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Phase Inversion and Coverage Analysis of H/E Pickering Emulsions Stabilized by Eudragit® L100 Nanoparticles

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Abstract

In a landscape marked by the widespread use of surfactants, there has been a renewed interest in Pickering emulsions stabilized by solid particles for two principal reasons: the imperative to minimize the utilization of synthetic surfactants due to environmental concerns, and recent progress in particle functionalization. The primary objective of this study was to formulate an H/E Pickering emulsion stabilized by Eudragit®L100 nanoparticles and to investigate the phenomenon of instability associated with phase inversion, while concurrently examining the coverage rate. Conductivity measurement was employed to ascertain the volumetric composition of emulsions during phase inversion, and microscopic analysis facilitated the assessment of coverage rates, enhancing our understanding of phase inversion. The findings reveal an approximate volumetric phase inversion composition of 85% in oil within the scope of our research, confirming the capacity of Eudragit® L100 nanoparticles to stabilize the formulated emulsions. These results signify a promising step forward in the development of sustainable Pickering emulsions, emphasizing the critical significance of environmentally mindful stabilization approaches across diverse industrial sectors.

Keywords: Pickering Emulsions Nanoparticles; Eudragit® L100; Phase Inversion Composition; Coverage Rates

Introduction

Pickering emulsions stabilized by solid particles, discovered by Ramsden and Pickering in the early 1900s, developed slowly until the 1980s [1,2]. Unlike surfactant-stabilized emulsions, they offer robust stabilization and high resistance to coalescence, thanks to a dense layer of solid particles irreversibly adsorbed around water droplets. Additionally, they are non-toxic, environmentally friendly, and have lower production costs [3-13].

These emulsions have recently gained significant attention in various research fields, particularly in cosmetics, pharmaceuticals, and food applications, where surfactants can cause adverse effects such as irritation or hemolytic behavior [13]. As an innovative drug delivery system, they could facilitate transdermal drug absorption [14], enhance the oral uptake of poorly water-soluble drugs [15,16], and improve drug stability [17].

Recently, new Pickering emulsions with droplet sizes ranging from 50nm to 30 µm have been developed [18-24]. These fine droplets minimize creaming and gravitational sedimentation, even over long periods. Their large specific surface area enables highly active formulations, which are advantageous for applications in cosmetics [21], agri-food [23,25], drug delivery [20], and food production [18,26,27].

The irreversible and strong adsorption of solid particles at the water/oil interface creates a dense film, forming an effective barrier against droplet coalescence. Adjusting the properties of stabilizing particles, influenced by the continuous phase, can modify their wettability and allow control over interface stability or even enable controlled drug release. The energy associated with nanoparticle (NP) interactions at the interface, including adsorption and desorption, depends on their wettability by water and oil [21].

However, the precise role of particles and their interfacial properties remains a subject of debate [28-31]. Some studies suggest that submicron particles have a limited effect on interfacial tension, acting as a third phase between water and oil with negligible influence on interfacial energy [32]. For instance, Hunter et al. [32] observed that hydrophobic silica particles (~260 nm) did not affect surface tension at the air/water interface. Conversely, other studies report a significant effect of particles on interfacial tension. For example, Kutuzov et al. [29] demonstrated a reduction in interfacial tension with increasing concentrations of cadmium selenide (CdSe) particles at the toluene/water interface. Overall, depending on the nature of the particles and experimental conditions, NPs can have a substantial impact on surface tension or exhibit minimal effects [28-31,33-35]. In the context of Pickering emulsions, several NPs have proven to be highly effective stabilizers, including CdSe NPs (1–8 nm) [36], silver NPs (1–5 nm) [37], and clay particles (25–35 nm) [38]. The drop tensiometer is commonly used to study interfacial tension and rheology at liquid/liquid interfaces. Nevertheless, the debate continues regarding the interfacial effects and activity of NPs. Some researchers claim that their adsorption does not alter interfacial tension or rheological properties [28-30,32-35], while others argue that adsorption significantly modifies these properties [39-42]. This ongoing debate is likely due to the vast variability in the chemical and physicochemical properties of NPs.

