

Research Article

Synthesis of Lignin Nanoparticles and Their Application in the Stabilization of Water-in-Water Pickering Emulsions: A New Technology for Valorization of Lignin from Sugarcane Bagasse

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Water-in-water (w/w) emulsions can mimic biological environments, and their stability is ensured by adding nanoparticles capable of adsorbing at liquid-liquid interfaces. To enhance the properties of w/w emulsions, there is a search for new sources of nanoparticles that are attractive for the food and biomedical fields. Thus, the present study investigated the use of sugarcane bagasse lignin (a cheap, nontoxic, and biodegradable polymer) as a source of nanoparticles for Pickering emulsions with maltodextrin (MD) and polyethylene glycol 6000 (PEG 6000). The nanoparticles were prepared from alkaline lignin (ALNP) and oxidized alkaline lignin (OLNP), and their application was performed using different dosages in the w/w systems (0%, 0.1%, 0.3%, 0.5%, and 1%, wt/wt). The nanoparticles presented different sizes, with OLNPs (327.8 nm) being smaller than ALNPs (689.8 nm). The systems with OLNPs showed better emulsification indices and smaller droplet sizes than systems with ALNPs. The concentration of nanoparticles and the volume of the dispersed phase influence the stability of the studied emulsion. The most promising stabilization results were obtained at a concentration of 1% wt/wt of OLNPs with an emulsification index of up to 63%. These results, combined with the extensive availability of functional groups in lignin, make this polymer a potential candidate for advanced studies of w/w emulsions.

1. Introduction

In order to develop innovative processes under sustainability criteria, processes with emphasis in the conversion of waste into useful human products have been investigated [1]. In this context, the agroindustrial waste accumulates 2 billion tons per year, which poses a risk to the environment [2, 3]. Among these wastes, sugarcane bagasse represents the largest amount (1,044.8 million tons), being a byproduct of the sugar and ethanol industry (1st generation) [4].

Sugarcane bagasse is obtained through the milling process that extracts the sugarcane juice and represents approximately 50% of the initial amount of sugarcane. Most of the bagasse is burned and used in the production of thermal and electric energy [5]. In Brazil, it is estimated that between 5 and 12 million tons of bagasse are generated annually.

Sugarcane bagasse is a lignocellulosic material, consisting mainly of cellulose (35%–45%), hemicellulose (26%– 35%), and lignin (11%–25%) [6]. The interaction of these components, represented especially by the complex structure of lignin and polysaccharides, makes lignocellulosic biomass resistant to biochemical and biological decomposition [7]. With the pretreatment, the black liquor rich in lignin is removed as a residue. Pretreatment can be carried out to remove lignin because it hinders access to cellulose, compromising the biomass hydrolysis. Recently, researchers have sought to add value to lignin and explore its potential in technological applications, such as cosmetic ingredient [8], bioactive compound source [9], and production of biofertilizers [10]. Some groups have investigated the use of lignin as an emulsion stabilizer, which is a field that encompasses a large portion of the chemical industry. Research has found that not only lignin-based surfactants but also lignin nanoparticles (LNPs) perform well as emulsion stabilizers and have already been used as potential candidates for more environment-friendly stabilizers [11, 12].

Emulsions are mixtures of two immiscible liquids in which a dispersed phase (small droplets) forms in the other phase (continuous phase). These mixtures are thermodynamically unstable and phase separation is favored. When solid particles are used to maintain emulsions stable, or at least to delay phase separation, these emulsions are called Pickering emulsions in honor of the precursor of this stabilization mechanism [13-16]. In this stabilization technique, the particles form a physical barrier around the droplets of the dispersed phase, preventing coalescence and enabling the stabilization of the emulsions [17]. This stabilization mechanism was first used in conventional emulsions, that is, emulsions formed by an aqueous phase and another oily phase, which can be oil/water (o/w) or water/oil (w/o). Some studies have shown that solid particles have also shown a tendency to accumulate at the liquidliquid interface of aqueous two-phase systems, forming what is being called water-in-water (w/w) Pickering emulsions [18-20].

