114 | *Macromol. Symp.* **2013**, 324, 114–123 DOI: 10.1002/masy.201200075

Encapsulation of Jojoba and Andiroba Oils by Miniemulsion Polymerization. Effect on Molar Mass Distribution

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Summary: This paper aims to study the encapsulation of jojoba and andiroba oils via miniemulsion polymerization. The effect of different hydrophilic monomers (acrylic or methacrylic acid), co-stabilizers (hexadecane, jojoba oil or andiroba oil) and initiator concentrations were evaluated. Results indicated the formation of particles with nanocapsule morphology when either hexadecane or jojoba oils were used. When andiroba oil was used no phase separation could be detected in polymer particles and molar mass distributions showed a shoulder of intermediate molar masses between those of the oil and of the pure polymer.

Keywords: andiroba oil; jojoba oil; miniemulsion polymerization; molar mass distribution; nanocapsules

Introduction

Recently, several studies shown that polymeric carrier systems meet the requirements for the vectorization of hydrophobic compounds. In this context, polymeric nanocapsules (NCs) appear as promising candidates for the controlled and targeted delivery of actives compounds in a variety of biomedical, food, cosmetics and other applications.[1] NCs consist of submicron particles formed by a polymer shell and a core containing a non-solvent (for the polymer), which can be water or an organic compound to be encapsulated.

Many techniques have been devised for the synthesis of polymeric NCs, such as layer-by-layer self-assembly,[1] block-copolymer assembly,[2] solvent evaporation[3,4] and miniemulsion polymerization.[5] In miniemulsion polymerization, the monomer and the hydrophobe (to be encapsulated) are pre-dispersed in water, in droplets of 50–500 nm, stabilized by a combination of a surfactant and a co-stabilizer soluble in the organic phase.[6] The particle shell is formed during the polymerization, when the growing polymer chains become immiscible and phase-separate from the hydrophobe-rich phase.[5] In this way, miniemulsion polymerization is a versatile and efficient technique with high encapsulation efficiency[6] that can be obtained in a single step.[5] However, effects of thermodynamic and kinetic factors on the phase separation and morphology of the particles are of paramount importance to the effectiveness of the method.

For stable miniemulsions, the choice of a suitable co-stabilizer is necessary. Many works describe the use of hydrophobic agents as hexadecane,[7] a long-chain alkane, with 16 carbon atoms. However, the applicability of nanocapsules in the biomedical field can be enhanced when biodegradable and biocompatible materials are used. Bathfield et al.[8] used Miglyol 812, mixture of triacylglycerols of caprilic and caproic acids as co-stabilizer in vinyl acetate miniemulsion polymerizations. More recently, Romio et al.[9] carried out a comparative study of methyl methacrylate miniemulsion polymerization reactions for the formation of biocompatible

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nanocapsules using Miglyol 812 as co-stabilizer and lecithin as surfactant.[8] Steinmacher et al.[10] compared methyl methacrylate miniemulsion polymerizations with vinyl acetate miniemulsion polymerizations for the formation of biocompatible polymeric particles using either Miglyol 812 or castor oil and verified the effect of the co-stabilizer on polymerization kinetics and evolution of molar mass distributions. Guo and Schork[11] verified the potential of replacing the alkyd resin by either linoleic acid or sunflower seed oil in methyl methacrylate/butyl acrylate miniemulsion copolymerizations, in these reactions 0.4 wt.% of hexadecane were also used in the oil phase. And Capeletto et al.[12] studied the effect of the incorporation of alkanes as n-heptane and n-hexane in styrene/methacrylic acid miniemulsion polymerizations, using hexadecane as co-stabilizer.

Jojoba oil is an oil from the seed of the *Simmondsia chinensis* and is a pure liquid wax ester, and not a triacylglycerol as most seed lipids.[13] Its chemical structure, shown in Figure 1a, provides high stability and resistance against oxidation and degradation, enabling its storage for years in closed containers, in contrast to oils that become rancid and decompose with time.[13] Emollient,[14] healing,[15] anti-inflammatory[16] properties justify the growing scientific enthusiasm about jojoba oil.

