Tunable Pickering Emulsions with Polymer-Grafted Lignin Nanoparticles (PGLNs)

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Abstract

Lignin is an abundant biopolymer that has native interfacial functions but aggregates strongly in aqueous media. Polyacrylamide was grafted onto kraft lignin nanoparticles using reversible addition-fragmentation chain transfer (RAFT) chemistry to form polymer-grafted lignin nanoparticles (PGLNs) that tune aggregation strength while retaining interfacial activities in forming Pickering emulsions. Polymer graft density on the particle surface, ionic strength, and initial water and cyclohexane volume fractions were varied and found to have profound effects on emulsion characteristics, including emulsion volume fraction, droplet size, and particle interfacial concentration that were attributed to changes in lignin aggregation and hydrophobic interactions. In particular, salt concentration was found to have a significant effect on aggregation, zeta potential, and interfacial tension, which was attributed to changes in solubility of both the kraft lignin and the polyacrylamide grafts. Dynamic light scattering, UV-vis spectroscopy, optical microscopy, and tensiometry were used to quantify emulsion properties and nanoparticle behavior. Under all conditions, the emulsions exhibited relatively fast creaming but were stable against coalescence and Ostwald ripening for a period of months. All emulsions were also oil-in-water (o/w) emulsions, as predicted by the Bancroft rule, and no catastrophic phase inversions were observed for any nanoparticle compositions. We conclude that lower grafting density of polyacrylamide on a lignin core resulted in high levels of interfacial activity, as characterized by higher concentration at the water-cyclohexane interface with a corresponding decrease in interfacial tension. These results indicate that the interfacial properties of polymer-grafted lignin nanoparticles are primarily due to the native hydrophobic interactions of the lignin core. These results suggest that the forces that drive aggregation are also correlated with interfacial activities, and polymer-nanoparticle interactions are critical for optimizing interfacial activities. Controlled radical polymerization is a powerful tool for polymer grafting that can leverage the intrinsic interfacial functions of lignin for the formation of Pickering emulsions.

Keywords

Pickering emulsion; Nanoparticle; Lignin; RAFT; Polymer graft; Interfacial tension; Surfactant; Biosurfactant

Introduction

Lignin is a complex biopolymer found in the cell walls of trees where it functions as a binder for cellulose fibrils with high levels of natural interfacial activities. Formed from the radical polymerization of aromatic alcohols called monolignols (viz., *p*-coumaryl, coniferyl, and sinapyl alcohols), native lignin has a range of functional groups, including aryl, methoxyl, hydroxyl, and ether groups [1]. These three monolignols are shown in Figure 1 and occur in various ratios depending on the source of the wood [2]. As a byproduct of the paper industry, lignin can be extracted as charged lignosulfonates through the sulfite pulping process or as kraft lignin through the alkaline kraft pulping process, which introduces sulfonate and carboxylate functional groups, respectively. Although some of the millions of tons of lignin produced a year are used as dispersants, binders, and additives for oil extraction [3], the stockpile of lignin “waste” generated annually is largely untapped [2] due to its relatively weak interfacial activities in its processed forms, despite its potential to form advanced materials for scientific and industrial applications [4-6].

Unmodified kraft lignin in particular has generally been shown to be weakly surface active [7] and can form stable emulsions on at higher pH [8-10]. At neutral pH, softwood kraft lignin has low solubility in water and is insoluble in hydrocarbons due to the primarily hydrophobic structure. As a result of the inherent heterogeneity in lignin particles and hydrophobicity, they tend to form aggregates of a variety of sizes. The addition of polymer grafts renders these biosurfactants with new properties, the most essential of which is water solubility. We have previously shown [11,12] that, in particular, synthetic polymers can be grafted from a lignin macroinitiator, that polymer-grafted lignin nanoparticles are surface active at the air-water interface, and that they are capable of forming stable emulsions. Given a characteristic lignin molecular weight of 25,000 g/mol, polymer-grafted lignin nanoparticles (PLGNs) are expected to form Pickering emulsions in mixtures of water and hydrophobic liquids.

Unlike traditional nonionic or ionic surfactant-based emulsions, Pickering emulsions are stabilized by solid particles at the interface between the dispersed phase and the continuous phase. Although they were characterized in the early 1900's by Ramsden [13] and the scientist whose name they bear today [14], there has only been renewed interest in their study over the past one to two decades [15,16], perhaps as a result of the increased ability to produce nanomaterials with finely tuned properties. Pickering emulsions are inherently more stable against coalescence than their surfactant-based counterparts in part due to the generally large energetic barrier associated with particle desorption from the interface. Indeed, the desorption energy for nanoparticles can reach up to $10^7 k_B T$ [15]. Furthermore, it is estimated that particles that exhibit amphiphilicity (with Janus particles at the strongest end of the spectrum), can further increase this barrier by up to a factor of three [17].

