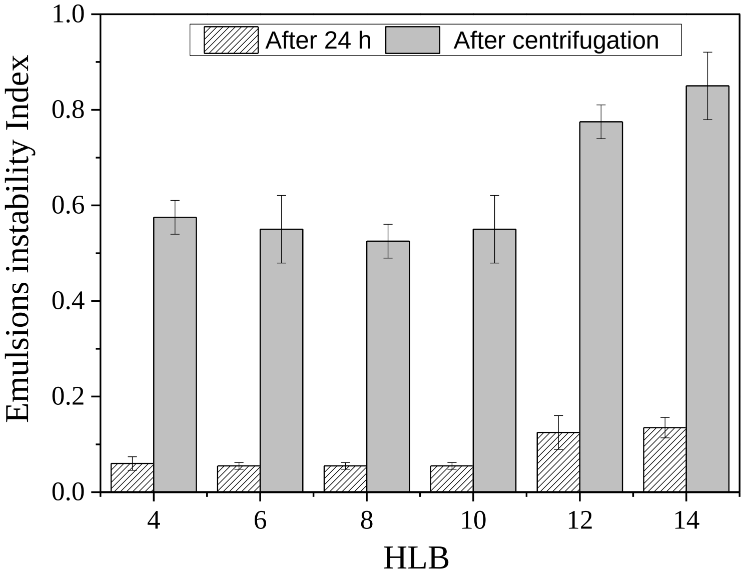


Figure 22. Stability of vegetable oil in water emulsions prepared at different HLB values, after 24 h and after centrifugation (at 2566 RCF for 10 min), the error bar is the product of three measurements.

The stabilization mechanism with the vegetable oils is mainly by adsorption of the surfactants at the oil–water interface reducing of surface tensions. The surfactants used in this study, being non-ionic, may also to stabilize the drops by steric hindrance. In addition, emulsions formulated with soybean oil have greater stability than emulsions formulated with mineral oil because the soybean components favour the adsorption of these surfactants at the interface, due to the presence of polar groups in the oil structure as shown in Table 1. The importance of formulating using vegetable oils lies in their possible replacement by essential oils, which have potential as repellents. However, for research purposes, we continued the tests with the vegetable oils.

Because we used 3 wt.% concentration of the surfactants to determine the optimal HLB and obtained stable emulsions, we inferred that it is necessary to use a low concentration of the surfactants. Therefore, we conducted tests to determine the minimum concentration of the surfactants to formulate stable emulsions.

3.3. Effect of surfactant concentration

Following the establishment of the optimal HLB 10 for the emulsions, another important compositional variable in the emulsion formulation to obtain is the minimum concentration of the surfactants that can stabilize the emulsions. To determine it, we formulated mineral oil-in-water emulsions using surfactant blends (Span®80/Tween®80) with various concentrations between 0.01 and 1 wt.%. As shown in Fig. 23, when the concentration of the surfactant mixture exceeds 0.1 wt.%, the amount of water drained is less than 20%. In contrast, when the concentrations are less than 0.1 wt.%, the amount of water drained is in the range of 40–70 v/v%, as shown in Figure 24.

These results reveal that the drainage of the aqueous phase decreases as the concentration of the surfactants increases. This is associated with more surfactants being available to better stabilize the oil–water interface, in addition to the delayed drainage due to the gravitational effect. The emulsions presented a cream formation; therefore, we conducted centrifugal field tests to determine whether a rupture or a phase separation of the emulsions occurred. The results showed that the emulsions did not present rupture; however, the amount of liquid drained was increased, which is in agreement with the results shown in Fig. 20 These results are consistent because the emulsions are subjected to an acceleration, which drains a larger amount of the fluid.

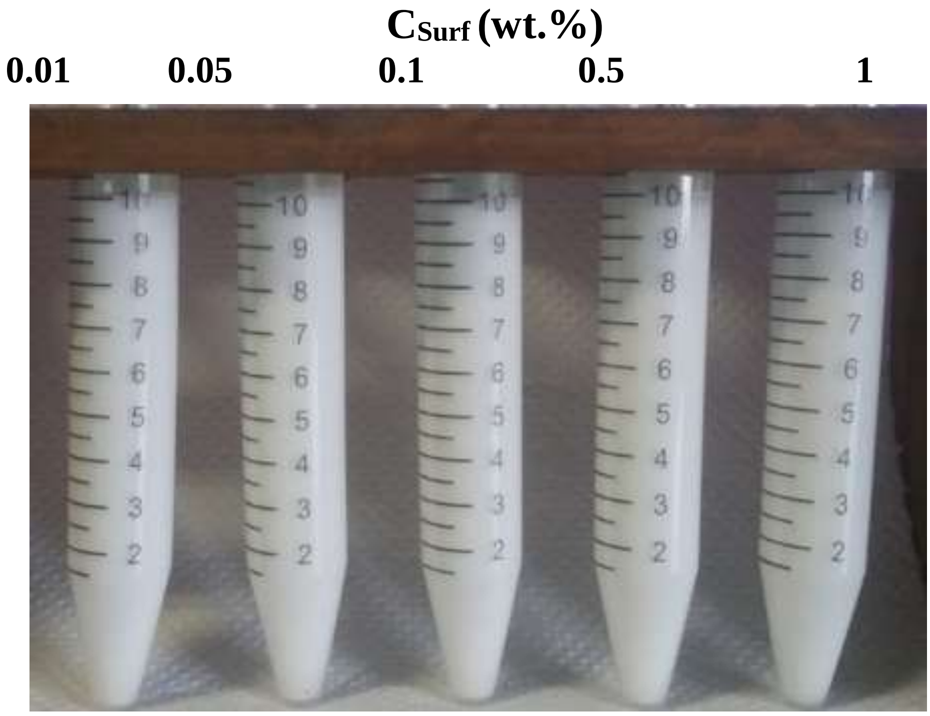


Figure 23. Initial stability of mineral oil in water emulsions using a mixture of surfactants Span®80/Tween®80 at different total surfactant concentration. Pictures taken immediately after preparation.

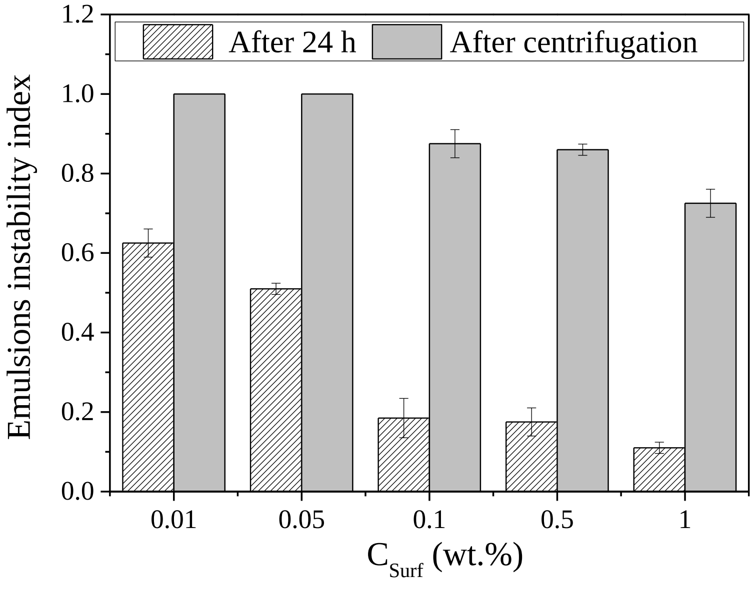


Figure 24. Stability of mineral oil-in-water emulsions prepared at different total surfactant concentration, after 24 h and after centrifugation (at 2566 RCF for 10 min), the error bar is the product of three measurements.

As before, the emulsions with a higher concentration of the surfactants showed higher stability, i.e., a smaller amount of the drained liquid. For further experiments, the concentration of 0.5 wt.% was chosen, despite the fact that 1 wt% presented better performance because it has more surfactants stabilizing the oil-in-water interface.

The results to determine the minimum concentration of the surfactants to stabilize the emulsions using the vegetable oils show that the emulsions present water drainage up to the first 24 h, at concentrations less than 0.1% by weight of surfactants, as displayed in Figure 25. The Span®80/Tween®80 mixture cannot stabilize the oil–water interface. Therefore, we hypothesize that surfactant concentration is not adequate to stabilize the at O/W interface, resulting in an unstable interface and the consequent rupture of the emulsions.

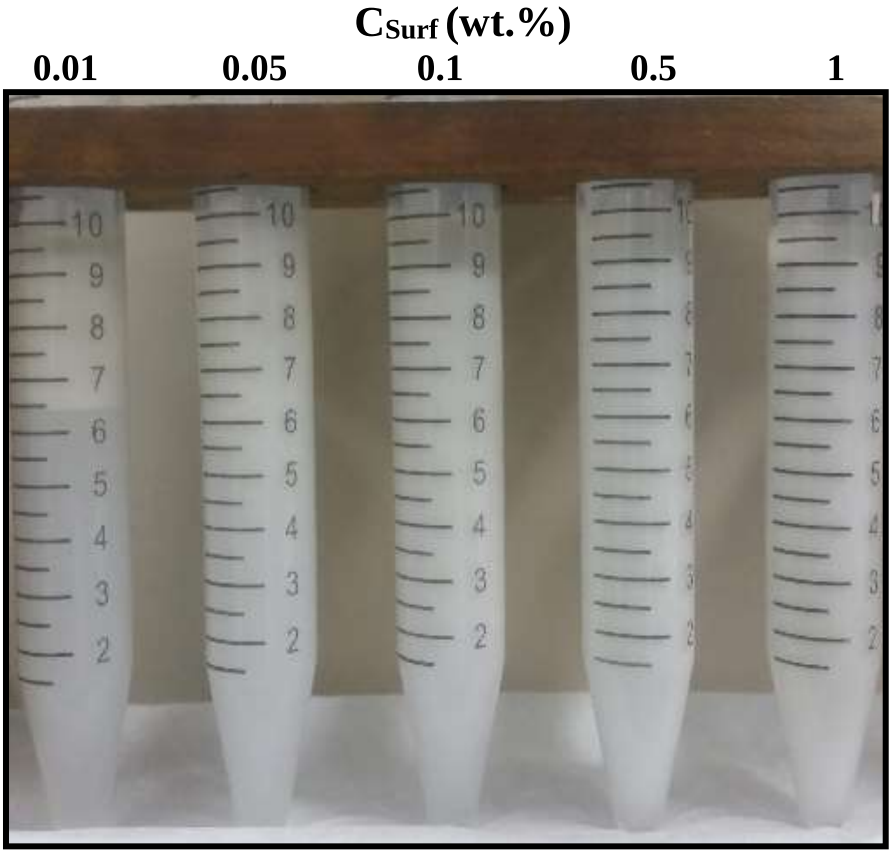


Figure. 25. Photographs of stabilized vegetable oil in water emulsions using different concentrations of surfactant (Span 80®/Tween®80).

Figure 26 shows that the amount of cream formed decreases as the concentration of the surfactants increases. This may be associated with the fact that more surfactants become available to stabilize the internal phase droplets, and thus, avoiding coursing and Ostwald ripening destabilization.

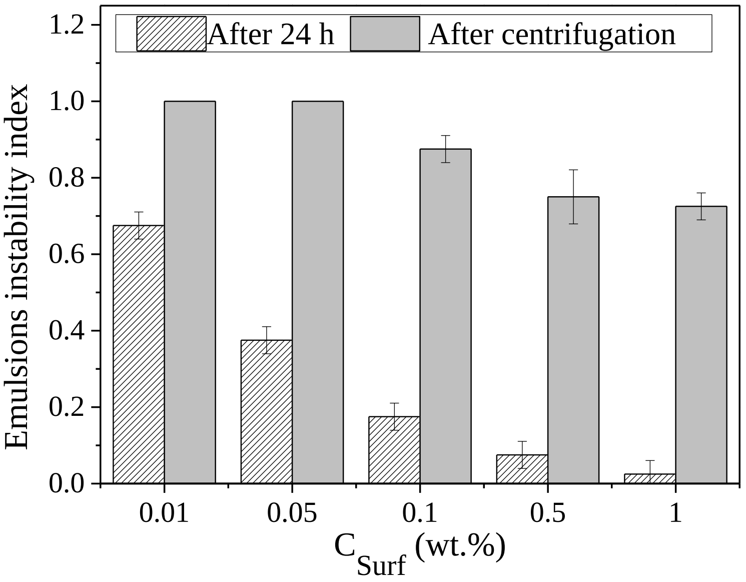


Figure 26. Stability of vegetable oil in water emulsions prepared at different total surfactant concentration, after 24 h and after centrifugation (at 2566 RCF for 10 min), the error bar is the product of three measurements.

Thus, to our best knowledge and understanding, this mixture of surfactants stabilizes the emulsions formulated with mineral and vegetable oils; however, those with the vegetable oils are more stable than the mineral oil emulsions. Therefore, we continued exploring the homogenization variable, specifically with mineral oil.

3.4. Exploration of mixing method to form emulsions

The homogenization method plays a fundamental role in forming emulsions and controlling their droplet size. Therefore, determining this formulation variables is expected to provide knowledge for formulating the emulsions. Consequently, in the study presented in this session, we examined three homogenization methods to identify the most appropriate one to use for the formulations.

The evaluated methods were manual, Ultra-Turrax homogenizer, and ultrasound, with each method offering its own advantages and disadvantages. The manual method has the advantage that it uses a small amount of energy to form emulsions; however, the droplets produced are large. The Ultra-Turrax homogenizer uses moderate energy and controls the sizes of the droplets based on the stirring speed.

Finally, ultrasound generates droplets with sizes of less than 1 µm, which favour absorption through the skin or rapid evaporation in the case of a volatile content. However, its energy consumption is higher than the other methods mentioned above, which will increase the production costs of the emulsions.

3.5. Evaluation of mixing method

The o/w emulsions in a ratio of 30:70 were homogenized using manual methods, an Ultra-Turrax homogenizer, and a sonicator. These emulsions were prepared at a concentration of 0.5 wt% of surfactants and HLB 10. The emulsions were stabilized by mixing non-ionic surfactants in synergy with SiNPs and modified SiO_2. SiNPs were added to formulate stable emulsions without the need to increase the concentration of the surfactants, transition towards the use of more sustainable materials, and develop controlled release emulsions. The emulsions were evaluated by characterizing the droplet size of the internal phase, macroscopic stability, morphology of the internal phase, and viscosity. These parameters are important because they define the functionalities and applications of the formulations.

3.5.1. Macroscopic stability of emulsions

We formed emulsions using the three homogenization methods, which are shown in Figure 27. The stabilization of the emulsions can be explained by the adsorption of the NPs and surfactants at the liquid–liquid interface. The emulsions homogenized manually and using the Ultra-Turrax homogenizer, after 1 h start to cream, which is an early stage of destabilization; this aggregation becomes more evident after 72 h. This process may be associated with a deficiency of the surfactant adsorption at the oil–water interface or difference between the densities of the phases.

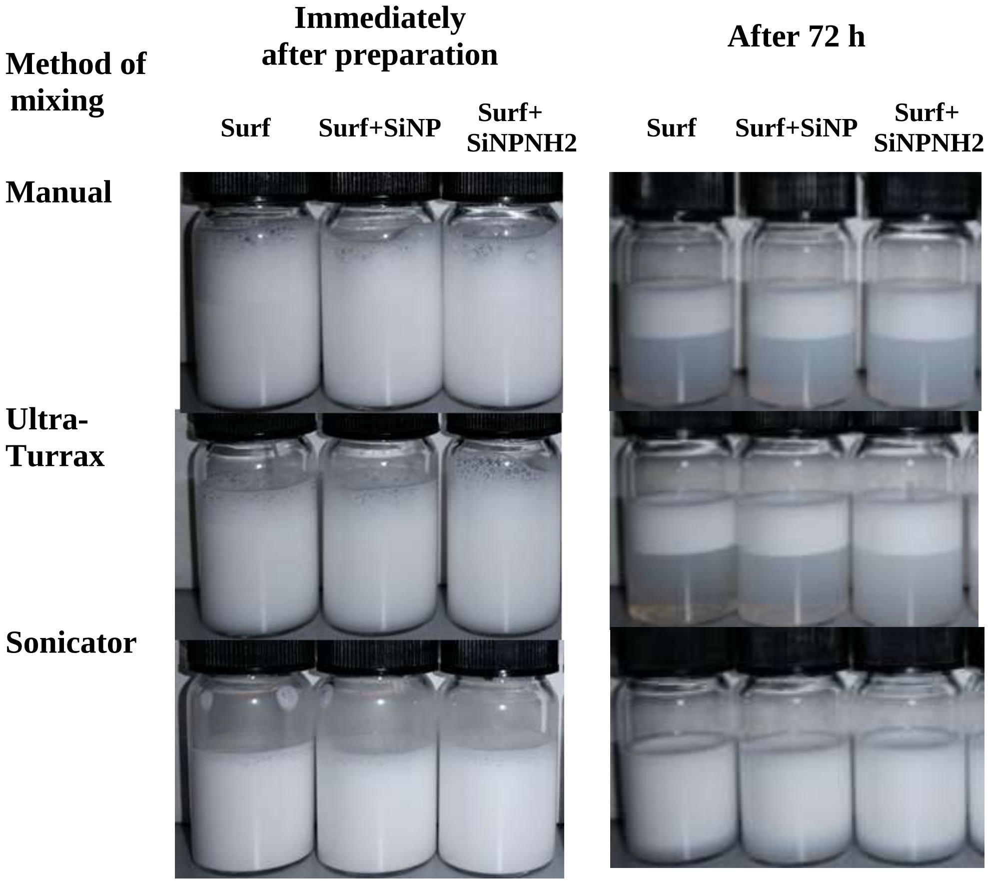


Figure 27. Stability of mineral o/w emulsions prepared with a mixture of 0.5 wt.% surfactants (Span®80/Tween®80) and 0.01 wt.% nanoparticles.

In contrast, the emulsions homogenized by the sonicator do not present cream formation over 72 h, owing to the adequate adsorption of the surfactants at the oil–water interface. This method favours the formation of an appropriate droplet size that delays the drainage of the aqueous phase. Ultrasonic emulsification has been extensively used in research studies owing to its ease of emulsion formation, high energy efficiency, and simple operation method.

Ultrasound emulsification forms droplets using acoustic cavitation, capillary surface waves, and liquid microjets, which fractionate the liquid, generating droplets of the internal phase, and thereby, achieving emulsification.

The emulsions containing SiNPs/Tween®80/Span®80 do not present macroscopic differences compared to those only formulated with the surfactants (Span®80/Tween®80). We hypothesize that this may be associated with SiO_2 being adsorbed at the o/w interface, stabilizing the interface, thereby avoiding the coalescence of the droplets and not playing the role of a rheological modifier. Therefore, our emulsions could be used as vehicles for the development of mosquito repellents.

The stabilization mechanism using colloidal NPs is the formation of a layer of densely packed particles at the oil–water interface and prevention of coalescence of the emulsified droplets by the generated electrostatic and steric repulsive forces.

However, the stabilization using NPs does not prevent the drainage of the aqueous phase (continuous), probably because the droplet size of the dispersed phase favours aggregation and separation according to the density. To verify the hypothesis that these emulsions form a cream when the droplet size exceeds 50 µm, we performed droplet size measurements.

3.5.2. Droplet size distribution analysis

The droplet size is a parameter for predicting the microscopic and macroscopic stability of an emulsion. It depends on several factors, among which the homogenization method and the surfactants or the particles used to stabilize the emulsions are relevant. If the homogenization method requires high energy (sonicator or more than 5 min in an Ultra-Turrax homogenizer), smaller droplets are generated compared to a low-energy method (manual or less than 5 min in an Ultra-Turrax homogenizer). Therefore, the droplet size depends on the stabilizers and the homogenization method.

We evaluated the droplet size distribution to determine the microscopic behaviour of the emulsions and predict their stability. We formulated co-stabilized emulsions with modified and unmodified NPs and SiO_2 in addition to the use of the surfactants.

Table 2. Effect of mixing method on the volumetric diameter (D_{90}) of emulsion droplets in o/w emulsions obtained with different stabilizers, reported results is the average of 5 measurements.

Mixing Method	D_{90} (µm)					
	Surf		Surf+SiNP		Surf+SiNPNH2	
	1 h	72 h	1 h	72 h	1 h	72 h
Manually	96.4	97.5	77.2	100.4	38.3	67.9
Ultra-Turrax	27.3	27.2	26.1	25.3	23.9	24.6
Sonicator	1.23	1.09	1.15	1.11	1.12	1.11

Based on Table 2, the droplet size decreases as the energy supplied to prepare the emulsions increases. This is associated with a higher energy favouring the adsorption of surfactants or NPs at an o/w interface, in addition to breaking large droplets into smaller ones until a size limit is reached.

From Table 2, we can infer that the NPs tend to stabilize smaller droplets, which may be associated with their coverage of the oil–water interface, which present a smaller area using the low-energy method (manual).

From the droplet size results, we can affirm that the manual method is more susceptible to modifying the droplet size when the NPs are added to the formulations. In contrast, when we homogenize using the Ultra-Turrax homogenizer or the sonicator, there is no significant difference with the use of the NPs. Therefore, we hypothesize that these medium and high-energy methods favour the dispersion of the continuous phase into droplets of a uniform size, regardless of the presence of the NPs.

We determined the droplet sizes of the emulsions after 72 h to determine if they had changed as shown in Table 2. The droplets of the manually homogenized emulsions increase in size, particularly of the emulsions formulated with the NPs. This may be attributed with the fact that this method does not favour the adsorption of NPs at the o/w interface. In contrast, when we use the Ultra-Turrax homogenizer or the sonicator, the droplet sizes do not undergo significant changes over time.

