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Preparation of Poly(3-hydroxybutyrate) Micro- and Nanoparticles as Hydrophobic Drugs Carrier Using Self-emulsifying Nanoemulsion Method

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Authors' contributions

This work was carried out in collaboration between all authors. Author RAB designed the study, carried out the colloidal study, the drug delivery study and wrote the first draft of the manuscript. Author DAM performed the microscopy and spectroscopy analyses. Author LCV managed the experimental process and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To correlate the different sizes and morphologies of polymeric particles prepared through the oil-in-water micro- and nanoemulsions.
Study Design: Poly(3-hydroxybutyrate) (PHB) micro- and nanoparticles were prepared using oil-in-water micro- and nanoemulsions based on the sodium dodecyl sulfate (SD) micellar system.
Place and Duration of Study: Chemistry Institute of São Carlos at University of São Paulo, São Carlos, São Paulo, Brazil, between September 2012 and June of 2014.
Methodology: The microemulsion domains of the sodium dodecyl sulfate (SD) micellar system were obtained by the pseudo-ternary phase diagram. The self-emulsifying nanoemulsions were formed by low-energy method by addition of NaCl to SD micellar system. Poly(3-hydroxybutyrate) particles were prepared using the in micro- and nanoemulsion domains determined. The

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encapsulation of Ibuprofen (IBU) was performed only via nanoemulsion method. The average size and size distribution of both the micellar systems and the polymeric particles were determined by dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern). The samples also were analyzed using the by Fourier transformed infrared spectroscopy (FTIR Prestige-21), PerkinElmer's LAMBDA 35 UV/Vis Spectrophotometer (Perkin Elmer) and by scanning electron microscopy (Zeiss LEO 440).
Results: Polymeric particles with different sizes and morphologies were prepared through the oil-in-water micro- and nanoemulsions. However, DLS measurements indicated that the precipitation of PHB did not occur inside micelles, but by an interfacial precipitation process. Despite, varying the oil content in microemulsion system, polymeric microparticles were obtained, while nanoparticles with size distribution around with 250-710 nm were prepared in nanoemulsion domain. The nanoemulsion method was used for encapsulating ibuprofen. Results indicated a non-Fickian release profile with biphasic pattern, with release around with 91.4% at 48 h.
Conclusion: The nanoemulsion method presented more suitable than microemulsion for preparing polymeric nanoparticles, as well to encapsulate lipophilic drugs, demonstrating versatility for future applications in biomedical area as versatile carrier.

Keywords: Self-emulsifying nanoemulsions; dynamic light scattering; poly (3-hydroxybutyrate) nanoparticles; ibuprofen-loaded particles.

1. INTRODUCTION

The polymeric colloids possess an attractive prospect in the biomedical and biotechnological areas. In the medicine field, polymeric particles (PP) have been widely investigated with different formulations due to their advantages in sustained drug release [1-3]. For such applications, the polymer should have as main property the biocompatibility with the biological system. Another important and desirable property is the biodegradability, which can occur enzymatic or hydrolytically and the products of the in vivo degradation must not generate toxic compounds [4]. Biodegradable polymeric microspheres for the parenteral controlled release, such as, poly (lactide-co-glycolide) (PLGA) and poly (lactic acid) (PLA) have been widely investigated for several therapeutic agents [5,6]. The PLGA and PLA are poly (α-esters), which are thermoplastic polymers with aliphatic ester linkages in their backbone structure, and these ester bonds are hydrolytically labile to degradation.

The poly (3-hydroxybutyrate) (PHB) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) polymers have attracted attention not only to research in controlled release systems, but also in tissue engineering [7]. Both, PHB and PHBV are microbial origin polyesters belonging to the poly (hydroxyalkanoates) family [8]. The chemical structure of the PHB and PHBV is very similar to PLA and PLGA polymers, but they degrade to a slower rate [9,10]. In in vivo, hydrolytic degradation of PHB and PHBV results in formation of D-(-)-3-hydroxy-butyric acid, which is a normal constituent of blood (concentrations between 0.3 and 1.3 mmol L-1) [4,11]. As the body reabsorbs PHB, it might be used as a surgical implant, in surgery, as seam threads for the healing of wounds and blood vessels. Fabrication of drug-loaded biodegradable micro and nanoparticles is being investigated for sustained drug release [12]. In pharmacology, PHB can be used as microcapsules in therapy or as materials for cell [13]. Elustondo and co-authors demonstrated that short-poly[(R,S)-3-hydroxybutyrate] is efficiently internalized by living cells [14]. PHB is a versatile material and presents application in others areas [15,16].

Polymer particles can be prepared from preformed polymers or by direct polymerization of monomers. Several methods have been developed for the preparation of polymeric micro and nanoparticles. Emulsions, microemulsions and nanoemulsion, this later called of miniemulsons or ultrafine emulsion, are often used to prepare polymeric nanoparticles from polymerization of monomers [17-19]. Atom transfer radical polymerization (ATRP) is a versatile route toward preparation and functionalisation of nanoparticles [20]. This method can provide a site-specific grafting to a variety of surfaces, and it is considered as great method for preparing polymer-grafted materials [21]. However, for the encapsulation of therapeutic drugs is more common the use of methods from preformed polymers. Emulsion/solvent evaporation, salting-out, nanoprecipitation, and dialysis are techniques widely utilized to prepare polymeric nanoparticles [22,23]. Emulsion and double-diffusion emulsion

followed by evaporating the solvent are the methods most employed to prepare micro and nanospheres [10,24-26]. On the other hand, the form and size distribution of the particles should be optimized to obtain a stable and effective sustained drug delivery system [26-28]. The effect of the homogenization conditions in emulsion systems determines the size distribution and morphology of the final particle, however the mechanism that governs these phenomena are not fully understood. The production of nanoparticles with uniform size and morphology is an actual challenge in many fields of science, especially in biomedicine [22,23].

The purpose of this study was preparing polymeric particles from preformed PHB by means of oil-in-water (o/w) micro- and nanoemulsion methods using sodium dodecyl sulfate (SD) as surfactant and 2-propanol as cosurfactant. The solubility of the PHB molecules however is basically limited to aprotic (tetrahydrofuran, dimethyl sulfoxide) and organochlorine (chloroform and dichloromethane) solvents [25]. Although the chloroform is not a common solvent in the microemulsion studies, in this paper it was chosen to use as solvent as well as oil phase in both micro and nanoemulsion systems. In order to stabilize the micelle formation, sodium dodecyl sulphate was preferred as surfactant due to its wide literature, low cost, non-carcinogenic, and as pharmaceutical excipient [29]. Sodium chloride was used for preparing the nanoemulsions at low-energy method. Different compositions of surfactant, cosurfactant and oil were investigated by electrical conductivity and dynamic light scattering techniques. The influence of the microemulsion and nanoemulsion domains in the PHB particles preparation was discussed as well. In order to test the applicability of the proposed method, Ibuprofen (IBU) was selected as a model drug, and in vitro sustained drug release was performed.

