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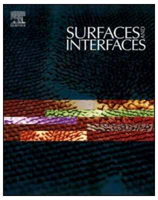

Development of water-in-oil Pickering emulsions from sodium oleate surface-modified nano-hydroxyapatite

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ABSTRACT

The stabilisation of water-in-oil (W/O) Pickering emulsions is still a challenge for the scientific community. In this work, sodium oleate (SO) surface modified nano-hydroxyapatite (n-HAp) particles were produced and tested as W/O stabilisers. The modified n-HAp particles presented a wettability dependent on the used SO content, switching from hydrophilic (initial state) to hydrophobic (one SO layer) and back to hydrophilic (two SO layers). The Pickering emulsions produced with the particles holding one SO layer (contact angle ~90 °) showed their tight adsorption at the water-oil interface (confocal analysis), and maintained the stability up to 15 days. Overall this work provides new developments in the field of W/O Pickering emulsions, a topic less studied than O/W, opening new avenues for several applications.

1. Introduction

Several food, pharmaceutical, and agrochemical products, among others, are emulsions [1,2]. Emulsions can be classified according to the disperse and continuous phase nature. When oil droplets are dispersed in water it is an oil-in-water emulsion (O/W), whereas if the opposite occurs, it is a water-in-oil emulsion (W/O) [1]. To ensure the stabilisation of this bi-phasic system, a third component, called surfactant or emulsifier, is needed [1]. The emulsifier molecules can be adsorbed at the oil-water interface decreasing the interfacial tension, thus preventing instability phenomena [1,3].

Pickering emulsions, identified by Ramsden [4] and Pickering [5], started to raise interest at the beginning of the 20th century, then increasing throughout the years. Pickering emulsions use solid particles as stabilisers, providing high physical and chemical stability due to their irreversible adsorption at the oil-water interface [6,7]. They have unique stability characteristics, and satisfy the lack of alternatives for clean-label emulsifiers in the food, cosmetic and pharmaceutical areas [8]. Moreover, they can act as encapsulation systems for bioactive compounds such as nutrients or drugs [9–12]. Most of the reported Pickering particles are directed to stabilize O/W emulsions, fact corroborated by the predominant number of studies addressing this type of emulsions [8]. Nevertheless, solutions to stabilize W/O Pickering emulsions start to raise interest with some recent studies emerging in the literature [13–16].

Hydroxyapatite (HAp), which is naturally presented in the human tooth and bones, is a surface-active material with chemical composition $Ca_{10}(PO_4)_6(OH)_2$ [17]. It is recognised as a biocompatible material, widely used in the biomedical field [17,18], and, recently, in tooth care products [19,20]. HAp started to enter novel fields of application, namely in the stabilisation of emulsions, which due to their predominant hydrophilic characteristics, is appropriate to stabilise O/W emulsions [21]. Namely, the results reported in our previous works showed that O/W Pickering emulsions prepared with n-HAp have good physical stability and interesting rheological properties, pointing out promising application prospects in several areas (e.g., food, pharmaceutical, and cosmetics) [22]. Moreover, the continuous production of n-HAp based emulsions was also demonstrated in a recent work of the group using NETmix technology, thus approaching the industrial production viability of these systems [23].

Pickering emulsions based on n-HAp are also being proposed as templates for microspheres or porous materials through combination with different polymers, with reported improvements on particle's adsorption at droplet's surface [21,24–26]. For example, Fujii et al. [25]

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and Liu et al. [27] used O/W Pickering emulsions as templates to obtain HAp microspheres coated with PCL and PLLA, respectively. Also, W/O templates based on HAp/PLLA, HAp/PCL particles were tested in the fabrication of porous materials (scaffold materials through oil phase evaporation), as reported by Zhang et al. [21] and Hu et al. [28], respectively.

Particle's wettability is an important parameter to consider when preparing Pickering emulsions and can be modulated by particle's surface chemistry. Two main approaches can be used, namely physical adsorption and chemical anchoring of small molecules or polymers to the particle's surface [29]. Reported studies focus the combination of the solid particles with emulsifier molecules to improve the physical stabilisation of the emulsions. Namely, Skelhon et al. [30] concluded that the addition of chitosan to silica particles favoured the adsorption of the particles at the W/O interface, and Sakazaki et al. [31] demonstrated that the wettability of silica-coated titanium dioxide particles can be tuned by soaking the particles in cyclic silicone oil, adjusting the particles to be used in the stabilization of O/W or W/O emulsions.

In this context, the objective of this work was to tune the wettability of the n-HAp particles to obtain stabilisers for W/O emulsions. Comparatively with O/W emulsions, works addressing W/O Pickering emulsions are less abundant in the literature, justifying the interest to conduct studies contributing to circumvent this constraint. W/O systems are interesting solutions for the encapsulation and delivery of hydrophilic active principles, e.g. polyphenolic extracts. The envisaged approach, uses sodium oleate (SO) to modify the n-HAp surface and improve the hydrophobicity of the pristine particles. SO is an ionic emulsifier with high hydrophobicity and a critical micelle concentration in water of 2.15 mM [32]. The modification was carried out taking into consideration an estimated number of layers covering the n-HAp particles. Their effect on physical (size and structure), surface (surface charge) and wettability (contact angle) properties were studied. Then, the produced SO modified n-HAp particles were validated to produce W/O Pickering emulsions, which were characterised by optical (OM) and confocal microscopy to check the droplet's morphology and particle's adsorption at the W/O interface. Furthermore, emulsion's stability was studied along one month of storage, and the emulsion type confirmed by the drop test and confocal microscopy. Overall, this study provides new insights concerning W/O Pickering stabilisers based on n-HAp particles, which are biocompatible systems with potential to be used in the delivery of hydrophilic active principles.