Polymer-grafted nanoparticles represent a diverse class of compounds with numerous applications, ranging from lubrication to improved composite materials. The graft monomer type, graft density, graft length, and architecture can all be controlled with high precision with the use of controlled radical polymerization techniques such as atom transfer radical polymerization (ATRP) [18], nitroxide-mediated radical polymerization (NMP) [19], and reversible addition-fragmentation chain transfer (RAFT) [20,21]. Nanoparticles grafted with surface active polymer chains have shown remarkable ability to decrease surface and interfacial tensions as well as create stable Pickering emulsions [22-24]. It is hypothesized that this stability

could be due in part to steric repulsion between the grafts, preventing droplet flocculation and subsequent coalescence, as well as penetration of the polymer grafts into the liquid phases. Furthermore, polymer-grafted particles could potentially exhibit an even higher energetic barrier to desorption because a single particle on the interface is associated with many polymer chains that could be surface active.

Thus, it is the aim of this work to demonstrate the ability of polymer-grafted lignin nanoparticles to form stable emulsions at neutral pH with various tunable properties as a function of initial oil (cyclohexane) volume fraction and ionic strength. Polyacrylamide was chosen as the grafted polymer because it is water-soluble and accessible by RAFT polymerization. However, its solvent interactions can be significantly affected by conversion of even 1% of the monomers to acrylic acid via hydrolysis, making it sensitive to monovalent salt concentrations as low as 1 mM [25]. Despite the complexities of these interactions, acrylamide is still one of the most commonly used water-soluble polymers prepared via radical polymerization, making it an important starting point for these studies. The results here will demonstrate the dramatic impact that polymer grafting has on kraft lignin and how uniquely these nanoparticles stabilize emulsions compared to other biosurfactants reported in the literature.

Figure 1: Chemical structures for the three monolignols.

Materials and Methods

Materials and Preparation of Polymer-Grafted Nanoparticles

Polyacrylamide grafts were polymerized from lignin nanoparticles using RAFT chemistry using the previously published method [11] and illustrated in Figure S1. All chemicals other than those specified were obtained from Sigma-Aldrich and used as received. Kraft lignin from softwood sources (TCI America) was neutralized before use with 0.1 M hydrochloric acid, rinsed with deionized water, and allowed to dry. Lignin macroinitiators were prepared by reacting lignin with potassium xanthate, 2-bromopropionic acid, and thionyl chloride in dry tetrahydrofuran (THF) under nitrogen at 70 °C overnight. Macroinitiator site densities were confirmed analytically using ¹H-NMR with a known mass of pentafluorobenzaldehyde (PFB). Polyacrylamide grafts were then grown off the RAFT macroinitiator using acrylamide and azobisisobutyronitrile (AIBN) in dimethylformamide (DMF) under nitrogen at 70 °C overnight, and were washed with dichloromethane and dried before use. Graft length was confirmed using ¹H-NMR after graft cleavage and was found to exhibit 98% conversion of monomer, as described previously [11].

Nanoparticles were prepared with average graft densities of 100 μmol and 700 μmol of grafts ($\pm 3\%$) per gram of lignin, which equates to approximately 2 and 17 grafts per particle, respectively. Polyacrylamide grafts were grown to yield an average degree of polymerization (DP) of 100.

Interfacial Tension Measurements and Particle Properties

In order to measure the effects of the polymer-grafted nanoparticles on the interfacial tension between water and cyclohexane, the prepared nanoparticles were first dissolved in deionized water (Millipore Milli-Q system, 18.2 M Ω cm) at a concentration of 2 mg/mL. In order to aid dissolution, solutions were ultrasonically agitated (Misonix S-4000 with Microtip) for 10 minutes, which consisted of alternating 85 W pulses at 70% amplitude and rest periods of one-second duration each. The solution was mixed with the proper amounts of NaCl (Fisher Scientific) and diluted further to yield final solutions containing 1 mg/mL of lignin nanoparticles in the presence of NaCl ranging from 0 mM to 10 mM.

The solutions were poured into glassware previously cleaned in a base bath in order to avoid contamination. Cyclohexane was then poured on top of the aqueous solutions. Lignin particles have been shown to exhibit slow diffusion to interfaces in bulk systems [7,26], so the mixtures were allowed to relax for a period of three days to reach equilibrium. Interfacial tension was then measured using a du Noüy ring apparatus (CSC Scientific Company) with a platinum-iridium ring at room temperature (25 °C).

