Scientific Research Publishing

Open Journal of Biophysics, 2025, 15(2), 19-32
https://www.scirp.org/journal/ojbiphy
ISSN Online: 2164-5396
ISSN Print: 2164-5388

Analysis of the Physicochemical Stability of Pickering Emulsions with Eudragit® RS100

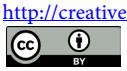
Papa Mady Sy[1*], Alphonse Rodrigue Djiboune[1], Sidy Mouhamed Dieng[2], Anne Dominique Niamké[1], Mari Alexia Touré-Tiglinatiénin[1], Boucar Ndong[3], Louis Augustin Diaga Diouf[1], Gora Mbaye[1], Mamadou Mbodj[3], Mounibé Diarra[1]

[1]Department of Pharmacy, Laboratory of Pharmaceutical Physics, Faculty of Medicine, Pharmacy and Odontology, UCAD, Dakar, Senegal
[2]Department of Pharmacy, Laboratory of Galenic Pharmacy and Legislation, Faculty of Medicine, Pharmacy and Odontology, UCAD, Dakar, Senegal
[3]Department of Medicine, Laboratory of Medical Biophysics and Nuclear Medicine, Faculty of Medicine, Pharmacy and Odontology, UCAD, Dakar, Senegal
Email: *papamady.sy@ucad.edu.sn, *madyspa@gmail.com

How to cite this paper: Sy, P.M., Djiboune, A.R., Dieng, S.M., Niamké, A.D., Touré-Tiglinatiénin, M.A., Ndong, B., Diouf, L.A.D., Mbaye, G., Mbodj, M. and Diarra, M. (2025) Analysis of the Physicochemical Stability of Pickering Emulsions with Eudragit® RS100. *Open Journal of Biophysics*, **15**, 19-32.
https://doi.org/10.4236/ojbiphy.2025.152002

Received: December 12, 2024
Accepted: February 23, 2025
Published: February 26, 2025

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Abstract

This work focuses on the stabilization of an oil phase (ORO® palm oil from *Elaeis guineensis*) using Eudragit RS100, thus creating a Pickering emulsion with water containing PEG 6000 as the dispersing phase. Ramsden then Pickering made it possible to understand in their work the role that solid particles can play in stabilizing the interfaces between two immiscible fluids. From these solid particles, we can formulate emulsions called Pickering emulsions. For more than a century, scientists have been increasingly interested in these types of emulsions. The stability that can be achieved with these types of emulsion is better compared to emulsions obtained with surfactants. The formulation was carried out using the spontaneous nanoemulsification technique. Physicochemical characterization was carried out using a series of physicochemical tests and surface tension measurements. Droplet size was determined using a Zeiss optical microscope. The direction of the emulsions was determined by conductimetry. The pH and viscosity of the emulsions were also measured. Tensiometry studies were carried out using the rising drop method with a tracker (Teclis, Longessaigne, France). The results showed that the emulsions were of the O/W type and the majority of them remained stable until the end of the tests. The pH measurements revealed an acidic character for all the formulations (pH between 2.7 and 4) and we also observed that the droplet size was controlled by the volume fraction of the dispersed phase. We also observed that the viscosity of the formulations increased as the volume fraction of dispersed phase became greater. This increase occurred rapidly for nanoemul-

sions and more slowly for emulsions. These Pickering emulsions and nanoemulsions stabilized by an acrylic polymer, in this case, Eudragit RS100, could be used to encapsulate peptides with a view to developing a potential vaccine or pH-dependent pharmaceutical form.