2. MATERIALS AND METHODS

2.1 Materials

Sodium dodecylsulfate (SD) (Fluka AG, purity > 99%), chloroform (CHF) (Sigma-Aldrich, HPLC grade, assay 99%), isopropyl alcohol (J.T.Baker) were used in all experiments without further purification. Poly (3-hydroxybutyrate) (PHB), (96% purity and weight-average molecular weight = 600,000 g/mol) from PHB-Industrial S. A. (Serrana, SP., Brazil). In all cases, ultrapure deionized water (specific conductivity lowers than 18.2 μS.cm-1) was supplied by Thermo Scientific Barnstead Ultrapure Water (D7381 Easypure II).The main text of the article should appear here with headings as appropriate.

2.2 Determination of Micro- and Nanoemulsions Domain

Micellar solutions of different water mass ratios (R) were prepared by mixing appropriate amounts of SD, cosurfactant and water. The mass ratio of SD/2-propanol (ρ) was kept fixed in 0.5. The water/SD-2-propanol (SP) systems with initial water fractions of 90, 80, and 70% (w/w) were named SP9, SP8 and SP7, respectively. Likewise, water-NaCl/SD-2-propanol systems (SPN) were named SPN9, SPN8 and SPN7. Conductivity measurements were performed by Orion 4-Star Plus pH/Conductivity (Thermo Scientific). In a typical experiment, a micellar solution of known mass was magnetically stirred at 25 ± 0.1℃ with water bath during the measurements. Then, chloroform (CHF, oil phase) was added into the micellar solution at fixed volume by using a manual microsyringe (Agilent Technologies, needle gauge of 0.47 mm) and the system remains under constant magnetic stirring until the complete solubility of the oil phase. Conductivity measurements were performed for all samples and for each oil additions; the weight percentages were estimated to investigate the micellar domains. Successive additions of the oil phase were continued until the mixture exhibited turbidity. Measurements were done for all samples in triplicate to determine the boundaries of the emulsion/micro (or nano) emulsion domains. In all cases, the oil fraction data was corrected by molar volume of the chloroform.

2.3 Preparation of Polymeric Particles of Poly(3-hydroxybutyrate, PHB)

PHB particles were prepared using the SP7 and SPN7 samples in micro- and nanoemulsion domains. The stock solution was carried out by the solubilization of the PHB in powder with chloroform in an erlenmeyer. For all samples, the final concentration of the PHB solution was kept at 1 mg mL-1. First method, two different water/SD-2-propanol solutions were prepared in erlenmeyers (SP7a and SP7b samples). Then, different aliquots of PHB solution were added in the both flasks suddenly under magnetic stirring. In each Erlenmeyer used different amount of oil,

but the final concentration of PHB molecules was kept at 1 mg mL-1. The quantity of chloroform added corresponds to 21.9% and 26.8% (w/w), SP7a and SP7b samples, respectively. The samples stayed under magnetic stirring overnight under continuous magnetic stirring at two different temperatures, at 25℃ and 40℃. Second method of preparing PHB particles, three water-NaCl/SD-2-propanol solutions were prepared in separate erlenmeyers (SPN7c, SPN7d and SPN7e samples). First flask, SPN7c sample, PHB solution was quickly added under magnetic stirring, which the quantity of added oil matches to 13.5% (w/w). Second flask, SPN7d sample, the quantity of added oil matches to 42.4% (w/w). Third flask, first, only CHF was added until to obtain the nanoemulsion domain (about 33% w/w) and in following the PHB solubilized in chloroform was also quickly added under magnetic stirring (SPN7e sample). All samples prepared from second method stayed under continuous magnetic stirring at room temperature; and final concentration of PHB molecules was kept at 1 mg mL-1. The samples were washed with deionized water and were centrifuged at 8000 rcf (relative centrifugal force) for three-fold. The polymer particles were re-disperse via ultrasound bath in deionized water for DLS and in 2-propanol for SEM analyses.

2.4 Preparation of IBU-loaded PHB Nanoparticles

The encapsulation of Ibuprofen (IBU) was performed via nanoemulsion method (SPN7e sample). A water-NaCl/SD-2-propanol solution was prepared and, only CHF was added for obtaining the nanoemulsion domain (about 33% w/w). Then, a PHB/IBU solution in chloroform was quickly added under magnetic stirring. The initial proportion of IBU was 5% (w/w) relative to PHB concentration. After addition of the PHB/CHF/IBU solution, the samples remained under continuous magnetic stirring overnight. The sample was washed with phosphate buffered saline solution (PBS, pH 7.4) and centrifuged at 8000 rcf (relative centrifugal force) for threefold. The samples were dried under glass vacuum desiccator in environment temperature. The encapsulation efficiency (EE) was determined through the unincorporated drug in the supernatant after the centrifugation process (UV absorbance at 221 nm, PerkinElmer's LAMBDA 35 UV/Vis Spectrophotometer). For in vitro Ibuprofen release, a certain weighted IBU-loaded nanoparticles with PHB:IBU ratio of 28:1 (w/w) was dispersed in 2 mL of PBS via ultrasonic bath for 15 minutes. The dispersion containing the IBU-loaded nanoparticles was sealed in a dialysis bag (cut-off MW 8–10×103) and immersed in 100 mL of PBS, which was continuously magnetic stirred at a fixed speed with around 50 rpm and 37℃. At a specified time interval, 2 mL was withdrawn from the release medium and IBU concentration was determined with UV absorbance at 221 nm. The fresh PBS solution was added to the release medium in order to maintain the medium proportions. In vitro release was taken from the average of three samples and the release mechanism study was carried out using the in Korsmeyer–Peppas model [26,28].

2.5 Characterization

The average size and size distribution of both the micellar systems and the polymeric particles were determined by dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern, UK) at 25℃. For sample with just on population, the sizes from autocorrelation function was obtained by cumulant method, while for multimodal distribution the CONTIN algorithm was used. The monitoring the preparation of polymeric particles was carried out by DLS analyzes, using the SPN7 sample. First, water-NaCl/SD-2-propanol/CHF system in nanoemulsion domain (without PHB) was prepared and analyzed by DLS. In followed, 100 µL of PHB/CHF solution at 1 mg/mL was added into nanoemulsion solution under magnetic stirring; after stabilization an aliquot of 800 µL was removed and put in a cuvette for DLS analysis. After 10 minutes under magnetic stirring, another aliquot of the water-NaCl/SD-2-propanol/PBH-CHF system was introduced into the cuvette for DLS analysis. The size distributions were analyzed by the correlation coefficients. The morphology and texture of polymer particles were evaluated by scanning electron microscopy (Zeiss LEO 440). Molecular structure of the polymeric nanoparticles was analyzed by Fourier transformed infrared spectroscopy performed in the FTIR Prestige-21 (Shimadzu Scientific Instruments). All spectra were obtained by averaging 64 scans at 2 cm-1 resolution over the spectral range of 4000– 400 cm-1 and the sample prepared in KBr pellets.

3. RESULTS AND DISCUSSION

The domains of formation of the oil-in-water microemulsion and nanoemulsion were studied by the partial pseudo-ternary phase diagrams.

