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Evaluation of short cycles of ultrasound application in nanoemulsions to obtain nanocapsules

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ABSTRACT

Ultrasound is widely used in several chemical reactions and other process, including production of nanocapsules by *in situ* polymerization. In this work, the main objective was to evaluate the impacts and viability of successive ultrasound application in nanoemulsions to obtain nanocapsules. Initiator potassium persulfate (KPS) concentration, number of ultrasound cycles and reaction time influences on polymerization efficiency and droplet size were evaluated. This work revealed the successful *in situ* production of nanocapsules using successive shorts cycles of ultrasound. Number of cycles was the only parameter that not exerted significant influence in polymerization yield. Particle size decay was observed in all nanoemulsions after the first ultrasound application, the same was not observed in further cycles. Gravimetric assessment showed remarkable increase of monomer conversion, indicating that once started polymerization continued at least until 28 days after ultrasound application. Concluding, ultrasound short cycles can be used with no harm to formulation, if carefully performed and, furthermore is a potential cost-effective route for polymerization reactions.

1. Introduction

Nanoemulsions can be defined as colloidal systems in which a lipid phase is dispersed in an aqueous continuous phase, as small droplets, stabilized by a thin interfacial layer of surfactant molecules [1,2]. Their nanometric size, frequently in the range of 10–200 nm, is an intrinsic characteristic giving them a variety of exclusives properties [3,4], one of which is the improved stability. Nanoemulsions are kinetically stable and are prepared with low amounts of surfactants (4–10%) [5,6] when compared with microemulsions, which are thermodynamically stable and require much higher concentration of surfactants. Due to the small droplet size of nanoemulsions, instability events, e.g. creaming, sedimentation and coalescence, tend to not occur as the gravity force is overcome by the Brownian motion of the droplets, allied to surface charge that inhibit droplets proximity and further aggregation [1,3,4,7,8].

All of these advantages make nanoemulsions suitable for applications in personal care, agrochemical, cosmetics, food and pharmaceutical industries. They have also been used as reaction media for polymerization [8,9].

Nanoemulsions are prepared by numerous methods. High-energy emulsification methods require high mechanical energy-input, generally provided by high shear forces to achieve small droplet sizes [4,7]. Emulsification is also achieved by low-energy methods that are based on physicochemical behavior of the system by altering the spontaneous curvature of surfactants. For example, the phase inverse temperature (PIT) method [10] consists of heating an oil-in-water (O/W) emulsion, prepared with nonionic ethoxylated surfactants. These surfactants are temperature sensitive molecules and undergo dehydration at elevated temperatures becoming more hydrophobic and resulting in a water-in-oil (W/O) emulsion. When the system is cooled, it goes through a point of zero curvature of the surfactant layer with minimum interfacial tension, promoting a phase inversion from W/O to O/W emulsion with nanometric droplet size [3,8]. Another method of changing spontaneous curvature of surfactants is by altering the water volume fraction. This approach is called the Emulsion Inversion Point (EIP) method. Adding water to an oil phase, initially, droplets are formed consisting in a W/O emulsion wherein becomes an O/W emulsion as the volume of water is progressively increased and overcomes oil volume fraction. The hydration of polyethoxylated surfactant chains also increases and, at the inversion point, it reaches a zero spontaneous curvature as well as minimum interfacial tension, forming small droplets [11,12].

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One use of nanoemulsion is as a template to obtain nanocapsules. Hydrophobic monomers are introduced in the oil internal phase and polymerization of these nanoemulsions leads to formation of nanoparticles with the oil entrapped within the polymer particles. The particle size of these solid colloidal systems ranges from 10 to 1000 nm [13]. Depending on their composition and structure, they are named: nanocapsules or nanospheres. Nanocapsules are vesicular systems composed of a cavity with an inner liquid core, usually lipophilic, surrounded by a polymeric membrane. Encapsulated material is dissolved in the inner core or adsorbed or dispersed in the polymeric shell. Nanospheres are devoid of a nucleus and consist of a polymeric matrix, in which drug is encapsulated in the matrix or adsorbed on the surface [7,14]. Nanocapsules and nanospheres are prepared using similar methods. They are used as carrier systems for targeted and controlled drug delivery as such or with surface modifications [15–17].