Particle size distributions and zeta potentials were measured using a Malvern ZetaSizer ZS with the same concentrations and ionic strengths used for the interfacial tension measurements. Solutions were allowed to relax for a period of two weeks before measurements were taken.

Preparation of Emulsions

Aqueous solutions with 1 mg/mL of polymer-grafted lignin nanoparticles with 0 mM, 1 mM, or 10 mM NaCl were prepared to study emulsion properties. Cyclohexane was then added to the solutions in varying volume fractions ranging from 0.1 to 0.9 v/v in 20 mL glass vials. The mixtures were then ultrasonically agitated again for 10 minutes to form emulsions and were allowed to equilibrate for at least 24 hours before being analyzed.

Analysis of Emulsions

To determine which phases were the continuous and dispersed phases, simple dilution and dye solubility tests with water-soluble methyl blue dye were performed.

The concentration of lignin nanoparticles in the aqueous phase was measured with a Cary 5000 UV-Vis-NIR spectrophotometer. Absorbance of the lower graft density nanoparticles (100 $\mu\text{mol/g}$) was measured at both 300 nm and 350 nm wavelengths. Absorbance of the higher graft density nanoparticles (700 $\mu\text{mol/g}$) was measured at both 250 nm and 260 nm wavelengths. These UV-range wavelengths correspond with those used in previous studies of lignin [27,28].

Optical microscopy (Leica DM IL LED optical microscope) was used to view emulsion samples. Several pictures of each emulsion sample were taken and image processing algorithms

(primarily the circular Hough transform as implemented by the function *imfindcircles* in MathWorks MATLAB software) were used to obtain associated droplet size distributions.

Results and Discussion

Figure 2: Dynamic light scattering data for (a) LPAM100 and (b) LPAM700 at salt concentrations of 0 mM, 1 mM and 10 mM NaCl.

Particle Behavior

In this work, we studied polymer-grafted lignin nanoparticles (PGLNs) at a concentration of 1 mg/mL, which is well above its critical aggregation concentration (CAC) that we found to be approximately 0.1 mg/mL [11], consistent with literature values for kraft lignin particles [7,27,28]. As a first step to quantifying particle behavior, dynamic light scattering was performed on PGLN solutions with graft densities of 100 $\mu\text{mol/g}$ of lignin (~ 2 grafts per particle) and 700 $\mu\text{mol/g}$ of lignin (~ 17 grafts per particle) with a graft degree of polymerization (DP) value of 100. Throughout the remainder of this work, these two graft densities will be denoted as LPAM100 and LPAM700, respectively. The results are shown in Figure 2.

As expected, with 0 mM NaCl, the PGLNs aggregated to form clusters of roughly 100 nm in radius at both graft densities, which is comparable to values reported for plain lignin particles [28]. There is also evidence that these clusters may even be spherical in shape [28]. The broad peak for LPAM100, however, does indicate some degree of polydispersity within the sample. PGLN solutions were ultrasonically processed and allowed to sit for two weeks before dynamic light scattering (DLS) measurements were taken, ensuring that the formation of these clusters did indeed represent stable conditions. The addition of salt, though, inhibited aggregate formation to a significant extent. Although larger aggregates were still present in the solutions with salt, LPAM100 with 1 mM and 10 mM NaCl exhibited maximum peaks at 14 nm and 10 nm, respectively. LPAM700 with 1 mM and 10 mM NaCl similarly exhibited maximum peaks at 7 nm and 12 nm, respectively. It is estimated that non-grafted lignin particles have a radius of around 5.0 nm [12], and with a DP of 100, we estimate that the polyacrylamide grafts should possess a radius of gyration 2.8 nm based on a molecular weight relation for polyacrylamide in water [29]. A first-order approximation, then, yields an estimated radius of 7.8 nm for individual

PGLNs. Thus, the peaks at smaller hydrodynamic radii associated with increased ionic strength/salinity most likely represented single PGLNs in solution.

The lack of aggregation in the presence of salt was unexpected. Monovalent salts are known to drive the precipitation of kraft lignin under alkaline conditions [30], so addition of NaCl was expected to further increase the hydrophobicity of the lignin core. Indeed, when compared to other biosurfactants such as rhamnolipids, which have similar disorganized structure to the PGLNs, aggregation was also observed with the addition of salt [31]. The increase in solubility observed here suggests that interactions of the hydrophobic lignin core with the polyacrylamide grafts are responsible for the reduction in aggregation, not necessarily through complete chain collapse onto the lignin core but possibly through greater associations that partially shield the water-lignin interactions.