The stabilization of w/w emulsions is an area that has recently attracted the interest of researchers and can trigger technological innovations, especially in the food industry [21-25], pharmaceutical [26], and biomedicine [27]. The formulation of functional foods with a low fat content from w/w emulsions can mean new applications in the food industry [28]. The w/w emulsions can also provide innovations in drug encapsulation, as well as in the controlled delivery of these bioactives, which could revolutionize disease treatments, such as some types of cancer [27, 29]. In order to enable such applications, it is necessary to stabilize these emulsions. Unlike o/w emulsions, w/w emulsions cannot be stabilized by molecular surfactants since they have a very thick interface (in the order of several nanometers) and low interfacial tension $(1 \mu N/m \text{ to } 1000 \mu N/m)$ [30]. In this context, some researchers have suggested several nanoparticles capable of promoting satisfactory stabilization of these emulsions. Investigations include protein nanoparticles [31-34], cellulose [35-37], latex [17, 31], and starch [38], among others. However, to the best of our knowledge, the literature does not report the use of LNPs in stabilizing w/w emulsions. The use of LNPs in these emulsions can offer

advantageous technological applications in the food, cosmetics, and biomedicine fields.

In this context, the present study evaluated the recovery of lignin from alkali pretreated sugarcane bagasse to produce nanoparticles. The nanoparticles were applied in the stabilization of w/w emulsions formed by the mixture of maltodextrin (MD) and polyethylene glycol 6000 (PEG 6000). Part of the lignin was oxidized to evaluate if the oxidation reaction improves the stabilization performance. Alkaline lignin nanoparticles (ALNPs) and oxidized alkaline lignin nanoparticles (OLNPs) were prepared and applied at different concentrations in the w/w emulsion stabilization. In addition, to nanoparticle concentration, the effect of phaseforming composition and temperature on stability was also investigated. The emulsions were monitored, and photos were taken to evaluate stability.

2. Materials and Methods

2.1. Reagents. Sodium hydroxide, sulfuric acid, hydrogen peroxide, ethylene glycol, and PEG 6000 were obtained from Synth (São Paulo, Brazil). The commercial MD was purchased at a local market (Natal, Brazil). The other reagents were obtained in an analytical grade.

2.2. Recovery of Lignin from Sugarcane Bagasse Using Alkaline Pretreatment. The sugarcane bagasse was provided by the company Miriri Food and Bioenergy S/A located in Santa Rita (Paraíba, Brazil). The material was naturally dried by exposure to the sun for 5 days. After drying, the bagasse was ground in a knife mill (model TE-680, TECNAL, Brazil) to a size of 20 mesh and stored in plastic bags.

The lignin was extracted through the alkaline pretreatment of sugarcane bagasse using sodium hydroxide (2% w/v) as a catalyst. In 500 mL Erlenmeyer flasks, 20 g of sugarcane bagasse (on a dry basis) was added together with 200 mL of 2% (wt/v) sodium hydroxide. The mixture was taken to the autoclave and kept at 121°C for 60 min. After this step, the lignin-rich liquor was recovered by cloth filtration and the pretreated bagasse was washed until neutral pH was obtained. The liquor went to the centrifuge (model SL-700, Solab, Brazil), and it was centrifuged for 3 min at $1,500 \times g$ and $25^{\circ}C$ to remove any particles that passed through the cloth. The supernatant was acidified to pH 2.0 using 5 M sulfuric acid, and the lignin was recovered via centrifugation. Finally, the lignin was washed twice with acidified water (pH 2.0) and dried in a circulating oven at 60°C for 12 h.

2.3. Lignin Oxidation. Based on the protocol of He et al. [39], the oxidation was initiated by dissolving 15 g of alkaline lignin in 100 mL of 2% (w/v) sodium hydroxide. The mixture was placed in a 500 mL three-neck flask equipped with a stirrer, condenser, and a thermometer. The system was heated to 80° C, and the dripping of 50 mL of 10% (v/v) hydrogen peroxide was started. The oxidation reaction was maintained at 80° C for 2 h. The temperature of the thermostat bath was kept at 15°C to ensure the condensation of

most of the formed vapors. After the reaction, the oxidized lignin was washed with acidified water (pH 2.0 with sulfuric acid) and dried in a circulating oven at 60°C for 12 h. Figure 1(a) illustrates the experimental setup employed in the oxidation of lignin.