A potential application of nanocapsules in pharmaceutical field is as nanocarriers to lipophilic drugs. Anton and coworkers [7] described three main advantages of nanocapsules for this use: high encapsulation efficiency, lowered tissue irritation at the administration site and protection against drug degradation; all of them are due to the great drug solubility in nanoparticle oily core while the solubility on the external phase is kept low. Another potential utilization as carrier is in cosmetic field, focusing in topical administration, once their nanometric size and larger superficial area favor drug penetration through the complex skin structure [4].

One way to perform *in situ* polymerization of nanoemulsions is using Atom Transfer Radical Polymerization (ATRP) technique. It is based on chain reaction caused by formation of a radical initiator and subsequent reaction with monomers. Potassium persulfate ($K_2S_2O_8$ or KPS) is described as one of the most common initiators [9]. Its characteristics of being hydrosoluble direct polymerization from the external phase toward the internal hydrophobic phase, inducing the formation of the polymeric shell. KPS undergoes an homolytic break forming radicals $[SO_4^{-}]^{\cdot}$ and starting a chain reaction [18]. Fig. 1 illustrates step by step of nanocapsule formation by *in situ* polymerization of monomers in an oil droplet.

The method suggested by Spernath and Magdassi [19] to produce polymeric nanoparticles requires heating and stirring of a nanoemulsion previously prepared by PIT method, using a solution of $FeSO_4$/KPS in a ratio of 1:1 to initiate polymerization. The Fe^{2+} ions cause the dissociation of KPS, activating the initiator. However Fe^{2+} ions can lead to undesirable complexes with other components present in formulation. Moreover, this process takes around 4 h to finish.

Ultrasound is used as another method to supply the energy required to start polymerization [20–22]. It is characterized as sonic waves of frequency between 2×10^4 and 10^7 Hz [23]. Many theories about its mechanism in chemical reactions are suggested, but they all propose acoustic cavitations, which occur when ultrasound waves propagate through a liquid media creating bubbles that grow and collapse. The temperature estimated during bubbles formation is in range of 750–6000 K and the breaking process emits pressures over 1000 atm. These conditions promote the rupture of initiator bonds, starting the polymerization reaction [21,24,25]. Since ultrasound is a high-energy method, the process time is in general faster than common methods. It is known that most of traditional methods used to supply mechanical energy to obtain nanostructured carriers frequently present an unsuccessful control of particle size distribution and, consequently, poor dispersion stability [26]. In this context, ultrasound cavitation has been pointed as a promisor method, once cavitation is able to provide a reliable and simple route for the control of both synthetic process and nanostructure. This method is also reported as feasible to afford chemical homogeneity and reactivity through atomic level [27].

In order to avoid long exposures to high energy provided by continuous ultrasound application but still assuring a fast method to obtain nanocapsules, we suggested the employment of successive short cycles. The present work is an initial step of development of proposed method and aimed to evaluate physicochemical impacts, behavior and viability of these successive ultrasound applications in nanoemulsions.

2. Experimental section

2.1. Materials

2-Ethylhexyl acrylate (2-EHA) (analytical grade) monomer was kindly provided by BASF. It was previously filtered with activated charcoal to remove the polymerization inhibitor (Hydroquinone Mono-Methyl Ether). Soybean oil was supplied by Cargill. Surfactants were Sorbitan Monooleate (Span 80 – Croda do Brasil – Campinas-Brazil) and PEG-40 Hydrogenated Castor Oil (Croduret® 50 Special – Croda do Brasil – Campinas-Brazil). KPS (analytical grade) was purchased from Merck.

2.2. Nanoemulsion production

Formulation was constituted of an oil and an aqueous phases. Oil phase was composed by 3.0% (w/v) of PEG-40 Hydrogenated Castor Oil, 2.0% (w/v) of Span 80 (both as surfactants), 5.0% (w/v) of Soybean oil and 5.0% (v/v) of monomer (2-EHA). Distilled water was used as aqueous phase. The O/W nanoemulsion was prepared using a method that combines PIT and EIP techniques. Oil and aqueous phases were heated separately to 80 ± 2 °C, thus aqueous phase was slowly added into oil phase under constant mechanical agitation of 600 rpm (Fisaton 713D) until cooling at room temperature (22 ± 2 °C) [28]. The monomer was added to oil phase during heating.

