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Tramadol Encapsulation in Aqueous Phase of Water/Oil Pickering Emulsion Stabilized by Magnesium Oxide Particles

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Abstract Pickering emulsions are systems without surfactants, stabilized by solid particles. These emulsions are experiencing a renewed interest, on the one hand, because it is preferable to limit the use of synthetic surfactants for toxicological and environmental reasons and, on the other hand, the need to make new formulations in order to control the drug release patterns by encapsulation or controlled release. Thus, we were interested in the formulation and evaluation of W / O Pickering emulsions stabilized by particles of magnesium oxide with tramadol hydrochloride in the internal phase. The Bancroft rule served as a model for the formulation. The emulsification was carried out by progressively adding an aqueous phase dispersed in an oil-dispersing phase using a turbo rotor stator mixer. The stability of these emulsions was studied using several parameters (droplet size, pH, viscosity, conductivity...) and the qualitative and quantitative analysis of the active ingredient by UV-visible spectrophotometry. The results obtained showed that the dye test and the conductivity measurement confirmed the W / O nature of these emulsions. Some parameters such as droplet size, pH and viscosity were strongly influenced by the amounts of Magnesium oxide particles and the active ingredient. The qualitative and quantitative analysis of the active ingredient confirmed the presence of tramadol in the internal aqueous phase. Thus, we have succeeded in developing a stable W / O Pickering emulsion with magnesium oxide particles. In addition, we were able to encapsulate tramadol in the dispersed aqueous phase.

Keywords Emulsion, Pickering, MgO, Tramadol Hydrochloride

1. Introduction

Pickering emulsions are dispersions of two immiscible liquids stabilized by solid particles. They are generally composed of three components: aqueous phase, oil phase and stabilizing solid particles. The stabilizing effect of emulsions by solid particles has been known for about a century. These emulsions are called "Pickering Emulsions" named after one of the earliest researchers who described this type of stabilization [1]. It has established that particles can act as surfactants and stabilize oil-in-water emulsions. However, the first description of this phenomenon is due to Ramsden; His work was cited by Pickering [1, 2]. Currently, there is renewed interest in Pickering emulsions. Their "surfactant-free" character makes them attractive for different applications where surfactants often show adverse effects (irritation, haemolytic behaviour, environmental problems...) [3]. In addition, one of the main advantages of Pickering emulsions is that they are more stable than other types of emulsions. The adsorption of solid particles at the oil-water interface is strong and irreversible. This leads to the formation of a dense film thus creating a barrier around the droplets giving them a high resistance to coalescence. The particle adsorption or desorption energy:

$$\Delta E = \gamma_{o/w} \pi r^2 (1 \pm \cos \theta)^2 \quad (1)$$

(r is particle radius, $\gamma_{o/w}$ is the oil-water interfacial tension and θ is the contact angle) is mainly related to their ability to be partially wetted by the two phases of the emulsion [4-6]. This wetting of the particles is characterized by an angle of contact between the aqueous phase, the oil phase and the solid particles, measured on the side of the

aqueous phase. Thus, the particles having a contact angle of less than 90° are usually called hydrophilic and preferably stabilize the O / W type emulsions whereas those whose contact angle is less than 90° are called hydrophobic and are used to stabilize W / O type emulsions [11].

Pickering emulsions also offer the possibility of being stabilized by varieties of particles such as metal oxides and hydroxides, silica, clays, laponite, etc. [6, 7-10].

This type of formulation can be a potential system for encapsulating active ingredients, allowing the controlled and targeted release of the asset from the internal phase. However, this type of emulsion is not yet marketed. There are numerous studies on the formulation and physicochemical properties of emulsions stabilized by solid particles, but to date studies with incorporation of an active ingredient have been very poorly described in the literature.

In this context, we have chosen to achieve emulsions stabilized by Magnesium oxide particles by incorporating tramadol into the internal aqueous phase. The choice of Magnesium oxide particles results in the fact of their non-toxicity and they can be used in several areas. In food, these particles are used as a food additive. They can also be used as an antacid in heartburn [12]. Tramadol has served as a model active ingredient for its hydro-solubility.