The smaller size and low polydispersity of the droplets of the internal phase can be attributed to the high energy released by the sonicator, equivalent to 2200 W, which causes a turbulent flow that uniformly disperses one of the phases. The Ultra-Turrax homogenizer is efficient because a high degree of homogenization is ensured at 13500 rpm for 2 min, which generates drops with small sizes and adequate polydispersity. The manual method, having the lowest energy released, produces droplets with large sizes and high polydispersity. The size distribution after 72 h can be seen from Table 2, which shows that the manually generated emulsions undergo a slow coalescence process, except those co-stabilized with SiNP and SiNPNH2.

The Ultra-Turrax homogenizer or ultrasound homogenized emulsions maintain their initial droplet sizes. From these results, we can affirm that the Ultra-Turrax homogenizer homogenized emulsions do not start the coalescence process, despite presenting cream formation. However, when we use the sonicator, the emulsions do not show any signs of destabilization.

The kinetic stability of the droplets is attributed to factors such as the high shear that they are subjected to during homogenization, which favours the adsorption of the surfactants and the NPs at the oil–water interface. The effect of ultrasound on emulsification is more effective than that of the Ultra-Turrax homogenizer because the cavitation energy generates more power than the turbulent flow generated by the latter.

One of the advantages of using a sonicator is that it allows formulating emulsions with a high content of the dispersed phase. Ni *et al.* used nanocellulose and a sonicator to formulate emulsions of high physical stability; however, they needed to use concentrations higher than 1 wt% to stabilize the emulsions. In this study, we used a low concentration of SiNPs (0.01 wt%) and 0.5 wt% of the surfactants, which allowed the stabilization of the emulsions. Therefore, the use of surfactants and materials can be an alternative for formulating stable emulsions for a long period of storage.

These droplet size results provide relevant information on the microscopic stability of the emulsions. Following the investigation of the droplet sizes of the emulsions, we determined the morphology of the drops.

3.5.3. **Characteristics of emulsion droplets**

The droplet sizes generated by the different mixing methods are discussed individually. Optical microscopy allows the morphology of droplets to be obtained, and thus, the microscopic behaviour of the corresponding emulsions can be predicted. Therefore, we evaluated the droplet morphology of the emulsions homogenized manually, using the Ultra-Turrax homogenizer and with the sonicator. We present the results of optical microscopy in the following order: manual method, Ultra-Turrax homogenizer, and sonicator. Fig. 28 shows the optical microscopy results of the emulsions produced using the manual method. For a better understanding, the emulsions formulated with the surfactants, surfactants plus SiNPs, and modified SiNPs are referred as A, B, and C, respectively.

The drops present spherical morphology and polydispersity, and the polydispersity decreases in the following order of emulsions: A, B, and C. The polydispersity is obtained through the graphs obtained with the equipment.

These results agree with those obtained based on the droplet size distribution analysis: the emulsion co-stabilized by SiNPNH2 is the least polydisperse. These results also suggest that the modified SiNPs favour the formation of smaller droplets, in contrast when the SiNPs are not used to formulate the emulsions, which contributes to the stability of the emulsions. Additionally, we can affirm that these are simple emulsions, because there are no drops within the drops. These results are favourable for application in various areas of cosmetics and personal care.

Fig. 28 shows the optical microscopy results of the emulsions homogenized by the Ultra-Turrax homogenizer, recalling that the operating conditions of the Ultra-Turrax homogenizer were a speed of 6500 rpm for 5 min. The droplets of the emulsions formulated only with surfactants **(A)** present polydispersity and spherical morphology.

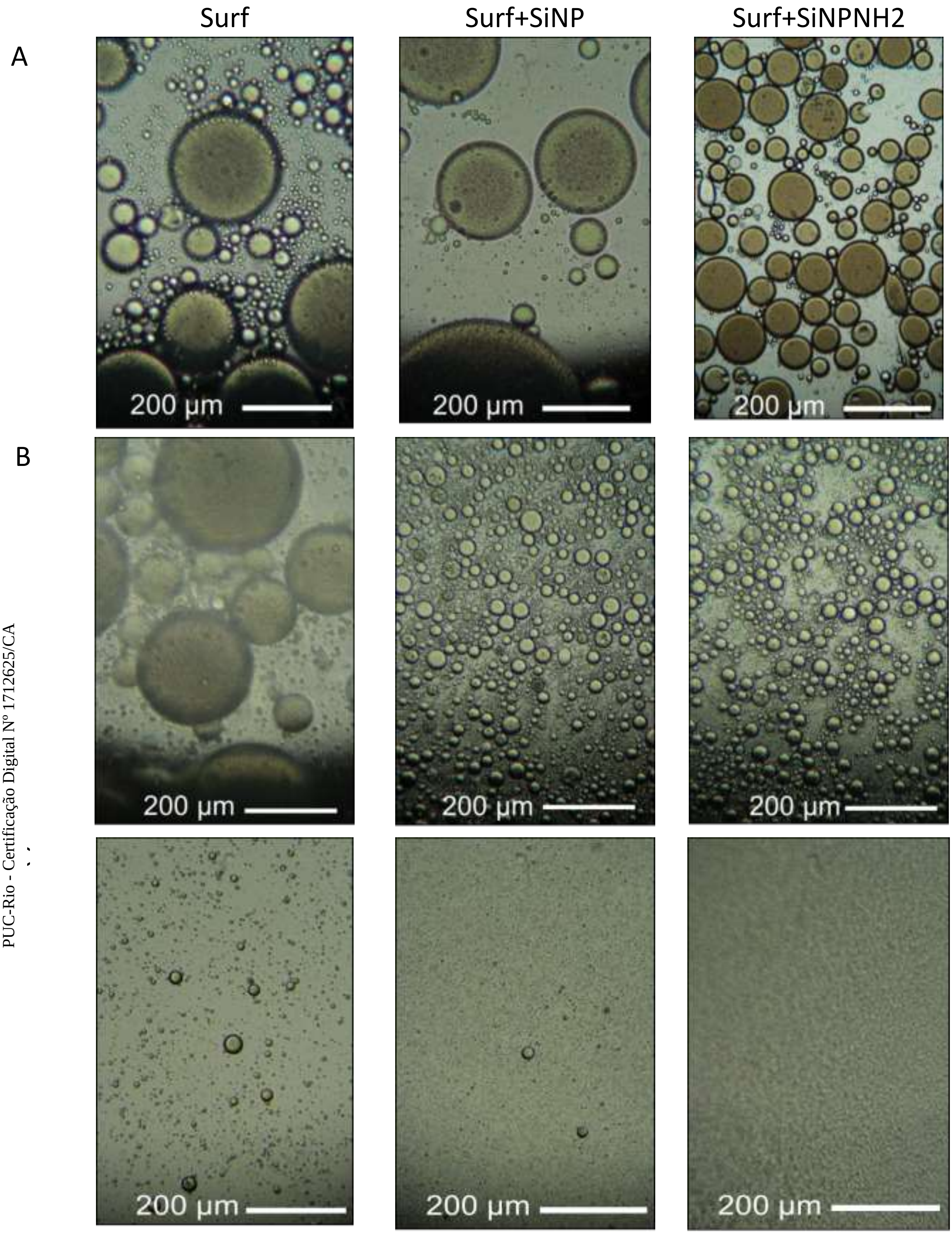


Figure. 28. Optical microscopy of mineral o/w emulsions stabilized using different stabilizers and mixing methods: (A) Manually, (B) Ultra-Turrax, (C) Sonicator. (Images were obtained 72 h after preparation).

In contrast, the emulsions formulated with the surfactants and NPs present monodisperse drops and spherical morphology, when we use the Ultra-Turrax and sonicator as homogenization methods.

Therefore, we can hypothesize that formulations **B** and **C** are more stable than formulation **A**. This greater stability is related to the fact that the NPs are adsorbed at the oil–water interface, which prevents droplet coalescence. These results are encouraging for the development of emulsions for use in cosmetics and personal care and for controlled or slow-release repellents.

Fig. 28 shows the optical microscopy results of the emulsions obtained using the sonicator for 2 min at 30% amplitude. The ultrasonically homogenized emulsions have spherical morphology and uniform particle size. There are no significant differences in the droplet sizes and morphology of the different formulations. When we emulsify with the sonicator, we obtain monodisperse droplet sizes owing to the high energy emitted by the ultrasound; this method disperses and breaks the liquid, which becomes the dispersed phase. These droplet sizes of less than 1.3 μm generate kinetically stable emulsions [66]. We find that when the sonicator is used, the smallest droplet size is obtained; these results are consistent with those for the droplet size distribution. It should be noted that because the droplets are extremely small, they cannot be clearly seen by optical microscopy.

The optical microscopy analyses results are in good agreement with the droplet size distribution (discussed above). The manual method offers the advantage of being inexpensive; however, it has the disadvantage of generating large droplets, which leads to macroscopic instability. In contrast, the sonicator method has the advantage of generating small droplets, which generates stable emulsions, whereas it has the disadvantage that these droplet sizes allow high absorption through the skin and rapid evaporation for the production of repellents.

Therefore, the method that presents the most advantage for the formulation of emulsions and development of repellent formulations is the Ultra-Turrax homogenizer. This is because it generates monodisperse droplets of sizes that should not favour adsorption through the skin, in contrast to the high polidispersity shown in the manual method or the very small size (more likely to permeate through the skin) obtained by sonication. The more homogeneous size distribution also contributes to a more stable emulsion, retarding the mechanisms of Ostwald ripening and coalescence, which is desirable for releasing the repellent in a more controlled fashion. In the next study, we evaluated the rheological behaviour of the emulsions to learn more about their performance.

3.5.4. **Rheology of emulsions**

Rheology allows the evaluation of how fluids behave at rest or under a shear rate. For the development of cosmetics, it is important because it provides information on how an emulsion will behave during storage (stock stability) and how it will move under stress application (application on surfaces). We evaluated the rheological behaviour of the homogenized emulsions mixed manually and using an Ultra-Turrax homogenizer and a sonicator. They were stabilized with mixed surfactants (Span®80 /Tween®80) (a) and mixed with SiNPs without modification (b) and modified SiNPs (c).

The rheology analysis showed that the emulsions generated under these conditions present Newtonian rheological behaviour, i.e., their behaviour is similar to that of water [67], as shown in Figure 31.

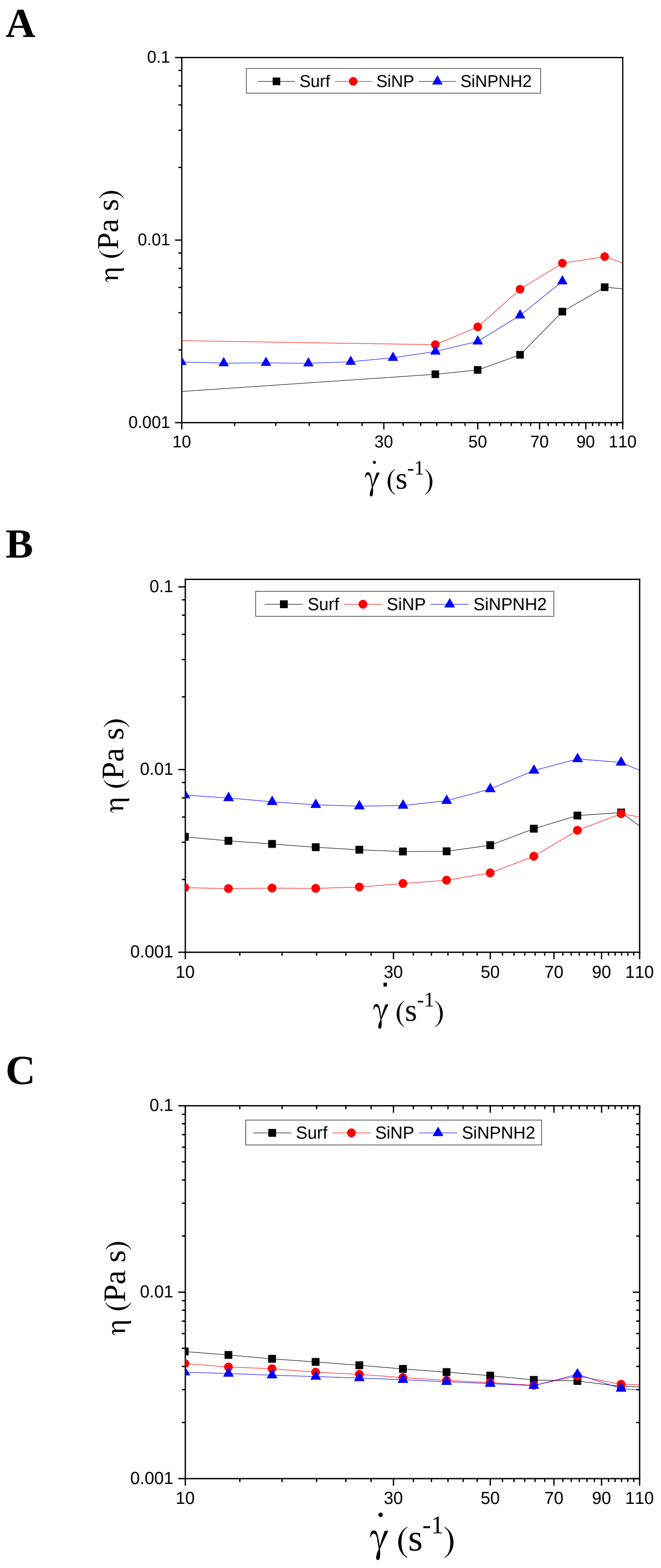


Figure. 29. Viscosity versus shear rate of mineral o/w emulsions generated with different mixing methods. A) Manually, B) Ultra–Turrax and C) Sonicator.

These emulsions were classified as Newtonian fluids because the viscosity remains constant as the shear rate increases. This is attributed to the concentration of the NPs being inappropriate to allow them to act as a rheological modifier. Their behaviour is similar to that predicted by Einstein for the viscosity of a diluted particle suspension [68].

It can also infer that the emulsions homogenized manually and using the Ultra-Turrax homogenizer present turbulence at high shear rate, which could be due to the laminar flow being interrupted due to the breaking of large aggregates. Based on these results, it possible incorporate an agent as a rheological modifier to increase the viscosity of the emulsions. We hypothesize that this rheological modifier will also increase the stability of the emulsions, as it will prevent the drops from moving freely and from creaming.

3.5.5. Adsorption of materials at the oil-water interface

We investigate the role of each component at reducing the interfacial tension of the mineral oil-water interface (Table 3). Due to their hydrophilic nature, SiNPs do not present any activity to reduce interfacial tension. The CCNF reduces the interfacial tension, but it is a slow process, taking more than 600 s for its stabilization, which would mean homogenizing for an extended period. In contrast, Tween®80 rapidly reduces interfacial tension because the ethoxylated chains have an affinity for the mineral oil phase, facilitating emulsion formation. We obtained the lowest interfacial tension using a mixture of all the components because the mix of surfactants increases the interactions between the aqueous and oil phases.

Table 3. Interfacial tension of aqueous solutions/dispersions containing the emulsion stabilizers (oil phase was mineral oil).

	IFT (mN m-^{1})		
	t = 60 s	t = 180 s	t = 800 s
Water	64.88	63.36	60.67
SiNP	66.2	66.33	66.01
CCNF	59.98	55.31	35.41
Tween80	12.77	11.77	9.19
Span80	5.90	4.31	6.73
Tween®80/Span®80 (1:1)	3.34	3.18	------
CCNF/SiNP/Tween®80/Span®80	2.71	2.48	------

Figures 30 shows the interfacial tension behavior over time of the emulsion components and their mixtures. With the exception of the CCNF alone, all other components reached the equilibrium very fast, which indicated a rapid diffusion and adsorption at the interface. In the case of CCNF, the high molecular weight of the particles retards these processes, needing longer times for stabilization.

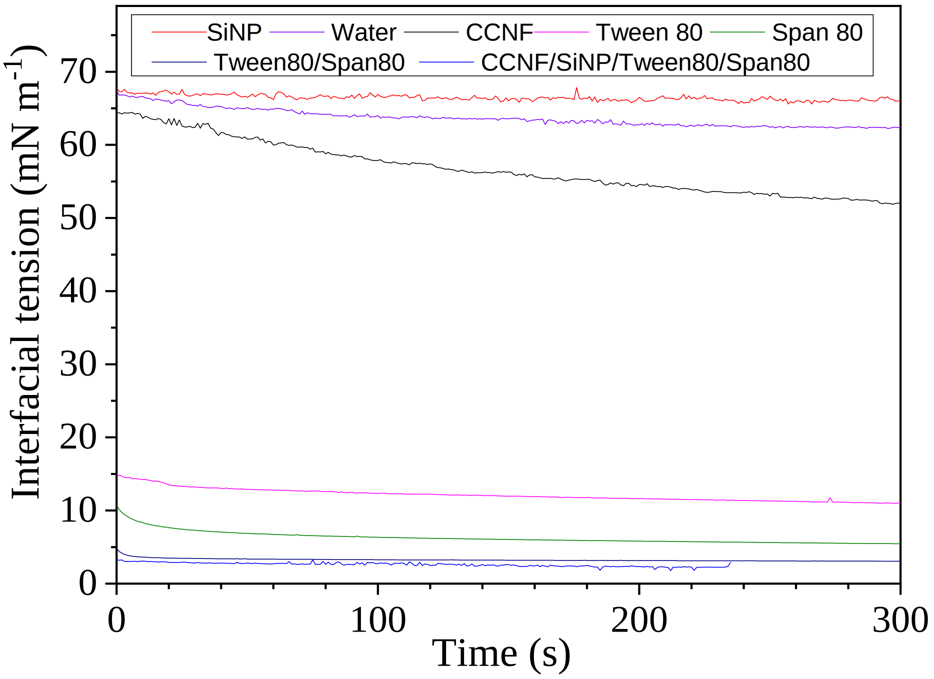


Figure 30. Interfacial tension profiles, as a function of time, of aqueous solutions/dispersions containing the emulsion stabilizers: SiNP (0.15 wt.%), CCNF (0.15 wt.%), Tween®80 (0.5 wt.%), Span®80 (0.5 wt.%). The oil phase was mineral oil, and measurements were performed at 25 °C.

In Figure 31 are shown the details of the drop shapes from the different interfacial tension experiments, at the beginning (1 s) and after 180 s. As can be seen, the surfaces of the systems containing the nanomaterials do not deform quickly, suggesting that these materials absorb slowly without deforming the oil in the water interface. For the surfactants, larger deformation was observed, typical of their fast interfacial adsorption and activity, leading to greater reduction of the interfacial tension.

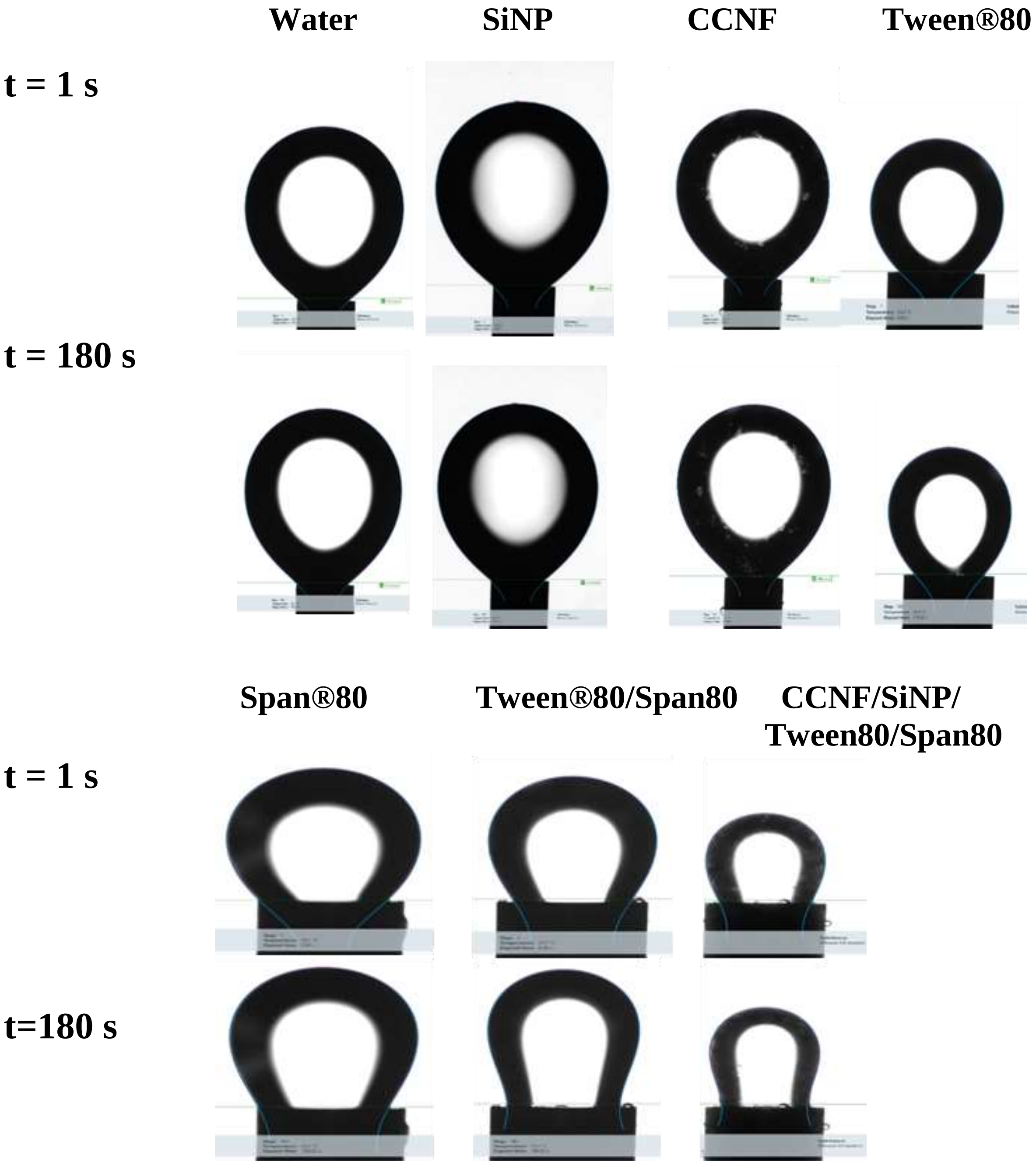


Figure 31. Drop shapes of mineral oil droplet surrounded by aqueous solution/dispersions of emulsion stabilizers.