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In this context, our study focuses on the development of innovative water-in-oil (W/O) Pickering emulsions stabilized exclusively by biodegradable Eudragit® L100 nanoparticles. These non-toxic, biocompatible emulsions, free of chemical surfactants, pave the way for new environmentally friendly opportunities. Our research centers on understanding the composition of emulsions during phase inversion, a fundamental aspect for designing stable emulsion systems applicable in fields ranging from cosmetics to food and pharmaceuticals.

To achieve the outlined objectives, the study dedicated to experimental research, focuses on the synthesis and characterization of Eudragit® L100 nanoparticles, the formulation of W/O Pickering emulsions, the study of phase inversion composition and coverage rate through conductometric monitoring, and microscopic characterization of the formulated emulsions.

Experimental Section

Materials

Eudragit® L100 (Ammonio Methacrylate Copolymer Type A NF) was sourced from Evonik Röhm GmbH Pharma Polymers (Darmstadt, Germany). Acetone and cyclohexane, both of analytical grade, as well as Kolliphor® ELP (a nonionic PEGylated surfactant), were obtained from Sigma-Aldrich. Medium-chain triglycerides suitable for parenteral use, specifically Labrafac® WL1349, were supplied by Gattefossé SA (Saint-Priest, France). Ultra-pure water was obtained from a Millipore Super-Q unit (Millipore, Illkirch, France). All chemicals were of analytical grade and used without further modification.

Methods

Eudragit L100 Nanoparticles synthesis: Eudragit L100 Nanoparticles synthesis Nanoparticles were synthesized using the nanoprecipitation method [45]. Eudragit RL100 was dissolved in the internal phase, which consisted of 50 mL of acetone at room temperature (25 °C). The organic-to-aqueous phase ratio was maintained at 3:1. The prepared organic phase was then injected into 150 mL of the aqueous phase, containing 1% Kolliphor® ELP, at a constant rate of 0.5 mL/min under gentle agitation at 300 rpm. Finally, acetone was evaporated using a rotary evaporator.

The nanoparticle suspension was initially concentrated by evaporating a significant amount of water (75 mL) and then washed over a 48-hour period. The nanoparticles were cleaned using MilliQ water through dialysis (Spectra-Por regenerated cellulose membrane, with an 8–12 kDa cutoff) in MilliQ water under gentle stirring. The dialysate was replaced once after 24 hours. This washing step aimed to remove any residual free surfactant (Kolliphor® ELP). The washed nanoparticle suspensions were subsequently used for the preparation of nanoemulsions.

Eudragit L100 Characterization:

Dynamic Light Scattering and Zeta Potentiel

Measurements of size distribution, polydispersity index (PDI), and zeta potential were conducted using a NanoZS (Malvern Instruments, Orsay, France). Size distribution and PDI were assessed via dynamic light scattering (DLS), while the zeta potential was determined by measuring the electrophoretic mobility of the nanoemulsion droplets. All experiments were performed in triplicate to ensure accuracy.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) characterization was carried out using a Philips Morgagni 268D microscope operating at 70 kV. No staining agents were applied, and the samples were diluted 100-fold with MilliQ water. A drop of the diluted sample was placed onto a carbon-coated copper grid (Type A, 300 mesh, Inc., Redding, PA) and dried at 40 °C prior to analysis.

Axisymmetric Drop Shape Analysis (ADSA)

Interfacial properties were evaluated using axisymmetric drop shape analysis with a dynamic drop tensiometer (Teclis, Longessaigne, France). In the first experiment, the behavior of a rising drop (8 µL of cyclohexane in water) was studied, maintaining a constant volume and temperature. The aqueous phase used was either a 40 nm nanoparticle suspension or MilliQ water as à control. The second experiment involved a pendant drop configuration, where a drop of aqueous nanoparticle suspension was placed in cyclohexane. Interfacial tension was determined by analyzing the drop profile using a CCD camera integrated into the setup. The drop profile resulted from a balance between gravitational forces, which elongated the drop, and capillary forces, which rounded it. The Laplacian shape of the drop was used to calculate the interfacial area and surface tension. All experiments were conducted at 25 °C and repeated in triplicate for accuracy.