2. Materials and Methods

2.1. Materials

The oily phase used throughout the study is a peanut oil Niani® from the market (mainly composed of mono-unsaturated, polyunsaturated fatty acid and saturated fatty acid) and petroleum jelly. The particles used for stabilization are Magnesium Oxide (MgO) Normapur® batch number 71329 from RHONE-POULENC laboratories. The aqueous phase used is distilled water. The active ingredient used is tramadol hydrochloride supplied by the pharmaceutical industry WINTHROP PHARMA SENEGAL (SANOFI AVENTIS Group). Various other chemicals were used during the different stages of this study: Nile red (Sigma-Aldrich), ethanol (SCHARLAU ET0005). All the chemicals were analytical grade and used as received.

2.2. Methods

2.2.1. Formulation of Pickering Emulsions

During the formulation, the direction of the emulsion formed is one of the most important properties and characteristics. The Bancroft rule, which states that the type of emulsion depends on the medium in which the particles are introduced initially, served as a model for the preparation of the formulations.

2.2.1.1. Preparation of the Dispersing Oil Phase

In the petroleum jelly, the magnesium oxide is progressively added by triturating until homogenization takes place. To this mixture was added peanut oil under agitation for 3 minutes at 1680rpm.

2.2.1.2. Preparation of the Dispersed Aqueous Phase

The amount of tramadol to be incorporated is dissolved in distilled water. The mixture is homogenized with a magnetic stirrer at 1000rpm for one minute.

2.2.1.3. Emulsification

In the suspension previously prepared, the aqueous phase is gradually added followed by the fragmentation of the drops of water with the aid of the mixer. Subsequently, the final mixture is homogenized vigorously for one minute at 1680rpm. The total time of preparation of the emulsion is five minutes. The preparation of all the emulsions of this work was carried out under the same operating conditions (stirring speed, stirring time, type of stirrer, temperature). Thus, an W / O emulsion containing tramadol in the internal phase was realised.

We prepared the Pickering emulsions in the following proportions (Table 1):

Table 1. Proportions of formulations

Tubes	T1	T2	T3	T4	T5	T'1	T'2	T'3	T'4	T'5
Oil (g)	15	15	15	15	15	15	15	15	15	15
Water (g)	15	15	15	15	15	15	15	15	15	15
MgO (g)	2	2	2	2	2	3	3	3	3	3
Vaseline (g)	15	15	15	15	15	15	15	15	15	15
Tramadol (mg)	0	100	200	300	400	0	100	200	300	400

2.2.2. Pickering Emulsion Stability

2.2.2.1. Bottle Test

The emulsions are left to rest in the absence of light and an ambient temperature in 50ml conical bottle tests. This visual inspection makes it possible to demonstrate certain phenomena of instability such as sedimentation, flocculation and coalescence.

2.2.2.2. Direction of the Emulsions

It is carried out by a measurement of the electrical resistance of the external phase of the emulsion. The measuring cell is introduced into a 50ml conical bottle test containing the emulsion. Be sure to place the electrode at the emulsified phase for the sediment tubes [13].

We also used the dye test. It is based on the determination of the solubility of the Nile red in the emulsion obtained. Place two milliliters of the emulsion to be tested in a watchglass. Add few grams of Nile red and mix. After assembly between blade and slide, observation will be done under optical microscope Axio Zeiss imager A1 coupled to a computer containing the Axio Vision release software Version 4.5 (Zeiss optical microscope).

2.2.2.3. Droplet Size Measurements

The technique used is based on the estimation of the mean number diameter of the droplets by individual counting. The light chamber microscope BBT KRAUSS was used for measurements.

2.2.2.4. pH of the Emulsions:

As for the conductivity, care must be taken to introduce the electrode up to the emulsified phase level for the sediment tubes. The reading time is set to three minutes after insertion of the electrode [13].

2.2.2.5. Viscosity of the Emulsions

The viscosity measurements were carried out using a BROOKFIELD viscometer pro VII. The principle of measuring the viscosity retained by Brookfield relies on the application of a force of movement to a product by rotating at a fixed speed a mobile of fixed size. The resistance of the product to the rotational movement of the mobile is recorded by means of an internal spiral spring and then converted into a viscosimetric unit.

