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# Nanoemulsion stabilized by $\beta$ -lactoglobulin: a promising strategy to encapsulate curcumin for topical delivery

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## Abstract

Curcumin has many interesting biological properties. However, it is poorly soluble and stable in aqueous media and its formulation is complex. Protein-stabilized nanoemulsions (NEs) are proposed as encapsulating systems to increase curcumin solubility while protecting it from degradations. Results showed that the incorporation of curcumin within the oily phase of NEs did not modify the size and the zeta potential of nanodroplets as well as NEs stability over time. A creaming phenomenon was observed in NEs over an 80-day storage period. However, droplet stabilization against coalescence was ensured by the formation of an elastic oil/water interface following the adsorption of  $\beta$ -lactoglobulin and/or curcumin. An *in vitro* study with Franz cells showed that curcumin diffused slowly through a synthetic membrane mimicking the skin. Thus, curcumin encapsulation in the dispersed phase of NEs seemed to be a promising strategy for the development of a topical formulation as NEs increased curcumin solubility and allowed its controlled diffusion through a synthetic skin model.

*Keywords: Nanoemulsions;  $\beta$ -lactoglobulin; curcumin; interfacial properties; Franz cell*

## 1. Introduction

Nanoemulsions (NEs) are colloidal dispersions characterized by very fine droplets with a mean diameter less than 200 nm and a narrow size distribution [1]. NEs gained great interest as drug delivery systems as they enhance the solubility of encapsulated active ingredients (AIs) and consequently their bioavailability. They also ensure the controlled release of AIs as well as their stability and their protection from enzymatic and chemical degradations [2]. Moreover, NEs have been reported to improve transdermal permeation of many AIs over conventional topical formulations [3].

NEs are thermodynamically unstable systems but thanks to their nanosized droplets, they are kinetically stable against gravitational separation, flocculation, and coalescence [1,4]. In the pharmaceutical field, synthetic surfactants are often used to ensure droplet formation and to maintain NEs stability. However, these emulsifiers directly or indirectly raise toxicity and environment issues [5]. Therefore, there is an increasing demand for natural and biodegradable products. Proteins are good candidates as emulsifiers. In fact, they are known to stabilize emulsions by forming a viscoelastic adsorbed layer on oil droplets [6]. They also contribute to droplets stability *via* steric and electrostatic repulsions [7]. Emulsion stabilization by proteins is already widespread in the food industry. However, this approach is not yet applied in the pharmaceutical industry.

The stabilization of NEs by  $\beta$ -lactoglobulin ( $\beta$ lg), the major protein of whey, is an alternative approach to replace synthetic surfactants by biocompatible polymers.  $\beta$ lg is a globular protein belonging to the lipocalin family. It contains 162 amino acid residues with a monomer molar mass of  $18.1 \times 10^3$  g/mol [8]. Its secondary structure is composed of eight antiparallel  $\beta$ -sheets forming a hydrophobic central cavity, called the lipocalin core or the calyx, and one  $\alpha$ -helix

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standing alongside the barrel [8]. The central cavity binding site can be occupied by several hydrophobic molecules, such as vitamin A and cholesterol [9-11]. Otherwise,  $\beta$ lg has good emulsifying properties [12,13]. Indeed, it rapidly adsorbs at the oil-water inducing a decrease in interfacial tension [14]. It forms an interfacial elastic film that limits emulsion destabilization by coalescence [15,16].  $\beta$ lg-stabilized NEs exhibit greater resistance to gravitational separation and better biocompatibility compared to NEs stabilized by whey protein isolate or by soybean protein isolate [17].

Curcumin is a powerful bioactive compound extracted from the rhizome of turmeric (*Curcuma longa*). Several studies have established that curcumin has many possible applications in the pharmaceutical field through its anti-inflammatory, anti-oxidant, antibacterial, antiviral, antifungal or anti-cancer properties [18,19]. However, the major disadvantages of curcumin are its poor water solubility, thus its low bioavailability, and its degradation in aqueous media making its formulation complex and its oral administration limited [20,21]. On the other hand, the low percutaneous permeation of curcumin does not favor its administration through skin. A possible solution to overcome these drawbacks can be the encapsulation of curcumin in the oily phase of NEs. This kind of drug delivery system can improve curcumin solubility and its absorption by the organism, its protection from chemical and photochemical degradations. In addition, many studies have been carried out to show the interest of using NEs as a system for encapsulating and delivering curcumin. For example, curcumin-loaded NEs have been shown to enhance the anticancer effect of curcumin over its free form [22,23] and even contribute in improving psoriasis healing [24]. Moreover, curcumin encapsulation in NEs enhances its *in vitro* and *in vivo* skin permeation [25]. Some authors showed a significant enhanced wound [25] and burn [26] healing effects comparing to pure curcumin.