Andiroba oil, extracted from seeds of *Carapa guianensis*, is basically composed of mixtures of triacylglycerols of oleic (53%), linoleic (8%), palmitic (26%) and stearic acids (9%), Figure 1b.[17] Due to its high concentration of unsaturated fatty acids (about 66%[17]), andiroba oil has an average number of 70 unsaturations in its chemical structure. Anti-inflammatory,[18] insect repellent,[19,20] anti-allergic and analgesic properties give a wide use of andiroba oil for pharmaceutical and cosmetic applications.[21]

The main focus of the present work is to verify the effect of using jojoba and andiroba oils as co-stabilizer on the kinetics of styrene miniemulsion polymerizations, the molar mass distributions of the formed polymer and the morphological characteristics of the nanocapsules.

Materials and Methods

Materials

Styrene (Sty) from Innova S.A., acrylic acid (AA, Águia Química) and methacrylic acid (MAA, BASF) were used as monomers. Sodium lauryl sulfate (SLS) and 2,2′azobisisobutyronitrile (AIBN, 98%), both from Vetec, were used respectively as surfactant and initiator. Hexadecane (Vetec), jojoba oil (Ferquima) and andiroba oil (Beraca) were use as co-stabilizer. To stop the reaction, hydroquinone (Nuclear) was used, and tetrahydrofuran (THF, Merck) was used as solvent in the gel permeation chromatography analyses. All materials were used as received. Distilled water was used in all experiments.

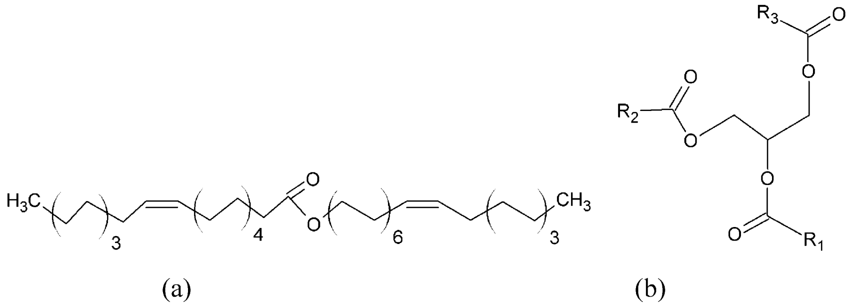


Figure 1.
Chemical structure of jojoba oil (a) and triacylglycerol (b).

Miniemulsion Polymerizations

Initially, organic (Sty, AA or MAA, co-stabilizer and AIBN) and aqueous (water and SLS) phases were prepared. After 20 min of magnetic stirring, the organic phase was added to the aqueous phase and the dispersion was stirred for 30 min prior to sonication (Fisher Scientific, Sonic Dismembrator Model 500) during 4 min with an amplitude of 60%. A cold water bath at 10 °C was used to reduce the temperature increase during the sonication. Batch polymerization reactions were carried out in a thermostatic bath at 70 °C during 6 hours. Samples were removed periodically and reaction was short stopped with the addition of a 1 wt. % hydroquinone solution. All reactions were carried out in triplicate.

Characterization

Styrene conversion was calculated based on gravimetric data. Intensity average diameters of monomer droplets (Dp_I) and of polymer particles (Dp_F) and their polydispersity indexes (PDI) were measured by dynamic light scattering (DLS - Malvern Instruments, Zeta Sizer Nano S). For these measurements, samples were diluted in distilled water saturated with monomer (1 mL latex in 5 mL of water saturated with styrene) in order to prevent monomer diffusion from the droplets to the continuous phase. Final reaction samples were also analyzed by transmission electron microscopy (TEM - JEOL JEM 1011) for the evaluation of the morphology of the particles. For this analysis, several drops of the diluted sample were placed on a 400 mesh copper grid. Several different regions of the TEM grids of at least two reactions for each experimental condition were analyzed to assure representative images. The molar mass distributions were determined by gel permeation chromatography (GPC). GPC analyses were carried out filtering a 0,5 wt% polymer solution (solvent tetrahydrofuran) through a Teflon-filter with a mesh size of 450 nmfollowed by automatic injection of 20 μL of the polymer solution into a High Performance Liquid Chromatograph (HPLC, LC-20A, Shimadzu) equipped with a refractive index detector (RID-10A) in THF at 35 °C. A column set was employed consisting of three 300 × 8 mm columns in series (GPC-801, GPC-804 and GPC-807). Average molar masses and molar mass distributions were calculated based on polystyrene (PS) standards in the range between 580 g.mol^{-1} and 3,800,000 g.mol^{-1}. Thermal characterization of the samples (loaded nanoparticles) was undertaken by differential scanning calorimetry (DSC) in a Perkin-Elmer (Jade DSC) calorimeter. About 7 mg of each sample was placed in an aluminum pan. The capsules were sealed and the materials were analyzed. All the scans were carried out under nitrogen stream (20 mL.min^{-1}), with zinc and indium as reference. The analysis was run as follows: heating ramp from 0 °C to 200 °C, cooling from 200 °C to 0 °C, and another heating ramp from 0 to 200 °C. The heating and cooling rates were constant (20 °C min^{-1}). The data obtained from the second heating ramp were used to construct the thermal curves and evaluate the results.