Keywords

Physicochemical Stability, Emulsion, Pickering, Eudragit® RS100

1. Introduction

Emulsions are pharmaceutical forms that can be used in many fields such as pharmacy, cosmetics, the food industry and chemistry [1]-[4]. When oil and water are mixed, the two phases separate because they are immiscible. However, the addition of surfactant molecules makes it possible to obtain a more or less homogeneous mixture of the two phases. Indeed, surfactant molecules have a polar head and a nonpolar tail. This configuration allows them to stabilize the oil-water interface resulting in pharmaceutical or cosmetic forms called emulsions. Emulsions are formed from an aqueous or oily phase dispersed in the form of droplets in another phase which is immiscible. The size of the droplets in these dispersions is of the order of a micrometer [5]-[7]. Surfactant molecules are widely used in the stabilization of emulsions [8]-[11]. They can be anionic like carboxylates and sulfonates, cationic like protonated amines or quaternary ammoniums, or zwitterionic like lecithin or nonionic. Surfactant molecules sometimes have the disadvantage of being toxic, particularly for the skin where some interactions have been recorded. One of the first proofs of the interaction of surfactants with the skin is the observation of clinical signs following prolonged or chronic exposure to formulas rich in surfactants. These molecules, now known to cause irritant contact dermatitis and inflammatory reactions, are the subject of a real public health problem concerning occupational diseases linked to detergents [8] [12]-[14].

Ramsden then Pickering [15] [16] made it possible to understand in their work the role that solid particles can play in stabilizing the interfaces between two immiscible fluids. From these solid particles, we can formulate emulsions called Pickering emulsions. For more than a century, scientists have been increasingly interested in these types of emulsions. The stability that can be achieved with these types of emulsion is better compared to emulsions obtained with surfactants. In addition, several disadvantages associated with the use of surfactants can be avoided. The particles used can be inorganic or organic in nature. In our previous work, we were able to show that magnesium hydroxide nanoparticles made it possible to stabilize an oil-water interface by lowering the interfacial tension. The adsorption of solid nanoparticles at the interface is therefore strong and almost irreversible. This adsorption protects the droplets from destabilization phenomena such as coalescence and provides them with long-term stability [17] [18]. The evolution of

the understanding of stabilization mechanisms by the Pickering effect has allowed the integration of other types of materials such as polymers or polysaccharides. In this work, the interest we have in acrylic copolymers lies in the fact that we can produce stable Pickering emulsions, in addition to being sensitive to variations in the pH of the study environments. These Pickering emulsions stabilized by acrylic copolymers could constitute a promising system for encapsulating therapeutically relevant molecules, facilitating the controlled and targeted release of the active pharmaceutical ingredient from the internal phase [5] [6] [17] [19] [20].

This study focuses of the analysis of the physicochemical stability of an O/W Pickering emulsion stabilized with Eudragit RS100. The formulation was carried out using the spontaneous nano-emulsification technique. Physicochemical characterization was carried out using a series of physicochemical tests and surface tension measurements.

2. Materials and Methods

2.1. Materials

PEG 6000, ethanol and acetone were purchased from Sigma-Aldrich. Poly(ethyl acrylate-co-methyl Methacrylate-co-trimethylammoniethyl methacrylate chloride) (Eudragit RS100) were sourced from Evonik Roehm, Darmstadt, Germany. Ultrapure water was generated using the Milli-Q filtration system (Millipore, Saint-Quentin-en Yvelines, France). We used ORO® palm oil as the oil phase. This oil is extracted from the seeds of the fruit of the oil palm (*Elaeis guineensis*). All the chemicals were analytical grade and used as received.

2.2. Methods

2.2.1. Dynamic Interfacial Tension Measurements

According to the axisymmetric drop shape analysis, the dynamic interfacial tension γ was measured with a drop tracker tensiometer (Teclis, Longessaigne, France). A rising drop of oil is formed and maintained in a thermostatically controlled cell filled with an aqueous phase containing the suspension of Eudragit® RS100 nanoparticles. The shape of the drop was recorded in real time with a video camera. Thus, its Laplacian form gave the values of the interfacial area and the surface tension. All experiments were carried out at 25˚C in triplicate.