The aim of this study was to encapsulate curcumin within a  $\beta$ lg-stabilized NEs for a topical application and to assess its diffusion through a synthetic membrane with a Franz cell device. The effectiveness of this formulation as a delivery system of curcumin was thus determined. To the best of our knowledge, NEs stabilized by a biopolymer have not been explored to encapsulate curcumin in the pharmaceutical field.

## 2. Experimental methods

### 2.1. Material

Curcumin (batch SLBH24003V) and  $\beta$ lg from bovine milk (batch SLBC2933V) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Miglyol 812 (batch 121228) was a gift from CREMER OLEO GmbH & Co., KG (Hamburg, Germany). Sodium azide (batch K305.2) was obtained from Carl Roth (Karlsruhe, Germany). Ultra-pure water (Millipore Synergy 185 apparatus coupled to a RiOs5TM) with resistivity of 18.2 M $\Omega$ .cm was used for the preparation of all solutions. Potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>) solution (batch SLBC5939V), potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>) solution (batch 068K8600), Tween® 20 (batch STBB3609), and ascorbic acid were purchased from Sigma–Aldrich Chemical Co., (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

### 2.2. Determination of curcumin solubility in Miglyol 812

Curcumin has been solubilized in Miglyol 812 in sufficient amounts to reach an excess of curcumin in the oil. Curcumin solution was prepared according to the following successive steps: magnetic stirring at 60 °C for 10 minutes then at 37 °C for 24 hours, sonication for 20 minutes, and centrifugation at 12,100 g for 30 minutes (Minispin, Eppendorf). Curcumin concentration was determined by UV/vis spectrophotometer (Jasco, V530) at 419 nm (wavelength of maximum of absorbance determined experimentally). Miglyol 812 served as reference for spectrophotometric measurements.

### 2.3. Preparation of nanoemulsions

The aqueous phase was prepared by dispersing  $\beta$ lg powder in 10 mM potassium phosphate buffer solution (PB at pH 7.0) under gentle magnetic stirring at room temperature. Sodium azide (0.05 wt%) was added to the aqueous phase as an antimicrobial agent. Protein solution was stored overnight at 4 ± 1 °C, then filtered through 0.22  $\mu$ m syringe filter. The final concentration of  $\beta$ lg solutions (1 wt%) was checked by UV/vis spectrophotometer at 278 nm, using the specific extinction coefficient of 9.6 dL.cm<sup>-1</sup>.g<sup>-1</sup> [27]. NEs were then prepared by first mixing 5 wt% of oily phase (Miglyol 812) with 95 wt% of  $\beta$ lg solution with a high shear mixer (Ultra Turrax rotor T10, IKA1-Werke GmbH &

Co.,Germany) by operating at 20,500 rpm for 2 minutes at room temperature. The pre-emulsion was then passed through a high-pressure homogenizer STANST ED (Stansted Fluid Power, Harlow, U.K.) at a pressure of 100 MPa and for 4 cycles at 40°C [28]. For curcumin-loaded NEs, curcumin was solubilized at 0.25 wt% concentration in the oily phase prior emulsification. All emulsions were stored at room temperature and protected from light.

#### 2.4. Characterization of nanoemulsions

The mean droplet size, size distribution, polydispersity index (PDI), and zeta potential ( $\zeta$ ) of NEs with and without curcumin were measured using a dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern). Measurements were performed at 25 °C at a scattering angle of 90°. All samples were diluted in PB 50-fold prior analyses.

NE viscosities were measured at 25 °C with a AR-G2 rheometer (TA Instruments, New Castle, U.S.A) equipped with a cone and plate geometry (diameter 40 mm, angle 1°, and truncation 28 mm). The shear rate was varied from 100 to 3000 s<sup>-1</sup>.