Results and Discussion

Effect of Comonomer Type

In miniemulsion polymerization, the morphology of the nanocapsules can be controlled by adding a comonomer to dispersed phase. Since the interfacial tension between PS and water is very high ($\gamma = 32$ mN/m),[22] the use of a hydrophilic comonomer for nanocapsule preparation by miniemulsion polymerization is recommended. The carboxylic acids commonly used as hydrophilic monomer or comonomer are acrylic, methacrylic, fumaric, maleic and itaconic acids.[23] In this work, acrylic (AA) and methacrylic (MAA) acids were used to verify their effect on latexes prepared by miniemulsion polymerization. The formulations used in these reactions are shown in Table 1. For comparison, besides reactions with jojoba oil as co-stabilizer (and encapsulated oil), reactions were also conducted with

Table 1.
Formulations of styrene miniemulsion polymerizations with different comonomers and co-stabilizers. The amounts of water (30 g), surfactant (0.5 wt% of organic phase) and organic phase (6.07 g) were kept the same in all reactions.

Reactants(%)	Reactions			
	AA-HD	MAA-HD	AA-J	MAA-J
Styrene	46.95	46.95	46.95	46.95
Acrylic Acid (AA)	2.47	–	2.47	–
Methacrylic Acid (MAA)	–	2.47	–	2.47
Hexadecane (HD)	49.42	49.42	–	–
Jojoba oil (J)	–	–	49.42	49.42
AIBN	1.16	1.16	1.16	1.16

hexadecane as co-stabilizer, since hexadecane is the most commonly used co-stabilizer in miniemulsion polymerizations.