It is known that kraft lignin particles at neutral pH contain a negative surface charge due to carboxylate and some sulfonate groups on its surface [32]. In fact, in order to confirm that the grafting process did not have an effect on the surface potential, the zeta potential, or potential at the shear plane, was measured for LPAM100 and LPAM700 solutions again with varying salinity. As shown in Figure S2, the zeta potential was found to be negative with values similar to those found for bare kraft lignin particles at neutral pH [32]. LPAM100 and LPAM700 values were within error of each other, further confirming that the surface charge of PGLNs is dominated by that of the lignin core. This is very atypical of biosurfactants, as the hydrophobic nature is strongly responsible for aggregation and minimal to no reduction is seen with salt effects. Additionally, the magnitude of the zeta potential decreased as ionic strength was increased, indicating that salt ions were screening some of the surface charge. This suggests that PGLNs behave like typical colloidal particles with electrical double layers that obey DLVO theory [33,34]. Yet, based on DLS measurements, salt *inhibited* aggregation/flocculation instead of promoting it, as would be expected with decreasing surface charge on colloidal particles.

We hypothesize that a more complex interaction with the grafts may account for the seemingly anomalous behavior. As the ionic strength is increased, the surface charge of the lignin core is screened, reducing interparticle repulsive forces and increasing the relative hydrophobicity of the core. This increase in hydrophobicity likely causes the largely hydrophilic polyacrylamide grafts to collapse onto the lignin core in order to shield it from the aqueous environment, interfering with some of the complex hydrophobic interactions that promote aggregation of lignin particles despite intrinsic high surface charges [27]. After all, similar CAC and cluster size values between bare lignin particles and PGLNs, despite the presence of grafts, indicate that hydrophobic interactions between the lignin cores drive aggregation. The presence of salt, then, would both decrease effective surface charge and decrease the extent of aggregation, which is exactly what is seen in Figures 2 and S2. (Salt concentration may also influence polyacrylamide graft conformation in solution [35,36], but these effects have been neglected in our analysis.) If true, this would also imply that the aggregated clusters are only weakly aggregated. This sort of complex interaction is not observed in other biosurfactants as noticed with the lack of a response to salt concentration. This shows that the hydrophobicity is responsible for reduction in aggregation even in PGLNs.

Interfacial Tension Lowering

We previously demonstrated that PGLNs were capable of reducing the surface tension of water [11]. In this work, we demonstrate the ability of PGLNs to reduce the interfacial tension between cyclohexane and water (more precisely the “effective” interfacial tension because PGLNs are considered to be solid particles, not molecular surfactants). The clean interfacial tension between water and cyclohexane has been measured to be between 48.5 and 50.0 mN/m [22,37]. We

Figure 3: Interfacial tension between cyclohexane and water in the presence of 1 mg/mL of LPAM100 and LPAM700 as a function of NaCl concentration.

confirm the ability of polymer-grafted nanoparticles to reduce interfacial tension substantially at low concentrations of 1 mg/mL, or 0.1 wt% [22].

Interfacial tensions were measured for LPAM100 and LPAM700 with varying ionic strengths in order to analyze how grafts may affect interfacial adsorption. A du Noüy ring apparatus was used, and measurements were corrected for meniscus geometry using the parameters of the ring. The samples were allowed to stabilize for a period of three days to account for the slow dynamics of lignin particle transport and adsorption [7,26].

As shown in Figure 3, the interfacial tension lowering appeared to be a strong function of both variables examined (graft density and salinity). LPAM100 lowered the interfacial tension more strongly than LPAM700, 24.5 versus 26.8 mN/m. Increasing salinity further decreased the interfacial tension to as low as 19.2 mN/m for LPAM100 and 23.3 mN/m for LPAM700. While these interfacial tensions may not be as low as those observed for other polymer-grafted nanoparticles (e.g., 10.8 mN/m for PDMAEMA-grafted silica nanoparticles [22]), perhaps due to the less surface-active polyacrylamide grafts used in this study, they nonetheless represent a significant degree of adsorption/penetration of the interface.

Although the interfacial tension is likely a complex function of graft density and ionic strength, the behavior of the PGLNs in Figure 3 can largely be explained if one considers them as disorganized hydrophobic particles with hydrophilic patches [38] and tunable hydrophobicity and wettability [39,40]. The lignin cores of the PGLNs can be considered mainly hydrophobic whereas the polyacrylamide grafts impart hydrophilic character. Certain particles may contain

greater or fewer grafts than the average, and the locations of grafts on the lignin particle are not controlled. Nonetheless, as few as two grafts per particle are shown to impart a degree of hydrophilicity that significantly affects colloidal properties and aggregation compared to bare, hydrophobic particles but still allows for significant reductions in surface tension.