Interfacial properties of  $\beta$ lg and curcumin at Miglyol 812/PB interface were studied with a pendant drop tensiometer (Teclis-IT concept, France). Interfacial tension ( $\gamma$ ) was measured over 24 h at 25 °C by forming a 40  $\mu$ L-oil drop into a glass cuvette containing 1 wt%- $\beta$ lg solution. Rheological properties of the  $\beta$ lg and/or curcumin film at the oil/PB interface were determined using the same apparatus (same operating conditions) by varying the volume of the oil drop sinusoidally ( $f = 0.1$  Hz, volume amplitude = 5% within the linear visco-elastic regime).

All results are expressed as averages and standard deviation of three measurements.

Finally, NEs stability was evaluated over 80 days by visual observations, by droplet size measurements, and by using a Turbiscan Classic MA 2000 (Formulaction) that measured transmitted and backscattered light intensities versus the height of the sample.

#### 2.5. In vitro diffusion experiments

*In vitro* diffusion studies were carried out in a Franz Cell system (at 37°C) over 21 hours. A Strat-M® membrane (Merck Millipore) was used to mimic human skin in this study. 1 mL of curcumin-loaded NEs or curcumin solubilized in Miglyol 812 was placed in the donor compartment. Aliquots were collected in the receptor compartment (composed of 90/10 (v/v) PB/ethanol, 0.1 wt% Tween 20, and 0.04 wt% ascorbic acid) at different times to follow the curcumin diffusion through the membrane. Curcumin concentration in the receptor compartment was determined by UV/vis spectrophotometer at 425 nm (wavelength of maximum of absorbance determined experimentally for curcumin solubilized in the receptor medium).

### 3. Results and discussions

#### 3.1. Curcumin solubility in Miglyol

The determined solubility of curcumin in Miglyol 812 was of  $0.2763 \pm 0.0073$  wt% ( $n = 12$ ) which is much higher than the solubility of curcumin in aqueous medium (0.000011 to 0.0007 wt% [29-30]). The curcumin concentration when solubilized in an oil can be related to the composition of the oil, mainly the length of triglyceride chains, which is an important parameter to control the solubility of hydrophobic molecules in oils [31]. Miglyol 812 is composed of medium chain triglycerides (caprylic and capric acids [32]), and it is commonly used in the pharmaceutical industry. The shorter the triglyceride chains, the better the solubility of curcumin since there are more polar groups per mass unit. In fact, solubilization tests of curcumin in oils containing long chain triglycerides have shown that Miglyol 812 solubilizes 2.2 to 4.2 times more curcumin than wheat germ oil and sweet almond oil, respectively.

Curcumin was subsequently prepared at 0.25 wt% in Miglyol 812 (for NEs formulation) in order not to exceed the maximum solubility of the curcumin.

#### 3.2. Nanoemulsions characterization

NEs (with or without curcumin) were formulated and characterized. The droplet size of NEs (with or without curcumin) was about 220 nm with a narrow size distribution (PDI < 0.2) indicating a monomodal size distribution (Fig. 1). In addition, the  $\zeta$  value was similar for both NEs (around -63 mV). Note that the global negative charge of the droplets was due to the adsorption of the negatively charged  $\beta$ lg (as pH > pI = 5.1) at the oil/water interface [33].

The presence of curcumin in the oily phase affected neither the size nor the surface charge of droplets. Rheological measurements have shown that all formulations exhibited a shear thinning behavior and whatever the formulation, the viscosity  $\eta_0$  value was around  $5.4 \times 10^3$  mPa.s (extrapolated value from the Cross model).

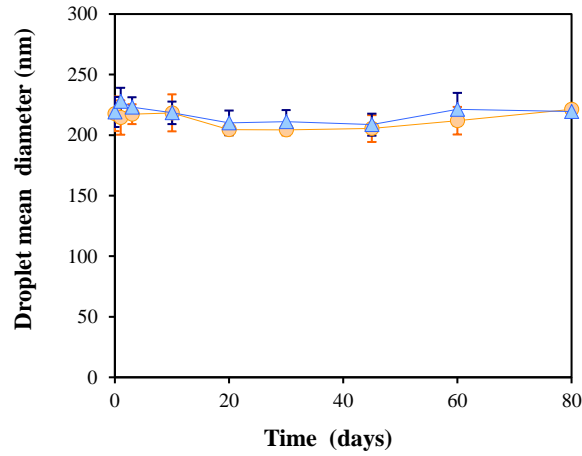


Fig.1. Time evolution of droplet mean diameter of unloaded nanoemulsion ( $\blacktriangle$ ) and curcumin-loaded nanoemulsion ( $\bullet$ ) over a storage period of 80 days.