3.6. Partial Conclusions

We formulated emulsions using both vegetable and mineral oils, regardless of their polarity, opening a route for developing repellent emulsions without alteration owing to their insolubility in the mineral oil. We determined the HLB required for a surfactant mixture of Tween®80 and Span®80 to formulate long-term stability emulsions containing DEET in the vegetable oils (HLB 8) and the mineral oil (HLB 10), using a surfactant concentration of 0.5 wt%.

The emulsions were stabilized using low concentrations of the surfactants and the NPs; however, these materials did not alter the rheological behaviour of the emulsions when we used manual and Ultra-Turrax methods. In contrast, the sonication method presented a different rheological behaviour, attributed to smaller droplets and therefore higher stability emulsions. The emulsions homogenized using an Ultra-Turrax homogenizer and a sonicator were stable, and did not show significant changes in the droplet sizes.

In the following steps in the development of this study, we evaluated the use of a rheological modifier to alter this property. For this purpose, cellulose was chosen as the rheological modifier.

The design and preparation of emulsions are fundamental in the current administration of repellents. We managed to vary and controlled different parameters such as the size and morphology of the drops by varying the homogenization method. The Ultra-Turrax homogenizer offered the best conditions because the obtained droplet sizes favour a controlled release, allowing a good dosage of the repellent. Future studies should be conducted to verify the repellence of mosquitoes as well as evaluate the acceptance of the final consumer by sensory tests. Additionally, tests are recommended to evaluate the penetration of the repellent into the skin.

3.7. Efficient encapsulation and controlled release of DEET from o/w emulsions stabilized by cationic cellulose and SiNPs

In the present study, we prepared o/w emulsions containing DEET stabilized using two oppositely charged nanomaterials (SiNPs and CCNFs), mediated by a mixture of food-grade non-ionic surfactants [69], 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. In the study below, it was shown that the strong stabilization of the droplets containing DEET can be obtained via enhanced interfacial adsorption of the nanomaterial complexes, and by the decreased diffusion due to a network formation in bulk.

3.7.1. Effect of pH on ζ-potential of aqueous dispersions of SiNPs and CCNFs

Both nanomaterials used in this study—SiNPs and CCNFs—contain functional groups, which determine their surface charge in an aqueous solution, and therefore, their possible electrostatic interactions. Thus, it is important to study the ζ-potential behaviour of the aqueous dispersions of the NP at different pH values, to understand the extension and nature of their interactions at the working pH and the possible behaviour under acid/basic conditions in the formulations. The ζ-potential measurements of the SiNP dispersions showed a predominance of a negative surface in the entire pH range studied, which was due to the deprotonation of the surface silanol (Si-OH) acid groups at pH values exceeding the isoelectric point (~2, Fig. 32) [56]. An opposite behaviour was observed for the CCNFs, because positive ζ-potentials were obtained, confirming the surface modification of cellulose with positively charged tetra-alkylammonium groups in the grafting with GTMAC. Interestingly, although the charge on the quaternized amine group in the CCNFs is pH independent, the ζ-potentials decreased with the increase in the pH.

This behaviour can be attributed to the presence of some carboxylic groups (pKa ~2.5) on the CCNF surfaces, originating from the residual α-cellulose in the pulp and some cellulose oxidation, which increase the dissociation as the pH increases [70]. Therefore, the decrease in the ζ-potential values is probably an effect of the compensation of the positive and negative charges on the CCNF surfaces [18]. In addition, the screening of charges due to the increasing ion concentration in the solution, as more NaOH was added, should be considered, since it could lead to the agglomeration of CCNF due to a decrease in intermolecular repulsions [71].

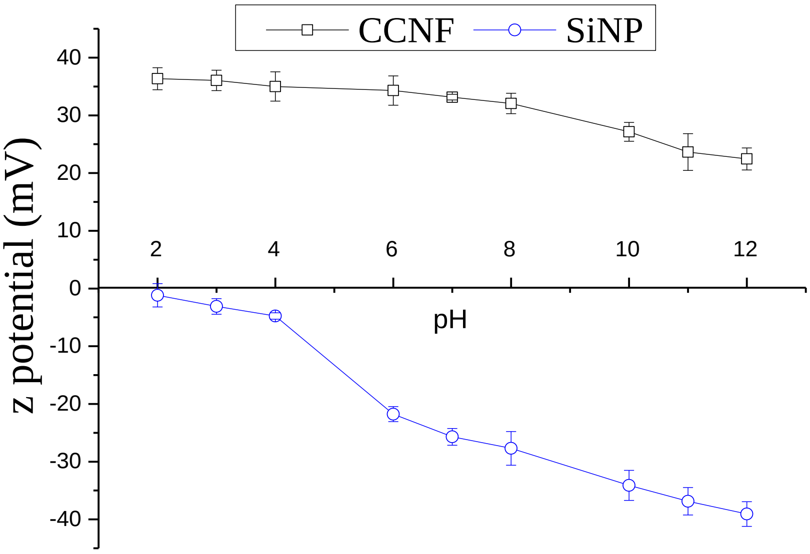


Figure 32. ζ-potential of aqueous dispersions of cationic cellulose (CCNF) and bare silica nanoparticles (SiNP) at different pH values.

The results of the surface charge behaviour of the nanomaterials in aqueous solutions as a function of the pH were in agreement with the differences observed in their hydrodynamic diameters (Figs. 33 and 34). In both cases, a tendency of increase in the size of the agglomerates in the solution as the ζ-potential decreases was observed following the changes in the pH. This also suggests that the DLS tests measured the sizes of the agglomerates, instead of the individual particle sizes.

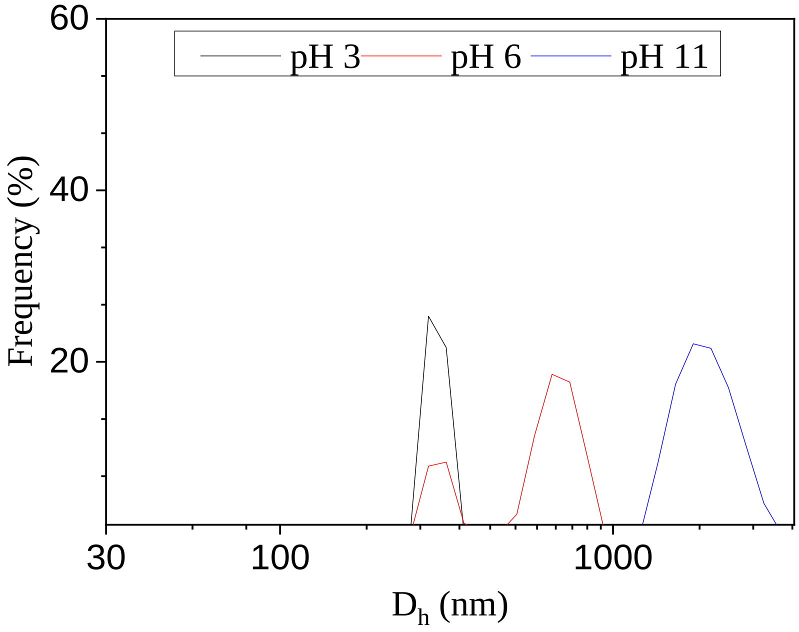


Figure 33. Size distribution profile of aqueous dispersions of CCNF at different pH values. (Dh: Hydrodynamic diameter).

Note that both the nanomaterials showed relatively high ζ-potentials (>|20| mV) at pH 6, which is the typical pH of the skin, [72] and therefore, the ideal condition for the current repellent formulations. The o/w emulsions produced in this study were prepared at pH 6, which was the resultant pH after incorporation of all components. The positive surface charge of the CCNFs, which is the opposite to that of the SiNPs at the working pH, can promote attractive interactions leading to electrostatic complexation, strengthening of network formation in bulk, and interfacial adsorption enhancement. Because strong complexation was expected between the charged nanomaterials used as stabilizers under these conditions, a mixture of non-ionic 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 emulsions during the preparation of the formulations.

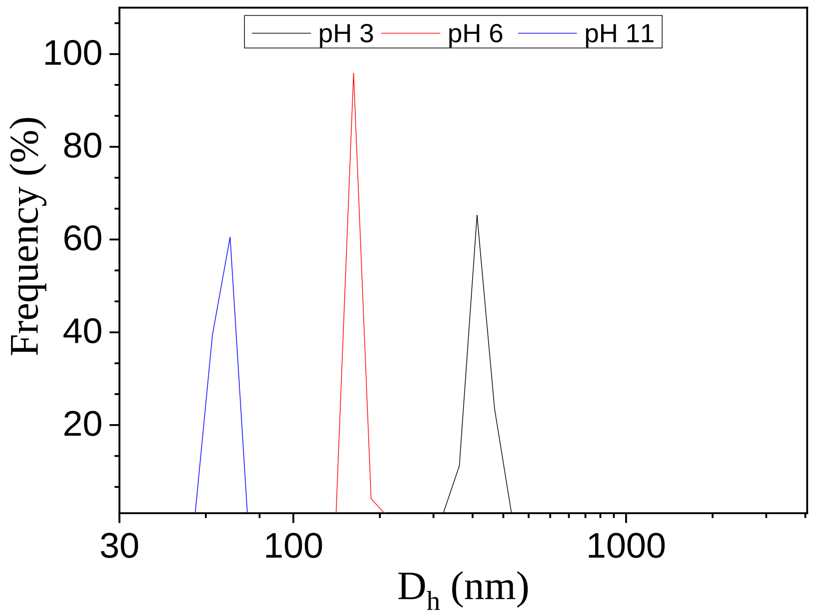


Figure. 34. Size distribution profile of aqueous dispersions of SiNP at different pH values. (Dh: Hydrodynamic diameter).

3.7.2. 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 formulations. High viscosity of the aqueous phase increases the stability of the corresponding emulsion by reducing the drainage and coalescence of the droplets. Because the CCNF aqueous dispersions can significantly affect the viscosity and rheological properties of the aqueous phase, a study to optimize the CCNF concentration in the emulsion formulation was conducted. Aqueous dispersions containing different concentrations of the CCNFs were prepared in surfactant solutions (0.5 wt% Tween®80), to resemble the external phase of the emulsions (Span®80 was excluded owing to its low solubility in water). [73] The results of the viscosity obtained at different shear rates showed that the presence of the CCNFs increased the viscosity of the aqueous phase. Moreover, at 0.3 wt%, the suspensions started showing a more pronounced shear thinning behaviour, i.e., a decrease in the viscosity with the shear rate (Fig. 35). 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, owing to a network formed of entangled fibrils [42]. In addition, above 0.8 wt% of CNNF there are no significant differences in the viscosity of the dispersions.

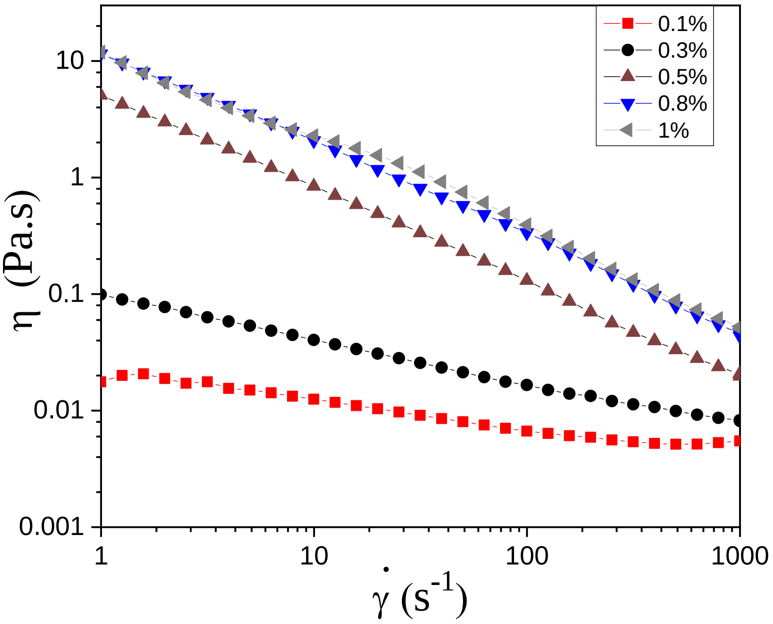


Figure 35. Flow curves for CCNF suspensions at different concentrations (wt%), suspended in a 0.5 wt% Tween 80 aqueous solution.

In general, the viscosities measured in this study are similar to the typical values reported in the literature for CCNF dispersions [61][70]. Based on these results, a CCNF concentration of 0.3 wt% was selected for the preparation of the emulsions containing DEET, because higher concentrations might lead to gelation, [74] which can affect the emulsion preparation protocol and the release of DEET.

The possible effect of SiNPs on the viscosity of the aqueous phase at the chosen CCNF concentration was evaluated. It was hypothesized that SiNPs can strengthen the network formation of the CCNFs in the aqueous solution via attractive electrostatic interactions. A SiNP concentration of 0.01 wt% was used to achieve synergy in the emulsion stabilization while avoiding extensive aggregation at the working pH (~6), where the CCNFs and SiNPs have opposite surface charges (as shown in the ζ-potential experiments). As can be seen from Figure 36, the presence of SiNPs, even at this low concentration, increases the viscosity of the aqueous solution containing 0.3 wt% CCNFs and Tween®80, maintaining the shear thinning behaviour.

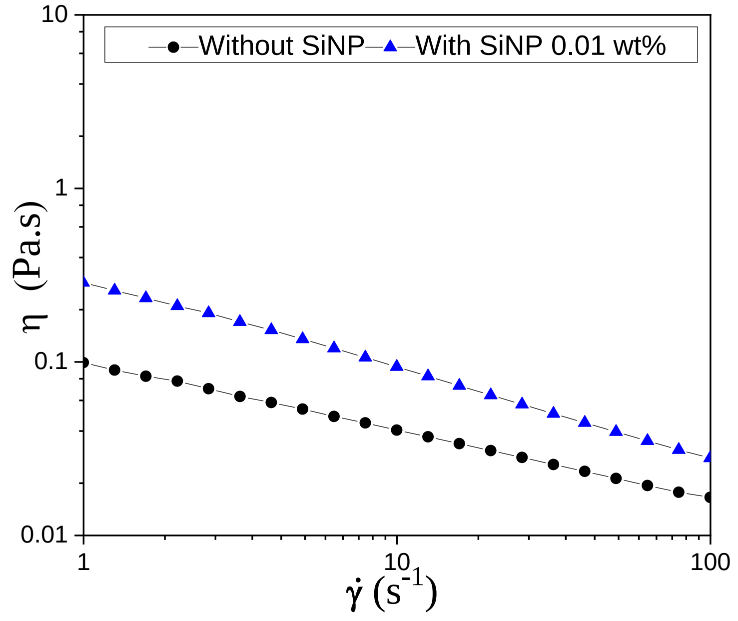


Figure 36. Effect of SiNP on rheological behavior of aqueous dispersions of 0.3 wt% CCNF, in 0.5 wt% Tween®80 solutions.

The latter is a desired characteristic for interesting formulations, because the viscosity can be decreased by skin rubbing, facilitating the spreading of the product and, thus, enhancing the delivery of the active compound. Based on the results obtained from the rheological characterization, CCNF and SiNP concentrations of 0.3 wt% and 0.01 wt%, respectively, were chosen for the formulation of the emulsions containing DEET. These were aimed at obtaining synergy in the interactions in the bulk and at the droplet interface to realize emulsion stabilization, without compromising the intended release of DEET from the emulsion droplets.

3.7.3. 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 mineral oil containing Span®80. This was followed by its high-shear mixing into the aqueous phase containing Tween®80 and CCNFs, SiNPs, or both nanomaterials. The use of DEET in repellent formulations is recommended in concentrations of 7–10% for short repellent action (up to 2 h) and 20–30% for longer periods (up to 6 h) [75]. In this study, a DEET concentration of 10 wt% was used to facilitate the evaluation of an extended release action and highlight the potential of the formulations for more efficient use of the active compound. For comparison, o/w emulsions were also prepared using only the mixture of the surfactants (Tween80®/Span80®), SiNPs, or CCNFs.

It was impossible to obtain an emulsified phase when using the nanomaterials (SiNPs, CCNFs, or their combination) in the absence of the surfactants, at the concentrations and conditions used in this study (Fig. 37). In the case of SiNPs, the very low concentration used in the formulation was insufficient to stabilize the oil–water mixture. A recent study by Silva *et al.* achieved stabilization of o/w Pickering emulsions using cationic nanocellulose at 0.5 and 1 wt% concentrations [42]. 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 the oil phase. However, these interactions cannot be considered for the light mineral oil used in the present study (and in common cosmetic and pharmaceutical formulations), which contains a mixture of saturated hydrocarbons. The partial polarity (log K_{ow} = 2.02; K_{ow}: octanol-water partition coefficient) [76] of the DEET molecules tends to reduce the partitioning of DEET in the mineral oil phases of the emulsions, decreasing the DEET encapsulation efficiency and the emulsion stability.

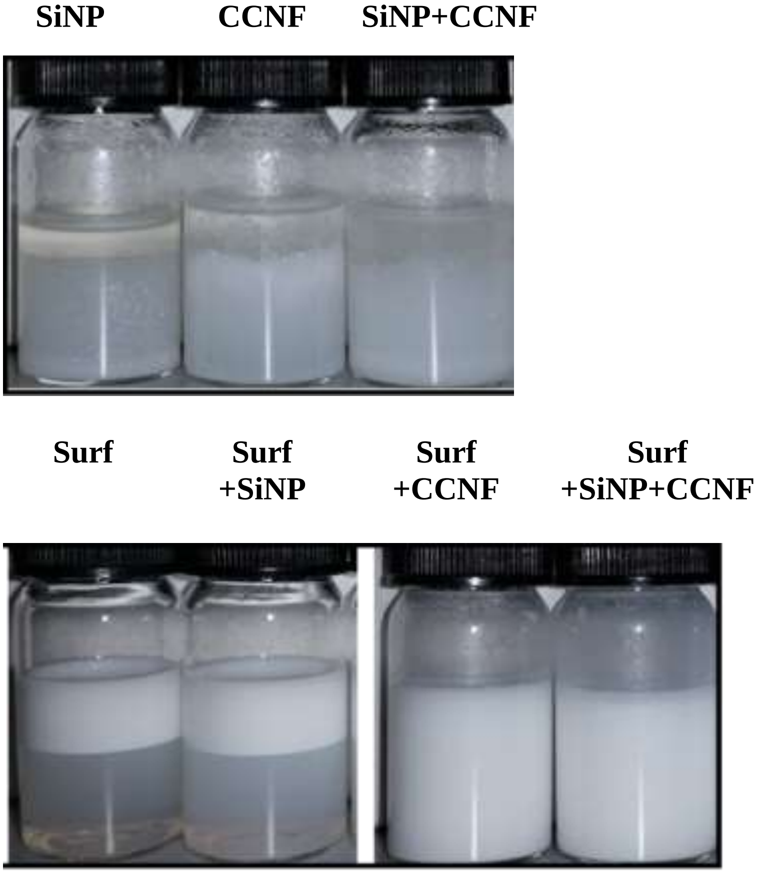


Figure 37. Photographs of mineral o/w emulsions containing DEET and stabilized with different stabilizers (0.5 wt% surfactants, 0.3wt% CCNF, 0.01wt% SiNP) after 24 h.

The combination of non-ionic surfactants allowed the formation of o/w emulsions; however, after 24 h, a creaming phenomenon was observed. This was 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 (Fig. 38). A similar result was obtained when combining the surfactants and SiNPs, exhibiting a phase separation after 24 h. However, the micrographs of the creamed phase showed smaller droplet diameters and a less polydisperse distribution (Figs. 38 and 39) than those of the surfactant-only stabilization (Fig. 38). The surface silanol groups in SiNPs can interact with the ethoxylated chains of Tween®80, and to some extent with the Span®80 headgroups, thereby strengthening the interfacial film and decreasing the coalescence. [77] However, these interactions are insufficient to maintain the emulsion stability over time.

The inclusion of the CCNFs in the formulations significantly improved the emulsion stability, both in the absence and presence of SiNPs (Fig. 38). These emulsions were stable for more than four months after preparation in the lab, not showing any indication of creaming or phase separation. The emulsion stability initially assessed by visual observation was confirmed by the reduced droplet size and increased homogeneous droplet distribution (Figs. 39 C, D). These microscopic properties are characteristics of stable emulsions, decreasing the coarsening and coalescence rates.