2.2.2. Formulation of Pickering Emulsions

The formulation method used is the spontaneous nanoemulsification method. First, a homogeneous organic solution is prepared, which may contain an oil phase, water-miscible organic solvents and polymers. This solution is then injected into an aqueous phase with stirring to form the emulsion. Finally, the various solvents are evaporated under reduced pressure to recover the emulsion/nanoemulsions [21].

1) *Composition of formulations*

The ternary diagram presented in **Figure 1** shows the different proportions

used for the formulations.

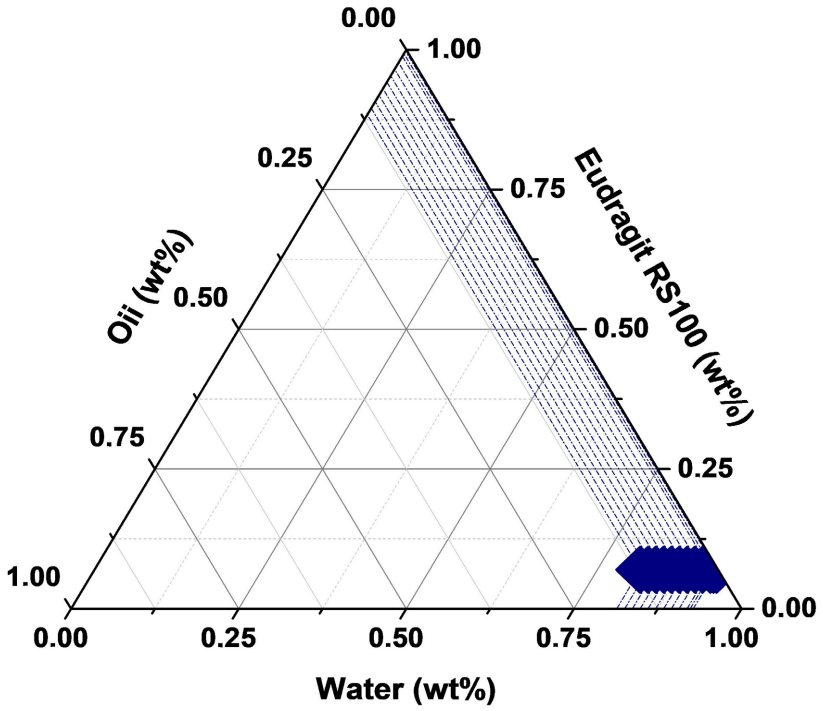


Figure 1. Ternary diagram of formulations.

Thus, we varied the proportions of oil (from 0.23% to 11.62%) and water (from 81.39% to 92.79%); the quantity of Eudragit RS100 was fixed (6.97%). This diagram will allow us to define 12 different proportions called tubes (T1 to T12) (Table S1). Furthermore, to get an idea of the coverage of the newly created interfaces, we calculated the volume fraction of the dispersed phase.

2) *Emulsification*

The organic solution was obtained by mixing *Elaeis guineensis* oil, organic solvents (acetone, ethanol) that are miscible with water and the Eudragit RS100 polymer. This solution was then injected into the aqueous phase, into which we had previously added Polyethylene glycol 6000. The abrupt transition of solvents such as ethanol and acetone to the aqueous phase leads to the formation of droplets or nanodroplets. The acetone and ethanol are then removed by evaporation using a rotary evaporator with a water bath.

2.2.3. Pickering Emulsion Stability Study

Physico-chemical characterization lasted 30 days.

1) *Bottle test*

The bottle test is a technique that allows you to check over the long term whether there are any instability phenomena such as creaming or sedimentation. Observations were carried out for 30 days. We used 50 mL conical bottles containing the emulsion.

2) *Conductivity measurements*

The conductivity measurement allowed us to verify the direction of the preparations. It was carried out using an electrode which measures the electrical resistance of the emulsions and for the nanoemulsions the electrophoretic mobility was measured.