Fig. 1 shows the domains investigated, in which the fixed ratio of the SD/cosurfactant (ρ = 0.5) is represented as a single component at one of the vertices. The Fig. 1(a) shows the appearance of the H_2O/SD/CHF emulsion (1), H_2O/SD-2-propanol/CHF microemulsion (2) and H_2O-NaCl/SD-2-propanol/CHF nanoemulsion (3). Addition of oil was carried out until the system displaying permanent turbidity, indicating the maximum amount of oil that can be solubilized in the system. Regions of continuous single-phase microemulsion were determined as being the compositions that showed permanent transparency. Fig. 1b shows a partial pseudo-ternary phase diagram of water/SD/chloroform system using 2-propanol as cosurfactant. On the abscissa, chloroform concentration increases from left to right, while on the ordinate, water concentration increases from top to bottom, and surfactant/alcohol concentration is increases from bottom to top. The concentration of each component is in weight percent. The four-component o/w microemulsions remain stable at 25°C. The use of 2-propanol as cosurfactant is not common for microemulsion systems [30] due to the fact that if it is used in high concentrations, it may form an isotropic solution with water and chloroform (data not shown). Therefore, only the water fractions of 90, 80, and 70% (w/w) were investigated for ensuring the presence of an immiscible phase in all the studied compositions.

Fig. 1b shows the partial pseudo-ternary phase diagrams of water/SD-2-propanol/chloroform (in black) and water-NaCl/SD-2-propanol/chloroform (in blue). Without the NaCl addition, just microemulsion domains were observed (black-shared area). When 1% (w/w) NaCl (relative to ~0.17 mol/L) was added to initial solution before oil addition, different behaviours were observed.

First, the presence of NaCl leads an increase of the o/w microemulsion domain when compared to a solution without NaCl. For example, using R = 80 for the water/SD-2-propanol system (SP7 sample), 26.8% of oil was necessary for the solution becomes cloudy, while in presence of NaCl (SPN7 sample) the weight percent was around with 36.8%. However, for samples with sodium chloride, after the addiction of oil until the formation of an emulsion system, the cloudy solution readily disappears and a bluish transparent solution was formed (like image 3 in Fig. 1a). This region is represented by the blue-filled area in the Fig. 1b, which corresponds to the nanoemulsion domain.

All data from phase diagrams were followed up by electrical conductivity measurements. For microemulsion systems, the most of the electrical conductance measurements are performed in water-in-oil regions because of the occurrence of the percolation phenomenon. Concerning the oil-in-water regions, however, are observed less studies relative to electrical conductance behaviour. For o/w emulsions, classical semi-empirical conductance theory is used, in which, the conductivity of polydisperse o/w emulsions is related to Bruggeman's equation, [31] while for monodisperse systems the data behave between the Bruggeman and Maxwell equations [32-35]. In order to put our results on a uniform basis, we employed the molar conductance (Λ) rather than the specific conductivity (κ), because the molar conductance is correlated to the measured conductivity and the molar concentration. The molar conductance of the water/SD/CHF sample followed the predicted behaviour by Bruggeman's equation at low oil fractions, which polydisperse o/w emulsions was verified. But for microemulsified system (water/SD-2-propanol/CHF), the molar conductance data did not follow Bruggeman's equation, an allometric fit was the best equation found for fitting the experimental data. Gunaseelan and co-works investigated the specific conductivity of several o/w microemulsions stabilized by sodium dodecyl sulfate and 1-butanol as function of the volume fraction of oil and the molar ratio of water to surfactant (R) [34]. The profiles of the conductivity data were explained by the modified Bruggeman's equation ($(\Lambda/\Lambda_0)^{2/3} = 1 + f\varphi$), in which the value of the slope of this equation (f) was found to depend on the concentration of surfactant and the nature of the oil. In this equation, the φ parameter corresponds to the oil fraction volume.

Fig. 2 shows the molar conductance data of all samples. The molar conductance data of the SP9, SP8 and SP7 samples presented the same behaviour as shown by Gunaseelan's studies. Nonetheless, electrical conductance measurements showed in all investigated system that the first oil addition provided an increase in the molar conductance data, which indicates a variation in the ionic mobility. In subsequent additions, the molar conductance data decreased, as expected. The molar (or equivalent) conductance has a maximum fixed number of charge conductors, therefore, the conductance value will increases with the dilution, because how much more dilute is the solution, greater it will be the moving of the ionic

species and, consequently, larger conductance values will be observed. In this case, the increase of the molar conductance in low chloroform proportions may be interpreted as an overlapping of the two distinguished phenomena: (i) small proportion of alcohol, which was not partitioned within the micellar phase, could form an isotropic solution ($CHCl_3$—$CH_3CH(OH)CH_3$—H_2O); and (ii) chloroform being to some extent polar, at low CHF proportions, may act both as very weak cosurfactant or as oil [36].

Fig. 2 shows the presence of two linear regions for the SP9, SP8 and SP7 samples. The values of the slope (f) obtained from the modified Bruggeman's equation were calculated for each linear region. First linear region showed that the f values did not present significant difference for SP8 and SP7 samples (0.2221 and 0.2348); however, SP9 sample presented a value almost twice larger (0.4017). Second linear region showed that the f values are dependent on surfactant ratio, SP9 > SP8 > SP7 samples, (1.5992 > 0.6228 > 0.4942, respectively). The value of the empirical parameter f is reported [32,33] to be greater than 1 in o/w microemulsion stabilized by non-ionic surfactants and is assigned to the hydration of the droplets. Thus, microemulsions containing unhydrated spherical droplets are consistent with the f parameter value equal or less to 1. Nonetheless, some works in the literature [33,34] observed a value of f less than 1 only in few exceptions based on the o/w microemulsions stabilized by anionic and non-ionic surfactants.

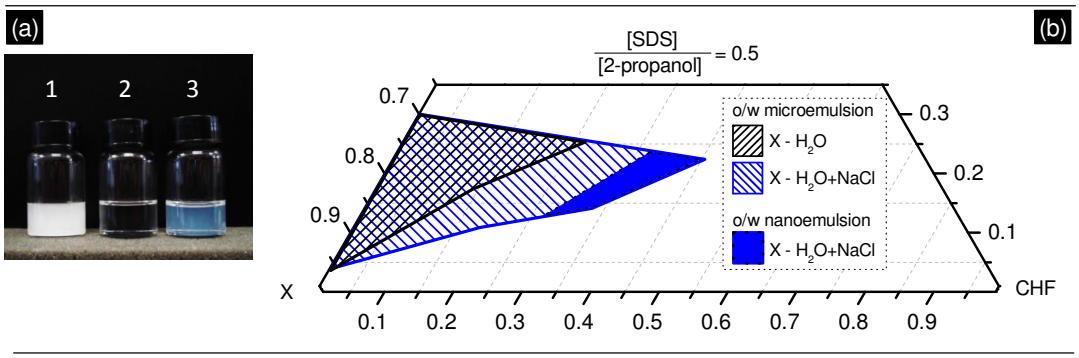


Fig. 1. Appearance of the O/W emulsion (1), micro (2) and nanoemulsions (3) obtained by the studied systems (a); partial pseudo-ternary phase diagram of investigated systems (b). Black-shared represents just o/w microemulsion domain to water/SDS-2-propanol/chloroform system. Blue-shared represents the o/w microemulsion domain to water-NaCl/SDS-2-propanol/chloroform system, while blue-filled represents the formation of nanoemulsion domain