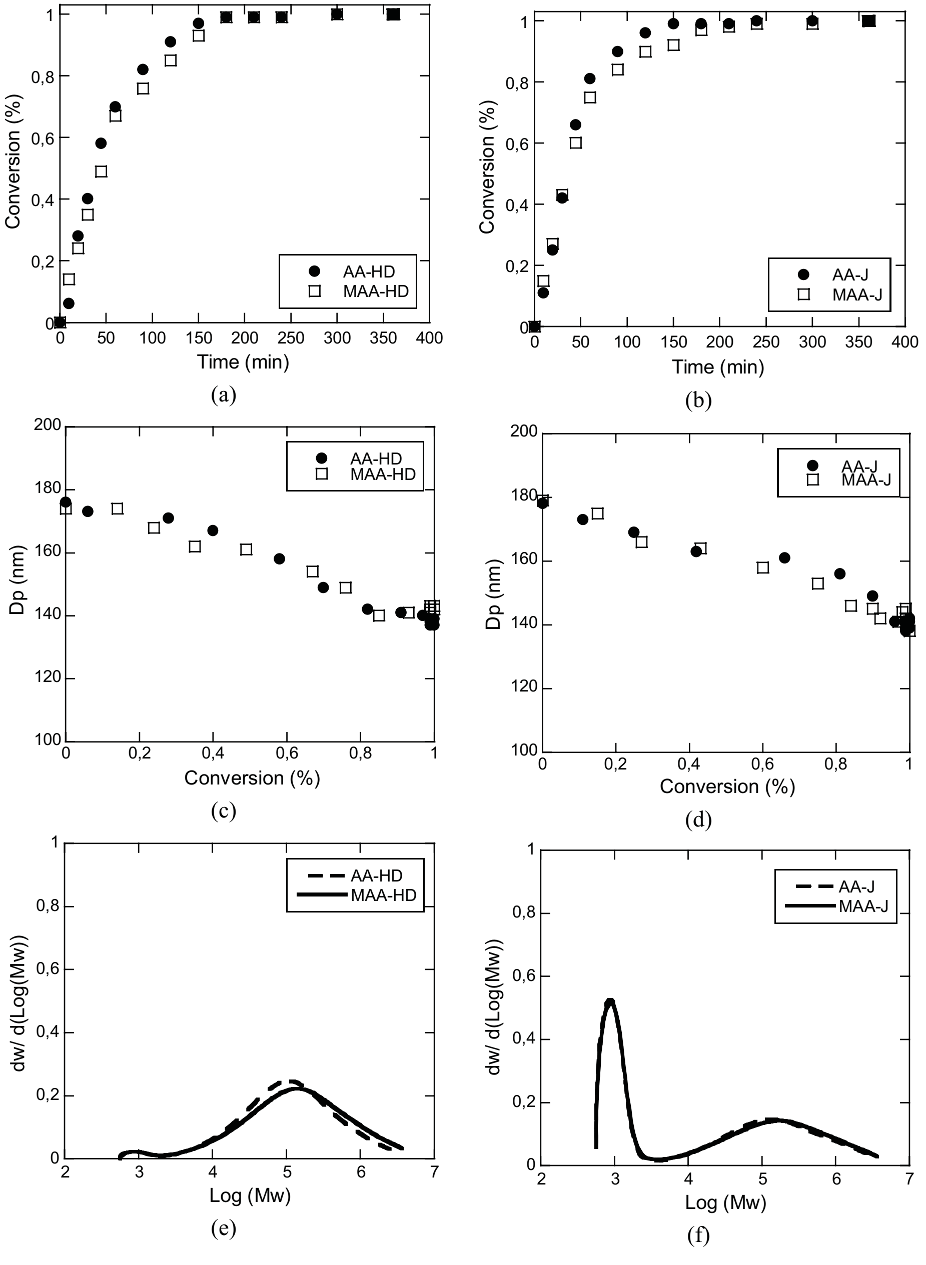
According to results shown in Figure 2 and Table 2, the type of hydrophilic comonomer, acrylic or methacrylic acid, did not affect the evolution of conversion and average particle diameters. However, a small reduction in the average particle diameter (above that expected due to the volumetric contraction by the difference in density between the monomer and polymer) was observed during all reactions. This behavior can possibly be explained by the co-existence of droplets of different sub-micrometric sizes immediately after sonication, responsible for the higher initial intensity average diameter of nanodroplets (this average value attributes a higher weight to larger droplets) resulting in higher initial polidyspersity indexes. Due to radical compartmentalization, reaction rate is higher in the smaller droplets/particles resulting in a monomer concentration gradient that leads to monomer mass transfer from larger to smaller particles.[12]

Molar mass distributions of reactions with acrylic acid were shifted towards slightly lower molar masses when compared to reactions with methacrylic acid. This effect may also be observed in the molar mass averages (weight average, Mw, and number average, Mn), shown in Table 2.

When comparing jojoba oil (J) with hexadecane (HD) as co-stabilizer no major effect may be observed on conversion, average particle diameter and molar mass distributions. The narrow molar mass peak observed in Figure 2f corresponds to the encapsulated oil (jojoba oil). Hexadecane, on the other hand, does not appear on the distribution (Figure 2e) since its molar mass is below that of the standards used in the calibration curve. In addition, hexadecane is evaporated during sample preparation.

Figure 3 shows TEM images of particles produced during styrene miniemulsion polymerizations. It may be observed that PS nanocapsules with cores of hexadecane or jojoba oil were successfully formed using both of the evaluated hydrophilic comonomers, acrylic or methacrylic acid, and those nanocapsules coexist with smaller particles that do not show the nanocapsule morphology. As mentioned in the discussion about the average particle size decrease during reactions (Figure 2), the presence of these smaller particles is attributed to the initial coexistence of droplets with different sub-micrometric sizes.

The higher reaction rate in the smaller droplets/particles results in monomer concentration gradient among particles and thus monomer is transferred from larger to smaller particles. In this way, the monomer-polymer/co-stabilizer ratio decreases in larger particles (leading to the formation of large capsules with relatively thin shells) while it increases in the smaller ones (leading to the formation of smaller particles, inside which no phase separation is observed due to the lower co-stabilizer concentration).