Pickering émulsion preparation: The oil-in-water (O/W) emulsions were prepared using a aqueous phase containing nanoparticles (NPs) and Ricin oil® WL1349 as the oil phase. The emulsification process was carried out with a mechanical mixer (Ultraturrax, IKA T-25 Digital) operating at 22,400 rpm for 3 minutes at 25 °C.

Pickering emulsion Characterization:

Macroscopic and Microscopique Examination

Bottle Test : The emulsions were stored in 50 mL conical bottles, kept away from light and at ambient temperature. Visual inspection was conducted to identify potential instability phenomena, such as sedimentation, flocculation, and coalescence.

Determination of Emulsion Type: The dye test was performed to determine the emulsion type based on the solubility of methylene blue in the prepared emulsion. Two milliliters of the emulsion were placed on a slide and mixed with a few milligrams of Sudan Red or methylene blue. After assembling the slide for examination, observations were carried out using an Axio Zeiss Imager A1 optical microscope. The microscope was coupled to a computer equipped with Axio Vision Release software (Version 4.5) for image capture and analysis.

Droplet Size Measurements: The droplet size was determined by estimating the mean diameter of the droplets through individual counting. Measurements were taken using an Axio Zeiss Imager A1 optical microscope, which was connected to a computer running Axio Vision Release software (Version 4.5) for data analysis.

Conductivity Mesurement: The method is based on measuring the electrical resistance of a solution placed between two platinumcoated plates. The conductivity of the solution varies depending on the concentration of ions present. In a 15 mL tube with a screw cap containing the preparation to be studied, the conductivity cell is inserted. If the preparation is conductive, the conductimeter displays a value corresponding to the conductivity, expressed in Siemens per meter ($S{\cdot}m^{-1}$). For tubes showing sedimentation, the conductivity cell should be immersed at the level of the emulsified fraction.

Phase inversion study: The conductivity measurement setup consists of the Vernier LabPro interface, a conductivity probe, and a computer equipped with the Logger Pro software. The LabPro is a versatile sensor interface designed for data collection and processing. For this study, it connects the conductivity probe to the computer, enabling the use of the data collection software.

Results and Discussions

Characterizations of NPs Eudragit L100

Dynamic Light Scattering and Zeta Potentiel: The size of the nanoparticles (NPs) is influenced by the composition of the aqueous and organic phases used during preparation. Eudragit L100 NPs were prepared, exhibiting a size distribution with a log-normal profile, centered around a diameter of 40 nm (see Figure 1). The polydispersity index (PDI) was measured at 0.098, indicating good monodispersity of the suspension. The measured zeta potential was -35 mV ± 3.1.

Transmission Electron Microscopy: Morphological characterization of the nanoparticles (NPs) was conducted using Transmission Electron Microscopy (TEM) (see Figure 1). The NPs (Figure 1 (a)) appeared as round spheres, with a size consistent with the dynamic light scattering (DLS) results, showing a distribution centered around a diameter of 40 nm.

Some nanoparticles were isolated on the carbon support, while others formed aggregates or clusters, a typical occurrence during sample drying. Since the nanoparticles were nanoprecipitated, they were in a glassy state and exhibited hypo-contrast, which contrasts with the typical behavior observed in lipid particles [43].

Characterization of Pickering Emulsion

Macroscopic Examination: The physicochemical and analytical parameters of the various emulsions were studied over a 28-day period, with the emulsions stored in the absence of light at room temperature. This allowed us to monitor the evolution of the formulations over time. The main results from the visual inspection indicated that the emulsions appeared macroscopically homogeneous and stable, with 100% of the emulsified phases. However, it is important to note that macroscopic observation alone does not determine the stability of the emulsion. In fact, this method cannot reveal the presence of oil droplets, which are on the scale of micrometers.

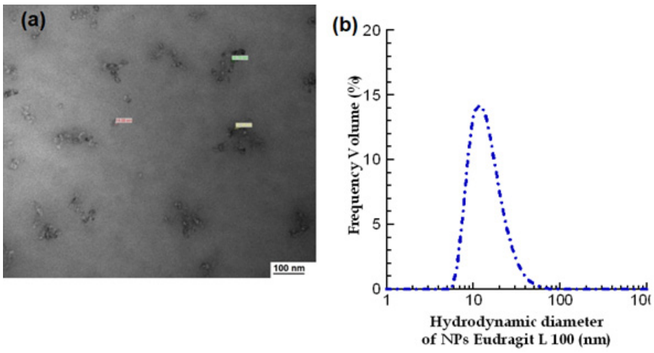

Figure 1: Physico-chemical characterization of the polymer nanoparticles (a), by transmission electron microscopy and dynamic light scattering (b).