Interfacial tension ( $\gamma$ ) at Miglyol (with and without curcumin)/PB (with and without  $\beta$ lg) interface was measured to determine the influence of  $\beta$ lg and/or curcumin on the stability of the interface, and consequently that of the droplets (Fig.2). For the Miglyol/PB system, the interfacial tension varied very little over 24 hours. The presence of 1 wt%  $\beta$ lg in PB immediately lowered the interfacial tension ( $12.9 \pm 0.4$  mN/m at 24 hours), as expected [33]. When solubilized in Miglyol, curcumin (at 0.25 wt%) was also able to diffuse, to adsorb at the interface and to lower the value of  $\gamma$ , but with a kinetics different from that of the protein. Indeed,  $\gamma$  remained relatively stable during the first 0.041 hours (150 seconds). Then, curcumin caused a sharp decrease in interfacial tension, reaching a value of  $13.6 \pm 1.0$  mN/m at 24 hours. To our knowledge, the interfacial behavior of curcumin has never been studied so far. In the presence of the two compounds ( $\beta$ lg at 1 wt% and curcumin at 0.25 wt%), the decrease in interfacial tension was very close to that of the protein. It seemed that the evolution of  $\gamma$  was governed by  $\beta$ lg. Subsequently, the adsorption of curcumin at the interface contributed to lower  $\gamma$  value (at 24 hours) compared to the values obtained with  $\beta$ lg alone.

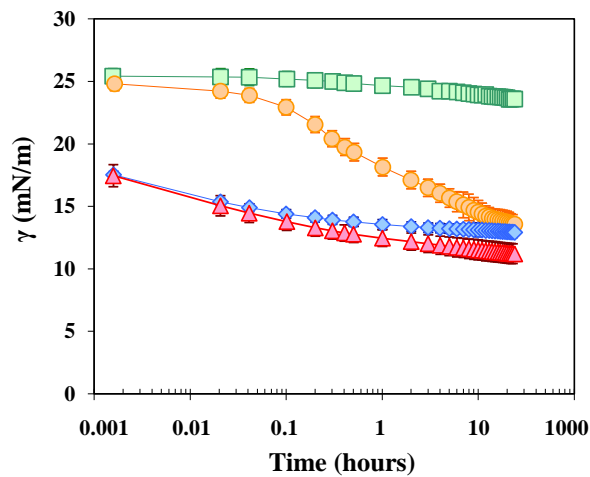


Fig. 2. Influence of  $\beta$ -lactoglobulin and curcumin on the interfacial tension of Miglyol 812/phosphate buffer interface over 24 hours ( $n = 3$ ).

■ 0%  $\beta$ lg-0% curcumin ; ● 0%  $\beta$ lg-0.25% curcumin ; ◆ 1%  $\beta$ lg-0% curcumin ; ▲ 1%  $\beta$ lg-0.25% curcumin

This result indicates that curcumin was able to insert between  $\beta$ lg molecules at the interface thus contributing to the decrease in  $\gamma$  compared to  $\beta$ lg system alone.

At 24 hours, the formation of a film at the interface (for all systems) was easily observed after reduction in the volume of the drop (see Fig. 3).

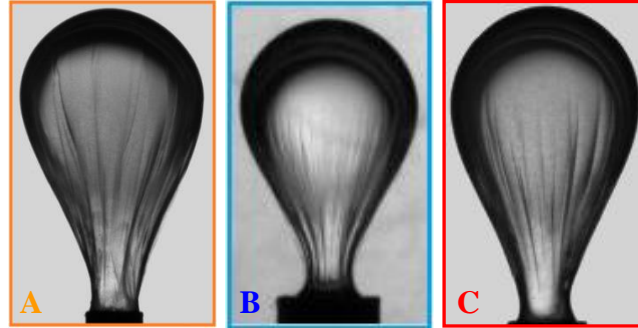


Fig. 3. Observation of the formed film at Miglyol 812/phosphate buffer interface after the reduction in the volume of the drop at 24 hours.  
A) 0%  $\beta$ lg–0.25% curcumin ; B) 1%  $\beta$ lg–0% curcumin ; C) 1%  $\beta$ lg–0.25% curcumin