2.4. Preparation of Lignin Nanoparticles. LNPs were prepared from alkaline lignin and oxidized alkaline lignin via the antisolvent precipitation method, according to Padilha et al. [12]. A quantity of lignin (1 g) was solubilized in 100 mL of ethylene glycol under magnetic stirring for 24 h. After this step, the mixture was vacuum filtered to remove possible impurities and the lignin-rich solution was placed in a 1.5 L beaker. Then, 1 L of acidified water (with sulfuric acid) at pH 2.0 was slowly dripped into the lignin solution using a peristaltic pump, leading to the formation of nanoparticles, as shown in Figure 1(b). The nanoparticles were washed and finally resuspended in acidified water (containing 0.01% (wt/ v) sodium azide and at pH 2.0) for use in Pickering emulsion tests. The concentration of the nanoparticle suspensions was determined by gravimetry.

2.5. Characterization of Raw Lignin and LNPs. The chemical compositions of lignins were determined based on the Klason lignin content, polysaccharide residues (cellulose and hemicellulose), and ash content [40]. Samples of these lignins were also subjected to thermal degradation (TGA), Fourier-transform infrared spectroscopy (FTIR) analysis, and evaluation of hydrodynamic size by zeta potential.

2.5.1. Thermogravimetric Analysis. Thermogravimetric analyses were carried out using the DTG-60 equipment (Shimadzu, Japan) in the temperature range of $25-600^{\circ}$ C, with 10 mg of initial sample, at a heating rate of 10° C/min and under an inert atmosphere.

2.5.2. FTIR Analysis. The FTIR spectrum of the lignin extracted from the sugarcane bagasse was measured using an FTLA 2000 spectrometer (ABB Bomem Inc., Canada) operating in the range of $4000-400 \text{ cm}^{-1}$, with a resolution of 4 cm^{-1} .

2.5.3. Analysis by Zeta Potential. The hydrodynamic size and zeta potential of the LNPs were measured using the 90Plus/BI-MAS ZetaPlus instrument (Brookhaven Inst. Co., USA) after sonication.

2.6. Obtaining the Binodal Curve and Tie Lines. Stock solutions of commercial MD (30% and 50% wt/wt) and PEG 6000 (50% wt/wt) were prepared. The phase composition of the aqueous system (MD/PEG 6000) was estimated based on the resolution of a system of equations for the calculation of the tie-line length (TLL), as described by Merchuk et al. [41]. The binodal curve of the system was constructed based on the cloud point titration method [42, 43]. In the experiment, 2 g of the PEG 6000 solution was placed in a 15 mL test tube,

3

and the MD solution was added dropwise until the solution became turbid and the mass of the added MD solution was measured. Then, 0.2 g of water was added to the solution and it returned to its initial (monophasic) condition, and the process was repeated until enough points were obtained to plot the binodal curve. Afterwards, the mass percentages of MD (X) and PEG 6000 (Y) were correlated to the binodal curves using the mathematical expression described in equation (1). The resolution of equations (2)–(6) allows for the determination of the mass percentage of MD in the upper phase (X_T) and the lower phase (X_B), as well as the mass percentage of PEG 6000 in the upper phase (Y_T) and the lower phase (Y_B). The TLL values in equation (6) are determined based on the MD and PEG 6000 compositions in the upper and lower phases.

$$Y = A.e^{B.X^{0.5} - C.X^3},$$
 (1)

$$Y_T = A.e^{B.X_T^{0.5} - C.X_T^3},$$
 (2)

$$Y_{B} = A.e^{B.X_{B}^{0.5} - C.X_{B}^{3}},$$
(3)

$$Y_T = \left(\frac{Y_M}{\alpha}\right) - \left[\frac{(1-\alpha)}{\alpha}\right] Y_B,\tag{4}$$

$$X_T = \left(\frac{X_M}{\alpha}\right) - \left[\frac{(1-\alpha)}{\alpha}\right] X_B,$$
(5)

TLL =
$$\sqrt{(X_T - X_B)^2 + (Y_T - Y_B)^2}$$
. (6)

In equations (1)–(6), the subscripts M, T, and B represent the initial mixture, upper phase, and lower phase, respectively. The term α is the ratio of the mass of the upper phase to the total mass of the system. The values of X_M and Y_M correspond to 20%/30% and 10%, respectively. A, B, and C are the parameters of the Merchuk equation. The Merchuk parameters were obtained through nonlinear estimation using Statistica 7.0 software (StatSoft, USA). The estimated values were 60.67% ± 2.90% (wt/wt) for A, $-0.75\% \pm 0.04\%$ (wt/wt) for B, and $10^{-5}\%$ (wt/wt) for C.