2. Experimental methods

2.1. Materials

The *nano*XIM-HAp102, hydroxyapatite aqueous paste, was supplied by Fluidinova S.A. *Nano*XIM-HAp102 is solely composed of 15.0 ± 1.0 (wt%) of n-HAp with particle sizes < 50 nm and water. This n-HAp was chosen due to absence of the salts or other materials in the formulation, making it more suitable to study the effect of the SO in the surface modification. Sunflower oil was purchased from a local supermarket. Sodium oleate and fluorescent dyes (Nile red and Nile blue A) were obtained from Sigma-Aldrich. Isopropyl alcohol was purchased from Riedel-de Haen. Distilled water, treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA), was used. All the other chemicals were of analytical grade.

2.2. Production and characterisation of modified n-HAp particles

2.2.1. Production of the modified n-HAp particles

The n-HAp (*nano*XIM-HAp102) particle's modification was done with sodium oleate (SO). Firstly, the SO powder was dissolved in distilled water at a predefined concentration aiming at achieve the desired number of layers to cover the n-HAp particles (0.5, 1, and 2), which was 0.09 g, 0.18 g and 0.35 g, considering 1 g of n-HAp102 particles. The methodology used to estimate the SO amount can be consulted in the supplementary material (S1 Section). Briefly, SO solution was prepared under stirring using a magnetic bar until complete dissolution in water was reached. In parallel, the n-HAp paste was dispersed in water using a rotor-stator homogeniser (Miccra RT D-9) operating at 11000 rpm for 2 min. Finally, the SO solution was injected to the dispersed n-HAp paste with a peristaltic pump at 240 rpm. The mixture continued to be stirred with the rotor-stator homogeniser at 11000 rpm for 2 min. The influence of the number of layers on the particle size, zeta potential, morphology (SEM analysis), and wettability of the modified particles was evaluated.

2.2.2. Characterisation of the modified n-HAp particles

Particle size and zeta potential. The particle size and the zeta potential of the SO modified n-HAp dispersions were determined by dynamic light scattering (DLS) and particle electrophoresis, respectively, using a Nano-ZS Zetasizer instrument (Malvern Instruments Ltd.). For the assays, the samples were diluted to an appropriate concentration (0.05 wt%, n-HAp basis) with distilled water. All measurements were carried out in triplicate at 25 °C using the value of 1.650 for the refractive index. The results are reported as average±SD.

Particle wettability. The wettability measurements were carried out with an optical contact angle device (Biolin Scientific - Theta Lite 100), according to method described by Li et al. [7]. To carry out the measurements, pellets of SO modified n-HAp particles were prepared, after drying the dispersion in an oven at 80 °C for at least 3 h to obtain the powders. Pellets with a diameter of 13 mm and an approximate thickness of 1 mm were prepared using a hydraulic press (PerkinElmer) at 10 tonnes for 2 min. Measurements were made right after pellets preparation by pouring a sessile drop of distilled water (5 μL) at their surface using a high-precision injector. The water droplet was recorded with a high-speed video camera coupled to the equipment, and the contact angle values between the droplet and compressed particles (left and right) automatically calculated by the software using the Laplace-Young equation. The measurements were performed in triplicate using different pellets, and the results are reported as average±SD.

Scanning electron microscopy analysis. Scanning electron microscopy (SEM) was used to check the morphology of the SO modified n-HAp particles. For the analysis, the dried SO modified n-HAp was mounted in a carbon adhesive, followed by coating with an Au/Pd thin film by sputtering, using the SPI Module Sputter Coater equipment. Then, the SEM/EDS analysis was performed using a high resolution (Schottky) environmental scanning electron microscope with X-ray microanalysis and electron backscattered diffraction analysis: Quanta 400 FEG ESEM/EDAX Genesis X4M.

2.3. Production and characterisation of the Pickering emulsions stabilised with the modified n-HAp particles

2.3.1. Production of the Pickering emulsions

The Pickering emulsions were prepared in batch mode using a rotor-stator homogeniser. For that, the SO modified n-HAp particle's dispersion, as prepared in Section 2.2.1, was added drop-wise to the continuous phase (sunflower oil) using a peristaltic pump operating at 240 rpm. The emulsification was carried out using a rotor-stator homogeniser running at 11000 rpm for 1 min. To avoid the temperature rise generated by the homogeniser, during the emulsification process the vial used for the preparation was submerged in an ice bath. Emulsions with a W/O ratio of 20/80, and 30/70, using, respectively 1.6%wt, and 2.7%wt of SO modified n-HAp particles were prepared. The methodology to estimate the amount of required particles can be consulted in the supplementary material (S2 Section).

2.3.2. Characterisation of the Pickering emulsions

Optical microscopy. Optical microscopy (OM) was used to check the morphology of the Pickering emulsions right after the emulsification process (0 days). For that, an aliquot of the sample was placed on a slide and gently covered with a coverslip. A Carl Zeiss Axiotech 100 HD optical microscope (Zeiss Instruments) fitted with a digital camera (AxioCam 105 colour) was used. The image acquisition and processing was made using Zen (2.3 blue edition) software.

Confocal laser scanning microscopy. Confocal microscopy was used to check the interfacial structure of the Pickering droplets. For that, the Pickering emulsion was stained with a mixture of fluorescent dyes in isopropyl alcohol, namely Nile red at 0.1% w/v and Nile blue A at 0.1% w/v. Nile red and Nile blue A are able to dye the continuous phase, i.e., oil phase (green colour) and the solid particles (red colour), respectively. These experiments were performed using a Leica TCS-SP5 AOBS (Leica Microsystems Inc. Heidelberg, Germany). The stained emulsion was put on a slide, and the fluorescent dyes excited by different wavelengths; namely 488 nm for Nile red and 633 nm for Nile blue A. The images were digitally recorded and processed with LasX software.

