

Formulation and Characterization of a Pharmaceutical Pickering Emulsion

Touzouirt S^{1*}, Zaïd TA², Nabiev M³ and Hadjsadok A⁴

¹Department of Chemistry, M. Ma mmeri University, Tizi-Ouzou, Algeria

²Department of Chemical Engineering, National Polytechnic School, Ave Pasteur, Hassen Badi, El-Harrach-Algiers, Algeria

³Department of Process Engineering, University of Boumerdes, Algeria

⁴Laboratory Functional Analysis of Chemical Processes, Department of Process Engineering, University of Blida, Soumaa Road, 09000 Blida, Algeria

*Corresponding authorTouzouirt S, Department of Chemistry, Faculty of Sciences, M. Ma mmeri University, Tizi-Ouzou, Algeria, Tel: +213669079853; E-mail: saida_touzouirt@yahoo.fr

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Abstract

The ability of solid particles to adhere to soft deformable interfaces, for example to the surface of emulsion droplets or bubbles, is currently the subject of renewed interest in material science. On the other hand, Clay minerals are among the most widely used materials in pharmaceutical formulation, because of their properties as excipients and/or their biological activities. These features depend on both their colloidal dimensions and high surface. The phenomenon that solid particles can reside at the interface of droplets, thereby providing them with resistance against coalescence or Ostwald ripening, is known as Pickering stabilization. In this study Algerian bentonite clay is used for this purpose. An evaluation of the antibacterial activity of the emulsion after incorporation of the essential oil of *Thymus fontanesii* (local plant) also showed a fairly good encapsulation ability.

Keywords Bentonite; Thyme; Antibacterial; Pickering emulsion; Encapsulation

Introduction

Emulsions play an important role in various fields such as in cosmetics, food, pharmaceuticals and drilling muds. They are heterogeneous mixtures of liquid droplets dispersed in a continuous immiscible liquid phase. In fact, the production and use of stable emulsions have been extensively examined in relation to the food industry, petroleum production, pharmaceutical and environmental applications. Indeed, clay minerals are among the most widely used materials in pharmaceutical formulation, because of their properties as excipients and/or their biological activities [1-3]. Understanding, the required conditions for the formation of physically stable emulsions is an important area of research [4,5].

Named after Spencer Umfreville Pickering [6], Pickering emulsions are nowadays widely used in different industrial fields. The interest of the pharmaceutical industry for this type of emulsions lies in the fact that they avoid or minimize the use of synthetic surfactants which are known to be toxic both to humans and to the environment. This is why Bentonite is considered as an environment friendly natural raw material and has been used in several Pickering emulsions stability studies [5-10]. Both the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP) contain monographs regarding clay mineral materials. Bentonite clay is included in the FDA Inactive Ingredients Database as well as in nonparenteral medicines licensed in the UK [11]. Algerian clay especially the Maghnia Bentonite has been the subject of numerous studies in the last decade [12].

Thyme, a member of the family Lamiaceae, is widely used in traditional medicine in relation to these activities, antibacterial, antimicrobial, antiviral, anti-oxidant, anti-infla mmatory, antiseptic and fungicidal activity. Several species of thyme are encountered in Algeria such as *Thymus fontanesii* [13-15].

Experimental

A study of the stability of a Pickering emulsion based on Algerian bentonite has been formerly carried using a Response Surface Methodology [16].

The optimal composition was composed of 7% Bentonite, 0,015% CTAB and 0, 015 mol/L NaCl. This formula is used in this study to evaluate the antibacterial activity of the Pickering emulsion after incorporation of an active substance into the oil phase.

Materials

The bentonite samples were graciously provided by The Algerian Bentonite Company (BENTAL). The samples are co mmercial grade (3% sodium bentonite, chemically activated). Raw Bentonite originates from the Ha mmam Boughrara deposit (Maghnia, Northwest of Algeria). The clay is mainly composed of montmorillonite (93%) and 7% illite (7%) and has a specific surface area of 872 m²/g, a swelling index of 35 cm³/g, a plasticity index of 120%, a cation exchange capacity (CEC) of 0.91 m²/g and an average particle size of 74 µm [12,17,18]. The cationic surfactant cetyltrimethyla mmonium bromide or CTAB (BIOCHEM Chemopharma, C₁₉H₄₂BrN, molar mass 364.45 g/mol) and sodium chloride NaCl (MERCK Eurolab, for analysis). Soybean oil is graciously supplied by the company CEVITAL (Algeria) and meets the specifications of the pharmacopoeia; its viscosity is 80 mPa.s at 20°C and its density is 916 to 922 g.cm⁻³ [9,11].

Emulsion preparation

Emulsions were prepared using a Heidolph RZR1 rotor stator. Bentonite and water are placed in plastic beaker for 24 h. Then, the surfactant and salt were added. The rotor-stator was then placed in the beaker and the mixture was stirred during 90 seconds at 670 rpm. The oil phase was slowly added under the same agitation. Then the mixture was further vigorously homogenized with a T10 IKA Ultra-Turrax homogenizer for 15 min at 14500 rpm.