2.3. Nanocapsule production

Nanocapsules were obtained from polymerization of nanoemulsions containing monomers. Polymerization was carried out by two methods: by successive ultrasound applications and by heating. The second one was mainly used as comparison.

2.3.1. Ultrasonic polymerization

Nanoemulsions were divided in 5.0 ml aliquots and 0.3 or 0.9 ml of 10% (w/v) KPS solution (respectively 6 and 18 mg/ml as final concentration) were added. Each aliquot was submitted four times to sonication using 25% of amplitude ultrasonic in processor (Vibra cell™ VC750 – Sonics-USA) with a metal rod titanium probe, 13 mm tip diameter, at 300 W for different cycle times: 0.5, 1.0, 2.0, 3.0 and 5.0 min.

2.3.2. Heating polymerization

The method was adapted from Spernath and Magdassi [19] and described by Goto et al. [27]. Different quantities of a 10% (w/v) KPS solution (corresponding to a final concentration of 6 or 18 mg/ml respectively) were added in the previously obtained nanoemulsions and kept in water bath Thermomix® (B. Braun Biotech International, model 18BU) at 40 °C under magnetic stirring. After 2 h of reaction, the same quantity of KPS was added to each formulation and mixed for 4 h.

2.4. Characterization of the formulations

2.4.1. Particle size measurements

Particles size distribution was obtained by Dynamic Light Scattering using Nanosizer® N5 Submicron Particle Size Analyzer

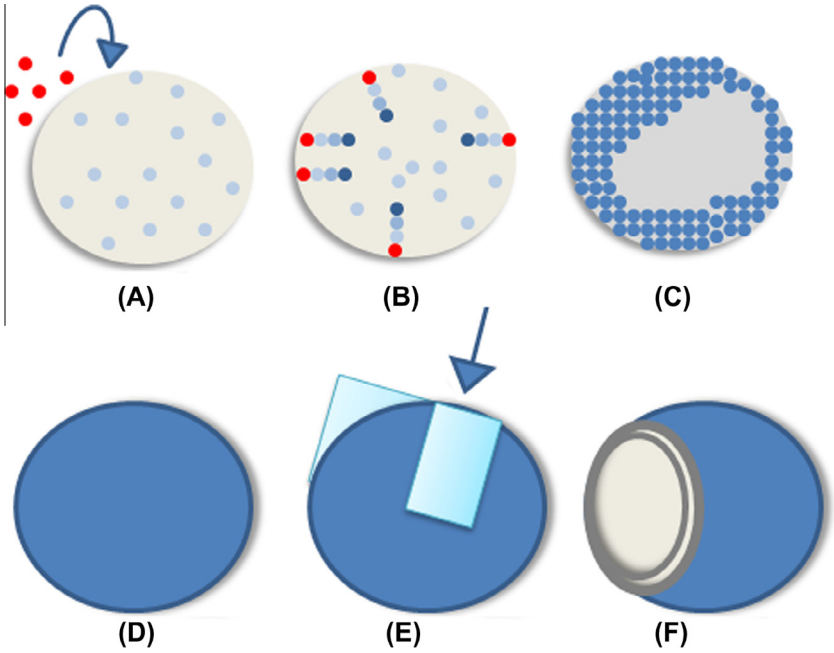

Fig. 1. Schematic representation of nanocapsule formation by *in situ* polymerization process. (A) Sulfate radicals (red dots) enters inside an oil droplet (gray bubble) containing monomers (light blue dots). (B) Radicals react to monomers, starting a chain reaction (polymerization) which leads to polymeric chain growth (dark blue dots). (C) Polymeric shell formation, surrounding the particle. (D) Polymeric reaction finishes forming the nanocapsule. (E) and (F) Cross section is done in order to detail nanocapsule inner structure composed of an oil core encircled by the polymeric layer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Beckman Coulter). Each sample was diluted 10^3 times with purified water. The measurements were made after emulsification and each cycle of polymerization.