Microscopique Examination: The emulsion type was determined using the methylene blue test and conductivity measurements. The methylene blue test, conducted under an optical microscope, revealed heterogeneous droplets dispersed in a blue-colored external phase, confirming the oil-in-water (O/W) nature of the emulsions (Figure 2).

Size Droplet: At a constant nanoparticle (NP) size and concentration in the aqueous phase, various Pickering emulsion formulations were prepared with increasing medium-chain triglyceride (MCT) volumes. The sizes of these emulsions, shown in Figure 3, demonstrate a gradual increase in emulsion size, directly influenced by the rise in the dispersed phase volume fraction. Notably, for NPs of 40 nm, the curves extend to higher values because nano-emulsions continue to form even at higher volume fractions.

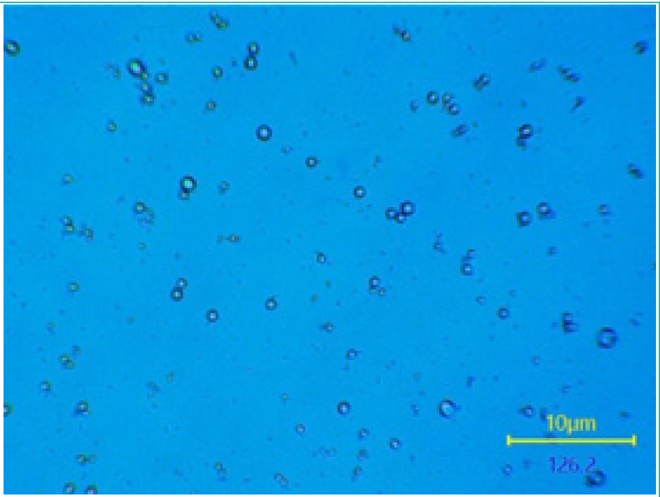

Figure 2: Microscopic examination of an emulsion stabilized by Eud L100 NPs (ratio 4:1).

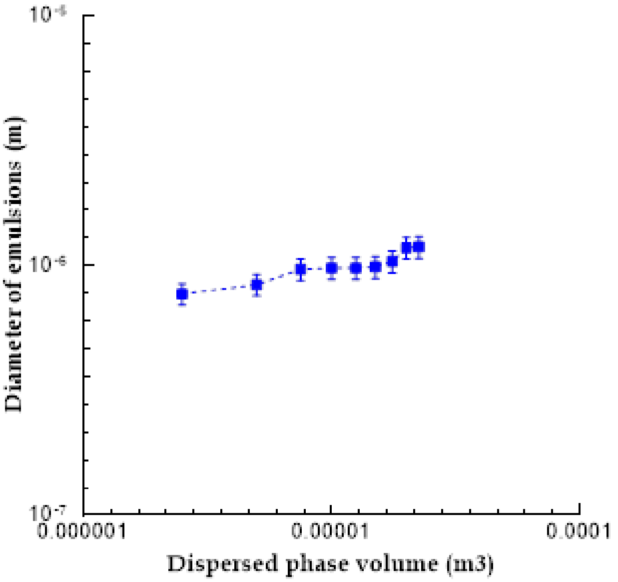

Figure 3: Evolution of the size of the droplets of emulsions depending on the dispersed phase volume (m^3).