Emulsion type. The drop test is a rapid test to check the formed emulsion type (O/W or W/O). This test is based on the fact that the emulsion will disperse when put in contact with the respective continuous phase. Briefly, if the Pickering emulsion drop rapidly spreads in water and remain agglomerated in the oil phase, the emulsion is of O/W type. If the Pickering emulsion drop rapidly disperses in oil and remain agglomerated in the water phase, the emulsion is of W/O type. This test is carried out by adding two or three emulsion drops in water and oil phases, followed by slight stirring and observation.

Rheological characterisation. The produced Pickering emulsions were analysed in terms of viscous and viscoelastic behaviour. The tests were performed using an Anton Paar rheometer (Anton Paar, GmbH, Austria) with a parallel plate and at 20 °C. The viscosity of the Pickering emulsions was determined as a function of the shear rate in a range from 0 and 3000 1/s. The frequency sweep was determined in a range from 0.3 to 100 rad/s, and all measurements were performed within the determined linear viscoelastic region (LVE) and made at 0.1% strain. Data is reported as storage modulus (G') and loss modulus (G'') as a function of frequency.

3. Results and discussion

3.1. Sodium oleate modified n-HAp particles

In this study, the effect of sodium oleate (SO) on the n-HAp particles surface was studied. The main objective is to increase the hydrophobicity of the solid particles modifying their wettability to render them appropriate for W/O Pickering emulsions stabilization. For that, the SO was added at an amount according to the number of desired layers (0.5, 1 and 2) covering the n-HAp solid particles (see supplementary material - S1 Section). The number of layers that cover the n-Hap particles was defined taking into account the number of SO molecules needed to cover the surface area of a n-HAp solid particle, which was then transformed in mass content. To apply this methodology, firstly, the number (mass) of particles to cover one layer was calculated. Subsequently, for two layers the mass was duplicated, and for 0.5 layers half of the quantity was used. This last case corresponds to a partial coverage of the particle surface as schematically represented in Fig. 1. The produced SO modified n-HAp particles were characterised in terms of size, potential zeta, morphology (SEM) and wettability, and the effect of the applied number of layers evaluated.

3.1.1. Effect of the layers number in particle's size and zeta potential

The particle size and zeta potential of the SO modified n-HAp particles were evaluated as a function of the used number of layers. These parameters can impact on Pickering emulsions development, namely, particles with smaller sizes can be better adsorbed at the droplet interface, as stated by Xi et al. [33]. In this study, a commercial n-HAp formulation (n-HAp102) was modified with SO (0.5, 1, and 2 layers). Table 1 shows the results obtained with the particles right after production. It is possible to verify that, as the number of layers increases, a significant impact on both size and zeta potential parameters was observed, namely the size decreases and the zeta potential increases (in magnitude), in accordance with an effective surface modification. The magnitude of the zeta potential gives an indication concerning particles stability in suspension. Namely, values higher, in magnitude, to 30 mV, i.e., ≤ -30 and ≥ 30, give rise to particles with enough electrostatic repulsion forces to prevent the formation of aggregates [34]. The

Table 1
Particle size and zeta potential of different SO modified n-HAp102 according to the number of layers.

SO layers	Size (μm)	Zeta potential (mV)
0.5	1.72 ± 0.49	-25.2 ± 0.6
1	0.50 ± 0.03	-35.8 ± 0.1
2	0.12 ± 0.01	-45.5 ± 0.5

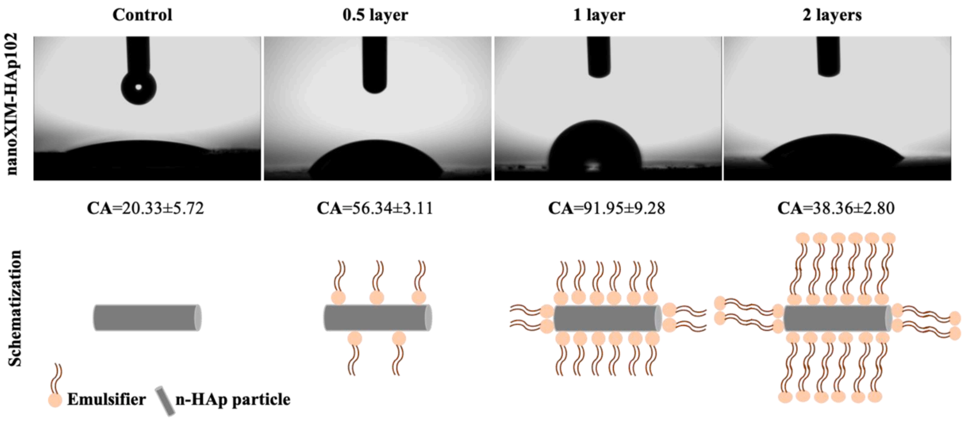


Fig. 1. Registration of the contact angle measurements of the original n-HAp (control) and SO modified n-HAp at different contents to achieve different number of layers (0.5, 1, and 2). Schematic representation reflecting the rearrangement of the SO molecules with the number of layers.