2.4.2. Phase Inversion Temperature – PIT determination

Conductivity of nanoemulsions was measured using a conductivimeter (Instrutherm CD-820) under slow and gradual heating in a water-bath from room temperature (25 °C) until 85 °C. PIT was determined as the average temperature between the highest and lowest conductivity value [5]. The experiment was performed in triplicate.

2.4.3. FT-IR measurements

The polymer was extracted from nanoparticles using chloroform, re-precipitated in methanol, dried and analyzed by FT-IR [30,31]. From each sample, 3.0 ml were withdrawn and the same volume of chloroform was added in a Falcon tube. The mixture was agitated with vortex (MS 1 minishaker Ika®) for 2 min and centrifuged for 10 min (Excelsa BABY I 206R – Fanem Ltda) to separate organic and aqueous phases. The supernatant phase was removed and 3.0 ml of methanol was added. Samples were homogenized and centrifuged again and maintained at 35 °C until solvents were evaporated. Extracted polymers were analyzed by Infra-Red with ATR device (Varian 640-IR, Spectrometer FT-IR), within the range of 4000–380 cm^{-1}.

2.5. Transmission electron microscopy (TEM)

Transmission electron microscopy was made in order to confirm the particle size and to characterize the shape and structure of the nanoparticles, using a Philips/FEI CM120 Biotwin Transmission Electron Microscope. Samples were diluted with purified water at the proportion of 50 µl of sample and 950 µl of water, dropped into the sample holder and let to dry overnight at room temperature (22 ± 2 °C).

2.6. Polymerization efficiency

Polymerization efficiency was calculated by gravimetric method. An aliquot (100.0 µl) of each polymerized sample was weighted and kept in an oven at 30 °C during 24 h for drying. Samples were kept at this temperature to enhance loss of volatile components and also prevent polymerization reactions that could occur at higher temperatures. After drying, each sample was weighted and the result correlated with residual monomers [33].

Polymerization efficiency was considered as percentage (w/v) of polymerized monomers and was calculated by the formula $PE\ (\%) = (Wp \cdot Wm^{-1} \times 100$, wherein *PE* is polymerization efficiency, *Wp* is polymer weight and *Wm* is the total weight of monomer added in formulation.

2.7. Statistical analysis

Statistical analysis was performed to evaluate the differences in particle size and polymerization efficiency of each ultrasound application. Analysis were made with one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical data analysis was performed using BioEstat (version 5.3) program, in triplicate with 95% confidence interval and values were reported as mean ± S.D.

3. Result and discussion

3.1. Nanoemulsion production

Nanoemulsion was obtained by a combination of PIT and EIP methods. This process avoids use of organic solvents, reducing potential toxicity [29]. PIT was determined to confirm the temperature resistance of the nanoemulsion, especially if it is used as a template for polymer synthesis involving heating and/or high energy application [3]. Once this formulation is composed by non-ionic surfactants susceptible to phase inversion caused by

temperature raise, PIT cannot be exceeded during polymerization reaction in order to avoid phase inversion. If PIT is overtaken, the oil phase becomes external phase, thus, polymerization reaction does not occur inside droplets and, consequently, nanocapsules are not formed. Thus, to obtain nanocapsules and preserve the formulation integrity, the system must be maintained in a range of temperature under PIT.

The conductivity profile (Fig. 2) showed a typical behavior of O/W nanoemulsions. During heating, there was a slight increase of conductivity along with temperature, which is a characteristic of hydrophilic systems, where the conductivity increases as the ionization of water and dissolved salts improve at higher temperatures. When the phase inversion takes place, oil becomes the external phase associated with a sudden decrease in conductivity reaching values close to zero [3,5]. PIT was calculated as 84.2 °C.

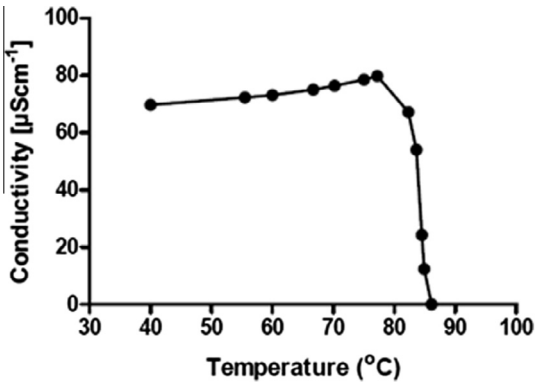

Fig. 2. Conductivity analysis for nanoemulsion.