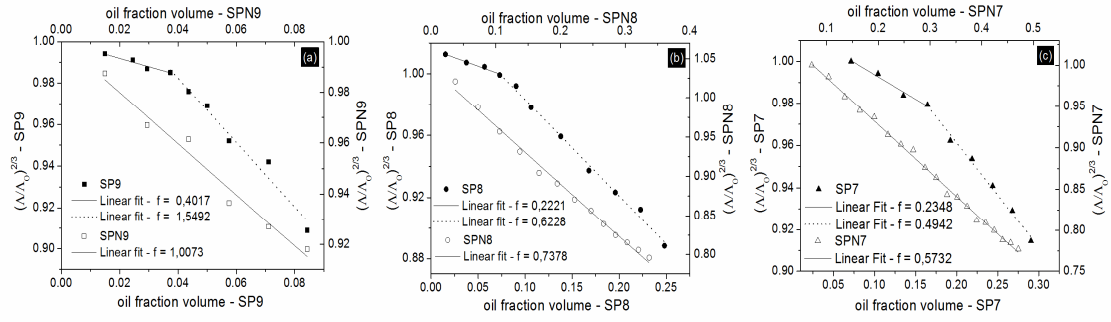


Fig. 2. Experimental molar conductance of the water/SDS-2-propanol/CHF and water-NaCl/SDS-2-propanol/CHF systems; (a) SP9 and SPN9, (b) SP8 and SPN8 and (c) SP7 and SPN7 samples

As it was observed by f values, just the SP9 sample presented hydrated micelles, and the increase of the surfactant/cosurfactant concentration provides non-hydrated micelles, SP8 and SP7 samples. This behaviour was not observed in the samples with sodium chloride. In the presence of NaCl, the SPN9, SPN8 and SPN7 samples showed linear profile in the entire experiment range. It was verified that the nanoemulsion did not provide any change in the molar conductance profile and it remained linear in all domain. The f values for the samples containing sodium chloride were found to be dependent on the surfactant/cosurfactant concentration as on the second linear profile of the SP9, SP8 and SP7 samples. The values of f decrease from the SPN9 to SPN7 samples (1.0073 > 0.7378 > 0.5732, respectively). SPN8 and SPN7 samples presented the formation of nanoemulsion with different oil fractions, whilst the bluish transparent solution was not observed for SPN9 sample. Therefore, both the hydration of the droplets and the counter-ion binding influenced the micelle stability, which had direct influence in the oil fraction to form different microemulsion and nanoemulsion domains through the low energy method.

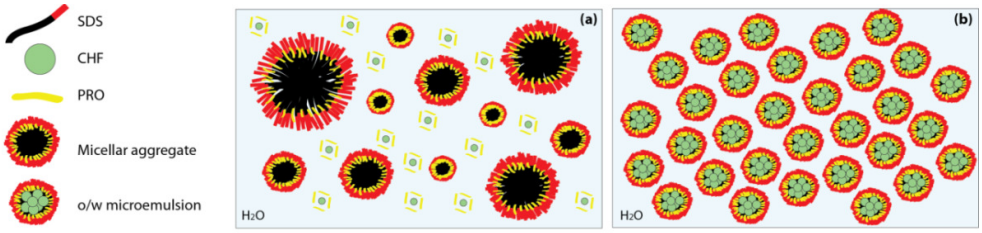
In order to comprehend these different behaviours in the electrical conductivity, dynamic light scattering (DLS) measurements were performed in all samples. The SP7 and SPN7 samples were chosen to illustrate the DLS results due to the high percentage of solubilized oil. In first linear profile of the SP7 sample, represented by a solid line in the Fig. 2c, DLS measurements were performed until the oil volume fraction around with 0.15 and showed a multimodal distribution with high polydispersity. Correlograms with high signal-to-noise ratio were recorded, indicating that results do not meet quality criteria. The heterogeneous distribution or formation of swollen micelles (or large aggregates) at low oil fraction can be attributed to the instability of the direct micelle, which is provided by the not well-ordered phase boundary between the Stern layer and the hydrocarbon core [37]. This behaviour can be understood by the “open" model, which was suggested by the studies of Russel and Whithen [38] about the SD use. This type of structure and the property of chloroform in act as very weak cosurfactant and oil provided the formation of a multimodal distribution. Based on results, Scheme 1(a) shows a possible formation of mix of SD micellar aggregates and, chloroform molecules structured with the alcohol and water molecules.

In the second linear profile of the SP7 sample, represented by a dashed-line in the Fig. 2c, the concentration of chloroform increases, and due to its nonpolar property, it begins getting into the micelles, forming stable o/w microemulsion. These phenomena are represented in Scheme 1(b). Increasing the oil content improved the micelle stability, as well as correlograms and cumulants analysis showed appropriate correlation function with monomodal distribution (as can be observed in Fig. 3b).

Fig. 3 shows the hydrodynamic diameter of the SP7 and SPN7 samples in different oil fraction and their relative correlation coefficients. SP7 sample showed that the increase of oil amount did not provide significant difference in the hydrodynamic diameters of the micelles. However, SPN7 sample showed dependent on the chloroform fraction. Oil addition into SD+NaCl solution provided the increase of micellar size from 1.6 to 17 nm, as shown in the Figs. 3(a,b). As aforementioned, the electrolyte had direct influence in the formation of the micro- and nanoemulsion systems. In fact, the electrolyte has the opposite effect of the alcohol; it increases the degree of counter ion association and, consequently, decreases the micelle charge [37]. The compression of the electrical double layer led to micelle destabilization and formation of the self-emulsifying nanoemulsions through the low-energy method. The nanoemulsion domains are represented by letter “N” in Fig. 3(b). Preparation of micro- and nanoparticles from poly (3-hydroxybutyrate) polymer were performed using the studied sodium dodecyl sulfate micellar systems. The samples with composition R=90 (SP9) did not present appropriated results, while sample with R=80 and R=70 showed similar results. Thereby the samples SP7 and SPN7 were chose to represent the preparation of polymeric particles.