Increased graft density would result in an overall more hydrophilic particle that is less wettable by the oil phase due to more hydrophilic “patches” on the surface. This, in turn, would lessen the extent to which it penetrates the oil-water interface and reduce interfacial tension compared to the more hydrophobic particles of lower graft density. Increased graft density would also lead to increased steric repulsive forces between particles. Increased repulsive forces would reduce packing at the interface and result in a higher interfacial tension as predicted by the Gibbs adsorption equation:

$$\Gamma = -\frac{1}{RT} \left(\frac{\partial \gamma}{\partial \ln c} \right)_{P,T} \quad (1)$$

where Γ is the surface concentration, R is the ideal gas constant, P is the pressure, T is the temperature, γ is the interfacial tension, and c is the bulk concentration.

Increasing salinity would have the opposite effect. First, decreased electrical double layer repulsion forces would allow for greater packing at the interface and lower interfacial tensions. Second, based on the data from Figure 2, increasing salinity prevents aggregation through graft collapse, which may allow more individual particles that would normally be trapped in aggregates to adsorb to the interface. Between 1 mM and 10 mM NaCl, the change in interfacial tension is less significant than that between 0 mM and 1mM because the grafts have already collapsed and there is no further change in graft conformation. At all salt concentrations, though, LPAM100 with a lower grafting density exhibited lower interfacial tensions compared to LPAM700, which was attributed to greater solvent exposure of the hydrophobic lignin cores that drives them to the air-water interface. In other biosurfactants, due to the strong aggregation of the system, different plateaus of surface tension values are recorded. This is a function of the size of the different aggregates in the system [31].

Rheological changes of the interfacial film may also play a significant role in interfacial tension lowering. In fact, while operating the du Noüy ring apparatus, the cyclohexane-water interface appeared to behave like a semi-solid viscoelastic skin, which has been documented elsewhere for lignin particles [8,41]. These effects warrant further investigation.

Emulsion Production and Efficiency

Figure 4: Images of emulsions formed with 1 mg/mL of (a) LPAM100 and (b) LPAM700. From left to right, the initial volume fraction of cyclohexane was 0.1 to 0.9 v/v.

Emulsions were prepared with cyclohexane and aqueous solutions of LPAM100 and LPAM700 at 1 mg/mL concentrations. Again, this concentration was well above the CAC. Besides graft density, initial volume fractions of cyclohexane and water as well as salinity were varied to study their effects on the emulsions formed. Ultrasonication was used to prepare emulsions [42] in pulses of one second duration for a total of five minutes. Although steady state droplet size can depend on the time used to homogenize samples [43], further ultrasonication beyond five minutes did not seem to have any effect on the structure of the emulsion. Indeed, it is well known that emulsion characteristics depend on preparation methods [8] and, in particular, ultrasonication power [42]. Thus, preparation methods were held constant under all parameters tested, allowing for ample comparison and analysis.

Representative images of LPAM100 and LPAM700 emulsions are shown in Figure 4. Note that the LPAM700 emulsions are lighter in color because LPAM700 has a lower molar absorptivity for visible light than LPAM100, even accounting for the larger fraction of polyacrylamide in the former. In fact, concentrations of LPAM700 in the emulsions were not significantly different than those with LPAM100. All emulsions prepared were oil-in-water (o/w) emulsions as confirmed by dilution and dye solubility tests. The dilution test consisted of placing a small amount of the emulsion in water and cyclohexane. All emulsions readily dispersed in water, indicating they were o/w. Furthermore, when added to the emulsion, methyl blue (a water-soluble dye) was found in the continuous phase of all samples when viewed under an optical microscope.

This behavior is expected based on the Bancroft rule, which states that the continuous phase of Pickering emulsions is the one in which the particles are most soluble [34].

Furthermore, the energy of desorption into the aqueous phase, ΔF , is given by

$$\Delta F = \pi r^2 \gamma_{ow} (1 - \cos \theta_{owp})^2 \quad (2)$$

where r is the radius of the particle, γ_{ow} is the interfacial tension, and θ_{owp} is the contact angle measured through the water phase. With a contact angle greater than 90 degrees, as is most likely the case with PGLNs, particles most easily desorb into the aqueous phase, which then usually becomes the continuous phase [15,39]. The fact that all emulsions were o/w despite a large range

of initial volume fractions indicates that PGLNs do not undergo a catastrophic phase inversion in the range of conditions studied [44].