2.2.3. Encapsulation Efficiency (E.E)

The evaluation of the encapsulation efficiency was carried out after destabilization emulsions in the oven at a temperature of 37°C for one (01) hour until liquefaction, followed by a centrifugation at 3500rpm for 20 minutes, the aqueous phase is extracted using a 5ml syringe. Then, we realize a second centrifugation of water at 3500rpm for two minutes for purification.

The tramadol hydrochloride concentration of each sample is determined following the measurement of their absorbance by an UV-visible spectrophotometer Agilent technologies Cary 60 at 284.5nm. The equation of the following line allowed us to determine the concentrations of tramadol:

$$Abs. = 0.0244\ C + 0.0081 \qquad R^2 = 0.9895 \qquad (2)$$

The encapsulation efficiency was calculated according to the following relation:

$$\%E.E = \frac{[\text{Tramadol encapsulated}]}{[\text{Tramadol Total}]} \times 100 \qquad (3)$$

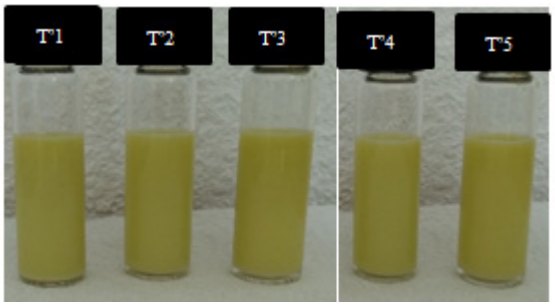
3. Results

3.1. Stability of Pickering Emulsions

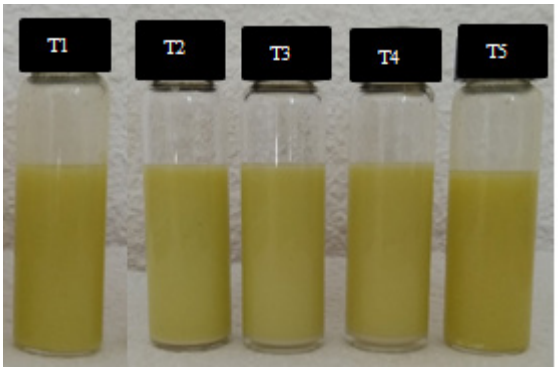
The study of the physicochemical and analytical parameters of the various emulsions formulated and stored in the absence of light at room temperature for 28 days, allowed us to follow the evolution of the formulations as a function of time**.**

3.1.1. Bottle Test

The emulsions obtained are beige to yellow, homogeneous (Figure 1). The emulsions are all stable, better stability was observed with the emulsions containing 3g of MgO.



(a)



(b)

Figure 1. Aspect of tubes after 28 days of storage: **(a)** emulsion with 3g of MgO, **(b)** emulsion with 2g of MgO

3.1.2. Emulsion Direction

The dye test carried out weekly during the 28 days of monitoring showed emulsions with aqueous droplets of heterogeneous sizes with a dispersant phase colored red by a lipophilic dye (Nile red) (Figure 2).

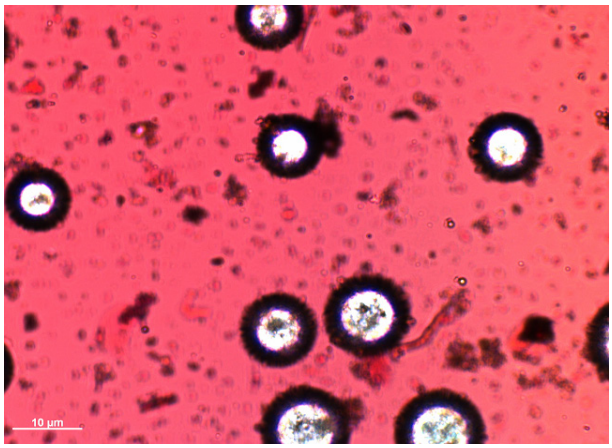
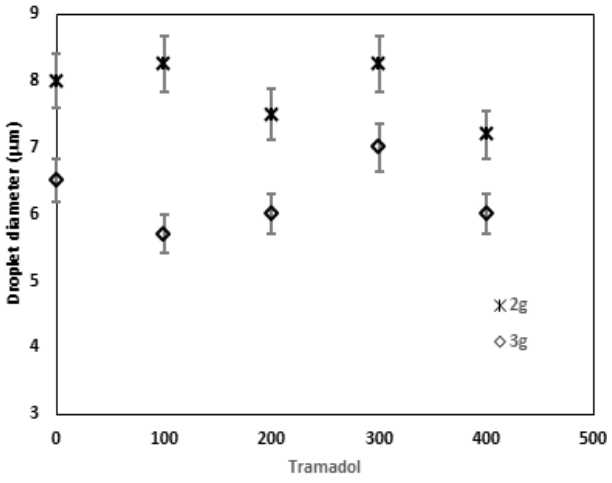