For interfacial rheology study, at the Miglyol/PB interface in the presence of  $\beta$ lg and/or curcumin,  $E''$  modulus was insignificant in all cases compared to the values of  $E'$  modulus (results not shown). A clear elastic interfacial behavior was observed for all systems (Fig. 4).  $\beta$ lg immediately gave an elastic behavior. This confirms the results obtained by Bouyer *et al.* [33]. Curcumin alone also led to an elastic interface but in a much more gradual manner than for  $\beta$ lg alone. This result is in line with the conclusions on the decrease in  $\gamma$  value of curcumin after a latency time. In addition, the interface became more elastic for curcumin alone compared to  $\beta$ lg alone after 8 hours. In the presence of both compounds, once curcumin adsorbs at the interface, the value of the  $E'$  modulus increased more slowly than in the presence of  $\beta$ lg alone. Curcumin therefore had a negative effect on the value of the  $E'$  modulus in the presence of  $\beta$ lg. By adsorbing at the interface, curcumin probably decreased the lateral interactions between  $\beta$ lg molecules or else disrupted the structural rearrangement of  $\beta$ lg [34], therefore decreasing the elasticity of the interface.

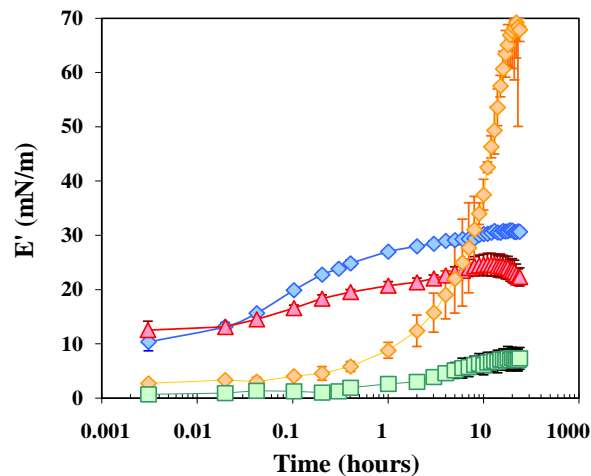


Fig. 4. Influence of  $\beta$ -lactoglobulin and curcumin on the elastic modulus  $E'$  of Miglyol 812/phosphate buffer interface over 24 hours ( $n = 3$ ).  
■ 0%  $\beta$ lg–0% curcumin ; ● 0%  $\beta$ lg–0.25% curcumin ; ◆ 1%  $\beta$ lg–0% curcumin ; ▲ 1%  $\beta$ lg–0.25% curcumin

A schematic picture of the influence of curcumin at the Miglyol/PB interface is proposed in the Fig. 5.

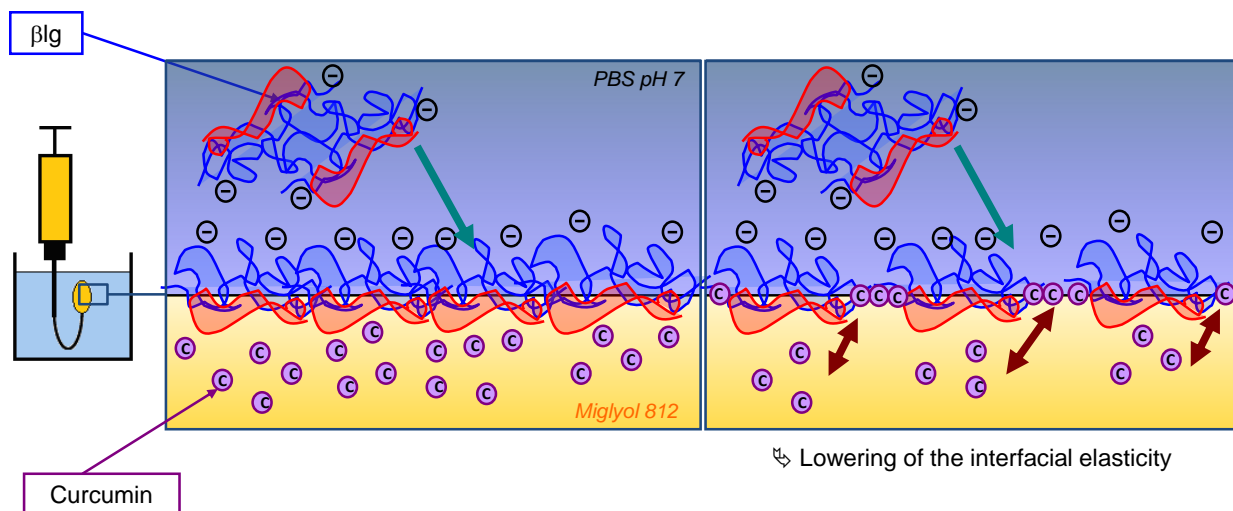


Fig. 5. A schematic representation of the influence of curcumin at the Miglyol 812/PB interface.