3) ***Droplet size measurements***

The technique used is based on the estimation of the mean diameter of the droplets by individual counting for emulsions. The optical microscope Axio Zeiss imager A1 coupled to a computer containing the Axio Vision release software Version 4.5 (Zeiss optical microscope) was used for measurements.

4) ***pH of the emulsions***

The measuring cell is introduced into a 50 mL conical bottle containing the emulsion. Be sure to place the electrode at the emulsified phase for the sediment tubes. The reading time is set to three minutes after insertion of the electrode.

5) ***Viscosity of the emulsions***

It is based on measuring the velocity limit reached by a ball falling into a container containing the liquid whose viscosity is to be determined. Based on Stockes-Einstein's law, this velocity can be used to determine the viscosity of the liquid.

3. Results and Discussion

By studying the physico-chemical and analytical parameters of the different formulations, stored in the dark at room temperature for 30 days, we were able to monitor their development.

3.1. Dynamic Interfacial Tension Measurements

In this part, we evaluated the impact of Eudragit® RS100 nanoparticles on the Water/cyclohexane interfacial tension using the axisymmetric drop shape analysis method. First, we measured the surface tension of the interface without nanoparticles to confirm the absence of impurities. The interfacial tension values are between 48 and 50 $mN \cdot m^{-1}$ (Figure 2).

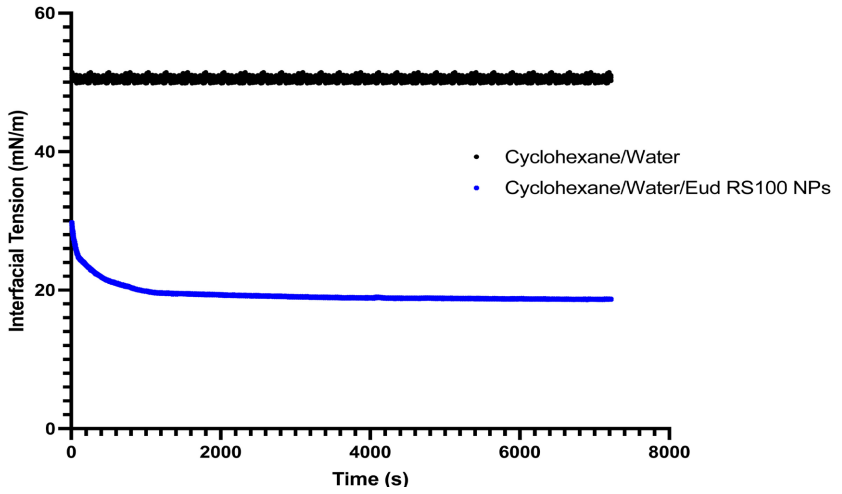


Figure 2. Dynamic interfacial tension in the presence of Eudragit® RS100 nanoparticles.

Analyzing Figure 1, a strong decrease in interfacial tension was observed for Eudragit® NPs. Thus, in the first phases of adsorption ($t \rightarrow 0$), the interfacial tension decreases rapidly. Subsequently, the decrease in interfacial tension slows down and finally reaches a dynamic equilibrium where the maximum coverage of the interface by the nanoparticles is achieved ($t \rightarrow \infty$). These results are identical to those obtained in our previous work with Eudragit® L100 nanoparticles [21]. Which in our opinion allows us to confirm a good stabilization of the interfacial tension and allows us to affirm that the Eudragit® RS100 is capable of stabilizing an interface between two immiscible liquids such as oil and water [22]-[26].

3.2. Pickering Emulsion Stability Study

3.2.1. Bottle Test and Conductivity Measurements

During the visual inspection of the dispersions, the main results showed macroscopically stable and homogeneous dispersions for the majority of the tubes with the appearance over time of an oily supernatant in tubes T8, T9, T10, T11 and T12. This oily supernatant was negligible in tubes T8 and T9 and more significant in tubes T10, T11 and T12. The appearance of this oily supernatant is due to the phenomenon of coalescence and increases in these tubes as a result of the gradual increase in the quantity of oil passing through these tubes. We also noted that tubes T1, T2, T3, T4, T5, T6 and T7 remained intact until the end of the 30-day storage period. However, it should be borne in mind that macroscopic observation alone does not prejudge the stability of the dispersion, as macroscopic observation does not allow us to see oily droplets in the micrometer range.