The top and bottom phases were also characterized by pH and density. The pH values were obtained using a pH meter 210B (Cap-Lab, Brazil), while the density values were obtained using a densimeter DDM 2910 (Rudolph Research, USA).

2.7. Preparation of w/w Emulsions. After determining the binodal curve, it was possible to prepare the emulsions. The emulsions were formed by mixing the aqueous solutions with 10% (wt/wt) PEG 6000 and 20% and 30% (wt/wt) MD, and LNPs concentrations of 0.1%, 0.3%, 0.5%, and 1% (wt/wt).

2.8. Evaluation of the Stability of Emulsions. The stability of the prepared emulsions was studied for two weeks. For this purpose, they were periodically monitored by photos of the



FIGURE 1: Experimental setup used in the oxidation of lignin (a) and preparation of LNPs (b).

test tubes and images were obtained with a BX51 optical microscope (OLYMPUS, Japan). In addition, the emulsification index (EI) was determined using the mathematical expression of equation (7) described by Cooper and Goldenberg [44]. The effect of the system composition was evaluated by preparing emulsions with different MD/PEG 6000 compositions (5%/14.16%, 12%/12.22%, 20%/10%, 35%/5.84%, and 45%/3.07%; wt/wt). In order to study the thermal stability of the emulsions, a system (20% MD, 10% PEG 6000, and 1% LNPs; wt/wt) was kept at 4, 35, and 40°C for two weeks using ALNPs and OLNPs.

$$EI (\%) = \frac{\text{Height of the emulsified layer}}{\text{Height of the total liquid column}} \times 100.$$
(7)

3. Results and Discussion

3.1. Characterization of the Phases. The binodal curve of the MD/PEG 6000 aqueous two-phase system was experimentally obtained at room temperature, as shown in Figure 2. The results were expressed in mass percentage. The twophase region covers a large region of the binodal curve, and the experimental data highlight the need for higher MD concentrations for the formation of two phases. Similar behavior was reported by Da Silva and Meirelles [45]. They used different types of PEG (1450, 8000, and 10000) in



FIGURE 2: Binodal curve of the system composed of MD and PEG 6000 in terms of mass percentage at $T = 25 \pm 3^{\circ}$ C. Experimental data obtained by the cloud point method; — Merchuk equation; — tie line: 20% MD/10% PEG 6000; — tie line: 30% MD/10% PEG 6000; \blacktriangle experiment conducted with different compositions of the emulsion in the same tie line.

aqueous two-phase systems with MD. Table 1 presents the characterization of the phases of the MD/PEG 6000 system with two initial mixtures of 20% (wt/wt) MD/10% (wt/wt)

Systems	Phase	%PEG 6000 (wt/wt)*	%MD (wt/wt)*	%Water (wt/wt)*	%TLL (wt/wt)*	pН	$\Delta \rho$ (g/mL)
20% MD/10% PEG 6000	Тор	14.55	3.59	81.86	49.10	2.00	1.07
	Bottom	1.47	50.91	47.66		1.99	1.17
30% MD/10% PEG 6000	Тор	17.81	2.64	79.55	57.15	2.10	1.09
	Bottom	2.11	57.60	40.29		2.00	1.21

TABLE 1: Characterization of the studied systems and estimation of the phase composition based on the Merchuk equation [41] at $T = 25 \pm 3^{\circ}$ C.

*Estimation of the composition of the top and bottom phases based on the Merchuk equation [41].

PEG 6000 and 30% (wt/wt) MD/10% (wt/wt) PEG 6000. The MD/PEG 6000 system generated an upper phase rich in PEG 6000 and a lower phase rich in MD. A tie line was determined for each chosen system.