Extraction of essential oil of thyme: The essential oil is extracted from the aerial parts of *Thymus fontanesii*, a plant species harvested from the region of Lakhdaria, north of Algeria.

The essential oil is extracted by hydro-distillation. One hundred grams of thyme samples were placed in a flask containing distilled water. The mixture is boiled for 2 hours. The vapors of essential oil are condensed and recovered in a separating funnel. The oil is separated from the water by simple decantation due to difference in density of oil and water [19].

Antibacterial effect assessment of the essential oil: Prior to its incorporation in the emulsion, the essential oil antibacterial activity has been evaluated against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus* and *Koch Bacillus* (KB), according to the qualitative aromatogram method. This technique consists in estimating the growth inhibition of microorganisms in contact with the product to be tested (diffusion in agar test).

Incorporation of active substance: Different amounts (1%-30%) of thyme essential oil are solubilized in the oily phase prior to its emulsification in aqueous phase. The final Pickering emulsion is then formulated using the procedure described in section 2.2.

Pharmacological study of final pickering emulsion: In order to assess the antibacterial activity of the formulated emulsions, we used the aromatogram technique as mentioned above. Small amounts of the tested emulsions are deposited on cellulose disk of 6 mm diameter. The disks were previously sterilized and then placed on the agar plate.

Measurement of the inhibition diameters is carried out after 24 hours of incubation of the Petri dishes at a temperature of 37°C. The scale of antimicrobial activity estimation classifies the zones of inhibition diameters of the microbial growth into 5 classes [20]:

- D \leq 30 mm Very strongly inhibitory
- 21 mm \leq D \geq 29 mm highly inhibitory
- 16 mm \leq D \geq 20 mm moderately inhibitory
- 11 mm \leq D \geq 16 mm slightly inhibiting
- D \geq 10 mm Non-inhibitory.

Results

Antimicrobial study of essential oil of thyme

The antibacterial effect of thyme essential oil is detailed in Table 1.

The bacterial strain	Diameter of inhibition zone (mm)		
Escherichia coli	70		
Staphylococcus aureus	47		
Pseudomonas aeruginosa	35		
Bacillus cereus	65		
Koch bacillus	50		

Table 1: Inhibition zone diameter of essential oil of Thyme.

Table 1 shows that the most dangerous bacterial strains (*E. coli, Bacillus cereus, Koch bacillus*) are very sensitive to the essential oil of thyme.

Antimicrobial study of the final Pickering emulsion

The antimicrobial study is performed on the formulation composed of 7% bentonite, 0.015% CTAB and 0.05 mol/L NaCl in which amounts ranging between 1 and 30% of essential oil of thyme were incorporated. Results are given Table 2 below.

EO Concentration (%)	1	5	10	15	20	30
Diameter of the inhibition zone (mm)	0	0	0	0	0	0

 Table 2: Inhibition zone diameter for final pickering emulsion.

Discussion

Results show that the antibacterial effect of Pickering emulsion containing Thyme EO is zero and this can be explained by the encapsulation phenomenon encountered in this type of emulsion [21,22].

The antibacterial effect of Pickering emulsions is related to nonencapsulated essential oil droplets or droplets dispersed in the aqueous phase [22].

Encapsulation efficiency

The volume of the encapsulated oil is determined visually by measuring the volume of supernatant oil after centrifugation of 10 ml of emulsion for 2 min at a speed of 500 rpm [22].

In order to determine the encapsulation efficiency, we have used the following formula:

 $\text{EE}{=}\,\text{V}_{\text{encaps}}{/}\text{V}\times100\%$

V_{encaps} is the volume of encapsulated oil.

V_{total} volume of the oil phase.

The antibacterial activity is negatively related to the encapsulation efficiency and so it is the delayed or controlled effect that increases as the encapsulation efficiency increases.

In our study the encapsulation efficiency is 100% which may be explained by the adsorption capacity of the Bentonite clay.

The essential thyme oil with its remarkable antibacterial effect and even its important anti-fungal effect [23] can be used as an active ingredient with controlled antibacterial and antifungal effect. When the emulsion is applied to the skin by the spreading movement, the shear necessary to break the emulsion in order to release the active ingredient is ensured. This can be verified by a practical study *in vivo* on animals.

Thus an interesting prospect for this study is to evaluate the diffusion of Thyme EO through the skin layers.

Conclusion

An application of Pickering emulsion in the pharmaceutical field has been envisaged by the incorporation of an active ingredient (thyme EO) into the organic phase of the Pickering emulsion. Preliminary results of the assessment of this EO antibacterial activity reveals high encapsulation power (100%), which makes this application useful when a prolonged release of the active substance is desired.

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