Figure 2. Pickering emulsion coloured by a lipophilic dye (Nile Red)

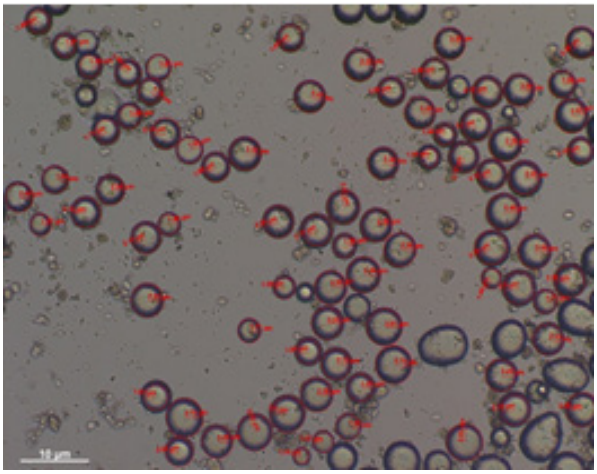
The determination of the direction of the emulsions by measuring the conductivity on the following days: D_1, D_7, D_{14}, D_{21}, D_{28}, gave zero conductivity (0.00 mS cm^{-1}) and a constant value of conductivity over time**.**

3.1.3. Size of Droplets

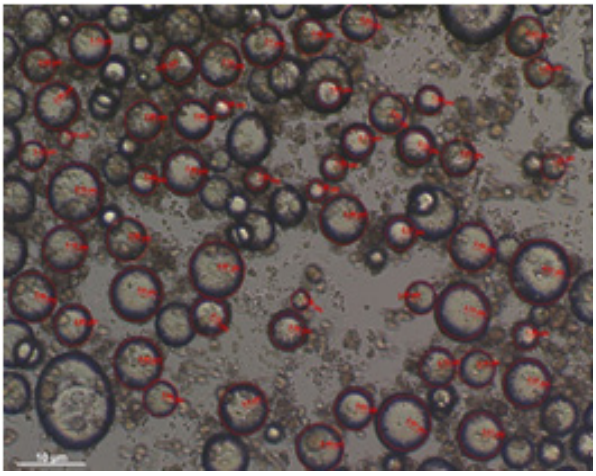
The size of the droplets varied according to the amounts of Magnesium Oxide used and the amount of tramadol (Figure 3). We found that the size of the droplets decreased when the amount of MgO was increased. The amount of tramadol did not affect the evolution of droplet size too much.



(a)



(b)



(c)

Figure 3. (a) Evolution of the size of the droplets as a function of the amount of Magnesium Oxide and tramadol, **(b)** Optical image (X10) of emulsions containing 3g of MgO, **(c)** Optical image (X10) of emulsions containing 2g of MgO

3.1.4. pH of the Emulsions

In Figure 4, we show the effect of the variation of the amount Magnesium Oxide and tramadol on the pH evolution of the emulsions. We found that the higher the amount of MgO, the higher the pH of the emulsions. All emulsions kept a basic pH throughout the shelf life.