Emulsions were remarkably stable for a period of months and did not exhibit any droplet coalescence or Ostwald ripening [45]. Emulsion stability at low particle concentrations has been observed before for polymer-grafted nanoparticles [22-24]. This stability has been attributed to steric repulsive forces between grafts, which would hinder droplet coalescence. Additional forces, though, may also aid in the stability of PGLNs. First, it is well known that particles of intermediate hydrophobicity are better emulsion stabilizers than those with strong hydrophobic/hydrophilic attractions [39,46]. As disorganized particles with hydrophilic and hydrophobic domains, PGLNs certainly fit this criterion. Second, it has also been shown that Pickering emulsions are most strongly stabilized when particles are weakly flocculated [15,46,47], which appears to be the case for PGLNs based on the DLS data in Figure 2. Finally, as mentioned with the interfacial tension measurements, a dense viscoelastic film formed by the lignin aggregates could provide the interface with rheological properties that deter coalescence [8,46].

Figure 5: Volume fraction of emulsion produced after creaming for (a) LPAM100 and (b) LPAM700 as a function of initial cyclohexane volume fraction and salinity. Lines are drawn to guide the eye. Error bars represent standard error.

Despite great stability, all emulsions exhibited relatively fast creaming over a period ranging from about 1 hour to 24 hours, with larger droplets creaming more rapidly due to greater buoyant forces. In fact, emulsions stabilized by lignosulfonates also exhibit creaming despite smaller droplet sizes [10]. The final volume of emulsion produced after creaming was found to depend on graft density, salinity, and initial volume fractions and is shown in Figure 5. Throughout the rest of this work, the term “emulsion partition” will be used to refer to this final, stable volume of emulsion droplets within the aqueous phase. In general, LPAM100 samples were able to produce more emulsion compared to LPAM700 samples, and the presence of salt increased the volume of emulsion formed at all initial conditions for both PGLNs. The fact that emulsion volume was maximized at intermediate cyclohexane volume fractions indicates that a balance exists between the amount of PGLNs and the amount of cyclohexane available to form

emulsions. At 0.8 v/v cyclohexane, the initial aqueous volume fraction evidently did not contain enough PGLNs to stabilize a large volume of emulsion under all conditions tested.

In order to determine the amount of PGLN incorporation into the emulsion partition, UV-vis spectroscopy was used to measure the concentration of lignin in the aqueous phase and calculate the concentration in the emulsion partition via the following simple equation that assumes volume and mass conservation:

$$c_{emul} = \frac{c_0\phi_{0,aq} - c\phi_{aq}}{\phi_{emul}} \quad (3)$$

where c_{emul} is the PGLN concentration in the emulsion partition, c_0 is the initial PGLN concentration (1 mg/mL), c is the measured concentration, and ϕ represents the volume fractions of the aqueous (aq) or emulsion (emul) partitions after emulsification or initially (0). The results are presented in Figure 6. Data points for emulsions with 0.1, 0.8, and 0.9 v/v initial cyclohexane were not included due to the substantial error introduced by the small volumes of emulsion formed. It is clear that the PGLN emulsions form two distinct groupings in Figure 6. That is, the samples in the presence of salt, regardless of graft density and salt concentration, exhibit a decreasing PGLN concentration with increasing cyclohexane volume fraction, and the samples without salt exhibit an increasing concentration. From a concentration standpoint, the addition of salt increases the PGLN affinity for the emulsion, especially at low initial cyclohexane volume fractions. This increased affinity for the emulsion phase in the presence of salt may be explained through arguments based on interfacial packing. Based on the data in Figure 2, the presence of any salt was found to prevent aggregation, which would render more lignin particles free to line the interface and is consistent with Figure 6.

Figure 6: The concentration of PGLNs in the emulsion partition as a function of initial cyclohexane volume fraction, graft density, and salinity. Lines are drawn to guide the eye. Error bars represent standard error.

Droplet size distributions for each emulsion were determined for all emulsion samples through optical microscopy. Several pictures of each emulsion were taken, and droplet circles were identified through image processing. As illustrated in Figure 7, droplet size was found to be a complex function of graft density, salinity, and initial cyclohexane volume fraction, but some general trends emerge. First, a lower grafting density corresponded to lower droplet sizes.