The diameter of the internal oil phase containing DEET was drastically reduced compared to that without CCNF. This supports the hypothesis of various mechanisms acting in synergy at both the interfacial film and in the bulk between the surfactants and CCNFs as well as the need for a multi-component system for emulsion stabilization. This is in agreement with the results reported for Pickering emulsions with CCNFs and coconut oil: increase in the emulsion stability and decrease in the droplet size after addition of a non-ionic surfactant [78].

Surf

D_{90}= 63 ± 50 µm

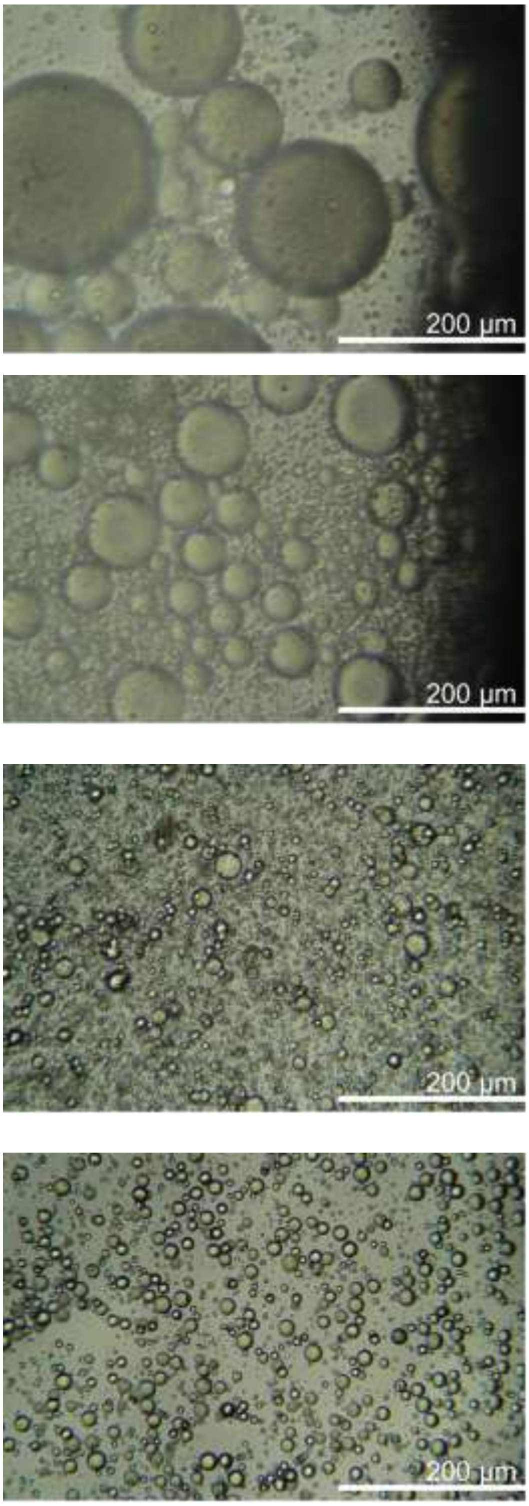


Figure 38. Images obtained by optical microscopy of mineral o/w emulsions containing DEET, that showed creaming phenomena (Surf and Surf+SiNP) or stable emulsion formation (Surf+CCNF and Surf+SiNP+CCNF). (Micrographs were taken 24 h after preparing the emulsions)

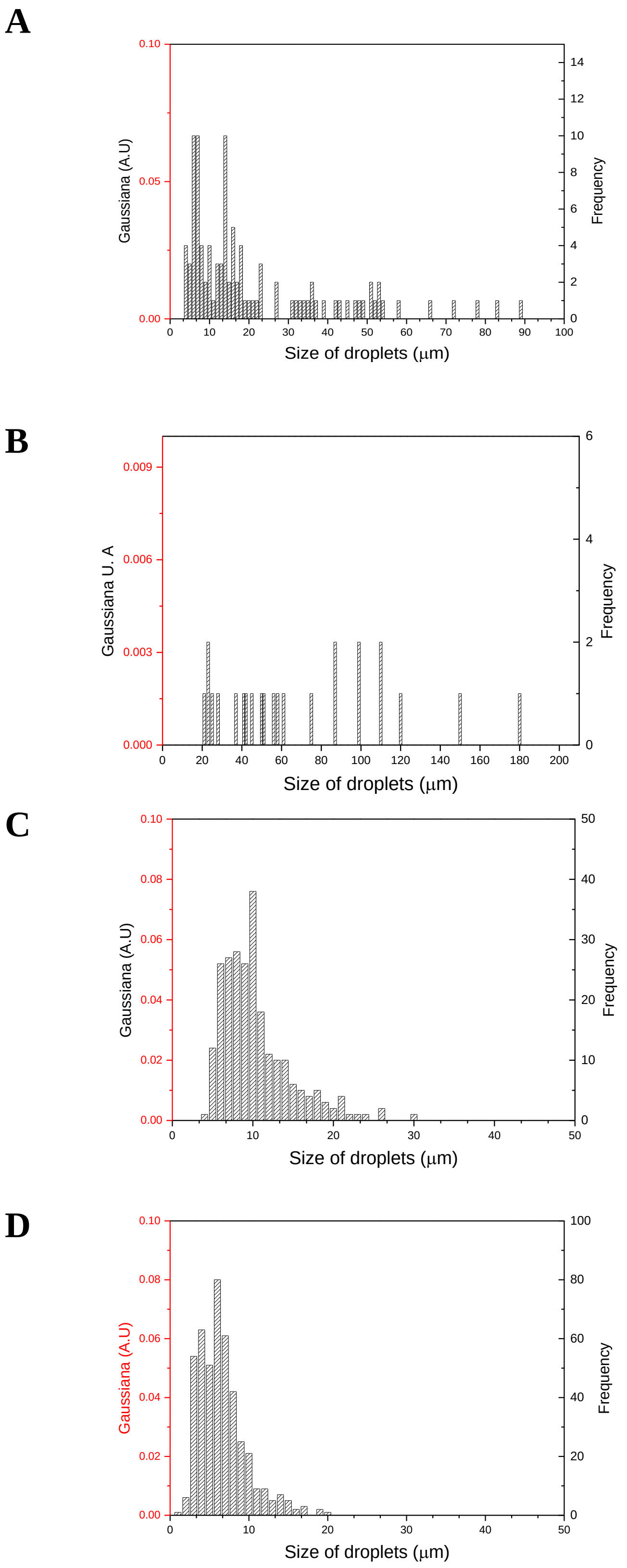


Figure 39. Histograms of size distribution of mineral o/w emulsions containing DEET that showed creaming phenomena (Surf and Surf+SiNP) or stable emulsion formation (Surf+CCNF and Surf+SiNP+CCNF). (Samples (A,B,C and D) were analyzed 24 h after preparing the emulsions.).

3.7.4. Effect of CCNF/SiNP interactions on rheological behaviour of emulsions

Interestingly, the presence of SiNPs, at the low concentration used in this study, did not significantly affect the macroscopic stability and droplet size and distribution of the o/w emulsions compared with the formulations containing only the surfactants and the CCNFs. In the former case, despite the electrostatic attractions 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 [55]. To further explore this effect, rheological measurements were performed for the stable emulsions obtained with the CCNFs, in the absence and presence of SiNPs (Fig. 40). Both emulsions presented a shear-thinning behaviour, and in the presence of SiNPs, a further increase in the viscosity occurred (ca. thrice), in agreement with the results obtained for the aqueous phases (Fig. 35).

The further increase in the viscosity of the emulsions observed in the presence of SiNPs indicates a strong association via electrostatic interactions with the CCNFs in the aqueous phase, even at the low concentration of these NPs. T. Gong *et al.* [74] found that the addition of colloidal SiNPs enhanced the viscosity of a dilute hydroxyethyl cellulose solution, leading to formation of gels by a mechanism involving physical adsorption of the NPs on the polymer [20]. In addition, electrostatic complexations were also expected to occur at the oil–water interface in the present study, increasing the emulsion stability via interfacial adsorption, which is shown in the emulsion stability results (Fig. 40).

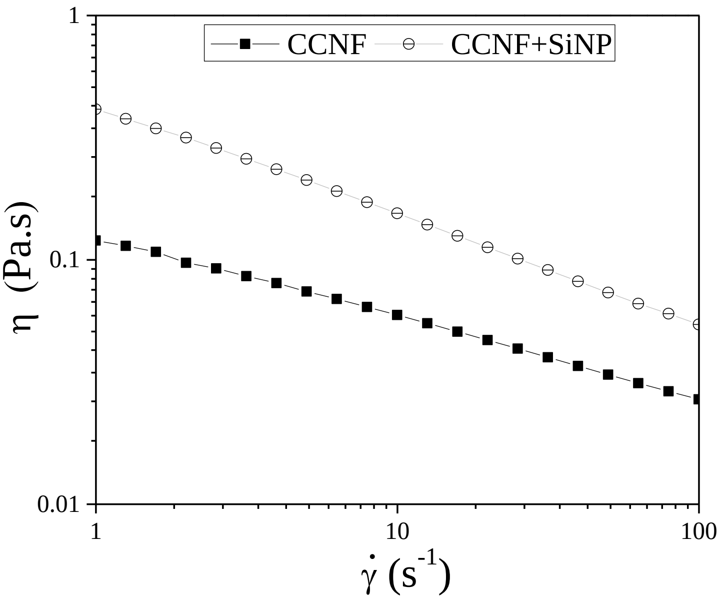


Figure 40. Effect of SiNP on rheological behavior of mineral o/w emulsions containing DEET and stabilized by 0.3 wt% cationic cellulose (CCNF) and nonionic surfactants.

Although, to our best knowledge, this combination of CCNFs and unmodified SiNPs has not been explored thus far for the stabilization of emulsions, similar systems that support this hypothesis are reported. Huan et al. recently studied using carbon nanofibrils (CNFs) and cationic nanochitin (NCh) to produce Pickering emulsions with sunflower oil. [79] They found that the interfacial adsorption of CNF/NCh complexes can be controllably tuned using the mass/charge ratio of the CNFs to the NCh. It can vary from a dominant dispersed system in the aqueous phase to a strongly associated system, both providing stable Pickering multiphase systems. In the present study, this concept was used to tailor the stability of the o/w emulsions containing DEET, using small amounts of SiNPs to retard the release of the active compound under ambient conditions (pH 6).

3.7.5. Physical destabilization of o/w emulsions by mechanical and thermal stress (heating)

The use of the nanomaterials with the non-ionic surfactant mixture produced very stable o/w emulsions that can retain DEET in the internal phase. Therefore, to investigate the impact of combining SiNPs with the CCNFs on the emulsion stability, accelerated destabilization tests were conducted. Specifically, the emulsions were submitted to centrifugation and heating, and the stability was assessed according to the extent of the separated phase. All emulsions showed a behaviour characterized by the release of free water; however, no oil was released after centrifugation and heating. This is in agreement with the results obtained by Aaen et al., [80] who found that emulsions stabilized by CNFs form a separated water phase after centrifugation, owing to the relatively stable network formed in the continuous water phase, trapping the oil droplets as well as adsorbing at the o/w interface. The results of these accelerated destabilizations tests also revealed that the emulsions containing SiNPs were more stable than those with the CCNFs and the surfactant mixture, owing to the reduced phase separation after centrifugation and heating at 45 °C (Fig. 41).

The difference in stabilities of the two systems was best observed following centrifugation, because the emulsion containing SiNPs had a smaller amount of the separated water phase (by ~20%) than the emulsion containing only the CCNFs and the surfactants [50]. This behaviour can be related to the additional stabilizing effect of the adsorbed electrostatic complexes formed between the CCNFs and SiNPs at the oil–water interface, hindering droplet coalescence. Lu et al. reported the stabilization of soybean o/w Pickering emulsions using an amphoteric lignin (modified with quaternary ammonium groups) and SiNPs, which synergistically adsorbed at the oil–water interface via electrostatic interactions. [50] The high stability of the emulsions was attributed to the coating of hydrophilic SiNPs with the polymer and the formation an interfacial gel-like structure with a certain rigidity, which effectively prevents the coalescence of the droplets.

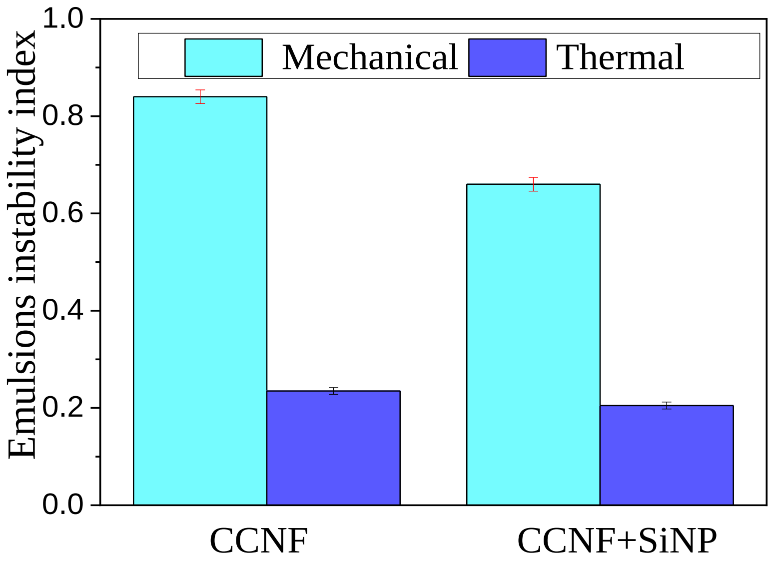


Figure 41. Destabilization of O/W emulsions containing DEET by mechanical (centrifugation) or thermal (T = 45 °C) stress (stability was assessed after 24 h of preparing the emulsions). Emulsion instability index represents extent of aqueous phase separation.

The effect of increasing the temperature on the emulsion stability was smaller than that of centrifugation, without significant differences between the two types of emulsions. The increase in the temperature increases the Brownian movement of the emulsion droplets. However, the presence of the CCNFs in both systems leads to a high viscosity of the aqueous phase (Fig. 36) and decelerating the migration speed and collisions of the droplets, which contribute to the stability of the emulsions.

3.7.6. Emulsion destabilization by pH and salt

In addition to the physical destabilization tests, emulsions were exposed to changes in their chemical conditions, to assess these destabilization effects on the interactions between the stabilizers, and thereby, on the 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), because these factors can significantly influence the extent of the electrostatic interactions responsible for the emulsion stabilization and the DEET retention. The results obtained in the presence of NaCl were similar to those found on centrifugation: the emulsion containing the SiNPs showed a smaller amount of the separated aqueous layer than the emulsion stabilized only by the CCNFs (Fig. 43).

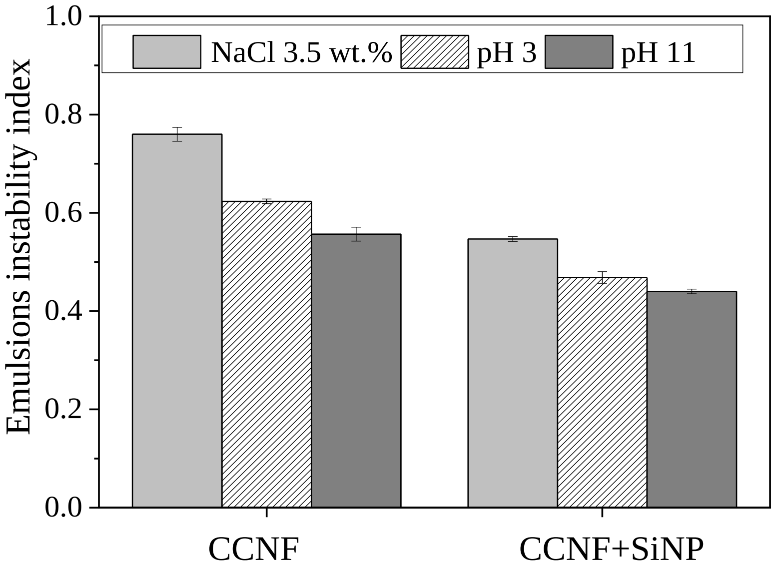


Figure 42. Destabilization of mineral o/w emulsions containing DEET upon addition of salt (NaCl) and varying pH (stability was assessed after 24 h of preparing the emulsions). Emulsion instability index represents extent of aqueous phase separation.

The presence of salt screened the charge on the cationic cellulose adsorbed at the oil–water interface, reducing the electrostatic repulsion between the droplets and promoting the droplet aggregation and coalescence (see ζ-potential values in Table 4).

Table 4. ζ-potential (Mean ± S.D.) of aqueous dispersions of cationic cellulose (CCNF) and bare silica nanoparticles (SiNP) at different pH values and in the presence of NaCl.

Sample	ζ-potential (mV)				
	pH 3	pH 6	pH 11	C_{NaCl} 1.8 wt.%	C_{NaCl} 3.5 wt.%
CCNF	37.1 ± 1.2	34.3 ± 2.5	23.6 ± 3.0	10.6 ± 1.4	9.8 ± 2.8
SiNP	-3.1 ± 1.4	-21.7 ± 1.3	-36.7 ± 23	-6.7 ± 0.5	-5.2 ± 0.7

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. This effect is probably counteracted to some extent by the presence of SiNPs at the droplet interface (~30% increase in the emulsion stability).

In experiments at different pH values, the emulsions also showed destabilization; however, the effect was smaller than that with NaCl. According to the ζ-potential results, the CCNFs have a high positive charge (> +30 mV) at pH 3 and a slightly lower pH at 11 (Fig. 32 and Table 4). The results showed that the extent of the aqueous phase separation is large at pH 3, because of the reduced adsorption at the oil–water interface, stronger repulsion among the adsorbed particles, and decrease in the stabilization of the droplets. Besides the effects on the interfacial adsorption, the pH can affect the strength of the entangled network in the aqueous phase, which is responsible for the increase in the viscosity [58]. At a higher pH, the probability of aggregation of the nanofibrils increases, causing a smaller amount of the structured water phase and reduced repulsion between the oil droplets, which contribute to the reduced emulsion stability.

In general, the emulsions containing SiNPs presented smaller instability indexes at both pH values than those containing only the CCNFs and the surfactants. This is explained in terms of the compensating effect of the SiO_2 surface charge (opposite ζ-potential behaviour with the pH) with that of the CCNFs, buffering the interactions at the droplet interface. As shown in Fig. 43, the emulsions maintained their macroscopic stability 6 h after salt addition and pH change; therefore, we can affirm that the formulations can be used as vehicles for the controlled release of repellents.

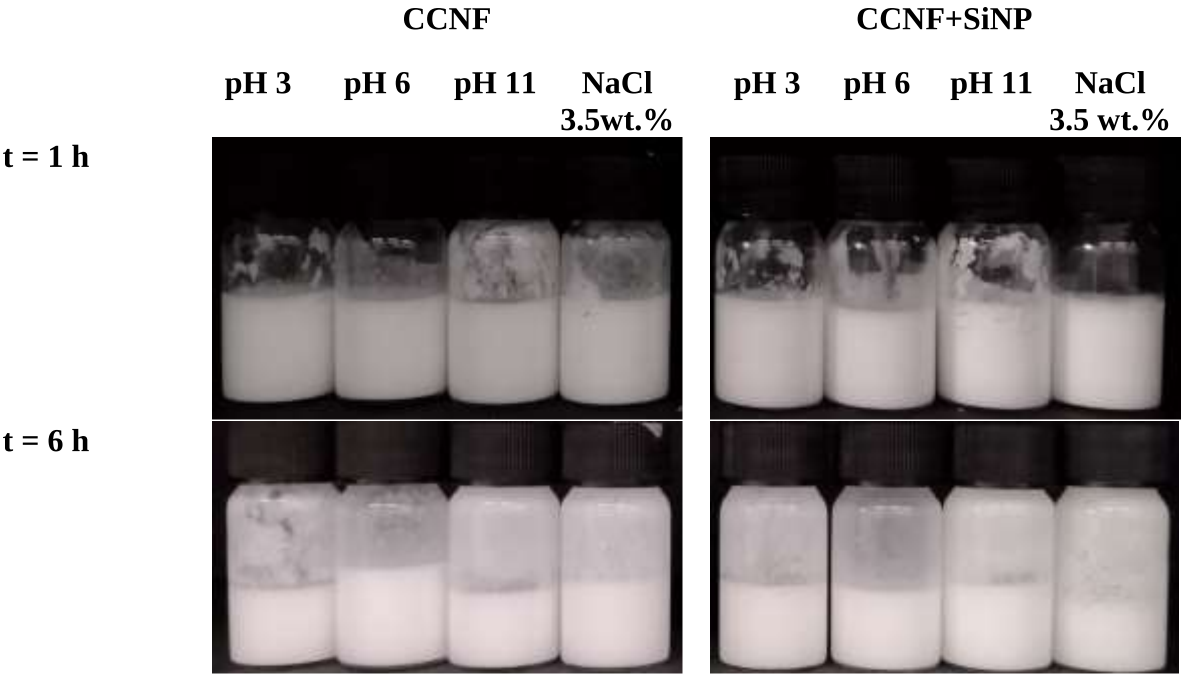


Figure 43. Digital images of mineral o/w emulsions containing DEET and stabilized with mixtures of surfactants, CCNF (0.3 wt.%) and SiNP (0.01 wt.%), upon addition of salt (NaCl) and varying pH.

According to the optical microscopy images in Fig. 44, when NaCl is added or the pH is changed from 3 to 11, the droplet size increase can lead to more release of DEET, under the study conditions. This increased release is a desired outcome because it leads to more protection when the formulation is exposed to these triggers.