The compositions of the phases of the studied systems (Table 1) were estimated based on the Merchuk equation [46, 47]. The system with 20% MD and 10% PEG 6000 generated a top phase with 81.86% water, followed by PEG 6000 as the second most relevant component in this phase, representing 14.55%. The second system (30% MD and 10% PEG 6000) also showed a large amount of water in the top phase (79.55%). This higher amount of water in the top phase is due to the presence of PEG in higher concentration in this phase. This behavior occurs because PEG is more hygroscopic. In both systems, the density of the MD-rich phase was higher than that of the PEG-rich phase and, therefore, it occupied the lower part of the test tube.

3.2. Characterization of Alkaline Lignin and Oxidized Alkaline Lignin

3.2.1. Chemical Composition of Lignin. The chemical composition of the alkaline lignin was $69.84 \pm 10.5\%$ (wt/wt) of Klason lignin, $8.17 \pm 0.66\%$ (wt/wt) of polysaccharide residues, and $8.08 \pm 0.11\%$ (wt/wt) of ash. Padilha et al. [12] used alkaline pretreatment on corn cob and green coconut fiber obtaining a Klason lignin content of $83.1 \pm 2.3\%$ (wt/wt) and $90 \pm 1.2\%$ (wt/wt), respectively. The oxidized alkaline lignin presented $72.24 \pm 8.57\%$ (w/w) of Klason lignin, $11.93 \pm 0.69\%$ (w/w) of polysaccharide residues, and $10.34 \pm 0.12\%$ (wt/wt) ash. After undergoing alkaline extraction and oxidation, there is still a significant concentration of polysaccharides present. Mancera et al. [48] also reported that xylans remained bound to oxidized lignins after sugarcane bagasse isolation.

3.2.2. Spectroscopy FTIR. The FTIR spectra of the alkaline lignin and oxidized alkaline lignin samples are shown in Figure 3. The obtained spectra show characteristic bands of lignin. In the region between 3435 and 3415 cm^{-1} , the spectral profile is characterized by the formation of broad bands, which are related to the stretching or deformation of hydrogen bonds (O-H). The band between 2980 and 2900 cm⁻¹ is related to the stretching of C-H bonds of al-iphatic groups [49]. These groups are present in greater quantities in lignin samples extracted from sugarcane bagasse, as reported by Singh et al. [50]. The region between 1696 and 1705 cm⁻¹ is common in lignin samples and



FIGURE 3: FTIR of alkaline lignin and oxidized alkaline lignin.

corresponds to the stretching of the C=O bond in unconjugated ketones, ester groups, aldehydes, and carboxylic acids. The vibrations of the aromatic skeleton occur in bands near 1600 and 1510 cm^{-1} [50]. The band near 1370 cm^{-1} corresponds to stretching of phenolic O-H bonds [51].

Analyzing the spectra, it is noticed that the peak of the 1600 cm^{-1} band has lower intensity for the oxidized alkaline lignin (red line) than for the alkaline lignin (black line), indicating that the oxidation process was effective, that is, resulted in the breaking of aromatic rings, indicating that at least one substituent is removed from the aromatic ring of alkali lignin [48]. This result is consistent with that of the study by He et al. [39]. The breakage of these aromatic rings makes the lignin structure more hydrophilic due to the increased presence of carboxylic groups. The peak of the band at 1230 cm^{-1} , corresponding to the angular deformations of the hydroxyl (O-H) bonds, is slightly higher for the oxidized alkaline lignin (red line) than for the alkaline lignin (black line), indicating an increase in the number of carboxylic groups after oxidation.

3.2.3. Thermogravimetric Analysis. Figure4 shows the result of the thermogravimetric analysis of both lignin samples. The alkaline lignin and oxidized alkaline lignin samples present relevant differences. The alkaline lignin (Figure 5(a)) presents three regions of thermal decomposition, with the



FIGURE 4: Hydrodynamic size distribution of alkaline lignin nanoparticles (ALNPs) and oxidized alkaline lignin nanoparticles (OLNPs) measured by DLS.