LPAM100 exhibited maximum droplet radii of 19 μm , 20 μm , and 49 μm , whereas LPAM700 exhibited maximum droplet radii of 24 μm , 89 μm , and 189 μm for 0 mM NaCl, 1 mM NaCl, and 10 mM NaCl, respectively. Second, droplet sizes increased for both grafting densities with increasing salt concentration. Third, intermediate initial cyclohexane volume fractions (usually between 0.5 and 0.8) produced the largest droplet sizes, especially in the presence of salt, under which conditions droplet size distributions transitioned from relatively uniform (seen in Figure 8a) to very wide and perhaps bimodal (seen in Figure 8b).

Droplet size trends can likely be attributed to interparticle and/or interdroplet interactions and not macroscopic interfacial tension lowering. If the latter were true, opposite trends would have been observed since a constant amount of work put into the system (as was the case with the ultrasonicator) would have yielded smaller droplet sizes for lower interfacial tensions based on the following simple equation [48]:

$$W = \gamma \Delta A \quad (4)$$

where W is the work of emulsification, γ is the interfacial tension, and ΔA is the new interfacial area created as a result of emulsification. Increased salt, however, decreased interfacial tensions while *increasing* droplet size significantly.

Figure 7: Emulsion droplet size distributions as a function of initial cyclohexane volume fraction for LPAM100 (a) without salt, (b) with 1 mM NaCl, and (c) with 10 mM NaCl and LPAM700 (d) without salt, (e) with 1 mM NaCl, and (f) with 10 mM NaCl.

The complex behavior illustrated in Figure 7, then, may be explained by noting that a higher hydrophile-lipophile balance (HLB) value for non-ionic surfactants (i.e., more hydrophilic) corresponds with larger droplet sizes and wider droplet distributions [43]. LPAM700 is more hydrophilic than LPAM100 due to more polyacrylamide “patches” and thus leads to larger droplet sizes. At 1 mg/mL concentrations for both grafting densities, however, LPAM100 might instead exhibit smaller droplet sizes because there are more particles in solution compared to the emulsions containing higher molecular-weight LPAM700, as an inverse correlation between particle concentration and droplet size for Pickering emulsions has been established [46]. Additionally, some emulsions have shown increasing droplet size with increasing dispersed phase volume fraction [8,45,49], although explanations for this effect vary.

For PGLN systems, it may well be that as the cyclohexane volume fraction increased, there were not enough PGLNs in solution to stabilize an increase in interfacial area associated with more cyclohexane. Droplet size consequently increased to compensate for the relative lack of PGLNs available to adsorb to the interface.

Figure 8: Emulsion droplets for (a) LPAM100 with 0.4 v/v initial cyclohexane and (b) LPAM700 with 0.5 v/v initial cyclohexane and 10 mM NaCl.

The salt effects are somewhat anomalous considering the interfacial tension lowering data. If salt does indeed allow more packing at the interface, one would expect droplet size to decrease with increased salinity in order to accomodate more possible particles at the interface. Although some studies have shown an increase in dispersed oil droplet size as salinity increased [47,49], the effect seems to be system dependent. We hypothesize that although PGLN packing increased at the oil-water interface, higher salt concentrations may screen the electrical repulsion between droplets enough to allow coalescence. This effect may be particularly significant for the especially large droplet sizes of LPAM700 with 10 mM salt. Due to the increased hydrophilicity of LPAM700 relative to LPAM100, the likely lower energetic barrier to desorption may also contribute to coalescence. Note that this coalescence during formation is not to be confused with long-term coalescence, against which PGLN emulsions were very stable. Finally, the decrease in PGLN aggregate formation due to the presence of salt (Figure 2) may also contribute to increased droplet size in that a decrease in interfacial area may occur to compensate for the lack of flocs available in solution for droplet stabilization since it is a general rule that larger particles (or in this case aggregates) stabilize larger droplets [50]. More data, however, are needed to confirm these hypotheses.

With regard to packing at the interface, interfacial surface concentrations were calculated for the PGLNs in a similar manner to other works [43,51] for each emulsion sample with the following equation:

$$\Gamma_{drop} = \frac{(c_0 - c)\phi_{0,aq}}{(\phi_{0,cyc} - \phi_{cyc})\left(\frac{3\sum r_i^2}{\sum r_i^3}\right)} \quad (5)$$

where Γ_{drop} is the PGLN surface concentration on emulsion droplets, $\phi_{aq,emul}$ is the volume fraction of the aqueous phase within the emulsion partition, $\phi_{0,cyc}$ is the initial volume fraction of cyclohexane, ϕ_{cyc} is the volume of cyclohexane after emulsification, and the summation terms are over the radii, r_i , in the droplet size distribution. It is implicitly assumed that PGLNs are completely insoluble in cyclohexane. Data points for emulsions with 0.1, 0.2, 0.8, and 0.9 v/v

initial cyclohexane were again excluded due to substantial error introduced by the small volumes of emulsion formed. As illustrated in Figure S3, surface concentration increased with increasing salinity, as expected. Additionally, the surface concentration was comparable between the two different grafting densities at the same salt concentrations except at 10 mM. At this salinity, the surface concentration of LPAM700 jumped significantly and was not comparable to LPAM100. This behavior supports the hypothesis of droplet coalescence during emulsification.