The percentages of oil used in the SP7 sample are in the second linear profile, as shown in Fig. 2; which the oil fraction added corresponds to 21.9% and 26.8% (w/w), SP7a and SP7b samples, respectively. Fig. 4 shows PHB polymeric particles prepared from the SP7a sample. Spherical microparticles with large size distribution were obtained from the water/SD-2-propanol/PHB-CHF system. Polymeric microparticles with smooth surface can be obtained (Fig. 4b) when the system was maintained under magnetic stirring at room temperature. When the system was magnetic stirred at 40°C overnight, spherical particles with

rough surface were formed (Fig. 4c). As shown before, microemulsion domain is obtained with the same oil fraction. However, the presence of PHB molecules became unstable the microemulsion, and the micelles were not able to act as templates for preparing PHB nanoparticle and, thereby, microparticles were formed. According to DLS measurements of the SP7b sample at 26.8% (w/w) of oil fraction, micelles of size around with 2.8 nm with narrow size distribution are formed. Nonetheless, in the presence of PHB molecules, a mixture of rods, wires and nonspherical particles was observed. This result infers that near the maximum of the oil load, the polymer destabilizes the totally microemulsion domain. As the effective forces are unbalanced, there may be the formation of interconnected channels instead of spherical drops (w/o or o/w), leading the microemulsion to a bicontinous zone, which is composed by non-organized medium and, thereby, complex structures can co-exist [39].



Scheme 1. Micellar systems formed in the SP9, SP8 and SP7 samples. First linear profile is composed by aggregates of surfactant molecules dispersed in water with chloroform molecules solubilized outside micelles (a); and the second profile, where homogeneous o/w microemulsion system is formed

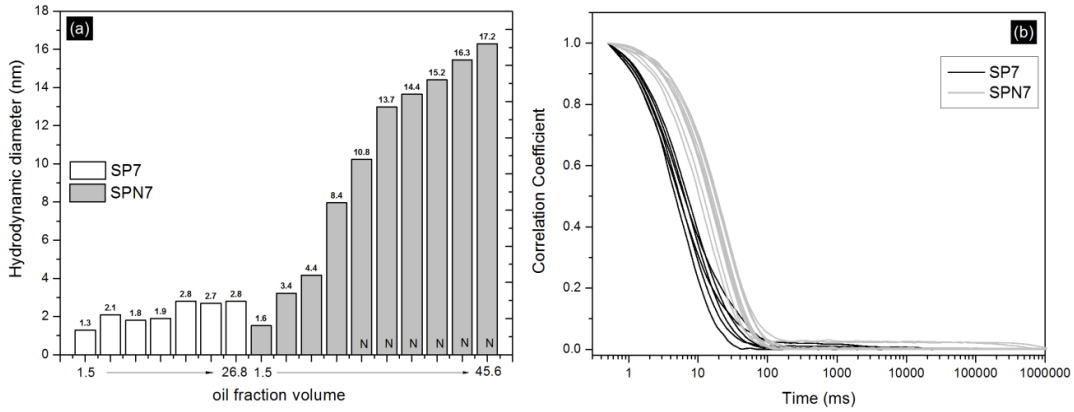


Fig. 3. (a) Hydrodynamic diameter obtained by dynamic light scattering measurements of the SP7 and SPN7 samples. The letter N inside base indicates the formatting of the nanoemulsion system. (b) Correlation coefficient of the SP7 and SPN7 samples

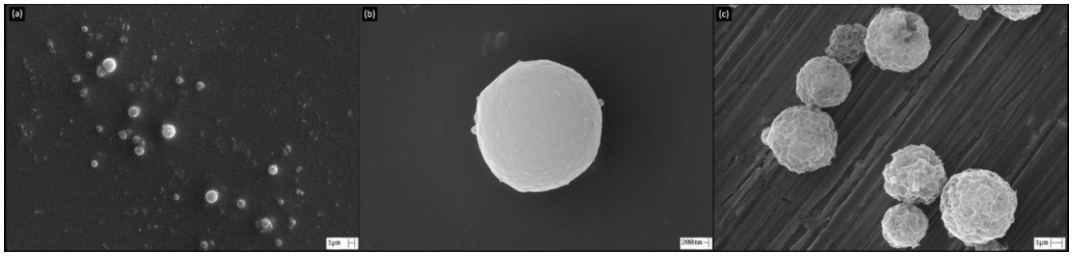


Fig. 4. SEM of the polymer particles synthetized in microemulsion domain; (a,b,c) SP7a

Fig. 5 shows the preparation of polymeric particles from SPN7 sample. SPN7c and SPN7d samples were prepared by direct addition of the PHB/CHF solution. The quantity of oil added in the SPN7c sample was at 13.5%, which this oil fraction is into microemulsion domain; and the SPN7d sample was prepared in the nanoemulsion domain with final amount at 42.4% w/w of oil. Figs. 5a and 5b show polymeric particles prepared in the microemulsion system (SPN7c sample). The micrographs show the formation of polymeric microparticles with rough surface and broad size distribution. For the SPN7d sample, large aggregates can be observed, which are composed by several polymeric nanoparticles together, Figs. 5c and 5d.

Figs. 5(c,d) and 5(e,f,g) represent the particles via nanoemulsion domains (SPN7d and SPN7e samples, respectively). For SPN7d sample, direct addition of a known volume of PHB/CHF solution was performed for reaching the nanoemulsion domain (at 42.4% w/w of oil fraction). Nonetheless, for SPN7e sample, first CHF was added to form the nanoemulsion domain and the PHB/CHF solution was added after, with final oil fraction at 42.4% (w/w) as well. The formation of nanoemulsion domain first, SPN7e sample, led to the spherical and lump-like polymeric nanoparticles as shown in Figs. 5 (e,f,g). Polymeric particles presented a polydisperse size distribution (255-712 nm), but with improved size control than that prepared using microemulsion system in either low or high oil concentrations. SPN7d and SPN7e samples showed different results, but both have the same PHB and oil concentrations. The main difference is that in the SPN7e sample the formation of nanoemulsion domain occurred first to the addition of PHB/CHF solution; and in the SPN7d sample, the formation of the nanoemulsion domain occurred in the presence of the PHB molecules.

The presence of PHB molecules modify the water/SD-2-propanol/Chloroform molecular structure, by making the microemulsion system does not act as a template for preparing polymeric nanoparticles. The presence of the monomer molecules in the micelle percolation threshold layer, changes the force interactions and, consequently, the local balance, destabilizing the micellar system and, thus, leading to the preparation of micro- and nonspherical particles. In fact, we suggest that the poly(3-hydroxybutyrate) macromolecules did not percolate the surfactant layer to the inside spherical droplet in the both studied systems. Micellar destabilization could be afforded by high molecular weight of PHB, in which this could not stabilize the polymer within the micelles and the precipitation of polymer could have occurred outside the micelle.