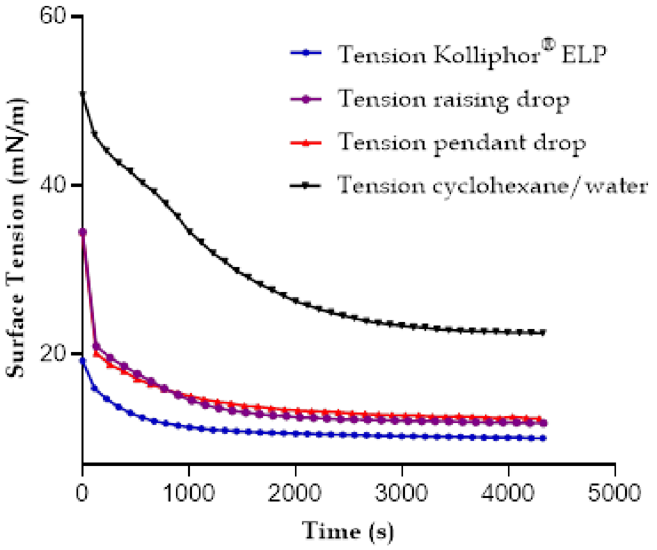

Figure 4: Study of the NPs adsorption of NPs Eud L100 on model C_6H_{12} / MilliQ water interface (aqueous suspension of NPs) at 25 °C. (a) Comparison of NPs Eud L 100 (40 nm), and free surfactants (Kolliphor® ELP at 3.10^{-3} mg/mL), for the rising drop configuration and pendant and drop configurations.

Conductivity Measurement: Regarding the conductivity values, all formulations exhibited values close to that of pure water. The conductivity of an emulsion is primarily determined by its external phase and the presence of electrolytes in that phase. The results obtained throughout the storage period indicate that the emulsions did not undergo any phase inversion. Furthermore, the conductivity values of the external phase were lower than the typical conductivity of water. This is because the oil phase is non polar, and the heterogeneous distribution of the oily droplets in the dispersing phase reduces the conductive role of water's electrolytes.

Phase Inversion Study

Interfacial Tension: To better understand the stabilization mechanism of emulsions and phase inversion, the adsorption kinetics of polymeric nanoparticles (NPs) onto a model interface were studied using both the rising drop configuration (O/W) and the pendant drop configuration (W/O). For this purpose, a model interface (C_6H_{12}/ water with aqueous NPs suspension) was created, and the interfacial tension was monitored. The first control experiment was conducted to confirm that the interfacial effect was indeed due to the NPs adsorbed at the interface and not the residual, unwashed surfactants. In this experiment (see Figure 4), a concentration of 5 µL/mL (i.e., 5 µL of NPs solution diluted in 1 mL of MilliQ water) of washed (i.e., before dialysis) and unwashed (i.e., after 48 hours of dialysis) NPs, along with surfactant (at 3.10^{-3} mg/mL – the concentration used in the NP formulation), were compared using the rising drop configuration and pending drop configuration.

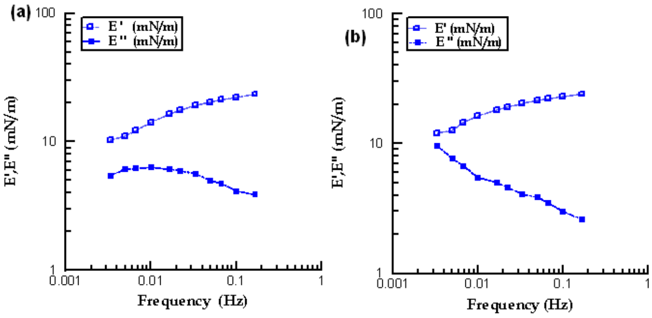

Figure 5: Interfacial rheology study on the model water (containing NPs) / C6H6 interface, rising drop configuration. Experimental values of conservative (E') and dissipative (E") modulus.

We demonstrate through these two configurations that nanoparticles adsorb and stabilize the interface effectively for both the pendant drop method and the rising drop method. This explains why nanoparticles remain adsorbed at the interface in the event of phase inversion.

Interfacial Rheology: The experiment aimed to measure the dilatational elastic modulus by analyzing the relationship between surface tension and interfacial area, which was modulated using a sinusoidal deformation. Measurements began only after surface tension stabilization and were conducted across a wide frequency range (0.0033 Hz, 0.005 Hz, 0.0066 Hz, 0.01 Hz, 0.016 Hz, 0.022 Hz, 0.033 Hz, 0.05 Hz, 0.066 Hz, and 0.1 Hz). To ensure consistency, the experiments were designed to maintain a Bond number between 0.2 and 0.25 and sinusoidal amplitudes at approximately 10% of the drop volume. All tests were performed at a constant temperature of 20 °C and repeated three times to guarantee reproducibility.