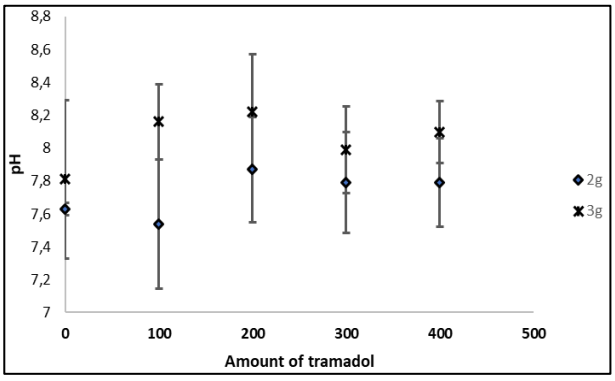


Figure 4. Evolution of the pH of the emulsions as a function of the amount of tramadol and MgO

3.1.5. Viscosity of Emulsions

The viscosity of the emulsions was strongly influenced by the amount of magnesium oxide particles and tramadol concentration in the internal aqueous phase (Figure 5). We have found that a high concentration of Magnesium Oxide has led to an increase in the viscosity of the external phase of the emulsions. The tubes with 3g of MgO have a much higher viscosity and are more stable.

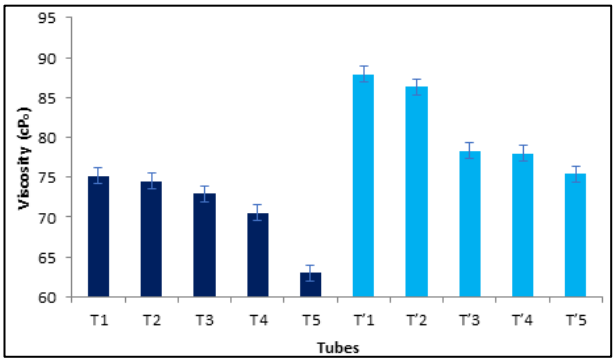


Figure 5. Evolution of the viscosity of the emulsions as a function of the amount of tramadol and MgO

3.2. Encapsulation Efficiency of Tramadol (E.E)

The evaluation of the encapsulation efficiency showed better rates in the most stable emulsions, i.e. at 3g of MgO (Figure 6).

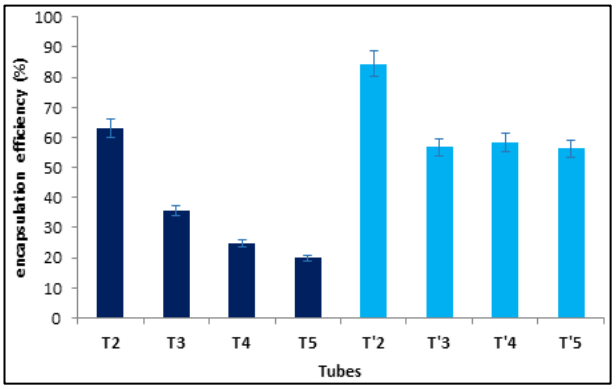


Figure 6. Encapsulation efficiency of emulsions as a function of the amount of tramadol and MgO

4. Discussion

The main results obtained showed that the emulsions were all initially homogeneous without creaming or sedimentation during a visual inspection and throughout the duration of the conservation. However, it is important to keep in mind that the absence of a perceived change to the naked eye does not prejudge the stability of the emulsion. Indeed, macroscopic observation does not allow seeing oily droplets smaller than 10µm [19].

The dye test carried out weekly during the 28 days of monitoring showed emulsions with aqueous droplets of heterogeneous sizes with a dispersant phase colored red by a lipophilic dye indicating the apolar nature of the external phase. The determination of the direction of the emulsions by measuring the conductivity on the following days: D_1, D_7, D_{14}, D_{21}, D_{28}, gave zero conductivity (0.00 mS cm^{-1}). Studies have shown that a constant value of conductivity over time is a determining criterion of stability [14]. The external phase of these emulsions is therefore apolar and therefore of the W / O type.

The size of the droplets plays an important role in the stability of the emulsions. This is one of the variables that influence the sedimentation rate described by Stokes' law.

Samples containing more particles have a smaller average diameter than samples containing a smaller amount of particles. Indeed, the most probable hypothesis would be the reduction in the diameters of the drops, which is a function of the MgO content leading to an increase in the interfacial area.