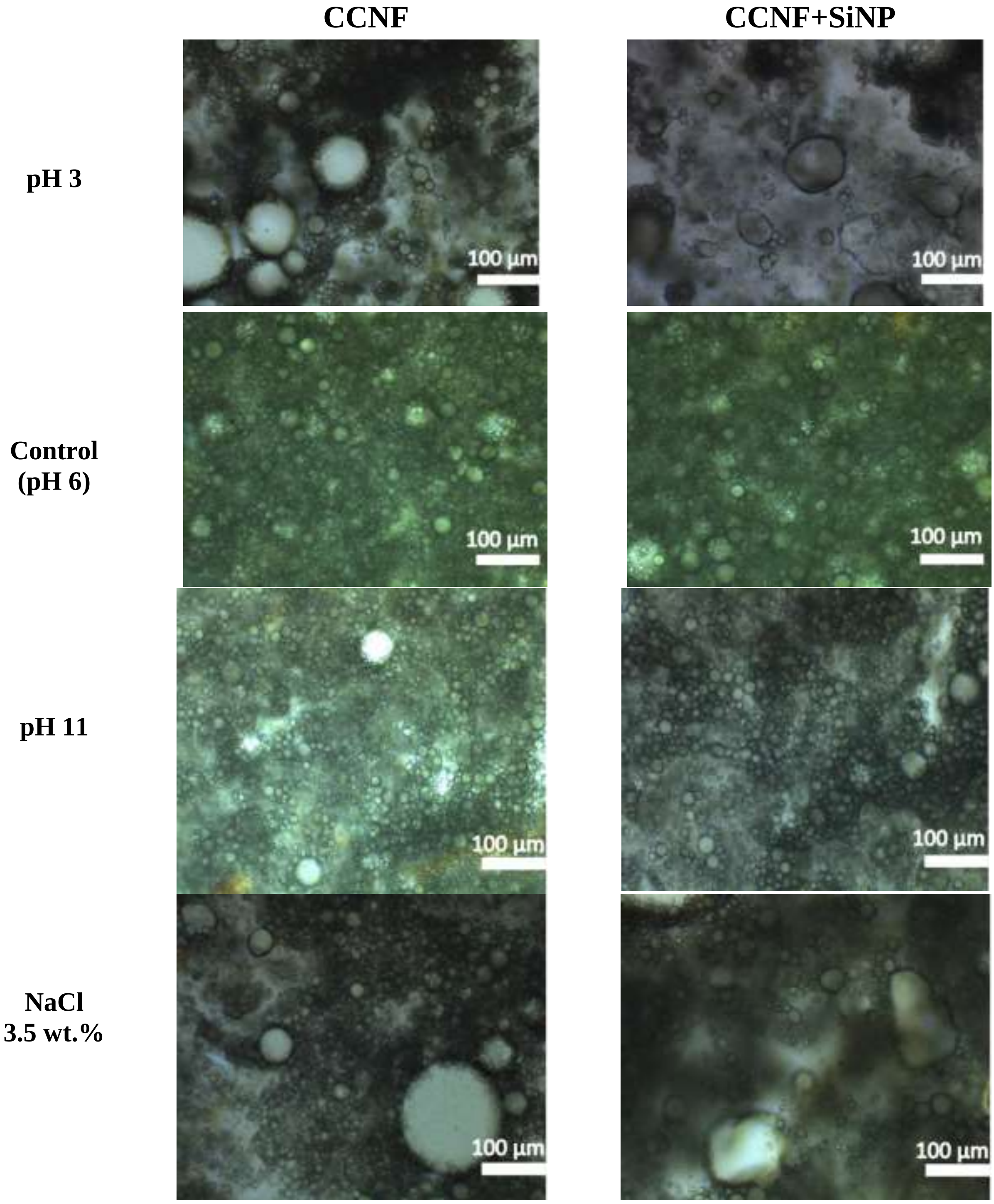


Figure 44. Optical microphotographs of mineral o/w emulsions containing DEET and stabilized with mixtures of surfactants, CCNF (0.3 wt.%) and SiNP (0.01 wt.%), upon addition of salt (NaCl) and varying pH.

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

The release of DEET from the emulsions prepared by combining the surfactants, CCNFs, and SiNPs was evaluated, and the results were compared with those of a release experiment conducted using a non-emulsified sample of DEET. The DEET released was quantified by UV–vis spectrophotometry, as described in the experimental section and presented in Table 5.

Table 5. Absorbance measurements (λ= 204 nm) of DEET standard solutions in 30% methanol/water mixtures by UV-vis spectrophotometry (3 replicates).

Concentration (mg L^{-1})	Absorbance				
	1	2	3	Mean	S.D.
0.264	0.174	0.172	0.171	0.172	0.002
0.528	0.253	0.252	0.252	0.252	0.001
2.640	0.675	0.673	0.672	0.673	0.002
5.280	1.234	1.233	1.235	1.234	0.001
8.423	1.858	1.857	1.856	1.857	0.001

The release of DEET was followed for 18 h. However, since the scope of this investigation was to show the potential of the developed emulsions to extend the repellent action after application, a period of 6 h was chosen for comparing the release profiles.

Delay in the release of DEET from emulsions is a key aspect for extended repellent effect, because high initial evaporation rates reduce the concentration of DEET available at longer times. This may be one of the causes of the short efficacy times associated with this repellent in several formulations, particularly for low-dose applications [81]. As can be seen from Figure 45, there is a drastic reduction in the initial release rates of DEET from the emulsions prepared in this study compared to that of a non-emulsified sample. In the former case, a very fast release occurs in the first 2 h, and after 6 h, almost all repellent migrates into the recipient phase (Fig. 46). In contrast, the two emulsions containing the CCNFs showed significantly slower release of DEET, exhibiting a sharp decrease in the burst release during the 6 h period (7–15% the cumulative release). In a similar study using polyurethane microcapsules reinforced with modified cellulose nanofibers (mCNFs) to encapsulate DEET, Kadam *et al.* also reported that the presence of the mCNFs significantly reduced the release rate of DEET (13–15% in 6 h) [55].

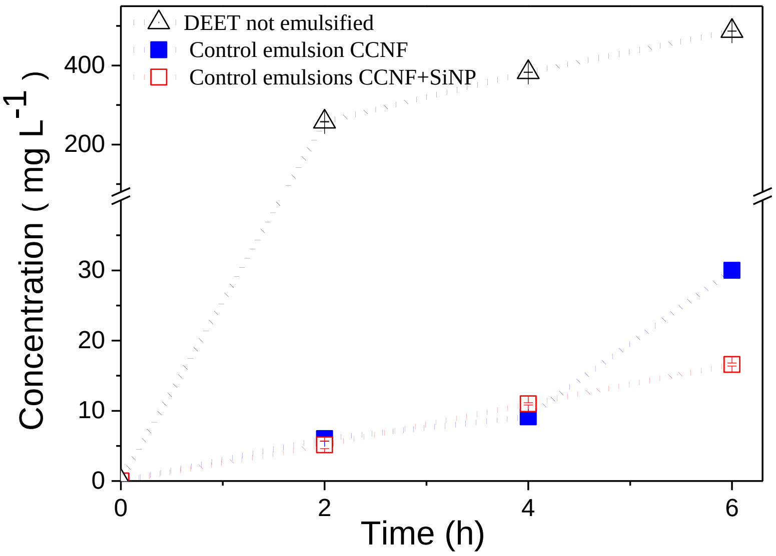


Figure 45. Profiles of DEET release as a function of time from a non-emulsified sample (pure DEET) and from mineral o/w emulsions stabilized by surfactants and CCNF (0.3 wt%), in the absence and in the presence of SiNP (0.01 wt%).

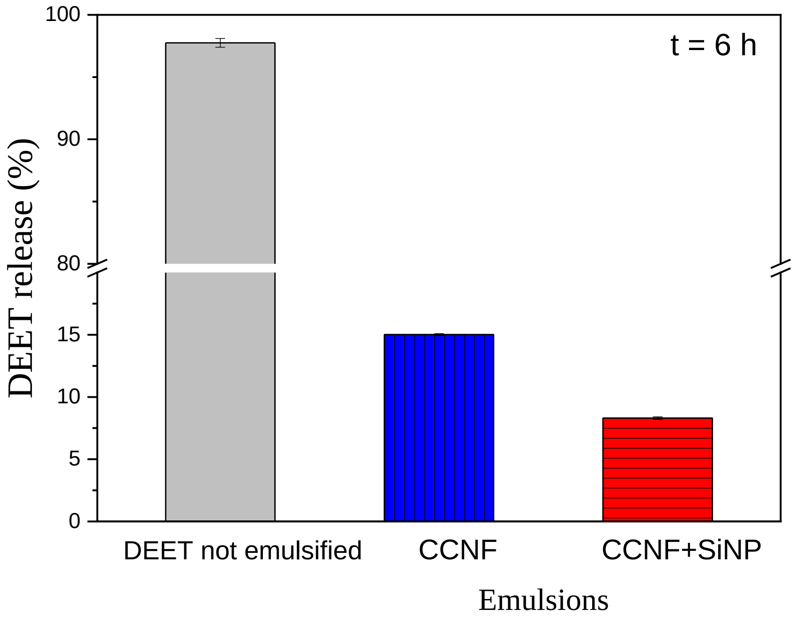
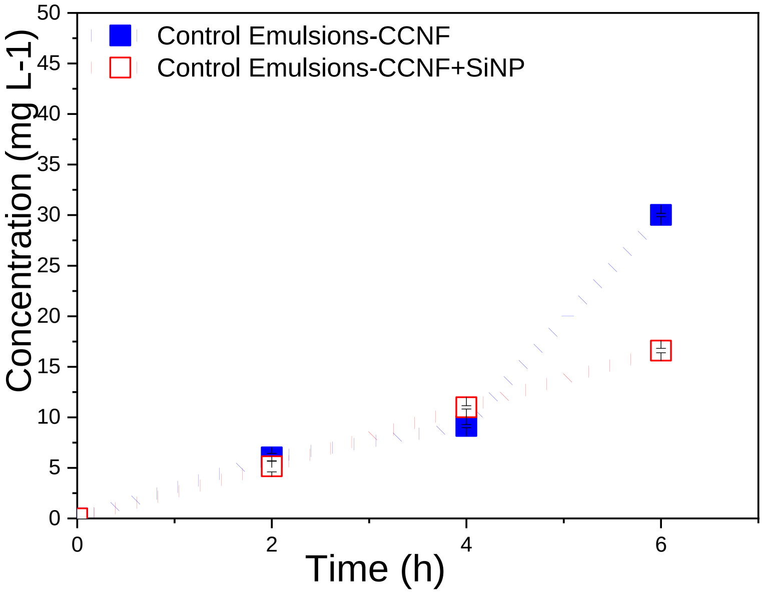


Figure 46. Profiles of DEET release after 6 h from a non-emulsified sample (pure DEET) and from mineral o/w emulsions stabilized by surfactants and CCNF (0.3 wt%), in the absence and in the presence of SiNP (0.01 wt%).

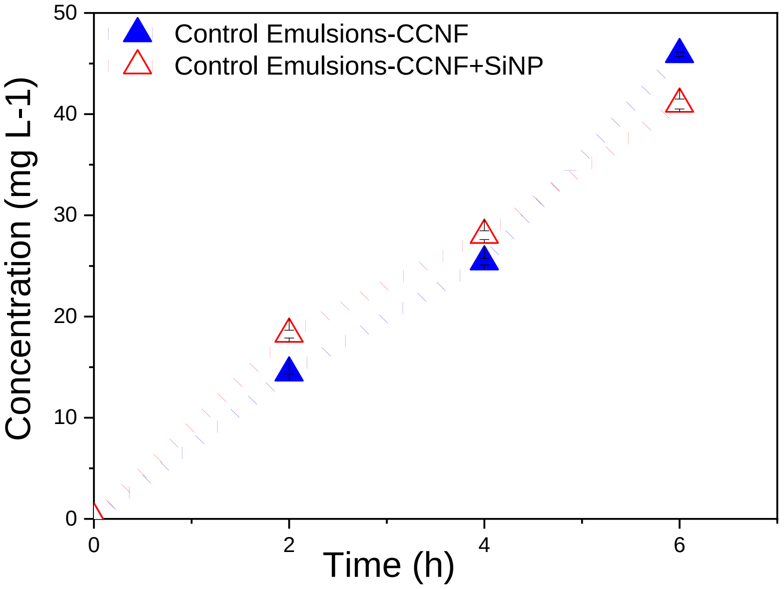
In the present study, the SiNPs act as an additional component in synergy with the CCNFs to obtain a linear release rate in the experimental duration (Fig. 47A). In the system containing only the CCNFs and the surfactants, a slow release of DEET was observed for up to 4 6, following a linear release. In comparison, 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 the microencapsulated species is controlled by their capability to permeate the capsule walls. [81] Therefore, we can assume that the increased stability of this emulsion due to the complexation at the interface, as observed in the accelerated destabilization tests, plays a key role in the retention of the active compound and extending its action. Noticeably, the differences 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. A more controlled, sustained release was achieved in the presence of NPs (Figs. 47C and D). These results confirm the role of electrostatic interactions between the CCNFs and SiNPs in the stabilization of the emulsions containing DEET. A more rapid DEET release was obtained with the salt (Fig. 47B), in agreement with the higher extent of emulsion destabilization under these conditions, as discussed in the previous section.

A



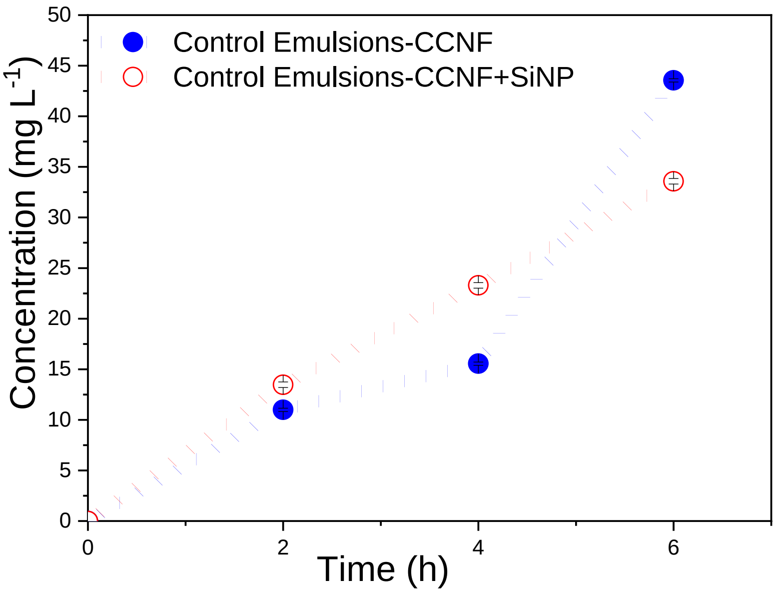
B

NaCl



C

pH 3



D

pH 11

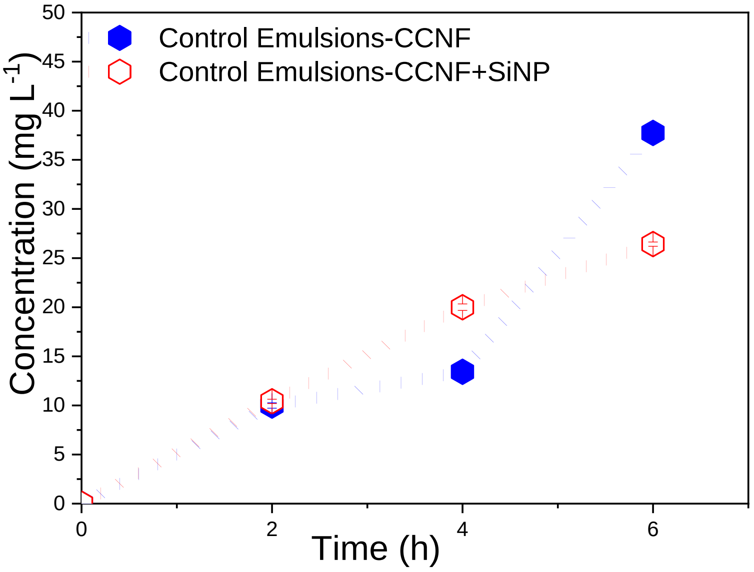


Figure 47. (A) Cumulative DEET release as a function of time from mineral o/w emulsions stabilized by surfactants and CCNF (0.3 wt.%), in the absence and in the presence of SiNP (0.01 wt.%). (B-D) Effect of salt and pH on DEET release from emulsions.

The presence of salt had a similar effect in both systems, because the principal effect was on the interfibrillar interactions related to the CCNFs in the solution and at the interface [19]. In this case, the increased the DEET release with the addition of NaCl can be used advantageously in formulations for controlled delivery of this active compound, to achieve an extended repellent action for a 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 promote an additional DEET release from the emulsions prepared in this study with the CCNFs and SiNP.

3.7.8. Partial Conclusions

Oil-in-water emulsions containing 10 wt% DEET were produced, showing long-term stability (up to four months from preparation, at room temperature) on combining the stabilization mechanisms associated with the improvement in the rheological properties of the emulsions with the CCNF. They also resist the coalescence by enhancing the interactions with SiNPs in the bulk and at the droplet interface. Both nanomaterials showed relatively high ζ-potentials at the working pH, which ensured their good dispersion in the aqueous external phase of the emulsion during the preparation of the formulations. The positive surface charge of the CCNFs, opposite to that of the SiNPs, promoted attractive interactions, allowing strengthening of the network formation in bulk. This was demonstrated by the further increase in the viscosity, observed even at the low concentrations of the NPs (0.01 wt%). The accelerated stability tests showed that the emulsions containing the CCNF/SiNP combination were more stable than those in which silica is absent, even on the addition of salt and at large pH variations. The emulsion stability was confirmed by the smaller droplet size and the more homogeneous droplet distribution. These support the hypothesis of various mechanisms acting in synergy between the two nanomaterials in the stabilization of the dispersed oil phase containing DEET, which is the requirement for delaying the 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. The results showed a drastic reduction in the initial release rate compared to that of the non-emulsified sample. In the latter, most of the active compound migrated into the recipient phase after 6 h, whereas there was a significantly slower release of DEET from the two emulsions containing the CCNFs. A sharp decrease was observed in the burst release during the 6 h period (7–15% cumulative release). In addition, the emulsions containing SiNPs showed more sustained release during the experiment, which confirmed the role of electrostatic interactions between the CCNFs and SiNPs on the stabilization of the emulsions containing DEET. This delay in the release from the emulsions is a key aspect for achieving extended repellence, increasing the concentration of DEET available at longer times. This property can be used advantageously for the development of formulations with long-term efficacy even at low-dose applications.

3.8. Development and evaluation of vegetable o/w emulsions stabilized by cationic cellulose and Tween 80/Span80 for controlled release of repellent

3.8.1. Roles of materials in stabilization of emulsions

We began the development of repellent emulsions by testing the materials individually to examine if they showed a good performance at the oil–water interface. After the optimization of the concentration of the materials for formulating stable emulsions using vegetable oils, we evaluated individually the effectiveness of the materials in stabilizing the vegetable oil/DEET–water interface.

The materials that stabilize the emulsions are the cationic cellulose, SiNP and surfactants, as shown in Figure 48. Previously, Silva *et al.* [42] and Shi *et al.* [41] stabilized emulsions using cationic cellulose, and correctly explained the mechanisms involved in their stabilization. We previously stabilized DEET-containing mineral oil based emulsions using CCNFs with mixed surfactants. However, in this part of the study, we addressed the stabilization of emulsions using vegetable oils, DEET and different stanilizers. To our best knowledge, this is the first time that vegetable oil/DEET-in-water emulsions have been stabilized using cationic cellulose and surfactants.

In contrast, the modified SiNPs modified (SiNPNH2) and unmodified ones (SiNP) did not stabilize the o/w emulsions in the concentrations studied (0.01% by weight). In a previous study conducted by our group [56], emulsions were stabilized using a mixture of SiNPs and polymers. However, when we use a mixture of the surfactants with the NPs, we obtain stable emulsions, owing to the synergy between these materials. These results are in agreement with those obtained by various studies [52,82,83], which achieved stabilization of vegetable oil and DEET emulsions. Notably, the stabilization of o/w emulsions is achieved by the action of the surfactants, which stabilizes the o/w interface. Moreover, the NPs act as a co-stabilizer, improving the stability of the emulsions. However, to our best knowledge, this is the first time that emulsions containing DEET solubilized in vegetable oils have been stabilized, opening the route for the development of repellent emulsions that can serve as alternatives for the development of new formulations of mosquito repellents.