We demonstrate through these two configurations that nanoparticles effectively adsorb onto and stabilize the interface in both the pendant drop 5(a) and rising drop 5(b) methods for rheologie experiment. This behavior explains their persistent adsorption at the interface during phase inversion.

Kinetics of Conductivity: The data collection software we used provides a conductivity value every second. Readings were taken for up to 8,000 or even 10,000 seconds, which is why we chose intervals of 500 seconds to reduce the size of the obtained data. The results are illustrated in Figure 7, showing the variation in conductivity over time. These figures present the evolution of conductivity over time, as captured by the data collection software.

The decrease in conductivity proves to be a critical indicator for refining the determination of phase inversion. Initially, we did not consider time-based measurements; however, thanks to Logger Pro software, we were able to record conductivity values every second for the formulation under study.

During this phase, we performed moderate stirring while gradually adding castor oil. In our aim to determine the phase inversion composition, we recorded the total volume of oil added at the point where conductivity dropped, enabling us to calculate the volume percentage of the dispersed phase corresponding to the phase inversion composition.

Table 1: Range of Pickering emulsions with varying proportions of castor oil.

N° Essais	1	2	3	4	5	6	7	8	9	10
Volume of NP Suspension (mL)	5	5	5	5	5	5	5	5	5	5
Castor Oil (mL)	2,5	5	7,5	10	12,5	15	17,5	20	22,5	25
Volume fraction of dispersed phase (%)	33,33	50,00	60,00	66,67	71,43	75,00	77,78	80,00	81,82	83,33

Table 2: Summary of initial and final proportions for the determination of phase inversion composition.

Initial proportions		Amount of oil added (mL)	Phase inversion composition		
Suspension NPs (mL)	Castor oil (mL)		Castor oil (mL)	Suspension de NPs (mL)	%PIC
34	51	141	192	34	84,96
25	75	63	138	25	84,66
20	80	33,8	113,8	20	85,05

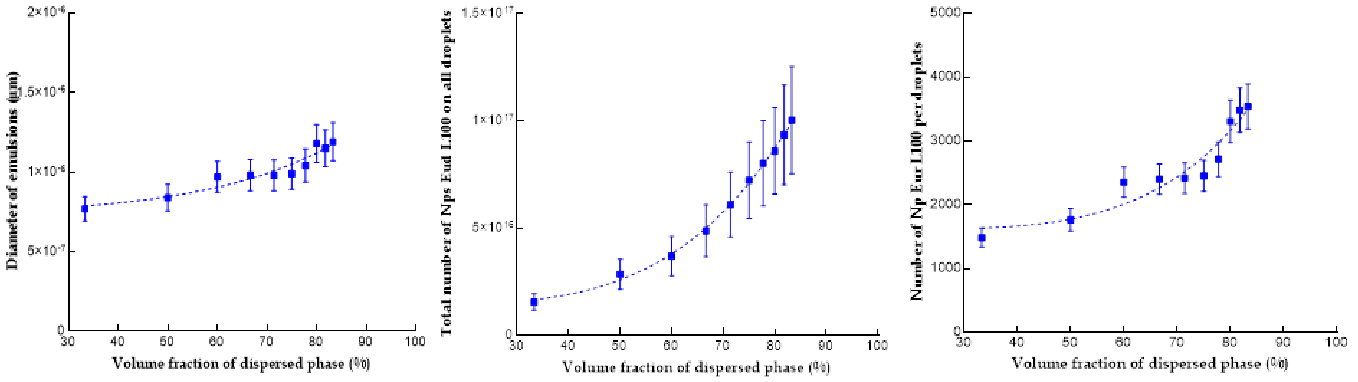

Figure 6: Study of the NPs Eud L100 coverage of the emulsion droplets, in function of the volume fraction of dispersed phase (continuous phase is water aqueous phase containing NPs Eud L100, and the dispersed phase is oil composed of ricin oil). (a) diameter of emulsions, (b) number of NPs Eud L100 adsorbed at the interface of a single emulsion droplet, and (c) number of NPs Eud L100 adsorbed onto all the emulsions droplets, n=3.