In order to investigate the preparation of nanoparticles, DLS measurements were carried out after the addition of PHB/CHF solution, as shown in Fig. 6. Water-NaCl/SD-2-propanol (SPN7e sample) was prepared and CHF was added to form the nanoemulsion first, which the total quantity of CHF in dispersion was about 33% (w.). Fig. 6(a) shows the correlation coefficient and the size distribution by intensity (7.5 – 13.5 nm) of the micelles in nanoemulsion domain (Water-NaCl/SD-2-propanol/CHF). The results presented a smooth and single exponential decay function, which are indicative for monosized particle dispersions. In following, 100 µL of PHB/CHF solution were added into nanoemulsion solution under magnetic stirring (Fig. 6b). DLS analyzes of water-NaCl/SD-2-propanol/PBH-CHF sample shows double exponential decay in correlation coefficient, which is indicative of population with different sizes, each one with their own decay. Fig. 6(b) shows two size distributions one around 7.5 – 8.8 nm and other around 1.5 – 4.8 µm. As observed by Fig. 6(a), the micelles represent the first distribution and the second distribution corresponds to an aggregate of polymeric particles, as it was observed by Figs. 5(e, f, g). These remarks indicate that precipitation of the particles does not occur inside the micelles. While the DLS analysis is being performed, the water-NaCl/SD-2-propanol/PBH-CHF solution stayed under magnetic stirring. Thereby, another aliquot was led to DLS analysis, with approximately 10 minutes of delay between them (Fig. 6c). DLS measurements presented a double exponential decay in correlation coefficient, however, some data showed multimodal fit error and noisy baseline, which are indicative of presence of large particles/agglomerates with high polydispersity degree. Due to the high polydisperse, this sample is not suitable for DLS analysis. Nevertheless, the correlation coefficients presented the same profile found in the Fig. 6(b). Thereby, this underpins that the polymer precipitation occurred outside the micelles and quickly.

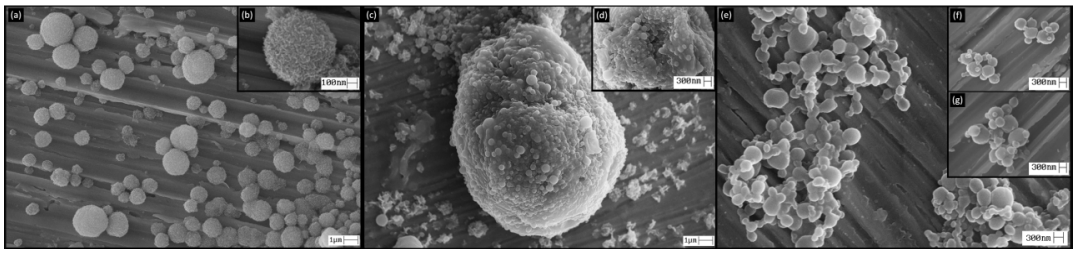


Fig. 5. SEM of the polymer particles prepared in microemulsion domain, (a,b) SPN7c, and in nanoemulsion domain, (c,d) SPN7d and (e,f,g) SPN7e samples

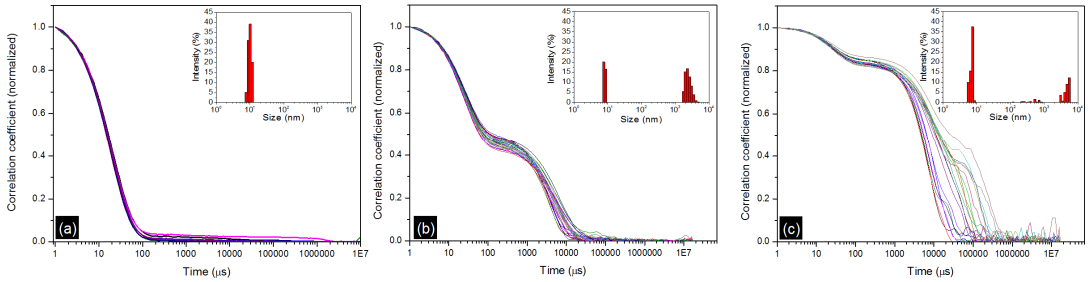


Fig. 6. Correlation coefficient and the intensity distribution of the (a) water-NaCl/SDS-2-propanol/CHF, (b) and (c) water-NaCl/SDS-2-propanol/PHB-CHF systems in different times.

In order to confirm the PHB phase was changed trough the nanoemulsion system, FTIR measurements were performed. Fig. 7 shows the FTIR measurements for the PHB as-received (commercial) and PHB polymer particles prepared by using the water-NaCl/SD-2-propanol/PHB-CHF systems (SPN7e sample). The absorption band at 1720 cm^{-1} and at 1276 cm^{-1} are assigned to stretch of C=O and C—O bonds, respectively and used for confirming the presence of PHB [40]. In both spectra, the 2970–2854 cm^{-1} region is dominated by the asymmetric and symmetric stretching bands of –CH3 and $–CH_2–$ of the hydrocarbon tail. The absorption band at 1456 cm^{-1} is due to the bending mode of $–CH_2–$, whereas the weak band at 1375 cm^{-1} corresponds to a $–CH_3$ deformation. From 1250 to 950 cm^{-1}, SD exhibits several distinct asymmetric and symmetric stretching bands of $–OSO_3–$. The strong doublet at 1220 and 1200 cm^{-1} corresponds to asymmetric S–O stretching, whereas the bands at 1053 and 977 cm^{-1} are assigned to symmetric S–O stretching. However, in the region that occurs the main SD vibrational bands also are presents a large number of bands due to polymer hydrocarbon chain. Thus, according to the FTIR results, the SD molecules used as surfactant during the preparation of polymeric particles were removed from the nanoparticles surface during the washing process; or they are present in a very small amount, practically undetected by FTIR measurements. Also, although CHF has been employed in the preparation of the nanoparticles, there is no evidence of the presence of CHF molecules in the final product after washing according to the data FTIR.

Ibuprofen (IBU) was encapsulated as model drug though the SPN7e sample in order to test the applicability of the purposed nanoemulsion method as one sustained drug delivery system. The IBU-loaded PHB nanoparticles prepared by the SPN7e sample presented encapsulation efficiency around 69%. The in vitro release profile of IBU-loaded nanoparticles in pH 7.4 PBS medium at 37°C is presented in Fig. 8. The IBU release curves exhibit a biphasic pattern. In the first 4h, an initial burst released around 50% of the drug was observed and 91.4% of drug loaded was released at 48h. Similar release profiles were observed with the poly (lactic acid) and poly-ε-caprolactone, which the time of release depends on the size of the polymeric particles [41-43]. The drug release data below to 60% were fitted in Korsmeyer–Peppas model [28]. The mechanism of diffusional release from IBU-loaded PHB nanoparticles prepared by the nanoemulsion method showed anomalous transport (non-Fickian), with diffusional exponent (n) with around 0.75. The release mechanism based on the anomalous transport, refers the combination of the both diffusion and erosion controlled rate release.