(a) (b) (c) (d) (e) (f)

Figure 2.

Effect of comonomer type (acrylic or methacrylic acid) on styrene miniemulsion polymerizations with hexadecane (HD) (a, c and e) and jojoba oil (J) (b, d and f) as co-stabilizer. (a and b) Conversion; (c and d) Average particle diameter; (e and f) Final molar mass distribution.

Effect of Co-Stabilizer Type

In reactions HD, J and AND, the effect of using three different types of co-stabilizers, hexadecane, jojoba and andiroba oils was evaluated using acrylic acid as hydrophilic comonomer. Hexadecane was chosen for comparison between vegetable oils because it is widely used as co-stabilizer

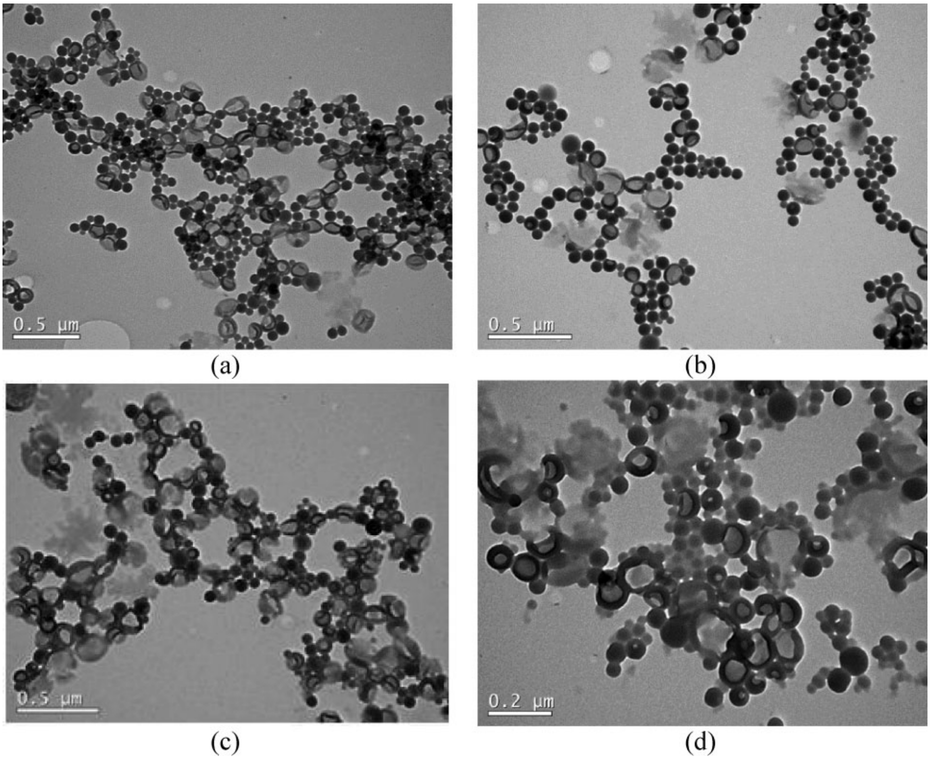
Table 2.
Conversion weight average molar mass (Mw), number average molar mass (Mn), polydispersity index (PI), average droplets (Dp_I) and particles (Dp_F) diameter and initial (PDI_I) and final (PDI_F) polydispersity index of styrene miniemulsion polymerizations with different comonomers and co-stabilizers.

Reactions*	Conversion (%)	Mw x10^3 (g/mol)	Mn x10^3 (g/mol)	PI	Dp_I (nm)	Dp_F (nm)	PDI_I	PDI_F
AA-HD	99.6	343	53.0	6.5	176	139	0.19	0.17
MAA-HD	99.3	447	63.5	7.0	174	142	0.19	0.16
AA-J	99.0	377	63.5	6.0	178	141	0.19	0.15
MAA-J	99.4	433	59.0	7.5	178	141	0.20	0.16

*Conversion and Dp results are average values of triplicate reactions. The maximum standard deviations were respectively ± 0,2% and ± 2 nm for conversion and Dp.

in miniemulsion polymerizations. Table 3 shows the formulations of the reactions.