3.2. Ultrasound application effect in particle size

The first objective was to analyze whether the repeated application of ultrasound compromises the nanometric particle size of the emulsions.

In Fig. 3, it was observed that, regardless of process time, nanoemulsions with monomer had a bigger initial droplet size which decreased after the first cycle of ultrasound application, although the continuous reduction of particle size was not observed after successive cycles. This contrasts with the results obtained when ultrasound was applied on chitosan nanoparticles [32], in which a continuous decrease of particle size was observed. Two main reasons that may occur simultaneously explain these results on Fig. 3: the first is due the stability provided by surfactants, as the nanoemulsion (formulation without monomer) did not show significant particle size reduction after successive applications. Surfactants reduce interfacial tension enabling an increase in superficial area and therefore particle size is limited by their concentration [4,33]. The second reason is that the polymerization occurred after the first ultrasound application, even at the lowest cycle time, as will be discussed in topic 3.4. Once the outer core of the droplets was polymerized, ultrasound did not have sufficient energy to break the solid polymer particles.

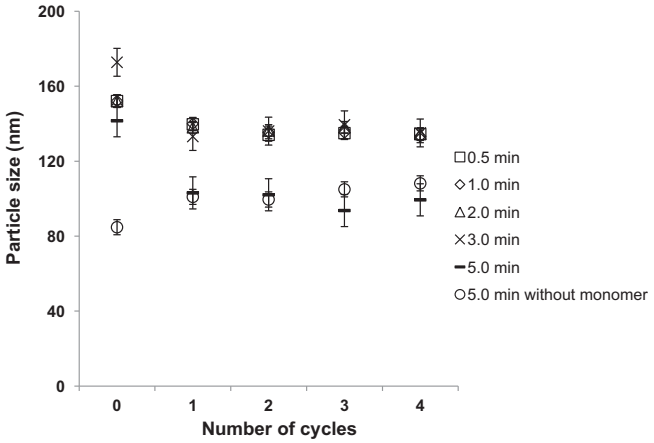

Fig. 3. Effect of successive ultrasound cycles in particle size (nm) using 6 mg/ml of KPS.

3.3. Effect of a higher KPS concentration in particle size

Fig. 4 shows the effect of successive application of ultrasound on particle size using KPS in a concentration 3 times higher (18 mg/ml). ANOVA showed no significant changes in particle size when compared with Fig. 3, indicating that KPS concentration had no influence on particle size. On the other hand, 5.0 min ultrasound cycles showed a substantial increase in particle size and phase separation was observed when the initiator was used at higher concentration. This significant change in particle size was only observed in these mentioned conditions at the third ultrasound application, indicating the existence of synergism of these variables in the process. It is worth noting that the sample reached a maximum of 64 °C after ultrasound application and although this is under PIT, evidence of instability was observed. The degradation observed may be related to non-ionic surfactants used in the formulation. Zhang et al. [23] also reported phase separation using ultrasound in formulations with non-ionic surfactants, but the same was not observed in formulations containing cationic or anionic surfactants. The authors hypothesized that both steric effect and charges present in ionic surfactants prevented the stability loss while non-ionic surfactants stabilize only by steric hindrance thus forming a less stable system.

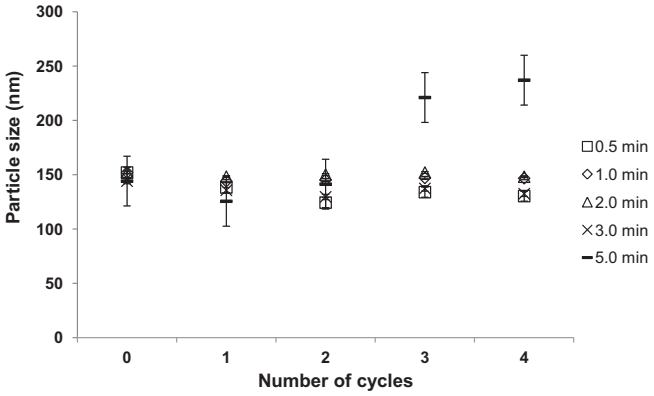

Fig. 4. Effect of successive ultrasound cycles in particle size (nm) using 18 mg/ml of KPS.