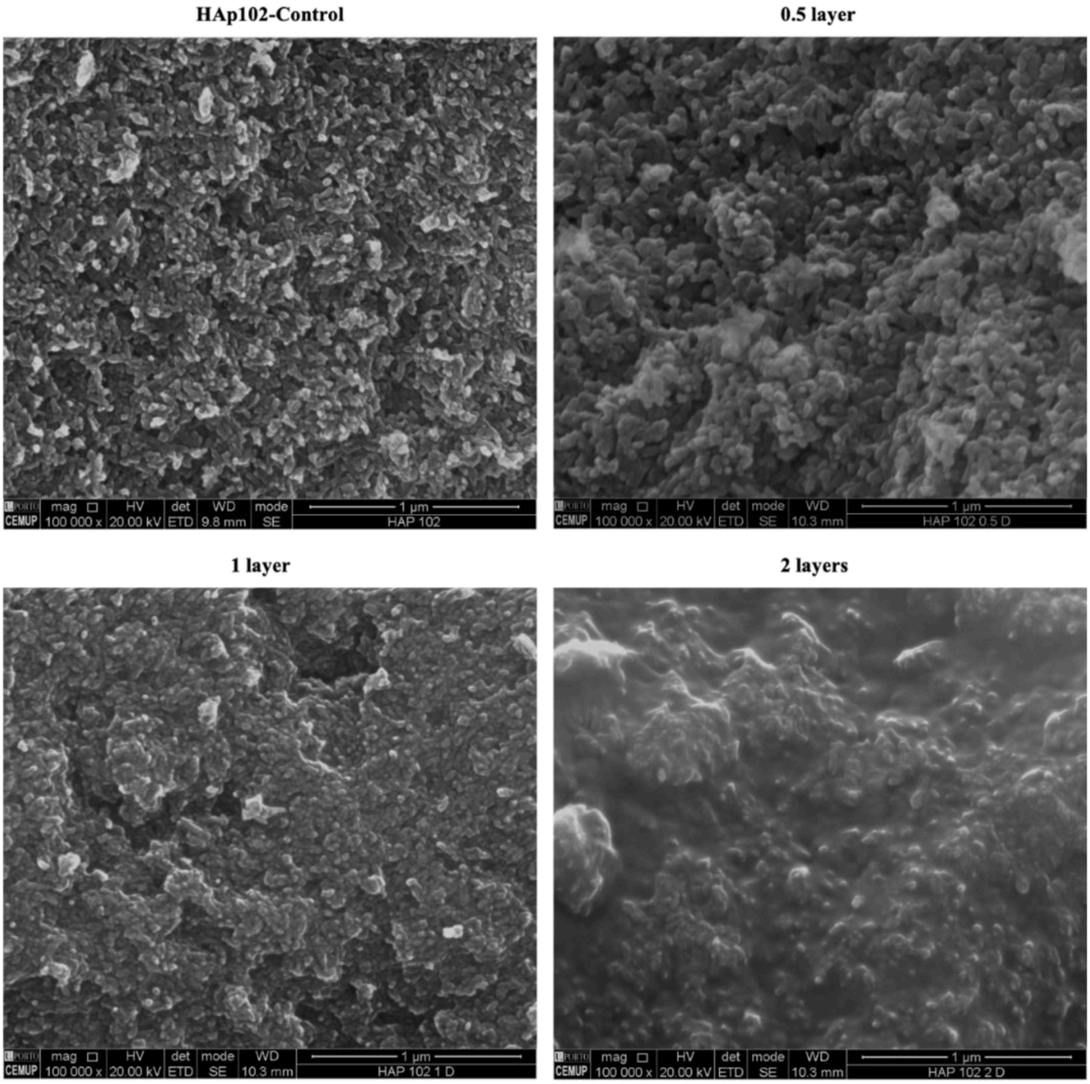
HAp102 (originally ∼25 mV) changes to -25 mV and -46 mV when the particles range from 0.5 to 2 layers showing an increase of stability (values lower than -30 mV). The measured apparent lower size can be justified by the increase of the electrostatic repulsion, preventing the formation of aggregates.

Other previous works reporting the influence of surface modifications on particle size and zeta potential have been published. In the work of Zhu et al. [35] zeta potential of silica particles was improved by increasing cetyltrimethylammonium bromide (CTAB) concentration, also reporting the adsorption of CTAB molecules at the particle–water interface at these concentrations. Li et al. [7] that studied gliadin nanoparticle's modification with octenyl succinic anhydride (OGNPs) reported changes in the particle size and zeta potential of OGNPs, comparatively with GNPs. Namely, an increase of the zeta potential (stability improvement) was observed as the octenyl succinic anhydride content increased; which was accompanied also by the production of larger size particles.

3.1.2. *Effect of the layers number in the particle's wettability*

To achieve Pickering emulsion stabilization, particles with a suitable wettability must be selected [6,36,37], similarly to traditional emulsifiers that are ruled by the hydrophilic-lipophilic balance (HLB) scale. The wettability measured by the contact angle determination, indicates the hydrophilic or hydrophobic character of the solid particles [6]. Thus, when solid particles have a contact angle $< 90^{\circ}$, the particles are considered more suitable to stabilise O/W emulsions; if the contact angle is $> 90^{\circ}$, the particles tend to stabilise W/O emulsions [36]. The wettability of the particles can be tuned through thermal [11,38], and chemical treatments [7], or through the combination with emulsifiers or polymers [21,37]. This can be advantageous for emulsion stabilisation when the original particles are highly hydrophilic or hydrophobic [2].

The contact angle of the n-HAp102 particles, untreated and SO surface modified (0.5, 1 and 2), was determined and the results are shown in Fig. 1, accompanied with a schematic representation reflecting the rearrangement of the SO molecules with the number of layers. For the untreated sample, the contact angle was around 20 °, indicating their higher hydrophilicity and suitability to stabilise O/W Pickering emulsions. As the number of SO layers increased, the contact angle of n-HAp particles increased until 1 layer, decreasing for 2 layers. Namely, when 1 layer was applied the contact angle increased to 92 °, and when 2 layers were applied the value decrease to 38 °. This behaviour is compatible with the fact that the second layer is formed over an existing layer (Fig. 1), switching the hydrophobic character achieved with 1 layer to hydrophilic, namely by exposing the emulsifier head (hydrophilic). This effect was also reported by Binks et al. [37] using silica particles and a di-chain cationic surfactant as the modifying agent. The authors reported a successive transition from hydrophilic, to hydrophobic, and again to hydrophilic as the concentration of the surfactant increased.