### 3.3. Nanoemulsions stability

NEs stability was determined over time. Eighty days after their formulation, NEs with and without curcumin appeared stable as neither creaming nor clarification were observed with the naked eye. Nevertheless, Turbiscan analyses highlighted a decrease and an increase of the backscattered intensity at the bottom and the top of the tube, respectively (Fig. 6), attributed to a creaming phenomenon. Droplet mean diameter did not evolve over 80 days (~ 220 nm) indicating that there was no coalescence of droplets. Thereby, the instability due to creaming can be reversible by simple manual shaking of the NEs. As seen in section 3.2,  $\beta$ lg adsorption at the oil/PB interface conferred elastic properties to the interface, which is likely to prevent droplet coalescence. Curcumin also adsorbed at the oil/PB interface and lowered the interfacial tension. The elastic behavior of the interface was obtained much more progressively than with the protein alone. However, it had no effect on NEs stability over 80 days.

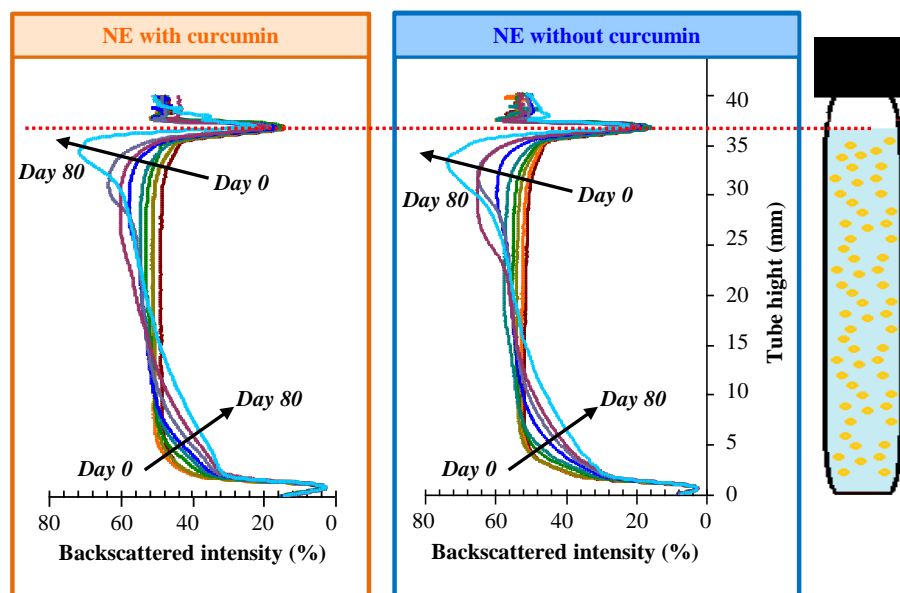


Fig. 6. Time evolution of backscattered intensity for NEs with and without curcumin (yellow frame and bleu frame, respectively). Arrows indicate the evolution over 80 days.

All these results suggest that the net negative charge on the droplets surface was sufficient to prevent them from flocculation. This could explain the size distribution stability over 80 days. Moreover, from a theoretical standpoint (Stokes equation), nanometric-sized globules promote Brownian motions more than the globules movement due to gravity. It probably limited a more important creaming than it should be (observable visually).

NEs with and without curcumin were physically stable over 80 days. However, the NEs did not provide curcumin a protection from photodegradation (results not shown). It is, thus, important to protect NEs encapsulating curcumin from light (for instance the use of amber bottles).