The direction of dispersions was established using conductivity measurements (Figure 3).

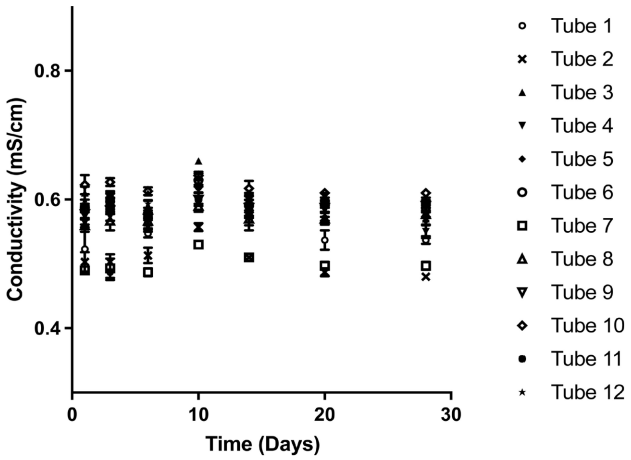


Figure 3. Conductivity of formulations as a function of time.

Since the conductivity of a dispersion depends on the nature of its external phase [5] [18] [24]-[27], the presence of electrolytes in the dispersion results in a non-zero conductivity (from 0.5 to 0.7 mS/cm), further justifying the aqueous nature of the external phase. The results obtained throughout the characterization

period show that the dispersions did not undergo any phase inversion. Also, the conductivity values obtained are higher than that of the water used (0.05 µS/cm) for all the formulations. To this end, a series of conductivity measurements were made in parallel with the water-PEG mixture only. We found that by varying the quantities of water for the same quantity of PEG, the conductivity of this mixture also varied. As the amount of water decreased, the solution became increasingly concentrated in PEG and the conductivity increased. We can therefore deduce from this that PEG is one of the factors that favours the increase in conductivity in our dispersions. It should also be added that our results show that the conductivity value for all the tubes varies within the same range over time. This could highlight the stability of the dispersions over time [28]-[31].

3.2.2. Size of Droplets

Figure 4 shows the image of the droplets from tubes T4 and T10 obtained with an optical microscope on the first day of characterization. We can see a big difference in size between the droplets from tube 4 and the droplets from tube 10.

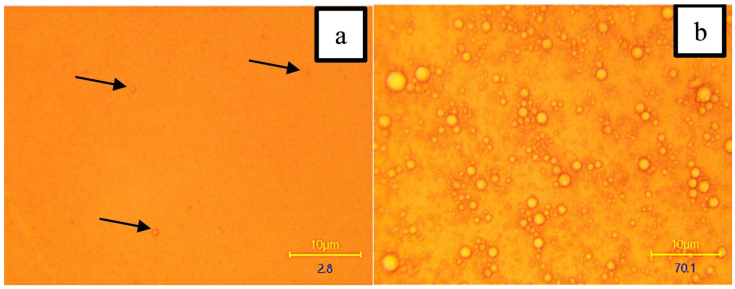


Figure 4. Image of droplets from tubes T4 (a) and T10 (b) under an optical microscope (20X) on Day 1.

Figure 5 shows the evolution of the droplet size of tubes 4 to 12 as a function of the volume fraction of dispersed phase. The horizontal line in red indicates the resolution threshhold (approximately) of the microscope. This means that the droplets from Tubes 1, 2 and 3 could not be observed because of their nanometric size.