Similar results were obtained with hydrophobic silica [8, 14]. However, the high particle concentration does not mean a dense overlap of the interface. Some authors have observed that particles are in the outer phase of the emulsion, even when the surface of the droplets is not completely covered [15-17].

The relation between the diameter of the droplets and the interfacial area per unit volume is illustrated by the following equation [8, 14]:

$$D = \frac{6\phi_v \quad V}{A}$$

D is the diameter of the droplets, A / V is the interfacial area per unit volume and $\emptyset_v$ is the fraction of the dispersed phase.

Frelichowska and al. had shown that the hydrophilic caffeine destabilized the emulsions by increasing the interfacial area [8, 14]. In our study, the hydrophilic properties of tramadol should ensure that it remains in the aqueous phase of the emulsion, i.e. inside the droplets. This should lead to an increase in volume of the aqueous phase causing an increase in interfacial air. However, this is not what was observed in our study.

The pH values of the emulsions depended on the amount of MgO particles. The greater the amount of MgO is, the lower the pH of the emulsions. Indeed, the MgO used is a powder of strongly basic character resulting in the basicity of the emulsions giving them a better stability. Studies have shown that a basic pH improves the stability of W / O emulsions [15-17, 19].

We have also found that a high concentration of magnesium oxide has led to an increase in the viscosity of the external phase of the emulsions. The tubes with 3g of MgO have a much higher viscosity and are more stable. Studies have shown that the excess of non-adsorbed particles contributes to the stabilization of the emulsions by the formation of a three-dimensional network of flocculated particles. This improves the stability by

interfering with the mutual contact of the droplets [8, 10, 18]. A similar conclusion was found by Luis Torres and his collaborators with clay particles **[20].** Thus, the increase in viscosity contributes to a better stability of the emulsions. However, it should be noted tramadol resulted in a decrease in viscosity for both 2g and 3g emulsions of MgO.

Regarding the encapsulation efficiency, generally the amounts of Tramadol hydrochloride incorporated in the aqueous phase are lower than the amounts that were initially dissolved in water. The emulsions with 3 g of MgO presented a better rate varying between 56.39 and 84.41% against 19.98 and 62.96% for the emulsions with 2g of MgO. In addition, the tubes that have less incorporated quantities have the best rates (T1 and T'1).

Two hypotheses can explain these differences: first, the stability of the emulsions plays a determining role in the encapsulation efficiency because the encapsulated active ingredient can be dispersed, dissolved or adsorbed to the surface of particles [21-22]. Secondly, the higher the quantities incorporated, the more these adsorption phenomena promote losses of active substances during destabilization because tramadol will sediment with the MgO particles.

However, the large amounts of tramadol found on all formulations show that the active ingredient is dissolved to a large extent in the dispersed phase.

5. Conclusions

In view of the results obtained, we can say that we have been able to formulate a stable W / O type Pickering emulsion encapsulated tramadol in the internal aqueous phase. Thus, these dispersed systems will protect an active ingredient from rapid degradation. They can increase solubility, therapeutic index by decreasing toxicity and increasing efficacy. In industry, Pickering emulsions have great potential for pharmaceutical and cosmetic formulation.

This study will be supplemented by in vitro release studies to obtain release profiles.

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REFERENCES

[1] RAMSDEN W. The separation of solid materials on the surface of solutions and suspensions. Observation concerning surface diagrams, foam blisters, emulsions and mechanical coagulation. *Proceedings of the Royal Society*, 1903, 72, p. 156-164.

[2] PICKERING S. Emulsions. *Journal of the Chemical Society*, 1907, 91, p. 2001-2021.

[3] CHEVALIER Y., BOLZINGER M., «Emulsions stabilized with solid nanoparticles: Pickering emulsions» *Colloids and Surfaces A: Physicochemical and Engineering Aspects, n° 439, pp. 23-34, 2013.*

[4] Binks B.P., Particles as surfactants–similarities and differences, *Curr. Opin. Colloid Interface Sci. 7, 21–41, 2002.*

[5] Hooghten V. R., Blair V.E, Vermant J., Interfacial Rheology of Sterically Stabilized Colloids at Liquid Interfaces and Its Effect on the Stability of Pickering Emulsions, *Langmuir, DOI: 10.1021/acs.langmuir.6b0436 5, 2017.*