FIGURE 5: TGA and DTG of alkaline lignin (a) and oxidized alkaline lignin (b).

smallest region between 130 and 180°C (4%) and the two larger decompositions occurring between 180 and 300°C (19%) and 300 and 575°C (31%). In the region between 180 and 300°C, the alkaline lignin underwent several

transformations, such as the breaking of the C-C bonds in the aliphatic chain of phenylpropane units, breaking of C-O-C bonds, and breaking of simple C-C bonds [52]. In the last thermal decomposition region of this lignin, the peak of the decomposition rate was obtained at 360°C; a similar result was reported by Naron et al. for lignin obtained from different sources [53]. Above 400°C, decomposition still occurs, probably from the aromatic rings, as reported by García-Pérez et al. [54]. Different behavior can be observed for the oxidized alkaline lignin (see Figure 5(b)), which presents a significant thermal decomposition region between 270 and 400°C (27%) and two less relevant regions between 225 and 270 (8%) and 400 and 525°C (9%). The thermal decomposition of the region between 270 and 400°C may be related to the cleavage of bonds of carboxylic and hydroxyl groups, a result of the oxidation process, also reported by He et al. [39]. At temperatures above 400°C, there was a reduction in thermal decomposition, which may indicate that the oxidation process caused the breaking of most of the aromatic rings in the lignin structure. Therefore, it can be affirmed that oxidized alkaline lignin has greater resistance to thermal decomposition than alkaline lignin.

3.3. Characterization of LNPs. Figure 4 shows the size distribution of alkaline lignin nanoparticles (ALNPs) and oxidized alkaline lignin nanoparticles (OLNPs) obtained by dynamic light scattering (DLS) analysis. Based on this analysis, OLNPs showed an average size of 327.8 ± 35.61 nm, while the average size of ALNPs was 689.8 ± 79.33 nm. The agglomeration of nanoparticles may have occurred under the studied pH condition (pH = 2.0), as indicated by the low recorded values of zeta potential (2-3 mV). Padilha et al. [12] reported the production of lignin nanoparticles with sizes of approximately 700 nm. In their study, lignin samples recovered from corn cob and green coconut fiber were not oxidized, and the particle size was similar to that of the ALNPs in the present study.

The focus of this study is the application of LNPs in w/w emulsions and, therefore, the antisolvent precipitation was chosen. The antisolvent precipitation is known for its simplicity and for not requiring more complex equipment. However, some issues with the method can compromise its scalability. After the formation of LNPs, organic solvents are diluted in water, so their recovery through evaporation will involve high energy consumption. When antisolvent precipitation occurs in the dialysis bag, several days are needed to obtain nanoparticles. In our study, nanoparticle collection involved successive centrifugation steps, which also implies higher energy consumption. Therefore, to produce LNPs on an industrial scale, other methods, such as aerosol, should be prioritized due to their high yield and absence of liquid byproducts [55–57].

3.4. Evaluation of the Stability of Emulsions. The emulsions were prepared by mixing MD and PEG 6000 solutions. After 30 minutes, a degree of phase separation was observed. After approximately 1 h, the system completely separated into two phases, with the MD-rich phase at the bottom and the PEG 6000-rich phase at the top. However, the addition of lignin nanoparticles delayed the phase separation, as shown in Figure 6. Figure 6(a) shows emulsions formed by MD (20%)

7

wt/wt) and PEG 6000 (10% wt/wt) in the presence of different concentrations of lignin nanoparticles monitored for two weeks. In general, increasing the concentration of nanoparticles delayed the phase separation. After 5 h, it was observed that the emulsified phase for the system containing 1% (wt/wt) of OLNPs had a visibly larger volume compared to the system with ALNPs, indicating that the oxidation reaction led to the generation of nanoparticles with greater stabilization capacity. After two weeks, the highest EI values were obtained in emulsions containing OLNPs. In the presence of different concentrations of OLNPs, the system showed EI of 30%, 32%, 44%, and 48% for concentrations of 0.1%, 0.3%, 0.5%, and 1% (wt/wt), respectively. While in the presence of ALNPs, the EI was 29%, 36%, and 42% for concentrations of 0.3%, 0.5%, and 1% (wt/wt), respectively. No emulsified region was observed during experiments with 0.1% (wt/wt) ALNPs.