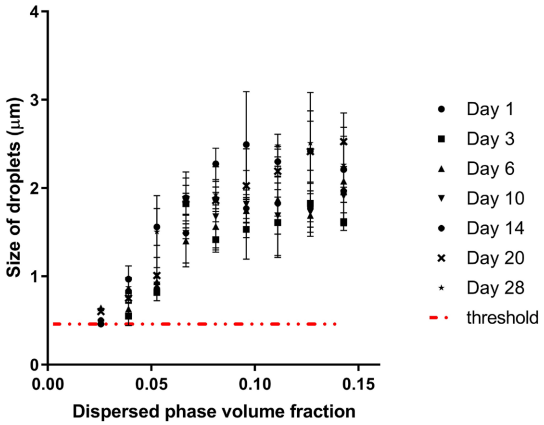


Figure 5. Droplet size of formulations as a function of dispersed phase volume fraction.

We notice that the size of the droplets increases with the evolution of the volume fraction of dispersed phase. The size of the droplets constitutes a very important criterion of stability. It is one of the variables that most influences the sedimentation rate described by Stokes' law [27] [28] [31]-[33]. Thus, we studied the evolution of the droplet size as a function of the volume fraction of dispersed phase. This volume fraction was obtained by making the ratio of the volume of the dispersed phase to the volume of the dispersing phase. We found that the droplet size increased as the volume fraction of the dispersed phase increased. Indeed, when the volume fraction of dispersed phase increases, new interfaces are created while the quantity of polymers in our dispersions does not vary. Thus, the droplets formed are not entirely covered with polymer. This causes limited coalescence of the droplets leading to an increase in their diameter [29] [30]. Furthermore, the size of the droplets could not be determined for tubes T1, T2, T3 and was difficult to determine for tube T4. Indeed, the optical microscope used for our study did not allow us to observe droplets smaller than around 500 nanometers. Thus, we obtained with the spontaneous nanoemulsification method, depending on the quantity of oil, two types of dispersions. On the one hand Pickering nanoemulsions for an oil volume less than 1 mL and on the other hand Pickering emulsions for an oil volume greater than or equal to 1 mL. All this confirms the principle of spontaneous nanoemulsification which allows nano-droplets to be produced only in the presence of a high solvent/oil ratio [28] [34]-[38].

3.2.3. pH

Figure 6 shows the evolution of the pH of the formulations as a function of the volume fraction of dispersed phase.

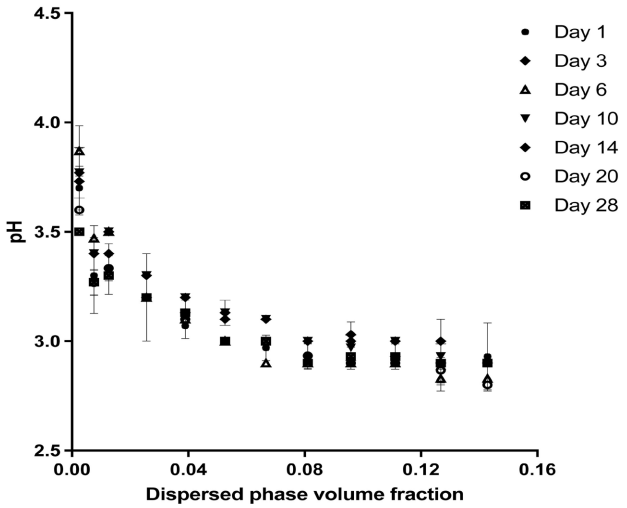


Figure 6. pH of formulations as a function of dispersed phase volume fraction.

We observe that the pH of the formulations decreases when we increase the volume fraction of dispersed phase. This for the entire storage period. We were able to observe through the results that the Pickering emulsions and nanoemul-

sions obtained all have an acidic character and that this acidity increases when the volume fraction of dispersed phase becomes greater. This decrease in pH is due to a progressive release of protons in the aqueous phase. These protons can be released from the polymer or oil used in the internal phase [28].