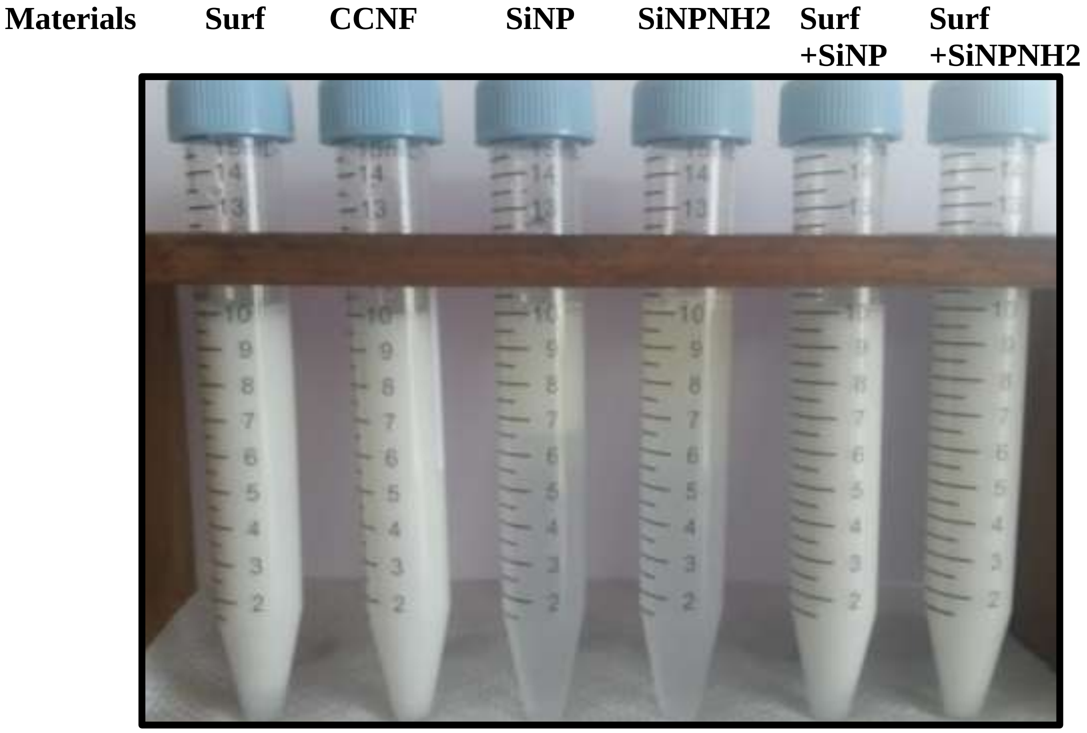


Figure 48. Digital photographs of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by different materials, taken after 24 h.

We quantified the amount of the liquid drained from the emulsions, and the results are shown in Figure 49. The emulsions stabilized by the CCNFs, surfactants (Tween80/Span80), and mixtures of the surfactants (Tween80/Span80) and with do not present drainage of the aqueous phase or phase separation. In contrast, the emulsions stabilized by the SiNPs show 100% phase separation, showing that they failled to stabilize the o/w interface, probably because the energy supplied was insufficient to lead the NPs to be adsorbed at the interface. We hypothesis that the NPs are very hydrophilic, which prevents their mobility towards the hydrophobic oily phase.

Moreover, it can be seen that SiNPs and SiNPHN2s, when used with the mixture of the surfactants, can stabilize emulsions, as shown in Figure 49. It is to be expected that the surfactants adsorb at the liquid–liquid interface and then facilitate the CCNF and SiNP do be adsorbed at the interface or to form a network. The network prevents droplet coalescence by cremation and flocculation phenomena [84] [85] [86]. Additionally, when mixed with surfactants, the NPs modify their wettability, becoming more hydrophobic, which also favours their adsorption at the oil—water interface.

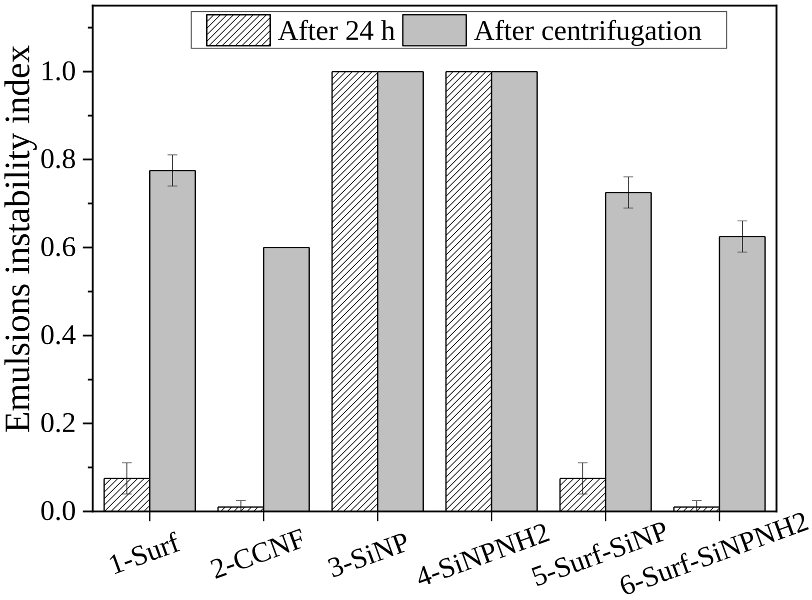


Figure 49. Destabilization by mechanical centrifugation of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by different materials, after 24 h and using centrifugation at 2566 RFC for 10 min, the error bar is the result of three measurements.

We also conducted rheological analysis to determine the viscosity of the stable emulsions, in addition to knowing their rheological behaviour. Figure 50 shows the viscosity as a function of the shear rate. We can see that the CCNF-stabilized emulsion presents a pseudoplastic behaviour and a higher viscosity at lower shear rates than the other emulsions.

We also expect that this CCNF-stabilized emulsion would tend to be more stable under long-time storage conditions.

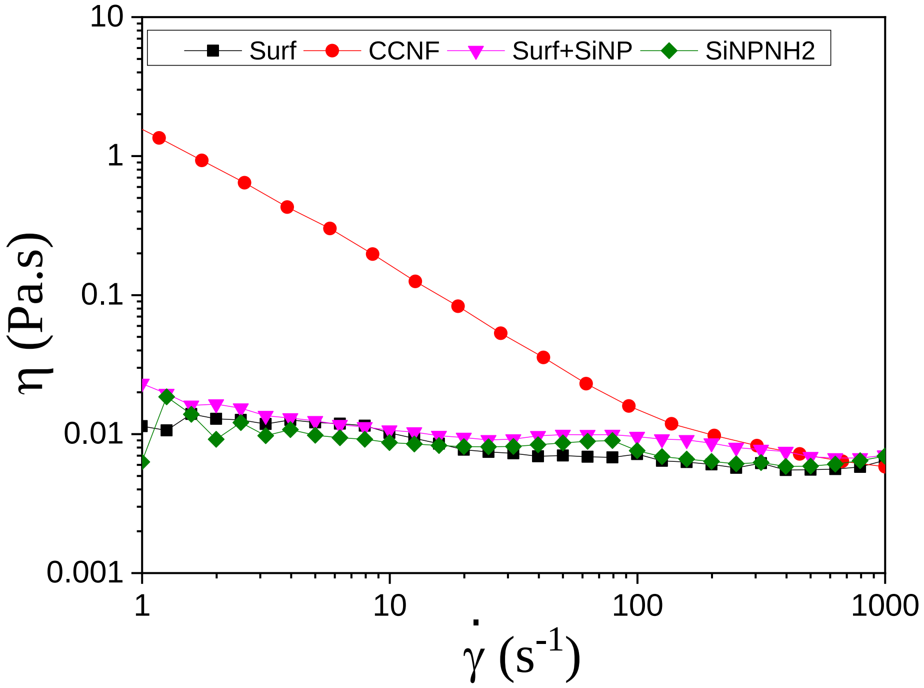


Figure 50. Flow curves of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by different materials, after 24 h.

In contrast, the emulsions stabilized using the surfactants and the NPs show Newtonian behaviour. Therefore, we can say that the NPs and the surfactants do not modify the rheological behaviour of the emulsions. This is because the surfactants and the silica NPs only adsorb the o/w interface, without forming a network in the continuous phase.

3.8.2. Formulation of repellents in emulsions

Once we determined the most efficient materials to stabilize the emulsions, we formulated repellent emulsions by mixing the materials that presented the best performance. We formulated three emulsions with 70% aqueous phase and 30% oily phase. We stabilized the emulsions with mixtures of (A) Tween®80/Span®80 and the CCNFs, (B)

Tween®80/Span®80, the CCNFs, and SiNPs, and (C) Tween®80/Span®80, CCNFs, and SiNPNH2, as shown in Figure 51.

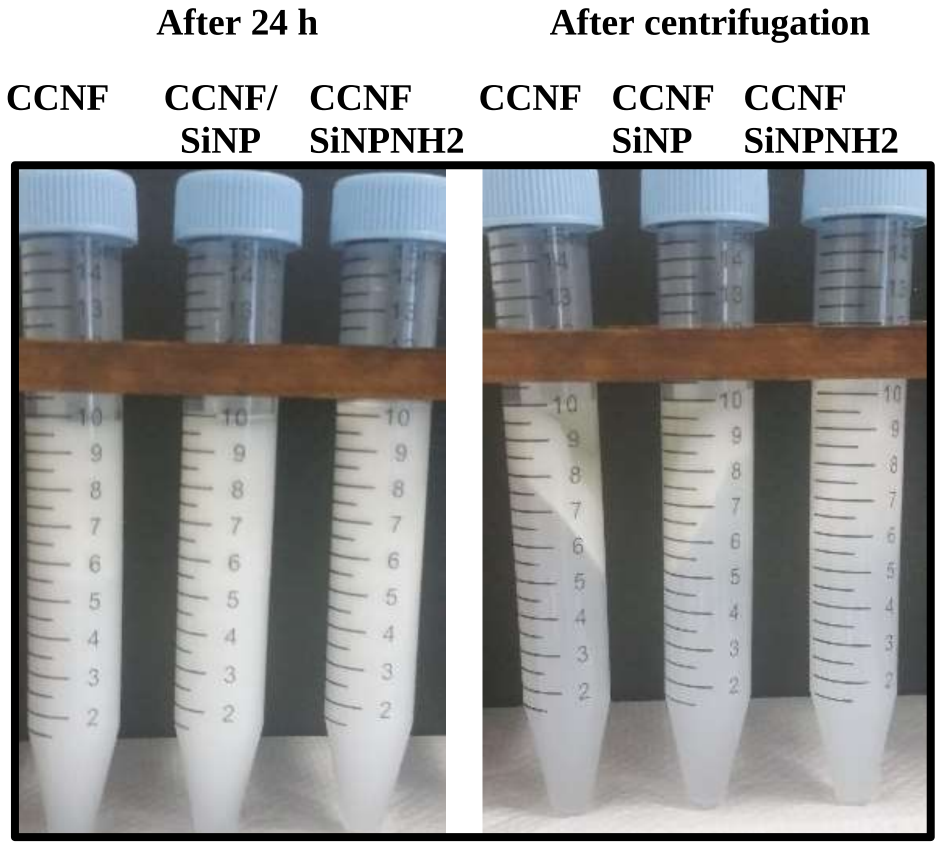


Figure 51. Digital photograph of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by Span 80/Tween 80 and nanomaterials. After 24 h and centrifugation at 5665 for 10 min.

All three repellent formulations were stable for more than four months. The stabilization is mainly attributed to the decrease in the interfacial tension by the surfactants. We hypothesize that there is a synergy between the nanomaterials and the surfactants, which increases the stability of the emulsions compared to when that they are used individually. As the emulsions were stable for a long time, they were subjected to centrifugal fields to conduct accelerated destabilization tests, as shown in Figure 52. The results of the centrifugal field application revealed that the emulsions containing SiNPNH2 were more stable than the others because the NPs interact with the CCNFs, enhanced by the action of Tween®80. This causes each droplet to experience a more significant impediment, which prevents the flocculation of the internal phase or coalescence within the droplets

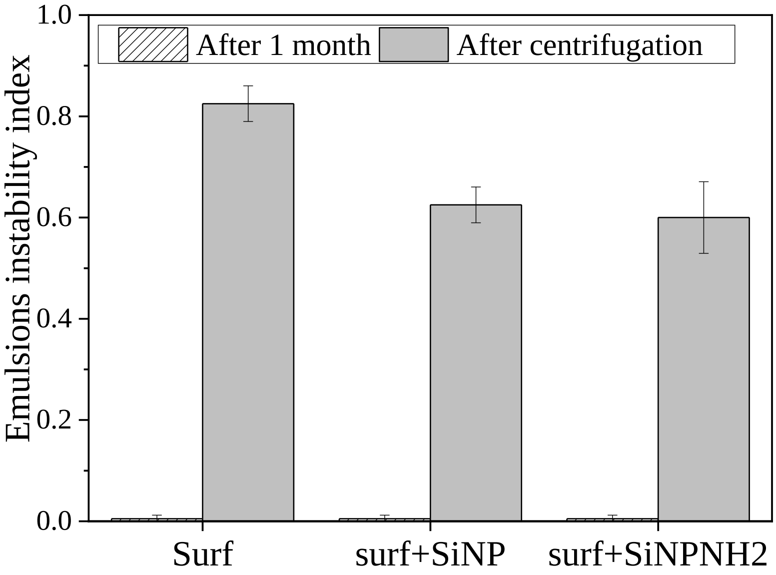


Figure 52. Destabilization by mechanical centrifugation of vegetable o/w emulsions containing DEET and stabilized by Surf (Span 80/Tween 80) CCNF 0.3wt% and SiNP 0.01wt.% and SiNPBH2 0.01wt.%. After 24 h and centrifugation at 5665 for 10 min, the error bar is the result of three measurement

These results are promising for the design and development of encapsulated repellents using this type of emulsion as the delivery vehicle. These emulsions offer advantages such as single-step formulation, when compared with those developed by Gomes et al. [63], who used a process involving different steps such as cold mixing followed by subjecting it to 70 °C in an autoclave to obtain the slow-release microspheres.

3.8.3. Rheological behaviour of emulsions formulated with vegetable oil

After evaluating the stability of the emulsions formulated with vegetable oil and stabilized with different nanomaterials in combination with the surfactants, we evaluated their rheological behaviour using flow curves, amplitude sweeps, and frequency sweeps.

3.8.3.1. Flow curves

All formulations were classified as non-Newtonian fluids and presented a pseudoplastic behaviour, in which the viscosity decreased with increasing shear rate (Figure 53). The pseudoplastic behaviour of the emulsions was attributed to the CCNFs, which form a three-dimensional network, preventing the free movement of the droplets. We hypothesize that the increase in the viscosity of the emulsions is due to the formation of a network between the CCNFs and Tween 80. The surfactant modulates the electrostatic repulsions of the CCNFs, resulting in attractions that favour the formation of this network, which traps the drops [87]. In general, the CCNFs favoured the rheological modification of the emulsions, increasing their stability of the formulation. These results are promising for the development of repellent capsules that use emulsions as templates.

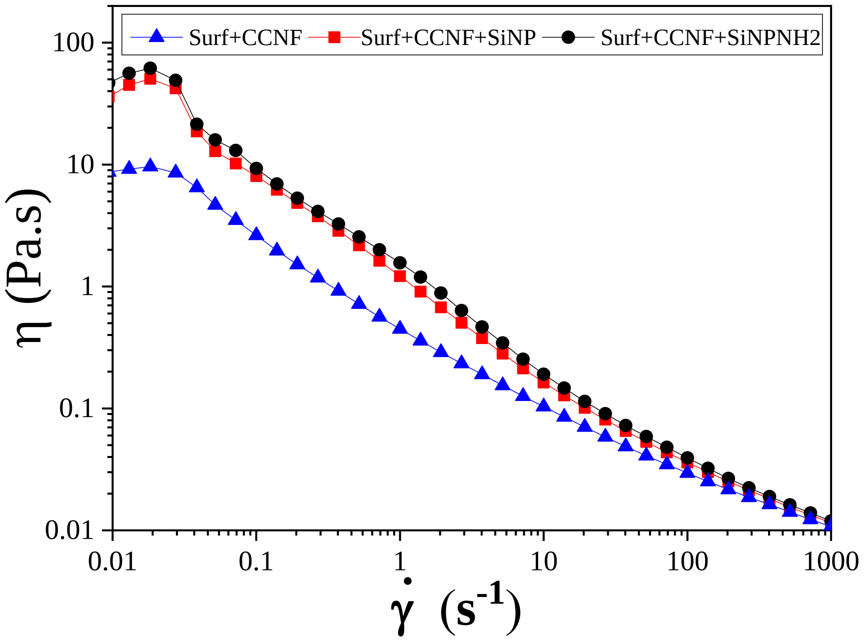


Figure 53. Flow Curve of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by Surf 0.5 wt.% (Span 80/Tween 80), CCNF 0.3wt% and SiNP 0.01wt.% and SiNPBH2 0.01wt.%.

The emulsions that contain SiNPs have a higher viscosity than those that do not use the NPs. This trend can be attributed to the interactions of these NPs with the CCNFs, which allows forming a more resistant network, generating emulsions having higher stability in storage [88] [89]. We did not find significant differences between the behaviour of formulations with modified (SiNPNH2) and unmodified (SiNP) silica nanoparticles; therefore, we decided to continue with the formulations co-stabilized by SiNPs. To determine the differences between the aqueous phase and the corresponding emulsion, we compared the viscosity, to identify the effects of the oil on the formulation of the emulsions. We observed that in all cases, the emulsions have a higher viscosity than their respective aqueous phases, as shown in Figures 54 and 55. In contrast, we noted that the viscosity of the aqueous phase in the region of low shear rate increased; this effect may be associated with a certain resistance of the network of the CCNFs formed with the surfactant. Under a certain shear rate, once the CCNF/surfactant network is aligned, the pseudoplastic behaviour begins.

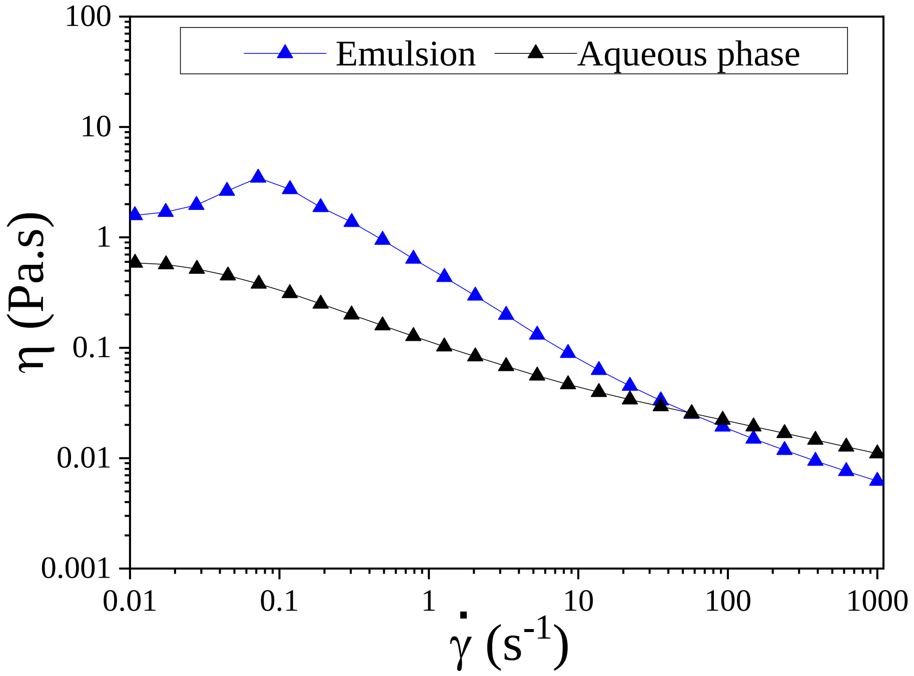


Figure 54. Flow curves of the aqueous phase of vegetable o/w emulsions containing DEET and stabilized by Span80/Tween80 and CCNF 0.3wt.% (after 24 h).

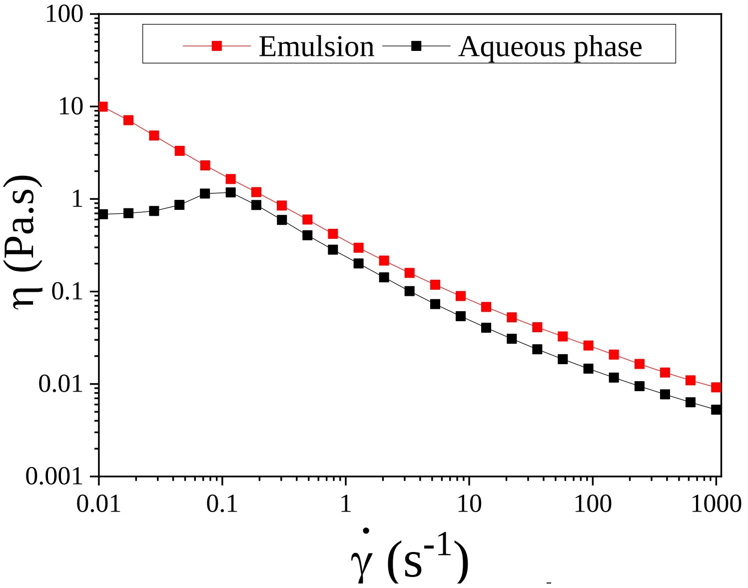
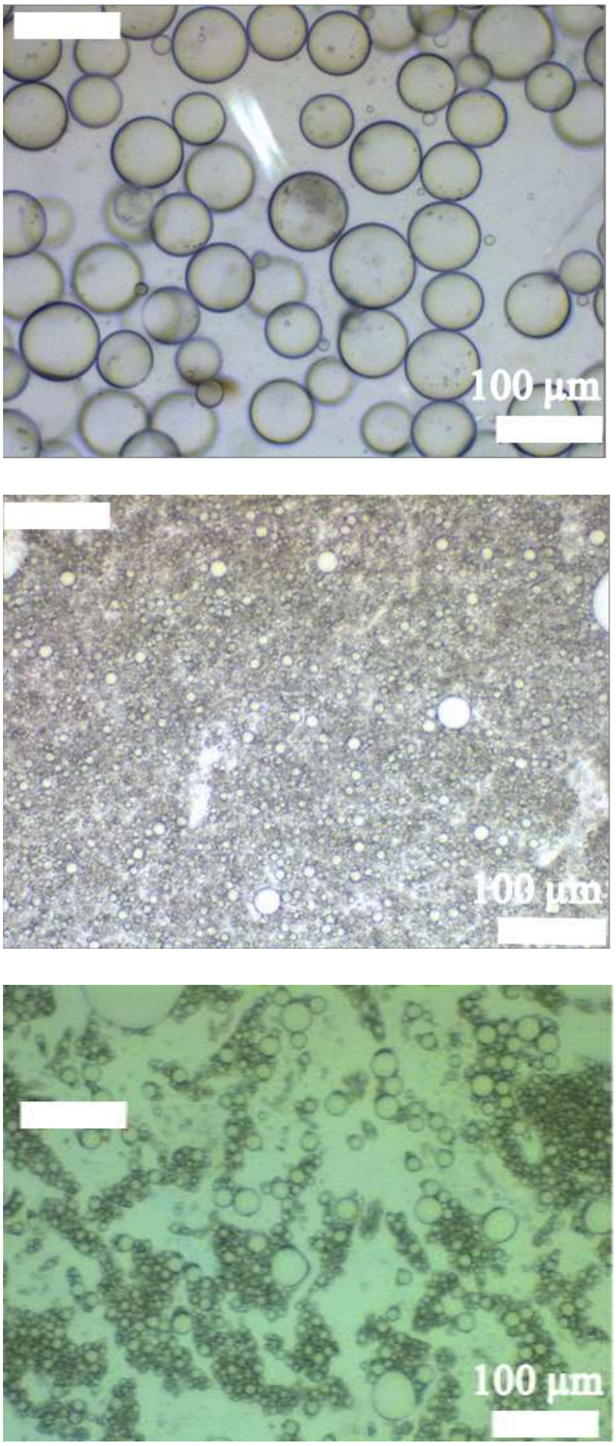


Figure 55. Flow curves of the aqueous phase and of vegetable o/w emulsions containing DEET and stabilized by Span80/Tween80 and CCNF 0.3wt.% and SiNP 0.01wt.% (after 24 h).