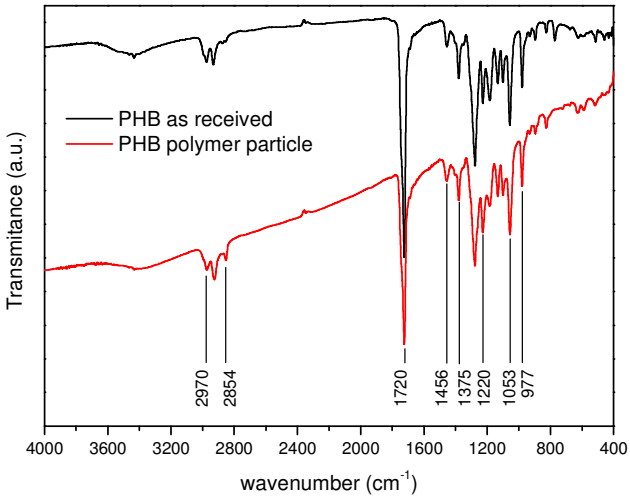


Fig. 7. FTIR measurements of the PHB as-received and PHB synthesized particles

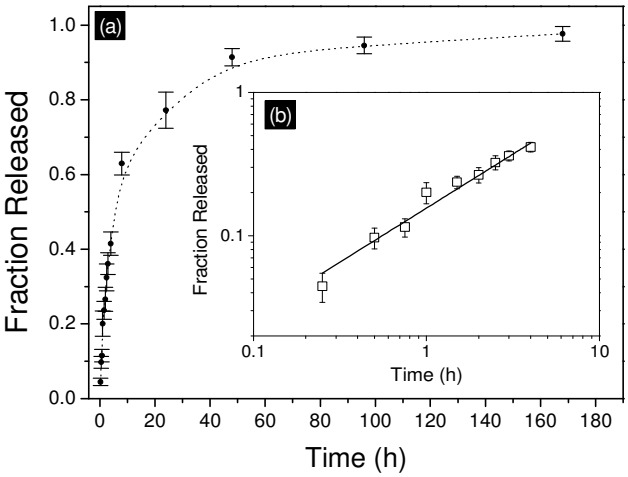


Fig. 8. Release data of IBU-loaded PHB nanoparticles (a) and log(fraction released) against log(time) of the experimental data, according Korsmeyer–Peppas model (b)

The initial burst with around 50% is related not only with the polymer porosity, but mainly with the drug molecules position in the solid polymeric phase. Drug molecules onto and closed to the particle surface present fast diffusion and release; the second phase was due to the release from polymeric matrix [44]. After 48 h a very low released IBU fraction was observed; which can be attributed to the polydisperse size distribution (255-712 nm). Polydisperse systems lead to a heterogeneous kinetic release. In a polydisperse size distribution, the mean sample size corresponds to a mean diffusion time for the system. As small particles have high superficial area than large ones, the acceleration of the early portion of the release curve is the result of release from particles smaller than the mean size. On the other hand, particles that is larger than the average size because the retardation of the transport at long times [27], as observed in Fig. 8, a low release between 48 and 168 hours. Therefore, for polydisperse systems, while small particles have already had their complete drug release, by diffusion or erosion, a low amount of drug remains in the large particles, providing a heterogeneous kinetic release.

4. CONCLUSION

Remarkable progress has been achieved in the polymeric particles area, which facilitate application in sustained and targeted delivery. The main technique for preparing poly(3-

hydroxybutyrate) polymeric particles is based on the double emulsion solvent evaporation/extraction. Consequence of the PHB molecules which are highly soluble by organochlorine solvents and, present poor solubility in other solvents, such as ethyl acetate. The size control of the PHB particles prepared by emulsification–diffusion technique is performed through ultrasonication steps or the use of ethanol for precipitating the particles [10]. We presented oil-in-water micro- and nanoemulsions based on the NaCl-water/SD-2-propanol/chloroform system for preparing PHB micro- and nanoparticles. In the absence of NaCl, only microemulsions were observed in whole range of concentrations, insofar as in the presence of NaCl, self-emulsifying nanoemulsions were formed by low-energy method. In microemulsion systems (SPN7 samples), polymeric microparticles with large particle size distribution are obtained. In the case of nanoemulsion, nanoparticles with enhanced size control were formed by using high oil concentrations. By nanoemulsion system, polymeric particles with size distribution of 255-712 nm could be prepared. As DLS measurements showed the formation of SD micelles below 20 nm, the insertion of PHB molecule destabilized the micellar system impeding it to act as template to prepare nanoparticles. DLS measurements indicated that the precipitation of polymer occurs outside the micelle through an interfacial precipitation process. Therefore, nanoemulsion considered as non-equilibrium thermodynamic systems with relatively high kinetic stability, demonstrated, for the sodium dodecyl sulfate micellar system, an improved control for preparing PHB particles than the thermodynamically stable systems. Through these investigations, the preparation of polymer particles using anionic surfactant, the nanoemulsion showed more adequate than microemulsion to prepare polymeric nanoparticles. The investigated nanoemulsion method can be used for encapsulating hydrophobic drugs and/or inorganic nanoparticles, like quantum dots, gold or magnetic iron oxide nanoparticles, thereby, demonstrating high versatility for future studies and applications. The nanoemulsion method showed good encapsulation efficiency using a lipophilic drug as ibuprofen. About 50% of the drug was released within the first 4 h and total release occurs after 48 h, indicating the potential of the system to be used in sustained drug delivery or others biomedical applications for hydrophobic drugs carrier.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coccolli V, Luciani A, Orsi S, Guarino V, Causa F, Netti PA. Engineering of poly(e-caprolactone) microcarriers to modulate protein encapsulation capability and release kinetic. Journal of Materials Science. Materials in Medicine. 2008; 19(4):1703-1711.
2. Park J, Mattessich T, Jay SM, Agawu A, Saltzman WM, Fahmy TM. Enhancement of surface ligand display on PLGA nanoparticles with amphiphilic ligand conjugates. J Control Release. 2011;156(1):109-115.
3. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V. PLGA-based nanoparticles: An overview of biomedical applications. J Control Release. 2012;161(2):505-522.
4. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Progress in Polymer Science. 2007;32(8-9):762-798.
5. Song XR, Zhao Y, Hou SX, Xu FY, Zhao R, He JY, Cai Z, Li YB, Chen QH. Dual agents loaded PLGA nanoparticles: Systematic study of particle size and drug entrapment efficiency. European Journal of Pharmaceutics and Biopharmaceutics. 2008;69(2):445-453.
6. Bhardwaj V, Ankola DD, Gupta SC, Schneider M, Lehr CM, Kumar MNVR. PLGA nanoparticles stabilized with cationic surfactant: Safety studies and application in oral delivery of paclitaxel to treat chemical-induced breast cancer in rat. Pharmaceutical Research. 2009;26(11): 2495-2503.