3.2.4. Viscosity

The viscosity values of the formulations as a function of the volume fraction of dispersed phase are represented in **Figure 7**.

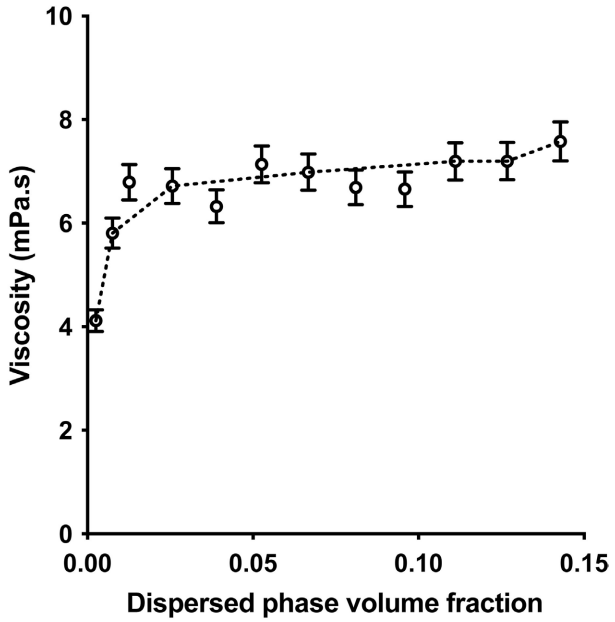


Figure 7. Viscosity of formulations as a function of the volume fraction of dispersed phase.

Finally, one of our work objectives was to study the viscosity of the formulations. We note that the viscosity is conditioned by the concentration of the formulation. It increases when the volume fraction of dispersed phase becomes increasingly important. Indeed, the viscosity value evolves as the volume fraction of dispersed phase increases. This is explained by the fact that as the volume fraction of dispersed phase increases, emulsions/nanoemulsions become more and more concentrated and more resistant to flow. In addition, it should be noted that from tube 1 to tube 4 the viscosity changes rapidly and goes from 4.1198×10^{-3} to 6.6818×10^{-3} mPa·s while from tube 5 to tube 12, it increases slowly and passes from 6.3275×10^{-3} to 7.5755×10^{-3} mPa·s. This difference in the increase in viscosity of nanoemulsions compared to emulsions is due to the stability of the NEs (limited absence of coalescence) and of the hydrodynamic layer surrounding the nanodroplets. These phenomena disappear when the volume fraction of dispersed phase is increased [39] [40].

4. Conclusion

In this work, we studied the physicochemical stability of an oil-water interface in

the presence of Eudragit RS100, thus allowing the production of a Pickering emulsion. Surface tension studies revealed a rapid and significant reduction in interfacial tension. For this purpose, the emulsions/nanoemulsions are of the O/W type and the majority of them remained stable until the end of the tests. The pH measurements revealed an acidic character for all the formulations and we also observed that the size of the droplets was controlled by the volume fraction of the dispersed phase. In addition, we were able to observe that the viscosity of the formulations increases as the volume fraction of dispersed phase becomes important. This increase occurs quickly for nanoemulsions and more slowly for emulsions. To this end, these emulsions/nanoemulsions stabilized by an acrylic polymer will be the subject of peptide encapsulation work with a view to developing a potential vaccine or a system with controlled release of active substance depending on pH.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Informations

Table S1. Proportions of formulations.

Tubes	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Oil (mL)	0.1	0.3	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Water (mL)	39.9	39.7	39.5	39	38.5	38	37.5	37	36.5	36	35.5	35
E. RS100 (g)	3	3	3	3	3	3	3	3	3	3	3	3
PEG (g)	1	1	1	1	1	1	1	1	1	1	1	1
Ethanol (mL)	20	20	20	20	20	20	20	20	20	20	20	20
Acetone (mL)	20	20	20	20	20	20	20	20	20	20	20	20