HAp102-Control

0.5 layer

1 layer

2 layers

Fig. 2. SEM images of the control n-HAp particles and SO modified n-HAp particles with different layers (0.5, 1 and 2). The scale bar represents 1 μm.

3.1.3. Effect of the layers number in the particle's morphology

The effect of SO in the particle's morphology was investigated by scanning electron microscopy (SEM), and the obtained images shown in Fig. 2 for the n-HAp102 particles without treatment (control), and the SO modified n-HAp102 particles with 0.5, 1 and 2 layers.

The n-HAp102-control particles exhibited the typical rod-like shape and the reported dimensions by Fluidinova [39,40], namely a typical length of 50 nm. With SO addition the morphology was modified; namely as the number of layers increased from 0.5 to 2, the n-HAp surface become smother. This effect evidences the successful adsorption of the SO at the n-HAp surface.

3.2. Pickering emulsions stabilized with the modified n-HAp particles

The production of O/W n-HAp based Pickering emulsions was previously reported by our group in batch [22], and continuous mode [23]. In order to increase the range of applications, namely for the loading of hydrophilic active principles the interest for the production of n-HAp based W/O Pickering emulsion was identified. Based on the previous section results the SO modified n-HAp102 with one layer, i.e., the particles characterized by a contact angle slightly higher than 90 °, were used. According to evidences reported in literature, particles with a contact angle around 90 ° facilitate their adsorption at the droplet's interface. They can also produce a steric hindrance against droplet's aggregation [7].

To produce stable Pickering emulsions, it is important to know the needed amount of n-HAp particles to stabilise the droplet's surface. In this work, the required amount of SO modified n-HAp particles was determined based on the findings of a previous work [22]. Briefly, the amount of particles was given by the product between the density, volume and the number of solid particles needed to cover droplets surface. Details of the used methodology can be seen in supplementary material (S2 Section). The W/O Pickering emulsions were produced according to the estimated mass of SO modified n-HAp that cover the total droplets. Two W/O ratios were used, namely 20/80 and 30/70 (W/O, v/v) and the emulsification carried out with a rotor-stator homogeniser. The 20/80 and 30/70 were produced with particles concentration of 1.6%wt and 2.7%wt, respectively. The optical images of both W/O Pickering emulsions, examined right after production, are shown in Fig. 3. It can be perceived the spherical shape of droplets, some level of aggregation, and size dispersity.

3.2.1. Type and storage stability of the W/O Pickering emulsions

After production, the emulsion type was determined through the drop test, and the stability evaluated along a storage period of 30 days (sampling at 0, 15 and 30 days) at room temperature. Fig. 4 shows the drop test made with the produced 20/80 Pickering emulsion. It is possible to observe that the drops of the Pickering emulsion spread in the oil phase and agglomerated in the water phase thus conforming the W/O type. This behaviour was also achieved for the Pickering emulsion produced with a ratio of 30/70.

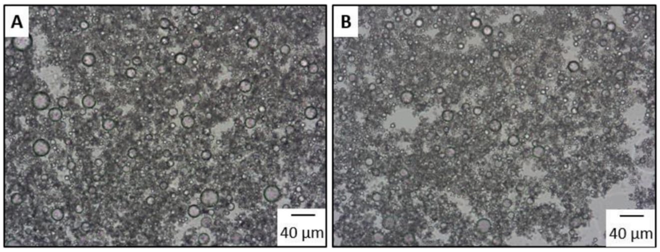


Fig. 3. Optical images of the W/O Pickering emulsions stabilised with the SO modified n-HAp: (A) 20/80 and (B) 30/70, acquired after production. Magnification: 20x.

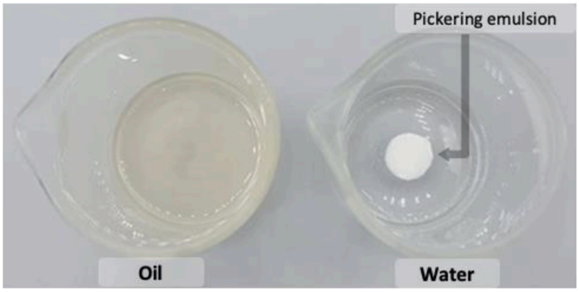


Fig. 4. Drop test behaviour for sample 20/80 (v/v). Pickering emulsion dispersed in the oil phase (left beaker) and Pickering emulsion agglomerated in the water phase (right beaker).

Fig. 5 shows representative photographs of the produced W/O Pickering emulsions (20/80 and 30/70) at different storage times (0, 15 and 30 days). The Pickering emulsions remained stable (without any phase separation) over 15 days at room temperature, as can be inspected in Fig. 5. However, some sedimentation phenomenon started to occur after this storage time (assigned by the black arrow). The stability of the W/O n-HAp based Pickering emulsions was also reported by Hu et al. [28] that prepared high internal phase Pickering emulsions (HIPEs). In this work, the authors reported a storage stability of 30 days. However, in this study, the emulsions had a gel-like structure, thus a higher viscosity, which can justify their increased stability over time. High viscosities in emulsions create mechanical rigidity helping emulsion stabilisation [1]. For W/O emulsions produced out of this range of compositions, instability was observed at early stages. This is the case of Zhang et al. [41] that produced Pickering emulsions based in n-HAp and PLLA achieving a stability not surpassing the 24 h. Zhang et al. [21] in their study addressing the effect of stearic acid modified n-HAp in Pickering emulsions and HAp/PLLA composites, reported stabilities of only 4 h. Furthermore, Hu et al. [32] reported rapid destabilisation of W/O Pickering emulsions stabilised with sodium oleate modified HAp. The destabilisation was associated with the antagonistic effect caused by high SO concentrations; i.e., under the used conditions the particles and surfactants lose their ability to stabilise the oil/water interface. Compared to the literature results, this work gave rise to satisfactory stability levels, which indicates that the Pickering emulsions produced with SO modified n-HAp particles with a monolayer results in an improved stability during storage time. This result also evidences the importance to analyse the effect of the used number of layers.