7. Chen GQ, Wu Q. The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials. 2005;26(33):6565-6578.
8. Hazer DB, Kılıçay E, Hazer B. Poly(3-hydroxyalkanoate)s: Diversification and biomedical applications: A state of the art review. Materials Science and Engineering: C. 2012;32(4):637-647.
9. Amass W, Amass A, Tighe B. A review of biodegradable polymers: Uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. Polymer International. 1998;47(2):89-144.
10. Poletto FS, Fiel LA, Donida B, Re MI, Guterres SS, Pohlmann AR. Controlling the size of poly(hydroxybutyrate-co-hydroxyvalerate) nanoparticles prepared by emulsification-diffusion technique using ethanol as surface agent. Colloids and Surfaces A-Physicochemical and Engineering Aspects. 2008;324(1-3):105-112.
11. Shishatskaya EI, Voinova ON, Goreva AV, Mogilnaya OA, Volova TG. Biocompatibility of polyhydroxybutyrate microspheres: *in vitro* and *in vivo* evaluation. Journal of Materials Science-Materials in Medicine. 2008;19(6):2493-2502.
12. Livshits VA, Bonartsev AP, Iordanskii AL, Ivanov EA, Makhina TA, Myshkina VL, Bonartseva GA. Microspheres based on poly(3-hydroxy)butyrate for prolonged drug release. Polymer Science Series B. 2009;51(7):256-263.
13. Kilicay E, Demirbilek M, Turk M, Guven E, Hazer B, Denkbas EB. Preparation and characterization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHX) based nanoparticles for targeted cancer therapy. Eur J Pharm Sci. 2011;44(3):310-20.
14. Elustondo PA, Angelova PR, Kawalec M, Michalak M, Kurcok P, Abramov AY, Pavlov EV. Polyhydroxybutyrate targets mammalian mitochondria and increases permeability of plasmalemmal and mitochondrial membranes. Plos One. 2013;8(9):e75812.
15. Phukon P, Saikia JP, Konwar BK. Bioplastic (P-3HB-co-3HV) from Bacillus circulans (MTCC 8167) and its biodegradation. Colloids and Surfaces B: Biointerfaces. 2012;92:30-34.
16. Yupapin DP, Pivsa-Art DS, Ohgaki DH, Pivsa-Art S, Srisawat N, O-Charoen N, Pavasupree S, Pivsa-Art W. 9th Eco-Energy and Materials Science and Engineering Symposium Preparation of Knitting Socks from Poly (Lactic Acid) and Poly [(R)-3-Hydroxybutyrate-co-(R)-3-Hydroxyvalerate] (PHBV) Blends for Textile Industrials. Energy Procedia. 2011;9:589-597.
17. Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F, Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2006;2(1):8-21.
18. Min K, Matyjaszewski K. Atom transfer radical polymerization in microemulsion. Macromolecules. 2005;38(20):8131-8134.
19. Co CC, Cotts P, Burauer S, de Vries R, Kaler EW. Microemulsion polymerization. 3. Molecular weight and particle size distributions. Macromolecules. 2001; 34(10):3245-3254.
20. Oh JK, Bencherif SA, Matyjaszewski K. Atom transfer radical polymerization in inverse miniemulsion: A versatile route toward preparation and functionalization of microgels/nanogels for targeted drug delivery applications. Polymer. 2009; 50(19):4407-4423.
21. Siegwart DJ, Oh JK, Matyjaszewski K. ATRP in the design of functional materials for biomedical applications. Progress in Polymer Science. 2012;37(1):18-37.
22. Mora-Huertas CE, Fessi H, Elaissari A. Polymer-based nanocapsules for drug delivery. International Journal of Pharmaceutics. 2010;385(1-2):113-142.
23. Rao JP, Geckeler KE. Polymer nanoparticles: Preparation techniques and size-control parameters. Progress in Polymer Science. 2011;36(7):887-913.
24. Zhao H, Gagnon J, Hafeli UO. Process and formulation variables in the preparation of injectable and biodegradable magnetic microspheres. BioMagnetic Research and Technology. 2007;5(2):1-11.
25. Terada M, Marchessault RH. Determination of solubility parameters for poly(3-hydroxyalkanoates). International Journal of Biological Macromolecules. 1999;25(1-3):207-215.
26. Quintanar-Guerrero D, Allemann E, Fessi H, Doelker E. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers.

Drug Dev Ind Pharm. 1998;24(12):1113-1128.
27. Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J Control Release. 1987;5(1):37-42.
28. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics. 1983;15(1):25-35.
29. Publication C. 7 Final report on the safety assessment of sodium lauryl sulfate and ammonium lauryl sulfate. International Journal of Toxicology. 1983;2(7):127-181.
30. Forland GM, Samseth J, Gjerde MI, Hoiland H, Jensen A, Mortensen K. Influence of alcohol on the behavior of sodium dodecylsulfate micelles. J. Colloid Interface Sci. 1998;203(2):328-334.
31. Bruggeman DAG. Calculation of various physics constants in heterogenous substances I Dielectricity constants and conductivity of mixed bodies from isotropic substances. Annalen der Physik. 1935;24(7):636-664.
32. Mackay RA, Agarwal R. Conductivity measurements in non-ionic microemulsions. Journal of Colloid and Interface Science. 1978;65(2):225-231.
33. Bisal S, Bhattacharya PK, Moulik SP. Conductivity study of microemulsions - evaluation of hydration of oil-water microemulsions applying bruggeman equation. Journal of Physical Chemistry. 1990;94(10):4212-4216.
34. Gunaseelan K, Umlong IM, Mukhim T, Ismail K. Electrical conductance behavior of oil-in-water microemulsions stabilized by sodium dodecyl sulfate and 1-butanol. Langmuir. 2003;19(18):7276-7281.
35. Maxwell JC. A treatise on electricity and magnetism. In Electricity and Magnetism, Oxford University Press: London. 1892;3:440.
36. Mukherjee K, Mukherjee DC, Moulik SP. Thermodynamics of microemulsion formation. 3. Enthalpies of solution of water in chloroform as well as chloroform in water aided by cationic, anionic, and nonionic surfactants. Journal of Colloid and Interface Science. 1997;187(2):327-333.
37. Georges J, Chen JW. Micellization study of sodium dodecyl-sulfate in water and microemulsion systems by conductivity and counterion-activity measurements. Journal of Colloid and Interface Science. 1986;113(1);143-153.
38. Russell JC, Whitten DG. Photochemical reactivity in organized assemblies .24. solute partitioning in aqueous surfactant assemblies - comparison of hydrophobic hydrophilic interactions in micelles, alcohol-swollen micelles, micro-emulsions, and synthetic vesicles. Journal of the American Chemical Society. 1982;104(22): 5937-5942.
39. Israelachvili J. The science and applications of emulsions - an overview. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 1994;91:8.
40. Pandian RK, Deepak S, Kalishwaralal V, Muniyandi J, Rameshkumar N, Gurunathan S. Synthesis of PHB nanoparticles from optimized medium utilizing dairy industrial waste using Brevibacterium casei SRKP2: A green chemistry approach. Colloids and Surfaces B: Biointerfaces. 2009;74(1):266-273.
41. Klose D, Delplace C, Siepmann J. Unintended potential impact of perfect sink conditions on PLGA degradation in microparticles. International Journal of Pharmaceutics. 2011;404(1–2):75-82.
42. Ye Z, Squillante E. The development and scale-up of biodegradable polymeric nanoparticles loaded with ibuprofen. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2013;422:75-80.
43. Carreras N, Acuña V, Martí M, Lis MJ. Drug release system of ibuprofen in PCL-microspheres. Colloid and Polymer Science. 2013;291(1):157-165.
44. Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(dl-lactic acid) size-dependence. Biomaterials. 1995;16(4):305-311.

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