[6] Yang H., Zhang H., Peng J., Zhang Y., Du G., Yu F., Smart magnetic ionic liquid-based Pickering emulsions stabilized by amphiphilic Fe3O4 nanoparticles: highly efficient extraction systems for water purification, *J. Col. Int. Sci. 485, 2017, 213–222*

[7] Chevalier Y., Bolzinger M. A., Emulsions stabilized with solid nanoparticles: Pickering emulsions, *colloids and surfaces A: Physicochemical and Engineering aspects, vol. 439, 23-34, 2013.*

[8] FRELICHOWSKA J. Émulsions stabilisées par des particules solides : études physico-chimiques et évaluation pour l'application cutanée. *Thèse pour obtenir le diplôme de Doctorat. Lyon : Université Claude Bernard Lyon 1, 2009, n°009, 211p.*

[9] ARDITTY S. Fabrication, stabilité et propriétés rhéologiques des émulsions stabilisées par des particules colloïdales. *Thèse de doctorat en physico-chimie de la matière condensée, Université de Bordeaux 1, 2004, n°2852, 211p.*

[10] FOUILLOUX S. Nanoparticules et microfluide pour un système modèle d'émulsions de Pickering. Etude des mécanismes de stabilisation et déstabilisation. Thèse de doctorat en physique et chimie des matériaux. Paris: Université Pierre et Marie Curie, 2011, 247p.

[11] AVEYARD R., BINKS B., CLINT J. H. Emulsions stabilized solely by colloidal particles. *Advances in Colloid and Interface Science*, 2003, 100, p.503-546.

[12] RAJGOVIND, SHARMA G., NAKULESHWAR, Phytoassisted synthesis of Magnesium Oxide Nanoparticles by *Swertia Chirayaita, Journal of Taibah University for Science, Vol. 11, Issue 3, 2017, P. 471-477.*

[13] FASCICULE DE TRAVAUX PRATIQUES DE PHYSIQUE 2éme année pharmacie, U.C.A.D, laboratoire de physique pharmaceutique, 2011, 91p.

[14] FRELICHOWSKA J, BOLZINGER M.A AND CHEVALIER Y. Effects of solid particle content on properties of o/w Pickering emulsions. Journal of Colloid and Interface Science 2010,351, p. 348-356.

[15] YAN N.X., CRAY M.R., MASLIYAH J.H. On water –in-oil emulsions stabilized by solids. *Colloids and surfaces A-physicochemical and engineering aspects*, 2001, 193, p.97-107.

[16] YANG F., NIU Q., LAN Q., SUN D. Effect of dispersion pH on the formation and stability of Pickering emulsions stabilized by layered double hydroxies particles. *Journal of colloid and interface Science*, 2007, 306, p. 285-295.

[17] KIM J.H., KIM S.E., KYONG K.Y., LEE E.J., CHO W.G., YOON M.S. Effect of aqueous phase composition on the stability of a silica-stabilized water-in-oil emulsion. *Ph.D. Cosmetic R&D center, LG Household & Healthcare Research Park*, 2004, p.343-350.

[18] DIOUF M.E., *Emulsion E/H stabilisée par des particules solides (Emulsion de Pickering) contenant du tramadol*. Thèse de doctorat d'état de pharmacie. UCAD laboratoire physique pharmaceutique, 2014.

[19] Mahé D. *Emulsion E/H stabilisée par des particules solides*. Thèse de doctorat d'état. UCAD laboratoire physique pharmaceutique, 2013.

[20] TORRES L., ITURBE R., SNOWDEN M.J., CHOWDHRY B., LEHARNE S. Pickering emulsion formation aid the removal of creosote DNAPL from porous media Chemosphere, 2008, 71, p. 12-132.

[21] JIE X., AIJING M., TIANQING L., CHUNLI L., DAYANG W. and HAOLAN X. *Ian Wark Research Institute, University of South Australia, SA 5095, Australia.*

[22] SCHAFFAZICK S., GUTERRES S., FREITAS L., POHLMANN A. Physicochemical characterization and stability of the polymeric nanoparticle systems for drug administration. *Quimina Nova, 2003, 26, p.726-737.*