The emulsions presented a pseudoplastic behaviour, and it is noteworthy, that this behaviour is desired in cosmetic formulations because it improves the sensory effects on application. In addition, we noticed that in the region of low shear rate, a plateau zone is observed, which corresponds to those emulsions which also presented colloidal stability during storage

Figure 56 shows that all the emulsions have a spherical droplet morphology. However, emulsions containing silica NPs have a smaller size. This smaller size may be associated with more effective packing of surfactants at the interface in the presence of NPs, and with the higher viscosity that prevents droplet coalescence. Accordingly, we hypothesize that the emulsions will be stable when they are in storage.



CCNF D_{90}= 68 ± 22 µm

Surf+CCNF D_{90}= 17 ± 8 µm

Surf+CCNF+SiNP D_{90}= 15 ± 10 µm

Figure 56. Optical micrographs of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by Span80/Tween80 and CCNF 0.3wt.% and SiNP 0.01wt.%. (after 24 h).

3.8.3.2. Amplitude sweep measurements

After determining the rheological behavior of the emulsions, we investigated their viscoelastic behaviour to identify the linear viscoelastic region (LVE). The aim was to determine the type of intermolecular interactions dominating the rheological behaviours of the emulsions. The LVE region is highly important because it is an area in which the formulations maintain the complete structure, from which we can determine the behaviour of the emulsions. The end of the LVE region was determined as 0.03 γ % for the two emulsions studied**,** as shown in Figures 57 and 58. Additionally, in the LVE region, emulsions stabilized by the CCNF/surfactant having viscoelastic behaviour are observed. In contrast, the emulsions stabilized by the CCNF/SINP/surfactant combination present a viscoelastic solid behaviour. We hypothesize that these characteristics provide more macroscopic stability of this emulsion than of that with only the CCNF/surfactant. Figs. 57 and 58 show that the aqueous phases in black lines present abrupt breaks as the amplitude increases. This is due to the disorganized breakdown of the network formed by the CCNFs. In contrast, these emulsions show an even and organized breakage. This breakage is ideal for the formulation in cosmetics and foods, because it uniformly maintains the structure without forming agglomerates.

Additionally, we observed that the two formulations have low structural resistances, which facilitate their application on a surface, mainly the skin, which is not a smooth surface. Briefly, the two formulations allow application on the skin and serve as vehicles for the encapsulation of the repellents, despite their differences in the elastic modulus, with CCNF/Tween/Span being a viscous liquid and CCNF/Tween/Span/SiNP being a viscous solid in the LVE region. Additionally, we note that the force necessary to disperse the emulsions is slightly higher for the formulation containing NPs; this factor generates a certain rigidity to the repellent formulation. The best feature of the CNF/SiNP/Tween/Span formula is that it does not flow at rest after application. In contrast, the CCNFs/Tween/Span formulations flow after the application.

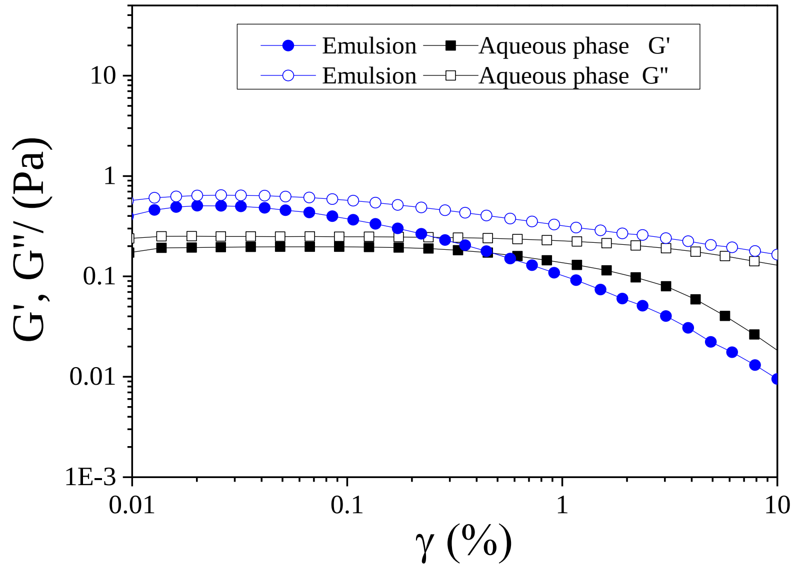


Figure 57. Amplitude sweep curves of the aqueous phase and of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by Span80/Tween80 and CCNF 0.3wt.% (after 24 h).

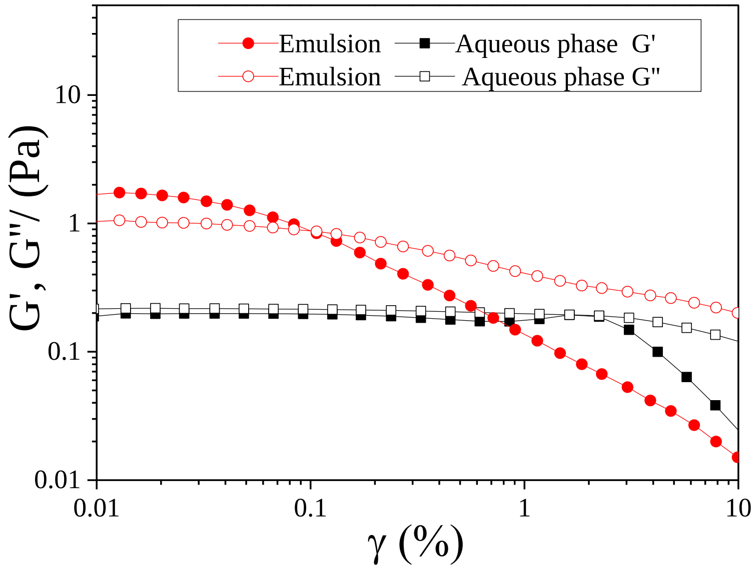


Figure 58. Amplitude weep curves of the aqueous phase and of vegetable o/w emulsions containing DEET (10 wt.%) and stabilized by Span80/Tween80 and CCNF 0.3wt.% and SiNP 0.01wt.% (after 24 h).

3.8.3.3. Frequency sweep measurements

To determine the long-term stability of the formulations, we conducted their frequency testing. The frequency tests were performed within the linear viscoelastic range (at a constant strain of 0.1%, determined by amplitude tests) at oscillation frequencies 01–100 Hz. The results of the frequency tests (mechanical spectra) are plots of storage modulus (G’) and loss modulus (G’’) against the pulsation x (2 pm). They provide information of the CCNF/Tween/Span and SiNPs forming a cross-linked or three-dimensional structure and the consistency of the formulations.

From Figs. 59 and 60, we note that the CCNF/Tween/Span combination does not form cross-linked networks under the conditions and concentrations studied. This can be affirmed because in the low-frequency zone, G" > G', which is a characteristic of non-cross-linked systems. When formulating the emulsions, the crossover point moves to a high frequency compared with its respective aqueous phase. This may be related to the decrease in the interactions between the CNF network, owing to the presence of the oil. However, this behaviour can favour the application of formulations as repellents using sustainable materials such as cellulose or NPs.

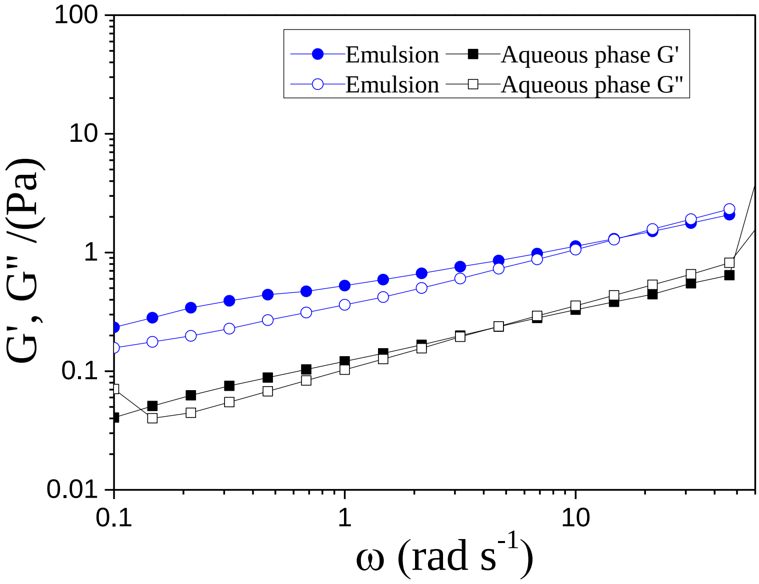


Figure. 59. Frequency sweep curves of the aqueous phase and of vegetable o/w emulsions containing DEET 10 wt.% and stabilized by Span80/Tween80 and CCNF 0.3wt.% (after 24 h).

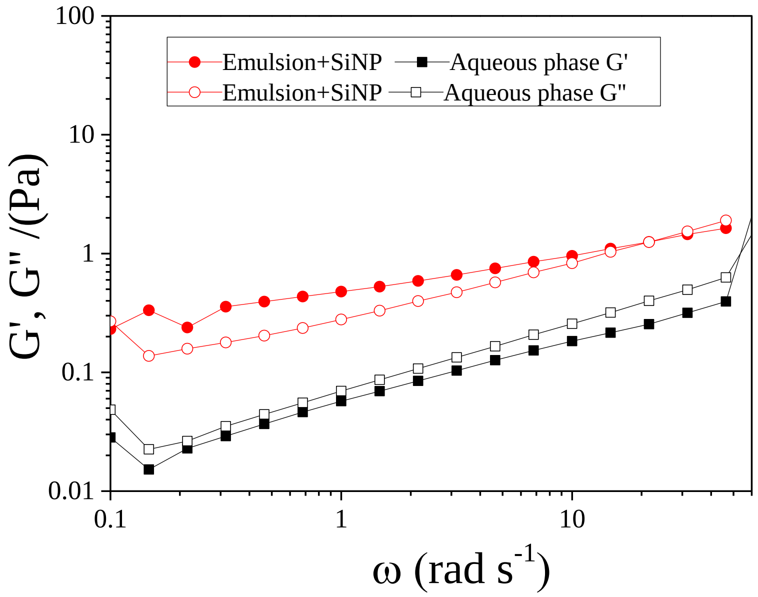


Figure 60. Frequency sweep of the aqueous phase and of vegetable o/w emulsions containing DEET 10 wt.% and stabilized by Span80/Tween80 and CCNF 0.3wt.% and SiNP 0.01wt.% (after 24 h).

3.8.4. Effect of pH and NaCl in emulsions

We aimed to investigate the rheological properties of the developed emulsions when subjected to changes in the pH and presence of salt, to evaluate the tolerance of pH and of the content of salt in the emulsions. The three formulations evaluated showed an increase in the viscosity when NaCl was added, and the results are as shown in Figs.61 and 62. We note that all emulsions show a pseudoplastic behaviour, which is that as the shear rate increases, the viscosity decreases. We note that at a low shear rate, the viscosity presents high values, which indicates that these emulsions present colloidal stability when they are in storage. We hypothesize that the increase in the viscosity is due to a screening of the cellulose charges, owing to adsorption of chloride ions on the surface of the CCNFs.

The addition of NaCl at concentration 1.8% by weight and pH changes from 7 to 4 and 10 to emulsions stabilized by CCNF and Tween®80/Span®80 (formulation A) was explored. They were aimed at determining the effects on the rheological properties. It was observed that the addition of salt and changes in the pH, change the rheological behaviour of the formulations. Figure 61 and 62 shows that there is an increase in the viscosity, which is major in acidic pH (pH 4). The increased ionic strength screens the electrostatic repulsive forces between the cellulose fibrils, improving fibril–fibril attraction, resulting in a denser fibrillar network and more viscous emulsion and therefore, generates emulsions with improved stability[90]. . This is in accordance with the curves shown in Fig. 62.

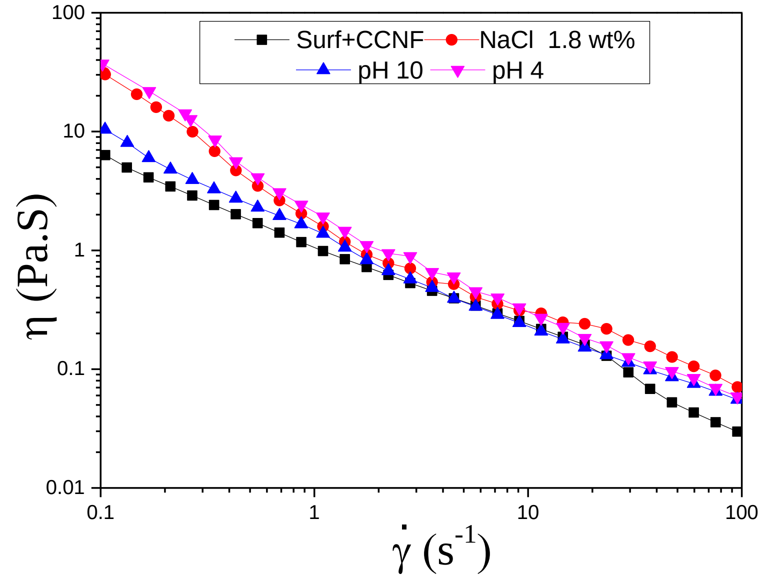


Figure 61.Flow curves of vegetable o/w emulsions containing DEET 10 wt.% and stabilized by Span80/Tween80 and CCNF 0.3wt.% (after 24 h).

The addition of salt and changes in the pH of the CCNF suspension drastically change the viscosity compared to the characteristics of the emulsions in absence of salt and at a neutral pH. Here, the addition of 1.8 wt% (~ 30 mmol) NaCl to the emulsions was sufficient to achieve stronger interactions between the fibrils without any noticeable precipitation or breakage of the emulsions

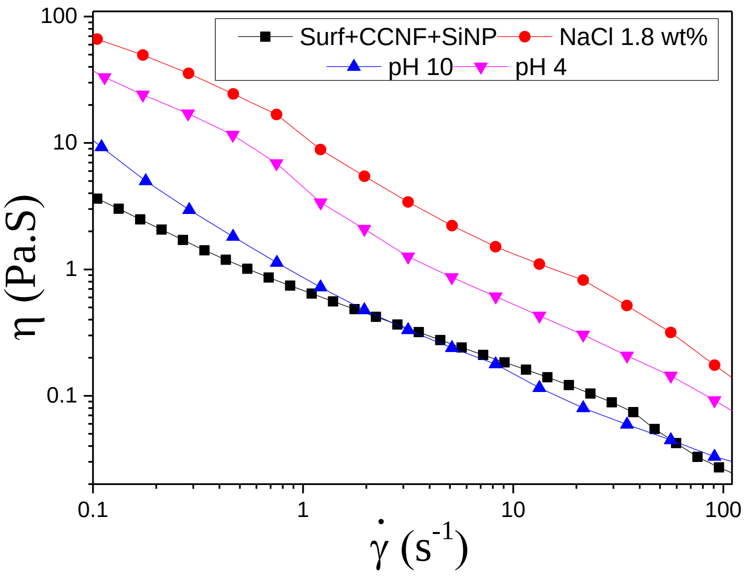


Figure 62. low curves of vegetable o/w emulsions containing DEET 10 wt.% and stabilized by Span80/Tween80, CCNF 0.3wt.% and SiNP 0.01 wt.% (after 24 h)..

3.8.5. Controlled release of DEET

We performed DEET release experiments by dispersing an amount of DEET in the formof emulsion on a paper exposed to air, and then quantified the remaining DEET every 2 h, for 8 h. The addition of NaCl and the pH changes from 6 to 4 and 10 promoted the delay of the release of DEET, as shown in Fig. 63. This is associated with a significant increase in the viscosity, making it difficult for the component of the internal phase of the droplets to move. These results are promising for the development of slow-release repellent emulsions.

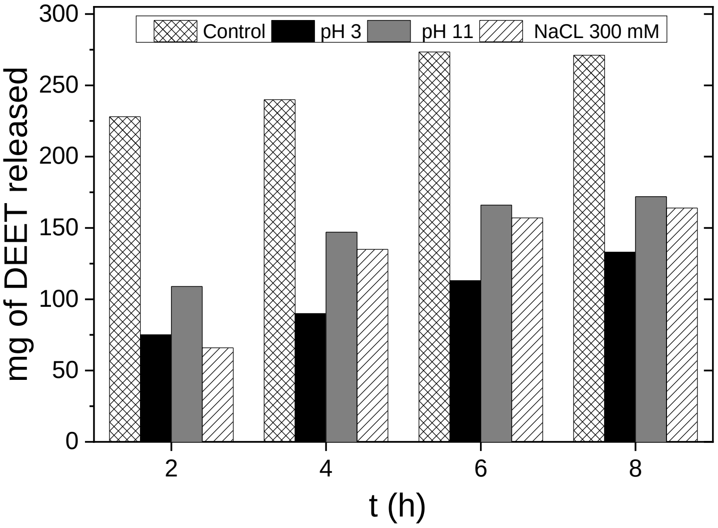


Figure 63. Release profile of DEET (10 wt.%) from vegetable o/w emulsions stabilized using Span 80/Tween 80 0.5wt.% and CCNF 0.3wt.%, upon different pH conditions and addition of NaCl (after 24 h).

3.9. Partial conclusions

Vegetable oil-DEET/Water emulsions were stabilized using CCNF showing superior stability when compared to emulsions formulated with Twenn/Span surfactants. CCNF confers a pseudoplastic behavior to the emulsions. The emulsions of repellent with a 10 wt.% DEET stabilize using a mixture of Tween/Span/CCNF/SiNP components, these formulations were stable for a period greater than 4 months. Additionally, the use of the mixture of materials induces the obtaining of a smaller droplet size of the emulsions in comparison when only CCNF is used. The rheological characterization by frequency and amplitude sweep shows us that the emulsions have a flow point of 0.01% and a viscous behavior indicating that it can spread easily when applied on a surface..

The amount of NaCl plays a vital role in the development of repellent formulations. When NaCl is less than 0.30 mol L^{-1}, the viscosity of the emulsions can be increased permanently without inducing the breakdown of the emulsions. Therefore, it is vitally important to control the amount of NaCl present in the formulations.

The repellent formulations presented a sustained and controlled release of DEET, in addition to a pseudoplastic rheological behaviour, which results in a good sensory performance when the emulsions are applied to surfaces. The surfactant/SiNP/CCNF system exhibited a higher viscosity than the surfactant/CCNF system; this result opens up the field for the development of formulations owing to the solidification of these emulsions with ageing.

4. General conclusions

Mineral oil and vegetable oil in water emulsions containing DEET were stabilized using sustainable materials such as cellulose (Cationic Nanocellulose) and silica nanoparticles, as well as Tween80 and Span 80 surfactants. These emulsions were stable for more than 4 months.

Formulations using mineral oil showed sustained release of DEET when exposed to NaCl 3 wt% and pH 4 and 10, with the receiving medium being a mixture of methanol and water. The emulsions formulated with vegetable oil also presented a slow release of DEET at NaCl 3 wt% and pH 4 and 10, and in this case the receiving medium was air.

The mixture of cationic cellulose and silica nanoparticle showed great potential for stabilizing emulsions regardless of the type of oil used. Additionally, cationic cellulose showed the ability to modify the rheological behavior of emulsions regardless of using an oily phase composed of immiscible oils..The silica nanoparticles interacted with the cationic cellulose increasing the viscosity and creating reticular networks, as demonstrated by the oscillatory rheology tests.

These results open the possibility of formulating sustained release emulsions containing other actives for delivery in acidic environments such as gastric environments, in addition to formulating different cosmetics or food to be used at pH close to neutral or moderate ionic strength. The emulsion formulations developed in this work showed a pseudoplastic behaviour, which would allow an easy spread that would facilitate the application on the skin.