In Figure 6(b), emulsions containing 30% (MD) and 10% (PEG 6000) for different concentrations of LNPs are presented. In this composition, the system has relatively similar volumes of phases and the emulsion showed lower stability. After two weeks of monitoring, the system containing 1% (wt/wt) of ALNPs still had an emulsified region. The system using OLNPs performed better, keeping part of the emulsion at concentrations of 0.5% and 1% (wt/wt). Higher EI values were also recorded for emulsions with OLNPs. In this condition, the EI was 55% and 63% for concentrations of 0.5% and 1% (wt/wt) OLNPs, respectively. In the presence of ALNPs, the EI was 57% at a concentration of 1% (wt/wt) and no emulsified regions were observed at other concentrations. The better performance of OLNPs may be related to the higher content of hydroxyl groups on the surface of these nanoparticles [39]. The oxidation process made the lignin structure more hydrophilic, which favors its interaction with aqueous phases and consequently leads to better stabilization results. The size of the OLNPs is also a factor to be considered. In Ago et al. [58], the authors observed that kerosene-in-water emulsions involving smaller LNPs showed greater stability than emulsions with larger LNPs. This finding opens up the possibility that even better stability results could be achieved through an improvement in LNP synthesis. Based on studies with other preformed polymers [58, 59], adjustments in the synthesis methodology, such as the initial concentration of lignin and the feeding method, may lead to even smaller LNPs and are interesting aspects to be investigated in future research.

In the abovementioned compositions, droplets of the MD-rich phase are dispersed in the PEG 6000-rich continuous phase. Comparing the system containing 30% MD with the one containing 20%, it can be observed that the higher composition of MD only showed emulsification at higher nanoparticle concentrations. This behavior may be related to the increase in the dispersed phase volume, which generated more droplets and consequently requires a greater number of nanoparticles to promote stabilization. At ALNPs concentrations of 0.1%, 0.3%, and 0.5% (wt/wt) and OLNPs concentrations of 0.1% and 0.3% (wt/wt) in system II, the emulsified layer is clearly reduced, indicating that the droplets are not stable and that the coalescence process was



FIGURE 6: Monitoring the stability of emulsions formed by mixing MD and PEG 6000. (a) Emulsions containing 10% (wt/wt) of PEG 6000 and 20% (wt/wt) MD in the presence of different concentrations of lignin nanoparticles (0%, 0.1%, 0.3%, 0.5%, and 1% (wt/wt) from left to right). The stabilizing effect of ALNPs (A) is compared with OLNPs (B) for two weeks. (b) Emulsions containing 10% (wt/wt) PEG 6000 and 30% (wt/wt) MD. The experiment was carried out at room temperature of $25 \pm 3^{\circ}$ C.

effective. The nanoparticle concentration has a great influence on the stability of emulsions [32, 35]. A similar behavior was obtained with another nanoparticle as the stabilizing agent since Qian and colleagues reported that the increase of the concentration of starch nanocrystals yielded promising results for stabilizing the emulsion formed by dextran and polyethylene glycol [38]. The results obtained from both systems studied can support the literature and suggest that increasing the concentration of ALNPs and OLNPs has a positive effect on stability of w/w emulsions. Figure 7 shows images obtained from an optical microscope of the selected systems at different concentrations of LNPs. In Figure 7(a), we have images of the system formed by MD (20%)/PEG 6000 (10%) and in Figure 7(b), the system formed by MD (30%)/PEG 6000 (10%) in the presence of 0.1%, 0.5%, and 1% (wt/wt) of ALNPs and OLNPs. In the images, it is possible to observe the coverage of the droplets from the dispersed phase (MD) by lignin particles. This behavior has an influence on the delay in phase separation, making coalescence difficult. Nguyen and



(b)

FIGURE 7: Images obtained under the microscope of emulsions formed by the mixture of MD and PEG 6000 in the presence of different concentrations of LNPs after 1 day. (a) 20% MD/10% PEG 6000 system and (b) 30% 20% MD/10% PEG 6000 system.

colleagues [32] also reported that increasing nanoparticle concentration leads to smaller dispersed-phase droplet sizes.