3.4. Polymerization efficiency

Gravimetric evaluation was employed to correlate cycle time and polymerization efficiency, and it was performed after each ultrasound application.

All curves showed similar polymerization behavior independently of cycle time (Fig. 5). Initially, it was observed a relative greater efficiency after first cycle followed by a smaller growth of polymerization on later applications. ANOVA evidenced no significant yield increase after successive cycles of ultrasound,

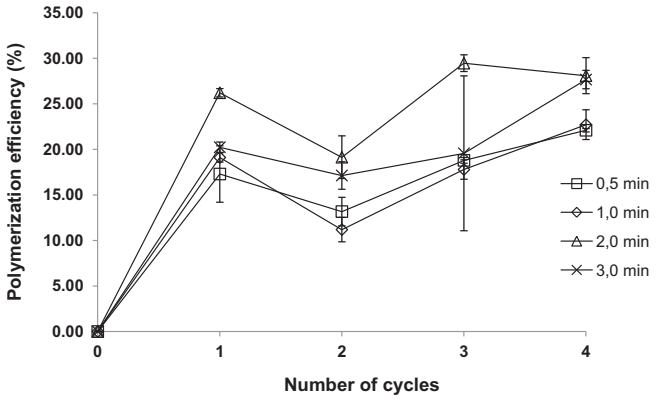

Fig. 5. Polymerization efficiency (%) after each cycle (1, 2, 3 and 4) of ultrasound application.

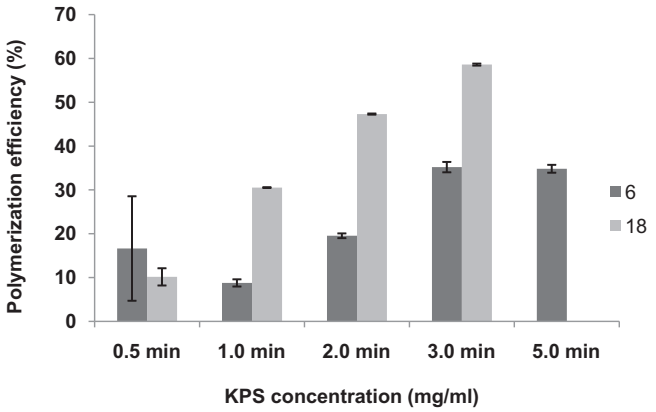

Fig. 6. Polymerization efficiency (%) using 6 and 18 mg/ml KPS concentrations. The result using 18 mg/mL in 5.0 min of cycles is not shown due to phase separation observed.

independently of length. Therefore, ultrasound was not able to reactivate radicals, either due to monomers or initiator depletion and, consequently, polymerization is not progressively increased after each cycle as expected. It was observed a general decrease in polymerization efficiency after cycle 2, although only in 1.0 and 2.0 min it was considered significant, which may be related to depolymerization caused by extreme conditions presented in sonic cavitation [24,25]. Polymers obtained with 0.5 min ultrasound application were less susceptible to depolymerization due to shorter cycle employed and higher temperature reached in 3.0 min compensate this phenomenon.

Higher process times were found to be more efficient than smaller ones. These results are explained by the temperature achieved after ultrasound application. 2.0 and 3.0 min cycles reached 47 and 56 °C, respectively while 0.5 and 1.0 min reached lower values (28 and 34 °C, respectively). Higher temperature induces more the activation of radicals due to the well-known thermosensitivity of KPS [34].

Polymerization efficiency analysis of nanocapsules polymerized using only heating, showed low results (5.0%) obtained when nanoemulsion was heated at 40 °C during 4 h of process. On the other hand, heating at 60 °C lead to 60.5% of polymerization efficiency, pointing that temperature has great influence in this process. Thus, exposing the nanoemulsion to ultrasound with no temperature control allows benefiting with ultrasound high energy and temperature reached reducing process time.