5. Future work

- Test the effectiveness of the repellent.
- Formulate repellent emulsions using essential oils.
- To test the formulations of emulsions stabilized with CNNF if they have an effect as a sunscreen.
- Using interfacial tension studies, interfacial rheology quantify the amount of adsorbed materials at the interface.
- Conduct studies if repellent formulations prevent DEET penetration through the skin.
- Mix the CCNF with different surfactants to determine its rheological behavior.

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7. Annex

7.1. Modification of silica nanoparticle and celulose

The cationic celluloses (CCNF) was obtained by mechanical disintegration are positively charged due to the presence of quaternary ammonium groups on their surface. Cationization reaction with glycidyl trimethylammonium chloride (GTMAC) converts C6 primary hydroxyls groups from cellulose alfa to hydroxypropyl trimethylammonium.

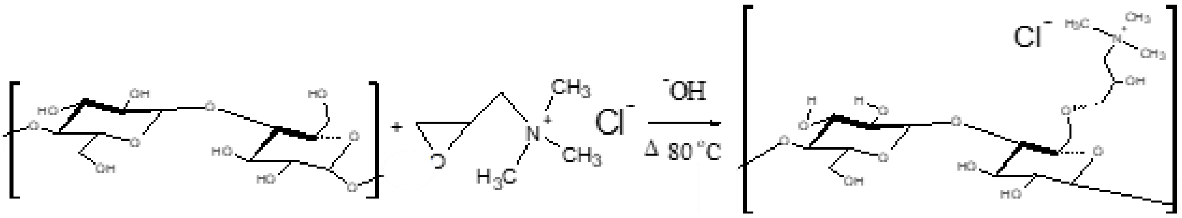

Figure A1. Cationization reaction of cellulose using GTMAC as cationic agent in alkaline media at 60–65 °C.

The mechanism involved in the modification of the silica, which occurs by a reaction of nucleophilic substitution in toluene, consists of an initial attack of the activated hydroxyl group on the APTMS silicon atom, with a tetrahedral inversion of the silanizing agent, with subsequent elimination of a methanol molecule and the siloxane group anchored on the silica surface .

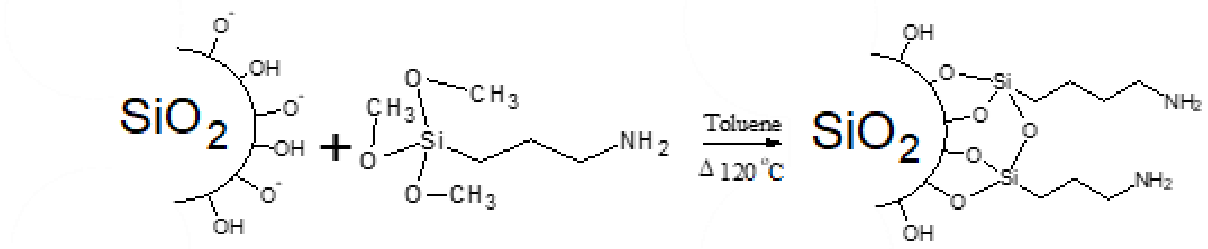

Figure A2. Schematic representation of chemical modification of silica surface to obtain hybrid silica nanoparticles.

7.2. Published paper

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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 NF) acted both as Pickering emulsifier and strengthened barrier properties of the microcapsules, resulting in nificant reduction of the DEET release rate.

As literature shows, there are different mechanisms t allow an efficient retention and release of the active npound; thus, the combination of several strategies n be advantageous for developing novel formulations ning extended DEET release. In O/W emulsions, the in mechanisms that control the release of the active npounds are the diffusion from the internal oil phase, as ll as the coalescence of the oil droplets, leading to phase paration.[3] Therefore, to obtain formulations with long-ting repellency the aim should be retarding the migration 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

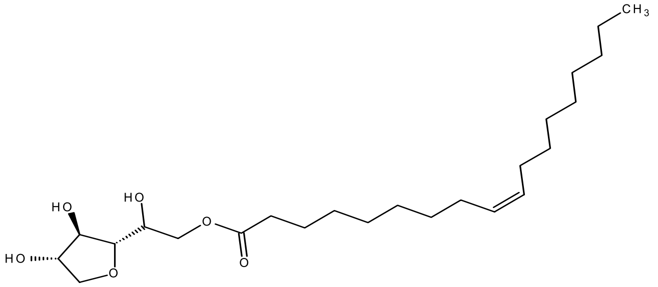
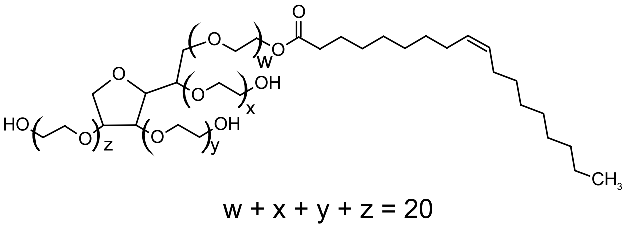
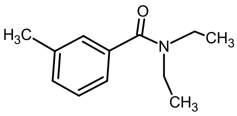
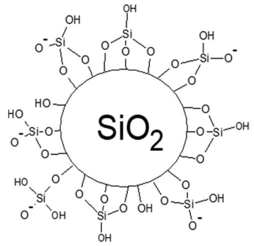
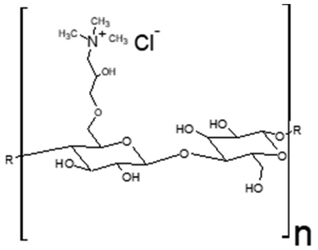
Materials

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 emulsion was diluted into 1000 µL of the aqueous phase, l dispensed on a glass slide prior to imaging. Experiments re carried out at room temperature (ca. 25 °C).

Droplet size and distribution were obtained using a stersizer 3000 Hydro with a small volume dispersion t, from Malvern Instrument (UK). The lens used can ect diameters ranging from 0.5 µm up to several mm. ulsions were diluted in the equipment's accessory avoid erroneous results due to the aggregation of the ps. The refraction index used was 1.33 and 1.47 for the ter and mineral oil, respectively. The reported particle e was obtained form an average of five measurements. presentative sizes were evaluated based on the D90, erred to as the particle size (diameter) below which the 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.

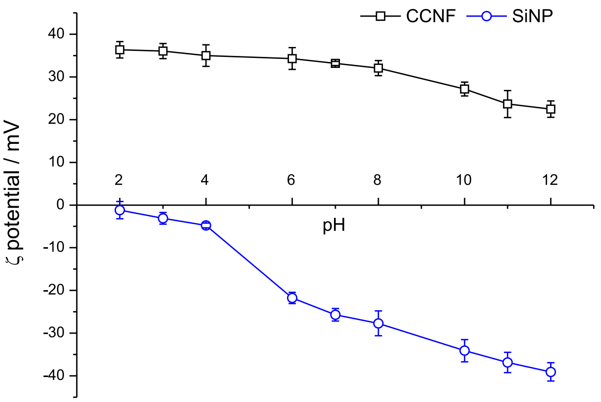


Figure 1. ζ-Potential of aqueous dispersions of cationic cellulose (CCNF) and bare silica nanoparticles (SiNP) at different pH values.

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

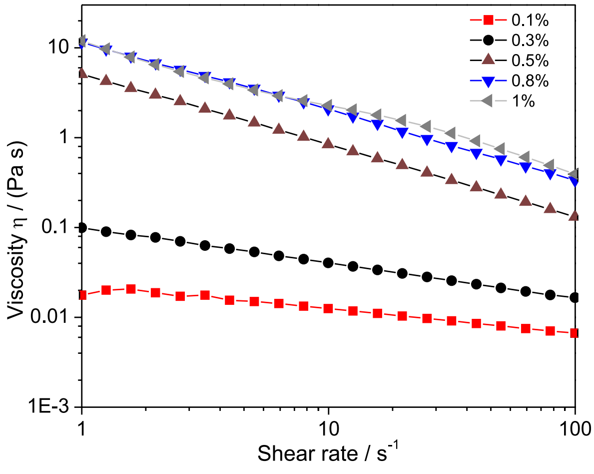


Figure 2. Flow curves for CCNF suspensions at different concentration (wt.%), suspended in a 0.5 wt.% Tween 80 aqueous solution.

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, ce a decrease in viscosity can be obtained by skin bing, facilitating the spreading of the product and, thus, ancing the delivery of the active compounds. Based the results obtained in the rheological characterization, NF and SiNP concentrations of 0.3 and 0.01 wt.%, pectively, were chosen for the formulation of the ulsions containing DEET, aiming to obtain a synergy in interactions in bulk and at the droplet interface towards ulsion stabilization, without compromising the intended ease of DEET from the emulsion droplets.

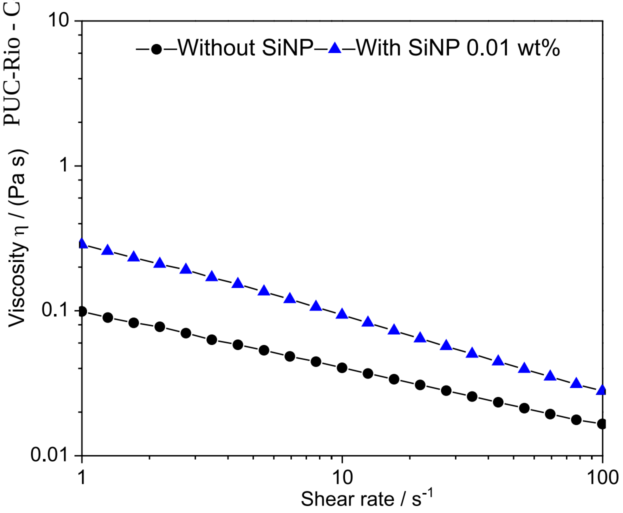


Figure 3. Effect of silica nanoparticles on rheological behavior of aqueous dispersions of 0.3 wt.% CCNF in 0.5 wt.% Tween®80 solutions.

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

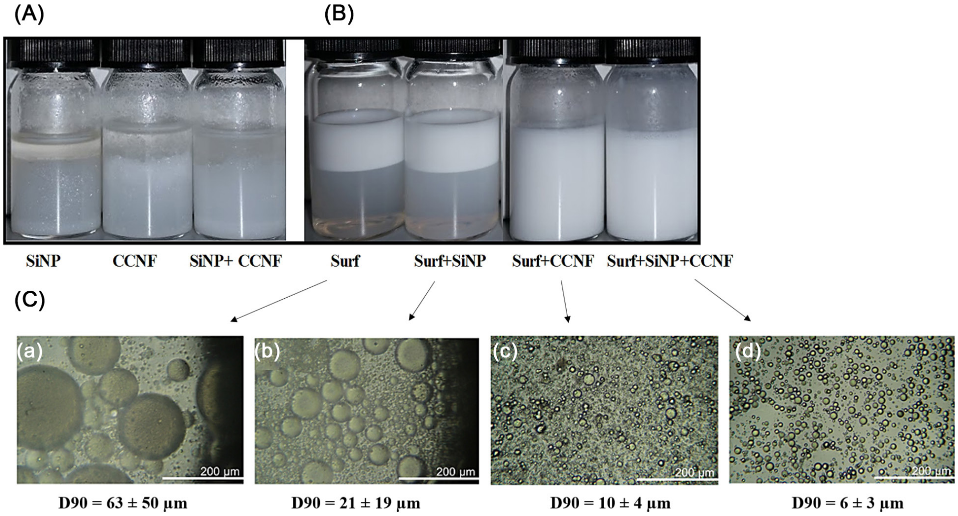


Figure 4. Photographs of O/W emulsions containing DEET and stabilized with (A) nanomaterials only and (B) mixtures of nonionic surfactants and nanomaterials (0.5 wt.% surfactants, 0.3 wt.% CCNF, 0.01 wt.% SiNP). (C) Images obtained by optical microscopy of formulations that showed creaming ((a) Surf, (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).

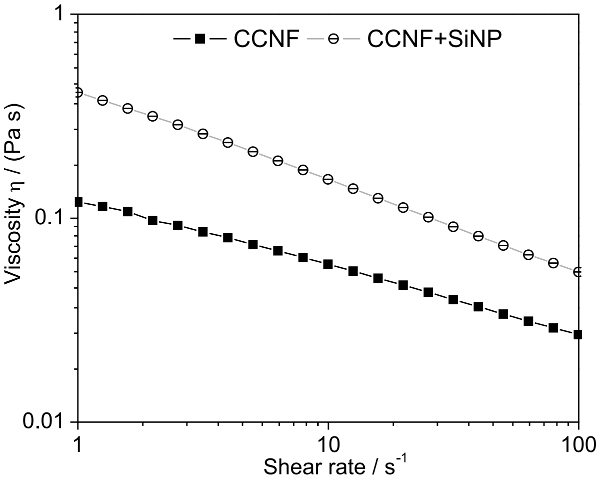


Figure 5. Effect of silica nanoparticles on rheological behavior of O/W emulsions containing DEET (10 wt.%) and stabilized by 0.3 wt.% cationic cellulose (CCNF) and nonionic surfactants.

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.

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

ysical destabilization by mechanical and thermal stress

The use of the nanomaterials with the nonionic factant mixture led to very stable oil-in-water emulsions, e to retain DEET in the internal phase. Therefore, to estigate the impact of combining SiNP with CCNF on emulsion stability, tests of accelerated destabilization re needed. For that, the emulsions were submitted to ıtrifugation and heating, and the stability was assessed the extent of the separated phase. All the emulsions ɔwed a behavior characterized by the release of free ter, 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

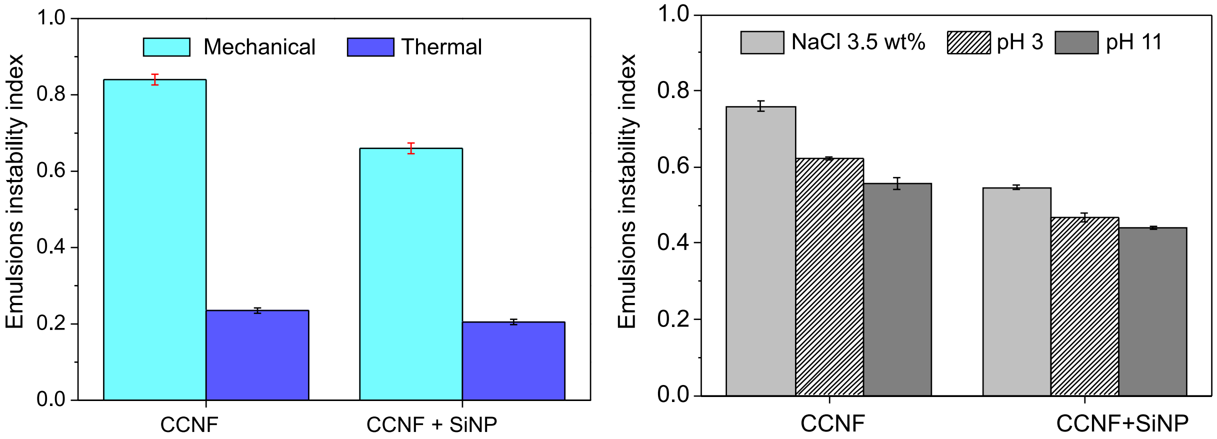


Figure 6. Destabilization of O/W emulsions containing DEET by (a) mechanical (centrifugation) or thermal (T = 45 °C) stress, and by (b) addition of salt (NaCl) or varying pH (stability was assessed after 24 h of preparing the emulsions). Emulsion instability index represents extent of aqueous phase separation.

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

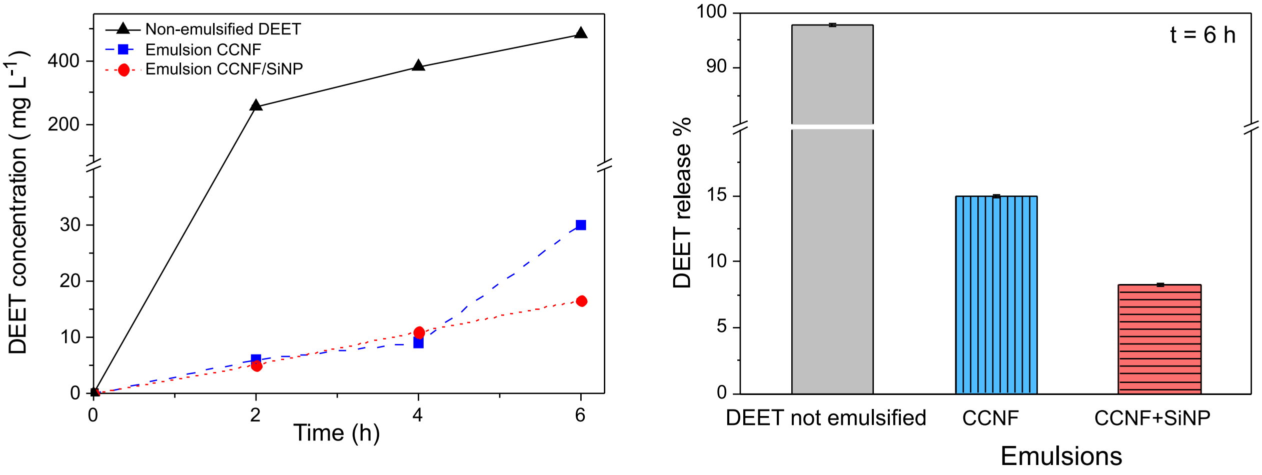


Figure 7. DEET release as a function of time from a non-emulsified sample (pure DEET) and from O/W emulsions stabilized by surfactants and CCNF (0.3 wt.%), in the absence and in the presence of SiNP (0.01 wt.%). (a) Release profiles. (b) Total amount of DEET released after 6 h (as percentage of initial 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 ntrolled by its capability to permeate the capsule walls.[34] erefore, 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

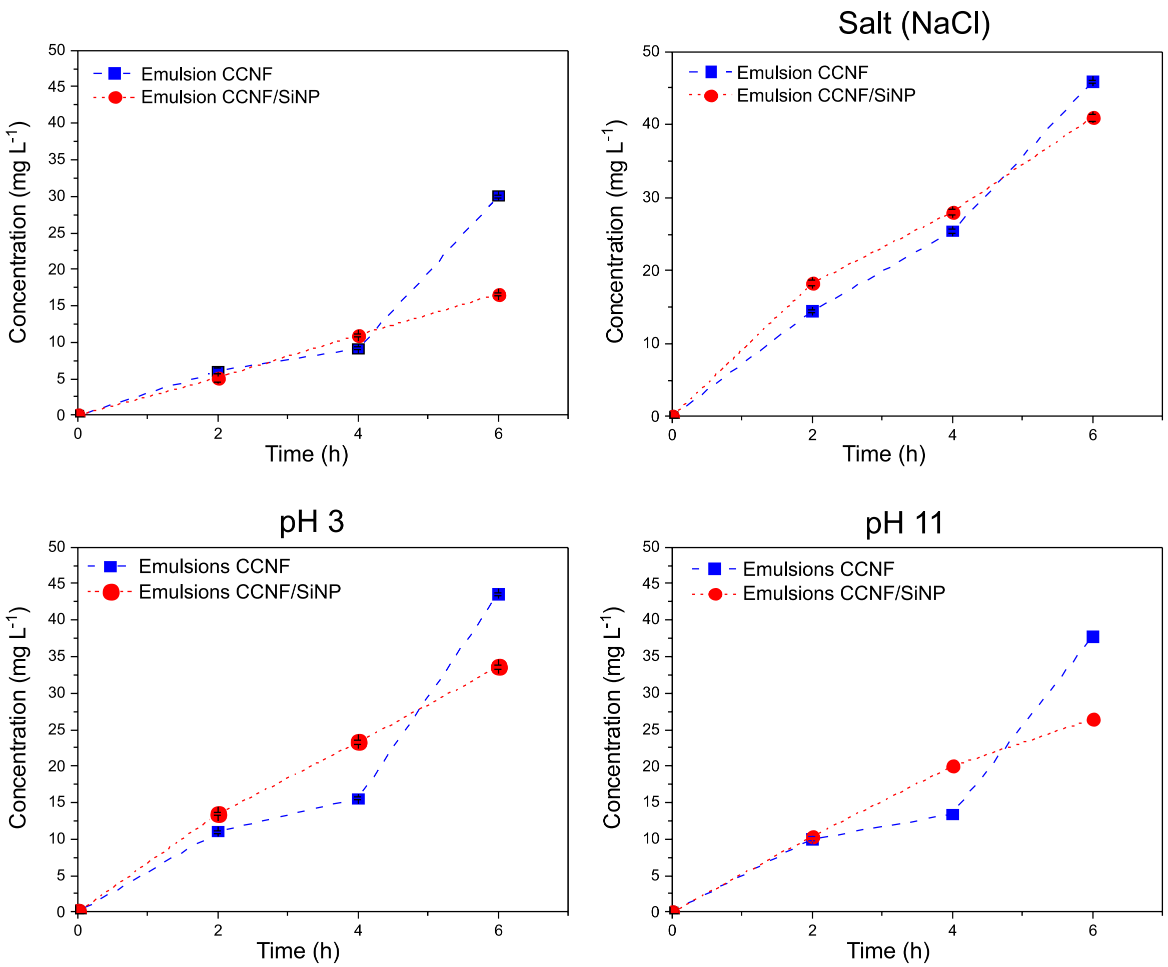


Figure 8. (a) Cumulative DEET release as a function of time from O/W emulsions stabilized by surfactants and CCNF (0.3 wt.%), in the absence and in the presence of SiNP (0.01 wt.%). (b-d) Effect of salt and pH on DEET release from emulsions. Emulsion pH was 6, unless otherwise stated. Standard deviation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