It can be observed (Fig. 4 and Table 4) that the use of different co-stabilizers did not affect the initial (droplets) and final (particles) average diameter of the miniemulsions. However, the use of andiroba oil resulted in a small decrease in polymerization rate. This reduction is attributed to reactions of the growing polymer chains with the unsaturations in the fatty acid chains of the andiroba oil, since this oil is a mixture of triacylglycerolswith 66% of unsaturated fatty acids.[17] In contrast, hexadecane is an alkane with saturated chain and jojoba oil consists of esters



(a) (b) (c) (d)

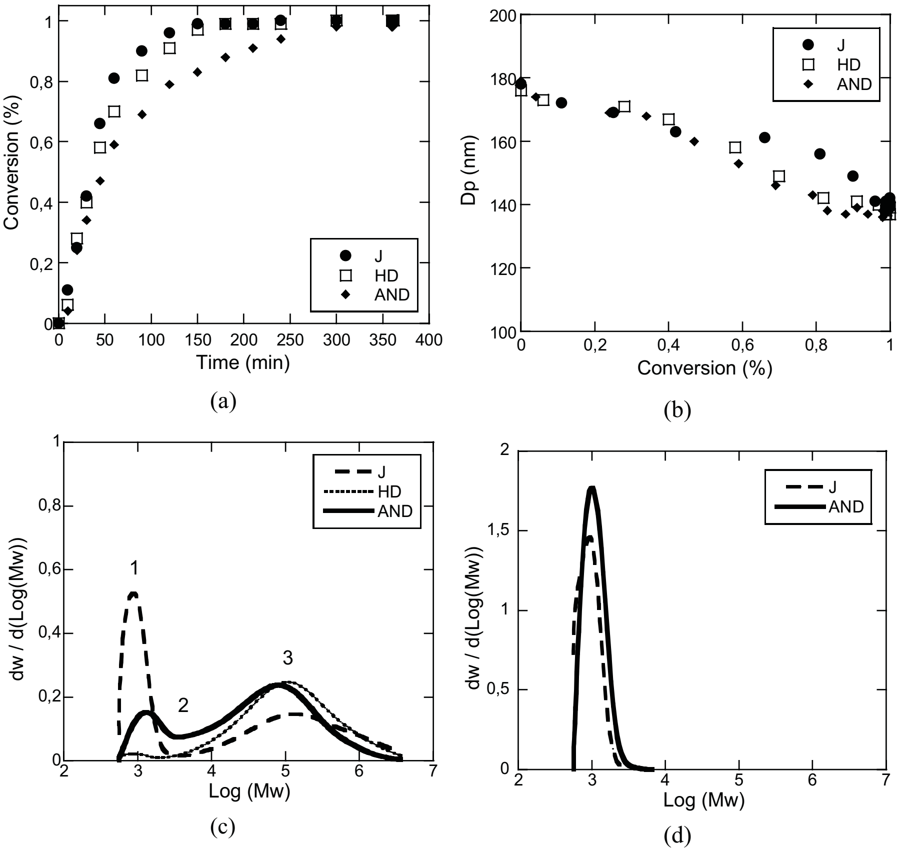
Figure 3.
TEM images of PS nanoparticles with hexadecane as co-stabilizer and acrylic acid (a) or methacrylic acid (b) as hydrophilic comonomer and with jojoba oil as co-stabilizer and acrylic acid (c) or methacrylic acid (d).

Table 3.
Formulations of styrene miniemulsion polymerizations with different co-stabilizers. The amounts of water (30 g), surfactant (0.5 wt% of organic phase) and organic phase (6.07 g) were kept the same in all reactions.

Reactants (wt%)	Reactions		
	J	HD	AND
Styrene	46.95	46.95	46.95
Acrylic Acid	2.47	2.47	2.47
Andiroba oil (AND)	–	–	49.42
Hexadecane (HD)	–	49.42	–
Jojoba oil (J)	49.42	–	–
AIBN	1.16	1.16	1.16

derived from monounsaturated fatty acids and alcohols. According to Guo and Shork,[11] the higher the concentration of double bonds in the co-stabilizer applied, the greater the probability of reaction between the polymer and the unsaturated radicals, resulting in more stable radicals, with lower propagation constant, retarding the polymerization.