Table 2 below lists the initial proportions used and the final values obtained during the evaluation of the phase inversion composition, providing a clearer understanding of the conductivity monitoring process. We determined that the final composition of the emulsions at the point of conductivity drop corresponds to the phase inversion composition. The results indicate a phase inversion composition of approximately 85%, regardless of the initial composition of the measurement.

Figure 7 and 8 below illustrates the variation in conductivity as a function of the volume fraction of the formulations. The results show a decrease in conductivity as the oil volume fraction in the emulsion increases. Conductivity drops to zero at a proportion of 85%, corresponding to the phase inversion. In other words, the oil-in-water (O/W) emulsion transitions into a water-in-oil (W/O) emulsion.

Interfacial Coverage: In this section, we aim to study the surface coverage of the emulsion by NPs through a methodology that investigates the relationship between the size of the formulated nanoemulsions and the volume fraction of the dispersed phase (i.e.of, oil). The emulsion coverage will be estimated by determining the maximum number of NPs trapped at the droplet interface.

The coating of a spherical droplet with smaller NPs typically results in thick and compact shells [42,44], which can help better understand phase inversion. To study and confirm this hypothesis, the surface coverage was monitored as a function of the volume fraction of the dispersed phase. Depending on the properties of the particles, the NP packing around a larger particle can either form a compact monolayer [45] or a thick multilayer shell [46].

At constant NP size and concentration in the aqueous phase, different Pickering emulsion formulations were prepared by increasing the volume fraction of the dispersed phase. The hydrodynamic diameter of the nanoemulsions (as shown in Figure 6 (a)) revealed a gradual increase in emulsion size, which is an expected result since the number of NPs in the bulk remains constant. Subsequently, the total number of emulsion droplets (E-tot) in the suspension (see Figure

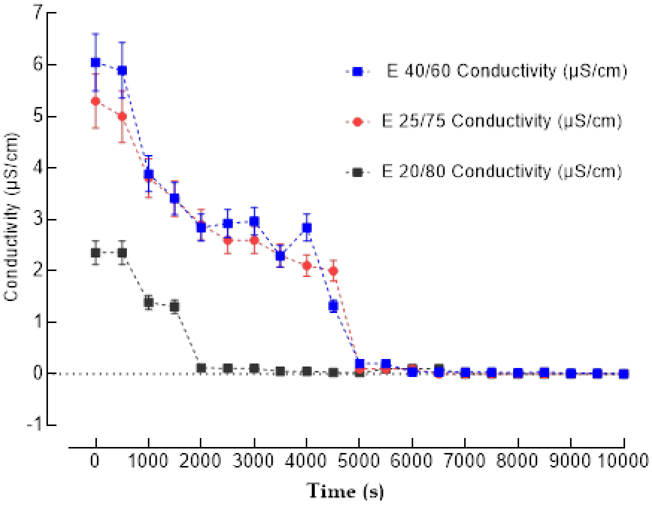

Figure 7: Monitoring of conductivity as a function of time.

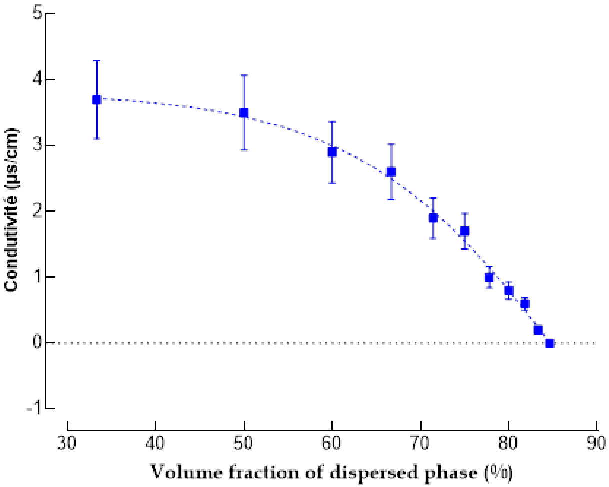

Figure 8: Variation of conductivity as a function of the volume fraction (%) of oil in the emulsion.