Since it consists in a high-energy method, ultrasound application works as a faster triggering of polymerization reaction, becoming possible to reduce the process time without an impact on system stability. During the ultrasound application, not only the temperature increase is important, but also the way that the energy is transferred to the system, it action very closely to the droplet surface and its role as a source of mechanical energy that keeps the particle size constant or even reduce it.

The influence of KPS concentration on polymerization efficiency is shown on Fig. 6. As mentioned in Section 3.3, samples with 18 mg/ml of initiator exposed to ultrasound for 5.0 min degraded; therefore they are not shown on Fig. 6. As expected, the samples containing initiator in higher concentration reached considerable better results for most of time evaluated, except for 0.5 min and 5.0 min. It was noticed that polymerization efficiency increase was not proportional to KPS concentration raise, indicating a limitation of this initiator to aid the reaction yield. Furthermore, higher KPS concentration showed to reduces the formulation capacity of withstand stronger reaction conditions. Thus, KPS concentration rise is beneficial until certain point, after that, it becomes very prejudicial.

The polymerization efficiency was evaluated after 7 and 28 days without any further ultrasound application, where the samples were kept at room temperature covered from light. The results (Table 1) showed a remarkable increase in polymerization efficiency after both times. This was due to the radical generated after ultrasound application that continued reacting during storage.

Table 2 compares polymerization efficiencies of ultrasound and heating polymerization methods in different KPS concentrations 28 days after polymerization. It was observed that KPS concentrations and cycle length had no influence on polymerization efficiency after this storage period, indicating a reaction equilibrium occurrence after a sort of time, independently of polymerization conditions. Ultrasound also exhibits yields closer to the ones obtained with heating method but with the main advantage of exposing the formulation to much less thermal degradation. Temperature reached after ultrasound application is time-dependent, where the maximum temperatures achieved after 0.5, 1.0 min, 2.0 min and 3.0 min ultrasound application were 28 °C, 34 °C, 47 °C and 56 °C, respectively. Whereas for the

Table 1
Polymerization efficiency (%) after cycle 4, 7 and 28 days.

Sample (min)	After application	7 days	28 days
0.5	22.1 ± 0.19	46.1 ± 0.24	83.3 ± 0.57
1.0	22.7 ± 1.63	52.3 ± 1.72	89.9 ± 0.01
2.0	28.1 ± 1.98	60.8 ± 1.60	78.6 ± 0.92
3.0	27.7 ± 1.01	74.1 ± 1.27	93.8 ± 0.42

Table 2
Polymerization efficiency (%) comparison after 28 days for different methods.

Sample	Polymerization efficiency (%)
KPS – ultrasound	
0.5 min	83.3 ± 0.57
1.0 min	89.9 ± 0.01
2.0 min	78.6 ± 0.92
3.0 min	93.8 ± 0.42
KPS 3× – ultrasound	
0.5 min	81.7 ± 4.10
1.0 min	82.4 ± 0.71
2.0 min	81.6 ± 2.47
3.0 min	76.8 ± 2.97
Heating	
KPS	78.5 ± 2.19
KPS 3×	84.5 ± 3.46
Without KPS	14.1 ± 2.76