The molar mass results (Table 4) corroborate the above statements. As expected, when polymeric radicals react with the double bonds of the co-stabilizer (andiroba oil) forming radicals of low reactivity, the molar mass of the polymer formed decreases. The molar mass distribution (MMD) of the latexes, Figure 4c, shows two peaks. The first peak, with Mw around 1,000 g/mol, corresponds to jojoba and andiroba oils (as can be seen in Figure 4d which shows the MMD of pure oils) and the higher molar mass peak, Mw >190,000 g/mol, corresponds to



(a)

(b)

(c)

(d)

Figure 4.
Effect of co-stabilizer type (jojoba oil, J, hexadecane, HD or andiroba oil, AND) on styrene miniemulsion polymerizations with acrylic acid as hydrophilic comonomer. (a) Conversion; (b) Average particle diameter; (c) Final molar mass distributions; (d) Molar mass distributions of jojoba and andiroba oils.

Table 4.
Conversion weight average molar mass (Mw), number average molar mass (Mn), polydispersity index (PI), average droplets (Dp_I) and particles (Dp_F) diameter and initial (PDI_I) and final (PDI_F) polydispersity index of styrene miniemulsion polymerizations with different types of costabilizers.

Reactions	Conversion (%)	Mn x10^3 (g/mol)	Mw x10^3 (g/mol)	PI	Dp_I (nm)	Dp_F (nm)	PDI_I	PDI_F
J	99.0	63.4	377	6.0	178	141	0.19	0.15
HD	99.6	53.0	344	6.5	176	138	0.19	0.17
AND	98.3	37.6	194	5.2	178	136	0.19	0.18

*Conversion and Dp results are average values of triplicate reactions. The maximum standard deviations were respectively ± 0,4% and ± 2 nm for conversion and Dp.