6 (b)) initially decreased but eventually stabilized as the oil volume continued to increase.

As the additional dispersed phase was added, the total specific surface area of the oil nanoemulsion decreased, and the number of particles reduced. The stabilization, indicating equilibrium, suggests the complete consumption of the NPs for interface stabilization. This result aligns with the absence of stabilizing agents, as all the NPs have already been trapped at the interface.

The study assumes that, at equilibrium, the oil/water interface is fully covered by nanoparticles (NPs) trapped at the interface with their maximum effective cross-sectional area (S_{NPs}). The maximum number of NPs that can adsorb onto the interface of a single emulsion droplet (N_{NPs}/E-1) is calculated as the ratio between the surface area of the droplet (A_{NE-1}) and the cross-sectional area of a single solid nanoparticles (S_{NPs}) (as illustrated in Figure 6(b)). Consequently, $N_{NPs/E-1}$ increases with the volume fraction of the dispersed phase a predictable trend, as larger droplet sizes result in a greater available interfacial area (see Figure 6(a)).

The total number of nanoparticles (NNPs/E-tot) adsorbed onto the nano-emulsion droplets, as shown in Figure 2(b), continued to increase consistently with the addition of more oil. This indicates that even at the highest volume fraction, maximum interfacial coverage was not achieved. The average droplet diameter was found to be directly proportional to the coverage rate and inversely proportional to the total number of stabilizing particles. A similar relationship between the final droplet diameter (Df) and the particle mass has been previously proposed in the literature (4), based on the assumption that the particles form a compact hexagonal monolayer at the water-oil interface.

The phenomenon of “limited coalescence” occurs when the total interfacial area of all droplets exceeds the surface area that stabilizing particles can cover. Since the adsorption of particles onto the interface is irreversible, coalescence occurs, reducing the total interfacial area until it matches the surface area that the adsorbed particles can effectively stabilize. As a result, when the volume fraction of the dispersed phase increases, the limited coalescence mechanism determines the final stabilized droplet size.

These experiments and the observed behaviors reveal that the emulsions formed are indeed stabilized by these nanoparticles, consistent with the structure of Pickering emulsions formed by a dense layer of NPs. Consequently, the stabilization of the droplets is primarily ensured by the nanoparticles. Increasing the volume fraction of the dispersed phase reduces the coverage rate, thereby facilitating a phase inversion through the phase inversion composition (PIC).

Conclusion

This work focused on the formulation of Pickering emulsions stabilized by biodegradable polymeric nanoparticles of Eudragit® L100. Pickering emulsions have gained popularity due to their ability to replace synthetic surfactants, offering significant environmental advantages. Moreover, recent advancements in particle functionalization have broadened their applications. We implemented a room-temperature preparation method using a nanosuspension of Eudragit® L100 obtained through nanoprecipitation to produce oil-in-water Pickering emulsions. The study of phase inversion composition (PIC) by conductivity measurements revealed a phase inversion composition of approximately 85% oil by volume. Microscopic observations showed a gradual increase in droplet size as the oil volume fraction increased within the emulsion range. This observation correlated with the phase inversion composition, thereby confirming the relationship between oil volume fraction and droplet size in our Pickering emulsions. Additionally, coverage rate analysis highlighted that Eudragit® L100 nanoparticles effectively and proportionally stabilized the emulsions. By determining the percentage of nanoparticles adsorbed at the droplet interface, we confirmed their critical role in maintaining emulsion stability, thus supporting our understanding of the stabilization mechanism of emulsions by nanoparticles. Ultimately, this work contributes to the formulation of inverse Pickering emulsions through phase inversion composition and their potential in various applications. It paves the way for future developments in emulsions stabilized by biodegradable polymeric nanoparticles, providing innovative solutions for environmentally friendly and sustainability-focused industries. To further explore the findings of this study, accelerated aging investigations will be conducted to assess the long-term stability of Pickering emulsions. This approach aims to better understand their evolution over time and evaluate their robustness for real-world applications. In parallel, practical case studies, such as formulations for medicinal syrups or emulsions in the agrochemical field, will be undertaken. These specific analyses will assess the relevance and efficiency of the emulsions in particular contexts, offering tangible perspectives on their potential industrial applications.

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