The physical stability of the Pickering emulsions can be achieved through different mechanisms, namely desorption energy, capillary forces, and particle-particle networks. One strategy to improve the stability is surface modification of the solid particles, which modifies their wettability and, consequently, their ability to be adsorbed and adhere to the oil-water interface [2]. In the present work, surface modification with SO was used to change particle's character from hydrophilic to hydrophobic. The obtained contact angle resulted close to 90 °, which conforms a situation where a high energy is needed to remove (desorb) the particles from the interface, i.e., the particles can be considered irreversibly adsorbed, thus pointing out for emulsion stability.

3.2.2. Confocal microscopy analysis

Confocal microscopy allows the visualisation of the droplet's interface, letting also to confirm the emulsion type. Following the results achieved for the storage stability (visual inspection), the 20/80 system was chosen for a detailed analysis by confocal microscopy (1 and 30 days after production). The corresponding fluorescence microscopy images are shown in Figs. 6 and 7. Both figures are composed of three images, which correspond to red fluorescence (showing the SO n-HAp modified particles), green fluorescence (showing the sunflower oil), and the overlap of the two fluorescence images. Fig. 6 shows the Pickering emulsions obtained after 1 day of production, where it is possible to

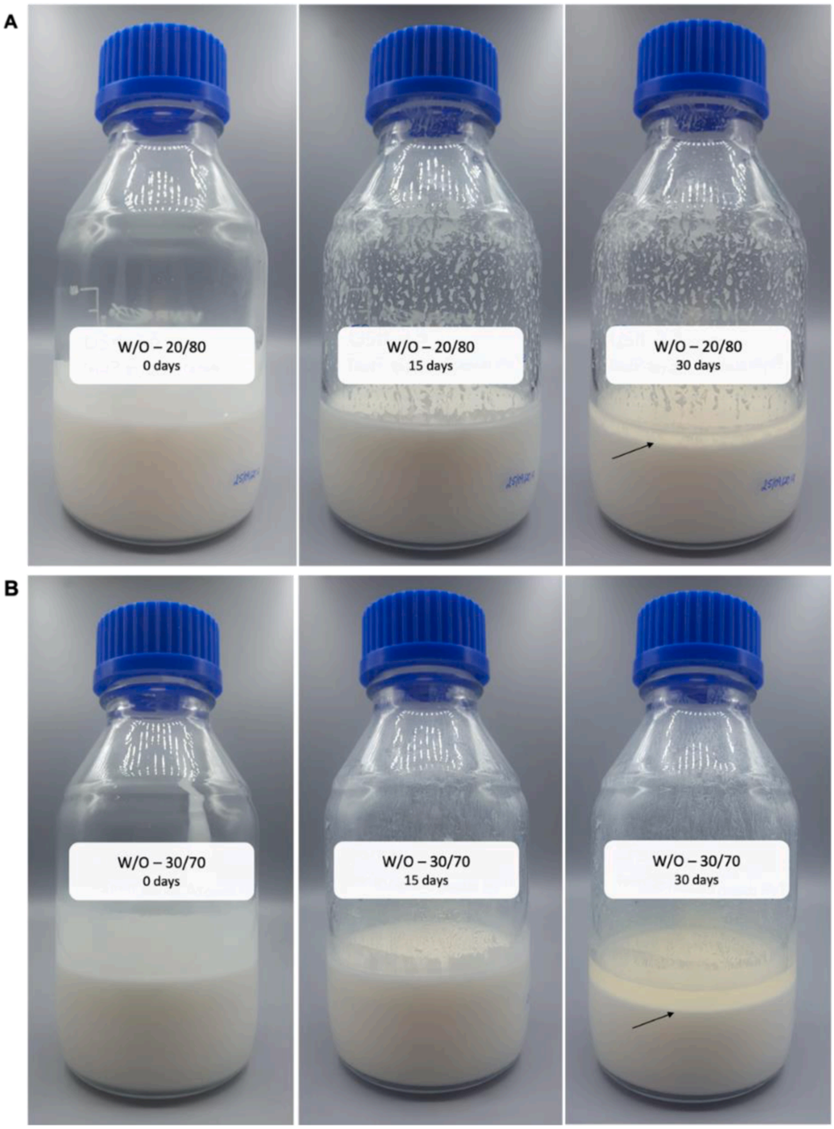


Fig. 5. Representative photographs of the W/O Pickering emulsions A) 20/80 (v/v) and B) 30/70 (v/v) as a function of the storage time (0 days – right after production, 15 and 30 days). The arrow indicates the emulsion phase separation.

identify the sunflower oil as the continuous phase (green fluorescence) and the modified n-HAp particles at the boundary of the droplets (red fluorescence). This observation is in agreement with the results given by the application of the drop test (Fig. 4). The higher magnification (bottom line) confirms, more clearly, the presence of the SO modified n-HAp particles covering the surface of the water droplet (evidenced as a red ring).

In Fig. 7 a comparison between the Pickering emulsion produced after 1 day (top line) and 30 days (bottom line) is made. From the set of images, it is possible to verify that there was no phase inversion, although some aggregation among the droplets is perceived (coalescence phenomenon). This observation is in accordance with the observations of Fig. 5 that put in evidence the occurrence of phase separation after 30 days of storage.