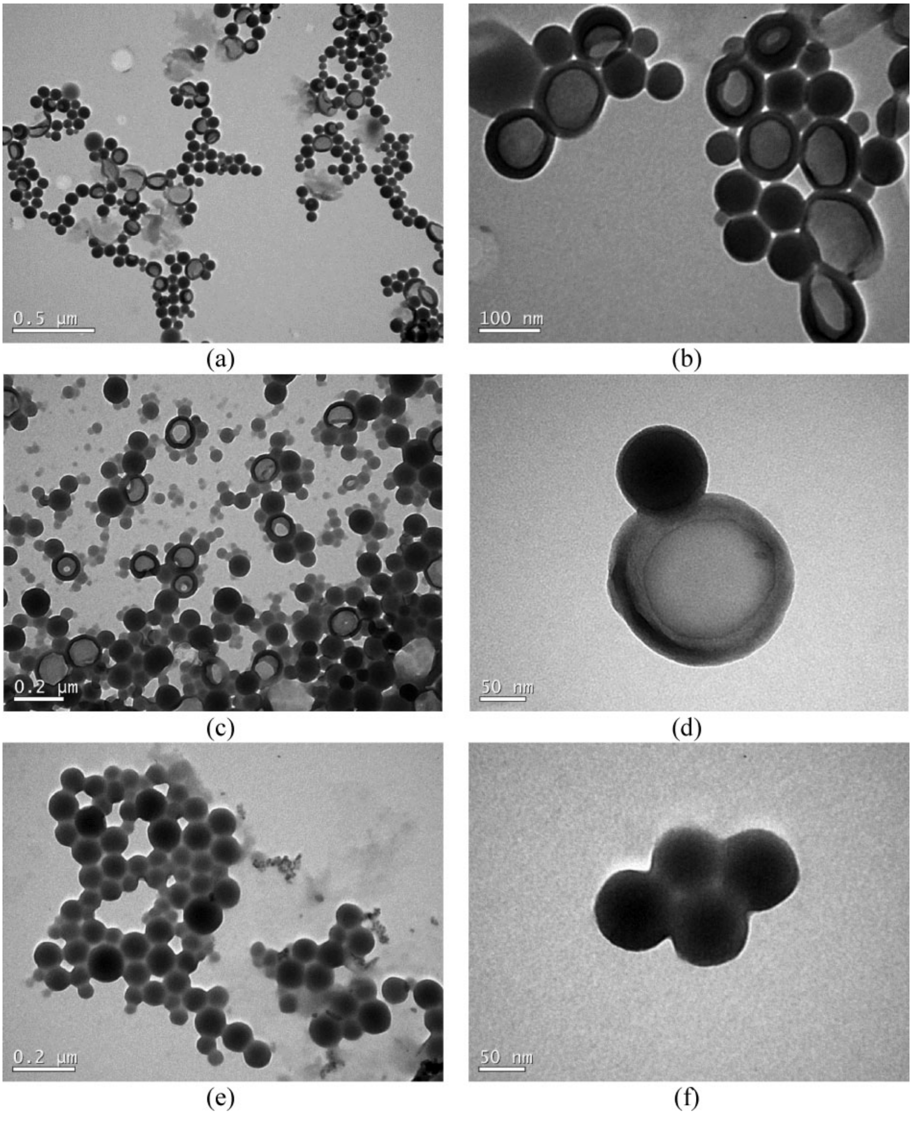


Figure 5.
TEM images of PS nanoparticles with hexadecane (a, b), jojoba oil (c, d) and andiroba oil (e, f).

PS formed during the polymerization. The shoulder formed between both peaks (at around Mw 14,000 g/mol) was observed only in the latex with andiroba oil, indicating that a group of chains with intermediate molar mass was generated from the reactions between the growing polymer chains and the double bonds of the oil.

Figure 5 presents TEM images of particles produced with the different types of co-stabilizers. While particles with hexadecane and jojoba oil exhibit a core-shell morphology with the oil in the core, no phase separation can be observed in particles with andiroba oil.

TEM images of the three reactions with andiroba oil showed the same type of morphology. In the same way the shoulder of intermediate molar masses was observed in the molar mass distributions of the three reactions. The lack of phase separation is attributed to the in-situ formation of a compatibilization agent due to reactions between the growing polymer chains and the double bonds of the andiroba oil.

In order to investigate further the structure of the PS nanoparticles with jojoba oil and with andiroba oil thermal characterization of the samples was performed by differential scanning calorimetry, as shown in Figure 6. For comparison, a PS sample was also analyzed. Thermograms (b) and (c) exhibit an endothermic peak at 14 °C and 7 °C, corresponding, respectively, to the melting points of jojoba and andiroba oils. The glass transition temperatures (Tg) of the polymers can be observed at 114 °C for PS (a), 71 °C for PS nanocapsules with jojoba oil (b) and 52 °C for PS nanoparticles with andiroba oil (c). These data indicate that these vegetable oils act as plasticizers for PS, since they have considerably reduced the Tg of the polymer.

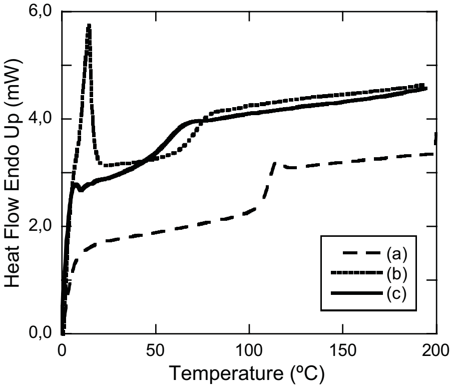


Figure 6.
DSC thermograms (second heating) of PS (a) and PS nanoparticles with jojoba oil (b) and andiroba oil (c).

Using the Fox equation[24] to estimate the Tg of the nanoparticles, assuming that PS forms a single phase with the oil, resulted in 57 °C for PS nanocapsules with jojoba oil and 52 °C for PS nanoparticles with andiroba oil. For PS nanoparticles with andiroba oil the result was the same as the experimental Tg indicating that no phase separation occurred inside these nanoparticles, corroborating TEM results. In the case of PS nanocapsules with jojoba oil the experimental Tg (71 °C) was higher than the theoretical one (57 °C) indicating that only part of the jojoba oil was miscible with PS, whereas another part has phase separated as also observed in TEM images.

Conclusion

Polystyrene nanocapsules of either hexadecane or jojoba oil were successfully formed by miniemulsion polymerization. When andiroba oil was used no phase separation could be detected in polymer particles according to TEM images and DSC analyses. The absence of phase separation was attributed to the in-situ formation of a compatibilization agent due to reactions between the growing polymer chains and the double bonds of the andiroba oil since molar mass distributions of samples with this oil showed a shoulder of intermediate molar masses between those of the oil and of the pure polymer.

Acknowledgements: The authors thank the financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and TEM analyses from Laboratório Central de

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