### 3.4. *In vitro* diffusion experiments

The *in vitro* diffusion study showed that curcumin could be released gradually from the dispersed phase of NEs and even from Miglyol 812 alone and diffuse through a synthetic membrane mimicking human skin (Fig. 7). This result was also visible by simple observation of the yellow color of curcumin in the receptor compartment of Franz cell at 21 hours (see photos of the device in Fig. 7). This study showed that there was a constant and low diffusion flux of curcumin through the membrane. Transmembrane flux values were of  $0.59 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  for curcumin solubilized in Miglyol and of  $0.47 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  for curcumin-loaded NEs. This can be explained by the small amount of curcumin present in the formulations (125  $\mu\text{g}$ ). The passive diffusion of curcumin through the membrane was driven by the concentration gradient that exists between the donor and receptor compartments. At 21 hours, the cumulative amounts of curcumin that diffused through the membrane were  $11.3 \pm 0.4 \mu\text{g}\cdot\text{cm}^{-2}$  for Miglyol solution and  $10.9 \pm 0.6 \mu\text{g}\cdot\text{cm}^{-2}$  for curcumin-loaded NEs. Note that no significant difference in the diffusion of curcumin was observed between the two systems. However, the diffusion study should be completed on real skin (*ex vivo* or *in vivo*). Indeed, the structure of skin is much more complex than that of the synthetic membrane used in the present study.

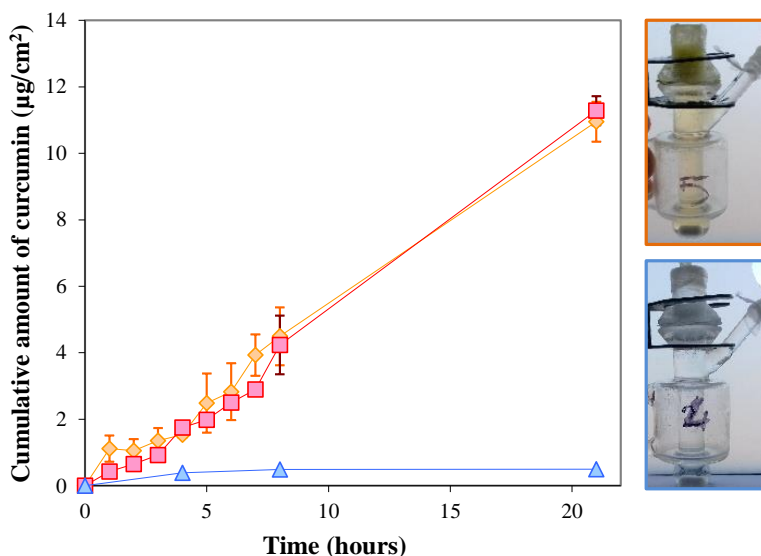


Fig. 7. Time diffusion of curcumin through Strat-M® membrane from different formulations ( $n = 3$ ): curcumin solubilized in Miglyol 812 (■) and curcumin encapsulated in NE (◆). NE without curcumin was the negative control (▲). Photographs of Franz cell device at the end of the experiment: noticeable yellowing of the receptor medium (top photo) following the diffusion of curcumin.

These results are in accordance with previous *in vitro* studies of curcumin diffusion through different membranes (snake skin, porcine cornea or porcine ear skin) and from different formulations (cyclodextrin complexation or microemulsions) [35-37]. Curcumin can thus diffuse through membranes when encapsulated.

The incorporation of curcumin inside nanodroplets (in NEs) has several advantages over its simple solubilization in an oil. Indeed, the stability of curcumin and its skin permeability can be improved by encapsulation [38,39]. Curcumin is protected from the conditions present in the biological environment (neutral pH, oxygen, enzymes, etc.) known to destabilize curcumin [38]. Furthermore, an oil is more easily subject to oxidation and rancidity.



## 4. Conclusions

Curcumin encapsulation within the oily dispersed phase did not modify the characteristics of NE as well as its stability over time. Nevertheless, the stability study revealed a creaming phenomenon within NEs which is reversible by hand agitation and will not be a limitation for the use of NEs.  $\beta$ lg and curcumin adsorption at the oil/PB interface conferred elastic properties to the interface, likely to prevent droplets coalescence. The diffusion study with Franz cells showed that curcumin diffused slowly through the synthetic membrane. Thereby, curcumin could potentially have a local effect. Thus, the encapsulation of curcumin in the dispersed phase of NEs seemed to be a promising strategy to increase its solubility and control its delivery through skin. Moreover, NEs should have the advantage over oil-solubilization to ensure the protection of curcumin against chemical and enzymatic degradations.

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