The confocal microscopy technique has proven to be a reliable technique to analyse W/O emulsions, which are characterized by a high oil content (continuous oil phase). Overall, it enables to not only to confirm the emulsion type but also to inspect the adhesion of the particles to the oil/water interface and thus to monitor the development of instability phenomena, being a valuable technique to study this type of systems.

3.2.3. *Rheological characterisation of Pickering emulsions*

The rheological properties of Pickering emulsions can be related with their performance and storage stability. The viscosity, storage (G') and loss (G'') moduli of the 20/80 and 30/70 W/O Pickering emulsions were determined after production (0 days) by steady-state flow and dynamic oscillatory measurements, respectively (Fig. 8). In terms of viscosity, it was possible to perceived a decrease as the water phase (dispersed phase) volume increased. For the moduli G' and G'', a frequency dependency was observed, with G' > G'' over the complete tested range, meaning that the requirements for a stable structure are fulfilled and thus physical stability for the produced Pickering emulsions is expected. This indicates a well consolidated structure derived from a stable net of forces.

4. Conclusions

This work addressed nano-hydroxyapatite (n-HAp) modification aiming at achieve suitable Pickering stabilisers for W/O emulsions, which was motivated by the lack of solutions in the field and their potential for several applications. The n-HAp surface was modified with sodium oleate (SO) to tune the wettability of the pristine particles,

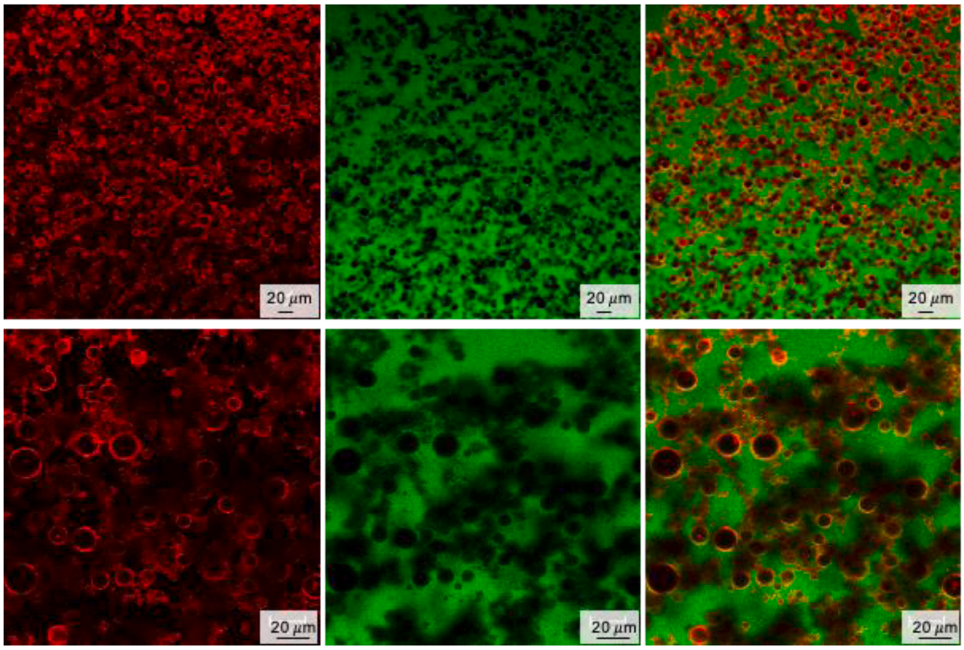


Fig. 6. Confocal microscopy images of W/O Pickering emulsions (20/80, v/v) after 1 day of production registered at two magnifications (Top – 63x and zoom= 1, Bottom - 63x and zoom= 3). The red and green fluorescence colours represent the modified solid particles (first column, dyed with Nile blue A) and oil phase (second column, dyed with Nile red). The third column corresponds to the superimposition of the two previous ones. The scale bar is equal to 20 μm.

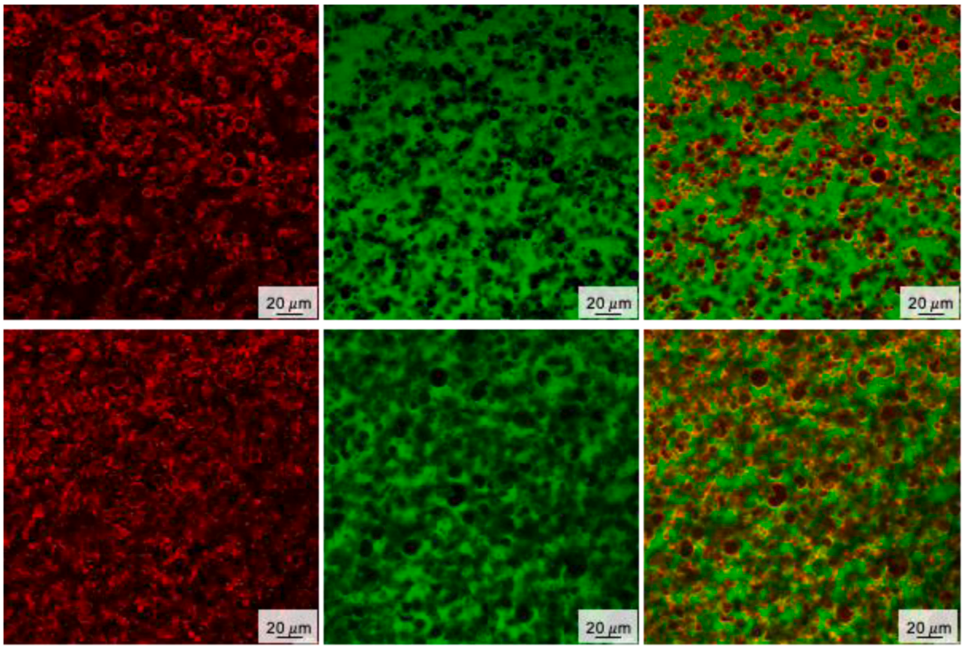


Fig. 7. Confocal microscopy images of W/O Pickering emulsions (20/80, v/v) after 1 day (top line) and 30 days (bottom line) of production registered at 63x of magnification. The red and green fluorescence colours represent the modified solid particles (first column, dyed with Nile blue A) and oil phase (second column, dyed with Nile red). The third column corresponds to the superimposition of the two previous ones. The scale bar is equal to 20 μm.