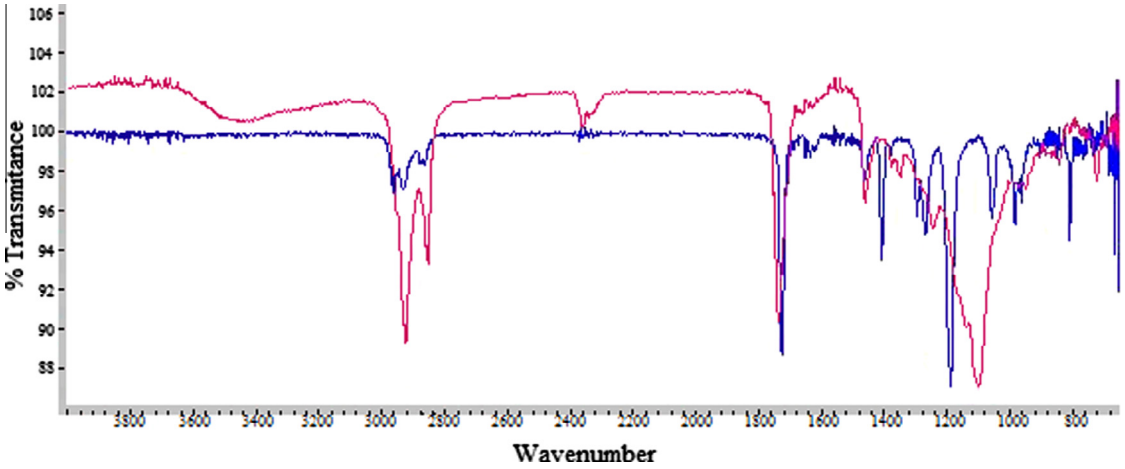

Fig. 7. Superimposed ATR infrared spectrums of monomer 2-ethylhexyl acrylate (blue) and poly (2-ethylhexyl acrylate) (red) extracted from nanocapsules. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

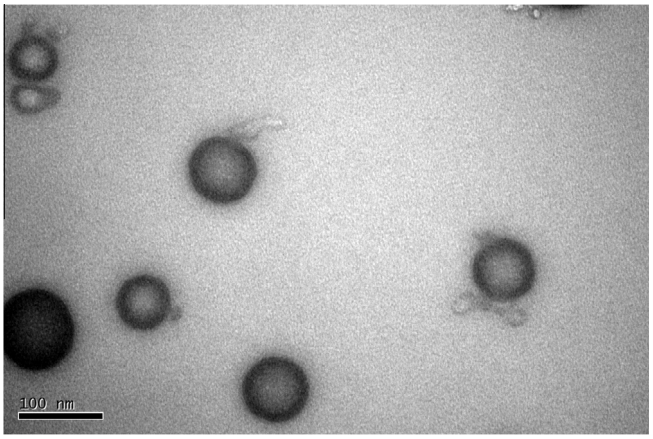

Fig. 8. Transmission electron microscopy image from nanocapsules.

polymerization initiated through heating, formulations were exposed to 40 °C for 4 h of reaction [19]. The shorter duration of exposure to higher temperature is expected to improve the stability of the dispersions. On the other hand, sample without KPS was subjected to 5.0 min of ultrasound and very low polymerization efficiency was obtained after 28 days (14.1% ± 2.76). This result shows that even applying a higher energy, polymerization will only be triggered after the activation of KPS and the self-polymerization is not favored by this method.

3.5. Confirmation of polymer formation after ultrasound application

FT-IR measurement was made to confirm the polymerization, after short cycles of ultrasound application. The characteristic bands of the monomer 2-ethylhexyl acrylate included a peak at 1600–1675 cm^{-1} pertaining to acrylate group's C=C bonds. However the extracted polymer spectrum showed the band related to acrylate group disappeared and bands relating to aliphatic C–H bonds (CH_2/CH_3) at 2900–3000 cm^{-1} increased in intensity (Fig. 7).

3.6. Morphology determination

TEM analysis mechanism consists in an emission of electron beam that crosses the sample and reflects its image in a different color scale: parts less dense are apparently clear than the denser ones. Images obtained by TEM allowed visualizing nanocapsules shape and morphology. As shown in Fig. 8, nanocapsules are spherical and their classical structures are well distinguished, wherein the clearer part is the oil core inside nanocapsules, whereas the darkest part corresponds to the denser product from polymerization reaction. By this, it could be seen that the polymer is formed around the oil core, being responsible for it encapsulation and showing that the ultrasound technique is able to form nanocapsules.

4. Conclusions

Evaluation revealed the successful *in situ* production of nanocapsules using successive shorts cycles of ultrasound. Physicochemical impacts undergone by formulation were related to strong reaction conditions, when variables evaluated were used in higher values, indicating an existence of a synergism. Thus, despite successive cycles were not considered significant, it may be important in the process as a whole. KPS concentration adjustment may be very beneficial, but it must be carried out carefully to not compromise the formulation capacity of withstand strong reaction conditions. Formulations stability was conserved with no significant increase in particle size at least for 28 days, even with continued polymerization. Therefore, ultrasound short cycles can be used with no harm to formulation, if carefully performed and, furthermore has a potential cost-effective route for polymerization reactions.

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