3.5. Effect of System Composition on the Stability of Emulsions. Figure 8 shows the monitoring of emulsions with different compositions on the same tie line in the presence of 1% (wt/ wt) LNPs. Although it is possible to observe fully emulsified systems in the first few hours, the emulsified layer becomes much thinner after two weeks. The LNPs seem to be insufficient to cover all droplets of the dispersed phase and maintain the system well emulsified for a longer time. It is also noticeable that the nanoparticles have a preference for the MD-rich phase. After two weeks, the systems where the dispersed phase was MD, in the presence of ALNPs (blue arrows) and OLNPs (white arrows), presented an upper phase (PEG-rich phase) and an emulsified lower phase. The systems containing OLNPs had a slightly larger emulsified layer. A different behavior was observed when phase inversion occurred. The last systems from left to right are formed by PEG-rich phases as the dispersed phase. The following three layers are clearly visible: the upper layer



FIGURE 8: Monitoring of the stability of emulsions formed by the mixture of MD and PEG 6000 in different compositions on the same tie line containing 1% (wt/wt) of ALNPs (a) and OLNPs (b). The MD/PEG 6000 emulsions have the following compositions: 5%/14.16%, 12%/ 12.22%, 20%/10%, 35%/5.84%, and 48%/2.24%.



FIGURE 9: Monitoring of the stability of emulsions formed by the mixture of 20% (wt/wt) MD and 10% (wt/wt) PEG 6000 under different temperatures (-4° C, 35°C, and 40°C). Control systems (without LNPs) correspond to tubes 1, while systems stabilized by ALNPs and OLNPs correspond to tubes 2 and 3, respectively.

(PEG-rich phase), the intermediate layer formed by the emulsion in the presence of both ALNPs (yellow arrows) and OLNPs (red arrows), and finally, the lower layer (MD-rich phase). As reported by Qian and colleagues [38], the theoretically expected behavior is for the emulsified layer to occupy the intermediate region at low nanoparticle concentrations.

3.6. Effect of Temperature on the Stability of Emulsions. The effect of temperature on the stabilization of the system formed by 20% (wt/wt) of MD and 10% (wt/wt) of PEG 6000 was evaluated, as shown in Figure 9. When the emulsion was placed at a temperature of -4° C, the emulsified layer did not thin out like the other explored conditions even after 2 weeks. At a temperature of 40° C, lignin clusters were

generated and settled at the bottom of the test tubes (green arrows) after 1 day. The third condition evaluated was 35°C, and after 1 week, the emulsion containing ALNPs was completely destabilized and the ALNPs formed an agglomerate at the bottom phase. On the other hand, the OLNPs still maintained an emulsion layer even after 2 weeks. The coalescence of the dispersed phase droplets was intensified in the systems maintained at higher temperatures (35 and 40°C), and this may be related to a viscosity reduction [60]. With the reduction of viscosity, the droplets gained mobility and the coalescence phenomenon is driven, forming increasingly larger droplets, leading to complete destabilization of the emulsions. On the other hand, the reduction of temperature increases the viscosity of the system and favors stability. These stability tests at different temperatures were carried out with the intention of possible development of products based on w/w emulsions stabilized by lignin nanoparticles.

4. Conclusion

The oxidation of lignin not only caused structural changes in the polymer but also resulted in a reduction in the size of the generated nanoparticles. The increase in nanoparticle concentration favored the stability of the emulsions, with the best results obtained for 1% (wt/wt) of the nanoparticles. OLNPs showed better stabilization performance than ALNPs, which is favorable due to the reduction in hydrophobicity. The emulsion with a composition of 30% MD and 10% PEG 6000 produced a larger volume of the dispersed phase, i.e., more droplets were produced, requiring a larger quantity of nanoparticles to prevent coalescence, which explains the greater destabilization of this system after two weeks. Overall, this study provides important advances in an unexplored theme, which is the use of lignin nanoparticles in the stabilization of w/w emulsions. The ability of lignin to absorb ultraviolet (UV) radiation, for example, may be beneficial for incorporating bioactives into this emulsion. Lignins are soluble in organic solvents and have multiple hydroxyl and carboxylic acid functional groups in their structure, making polymer functionalization straightforward. Experiments involving grafting of hydrophilic polymers (i.e., PEG) can yield nanoparticles with even better stabilization capabilities.

Data Availability

The data used to support the findings of this study are included within the article. The data are also available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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