which are characterized by a natural hydrophilic character. It was verified that the number of SO layers impacts the wettability of the n-HAp particles, which varies from hydrophilic (0.5 layers) to hydrophobic (1 layer), and again to hydrophilic (2 layers). Additionally, the presence of SO at the surface of n-HAp particles affects their physical characteristics, mainly the size and surface charge. As the number of layers increases, more stable the particles become in suspension, as evidenced by the measured zeta potential (< -30 mV). The particle size decreased as a result of the electrostatic repulsion increase, which presents advantages avoiding aggregates formation. The modified n-HAp particles with contact angle close to 90 ° were chosen to test in the preparation of W/O Pickering emulsions, corresponding to the ones treated with one SO layer, i.e., a saturated monolayer conferring a hydrophobic character. It was possible to confirm the ability of the particles to adsorb at the water droplets surface and their W/O typology. Besides, it was possible to achieve good emulsion stability over 15 days of storage with only slight signs of instability observed for 30 days.

Overall, the work presents a systematic approach to carry out surface

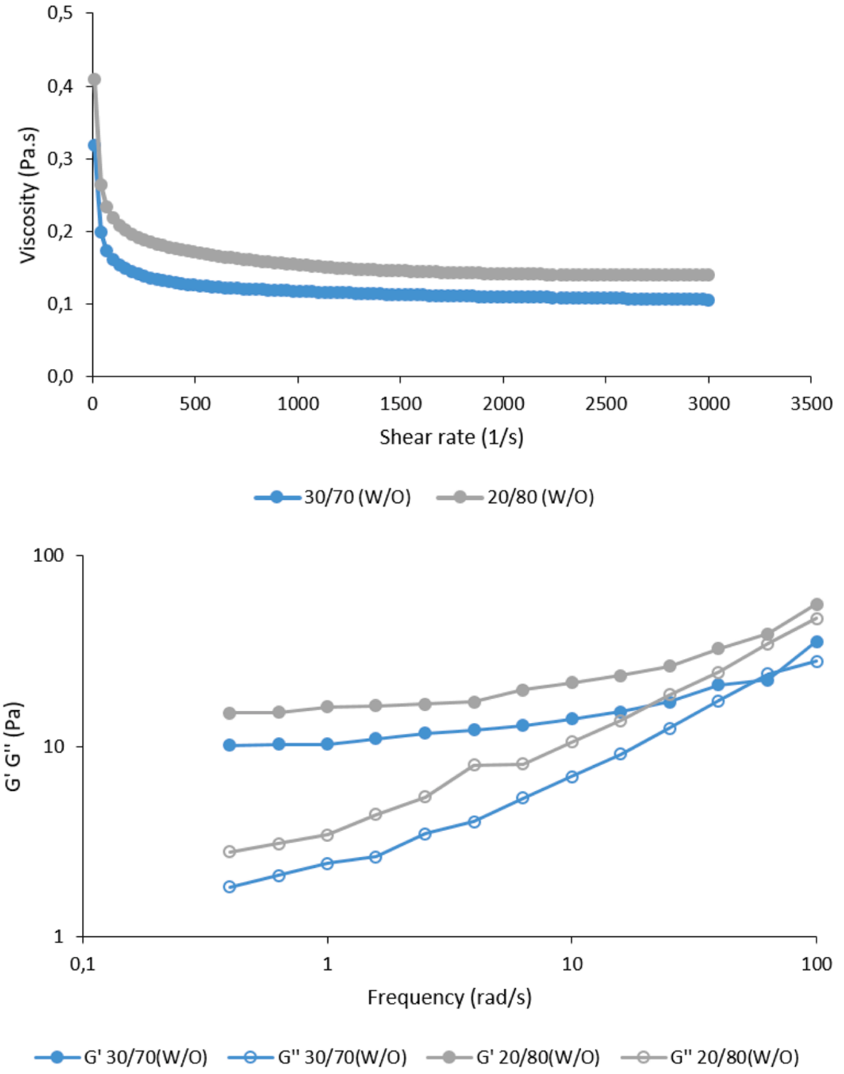


Fig. 8. Rheological properties of the 30/70 and 20/80 Pickering emulsions. Viscosity versus shear rate and storage modulus (G') and loss modulus (G'') versus frequency.

modifications of solid particles. The wettability of the n-HAp particles can be tuned according to the number of applied SO layers. Next steps will proceed with a more detailed study on the effect of the W/O ratio and particles concentration on the Pickering emulsion properties, including stability and rheological properties. By following this strategy, W/O emulsions were obtained opening new avenues for products based on n-HAp particles, which by nature stabilize O/W emulsions.

CRediT authorship contribution statement

Andreia Ribeiro: Methodology, Investigation, Validation, Writing – original draft. **Yaidelin A. Manrique:** Methodology, Writing – review & editing. **José Carlos B. Lopes:** Conceptualization, Methodology. **Madalena M. Dias:** Conceptualization, Supervision, Writing – review & editing. **Maria Filomena Barreiro:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.surfin.2022.101759.

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