Figure 9: The estimated area occupied per PGLN particle for (a) LPAM100 and (b) LPAM700 as a function of initial cyclohexane volume fraction and salinity. Lines are drawn to guide the eye. Error bars represent standard error.

The following equation was used to estimate the average area occupied per particle at the interface:

$$A_{particle} = \frac{M}{\Gamma_{drop} N_A} \quad (6)$$

where M is the molecular weight of the PGLNs and N_A is Avogadro's constant. The lignin cores of the PGLNs were estimated to have a molecular weight of 25,000 g/mol [12]. LPAM100 and LPAM700 were thus estimated to have molecular weights of 39,216 g/mol and 145,836 g/mol, respectively. Figure 9 displays the calculated areas and does not account for the error in estimating molecular weights. As one can see, the area occupied per particle decreased with increasing salt, which is expected based on electrical double layer repulsion and graft collapse, and further validates the interfacial tension lowering phenomena. LPAM700 also exhibited greater areas, which is, again, expected due to steric repulsive forces. Interestingly, a cross-sectional area of 190 nm² was found for aggregates of lignin particles using a Langmuir-Blodgett film technique [28] and is very similar to the calculated areas in Figure 9 for PGLNs with 0 mM NaCl, indicating that PGLNs likely existed as aggregates on the interface. In the presence of salt, calculated areas as small 10 nm² indicate that 5.0 nm may be an overestimate of particle size and, more importantly, that PGLNs likely did not exist as aggregates on the interface. This difference is completely consistent with the aggregation data and analysis presented here.

The extent of aggregation, interfacial tension lowering, volume fraction of emulsion formed, and emulsion droplet sizes have been shown to be strong functions of salinity and graft density, all of which can ultimately be explained in terms of hydrophobic and hydrophilic interactions among lignin cores and polyacrylamide grafts. Higher graft densities correspond to increased hydrophilicity of disorganized PGLNs, and increased salinity is proposed to drive graft interactions with lignin and decrease aggregation. By incorporating hydrophilic grafts onto hydrophobic lignin particles at low to intermediate grafting densities, interparticle interactions can be tuned in a way that critically affects emulsion properties.

Conclusions

Polymer-grafted lignin nanoparticles (PGLNs) represent a new class of nanomaterial engineered using environmentally sustainable resources [2,3] with tunable amphiphilicity and hydrophobicity. A study was performed to investigate the ability of PGLNs to form Pickering emulsions and the colloidal forces responsible for such behavior. PGLNs at only 0.1 wt% were shown to be capable of producing Pickering emulsions composed of cyclohexane and water that were stable against coalescence for a period of months, consistent with other polymer-grafted nanoparticles [22,24]. Within the range of graft densities and salinity investigated, all emulsions were of the o/w type and exhibited creaming within 24 hours of formation, which was likely due to relatively large emulsion droplet sizes.

PGLNs were shown to reduce the interfacial tension between water and cyclohexane. The interfacial tension was found to decrease with decreasing graft density and increasing salinity. This trend was attributed to the amphiphilic nature of PGLNs [15,39], electrical double layer repulsive forces, and the collapse of grafts onto the core in the presence of salt.

The emulsion properties of Pickering emulsions were found to depend heavily on graft density, initial cyclohexane volume fraction, and salinity. The following trends were observed. First, lower grafting density of polyacrylamide on a kraft lignin core resulted in higher levels of interfacial activity, as characterized by higher concentration at the water-cyclohexane interface with a corresponding decrease in interfacial tension. Second, the volume of emulsion produced was maximized at intermediate cyclohexane volume fractions. Third, the presence of salt caused the concentration of PGLNs in the volume of emulsion produced to increase, especially at low cyclohexane volume fractions. Additionally, droplet sizes increased and droplet size distributions widened with increasing salinity and increased graft density. Finally, droplet sizes were maximized at intermediate cyclohexane volume fractions.

Like other biosurfactants, such as rhamnolipids [52,53], lignin exhibits complex aggregation, and this work investigates how polymer grafting and salinity can affect such aggregation and, consequently, tune the properties of emulsions in the face of chemical variability. Many rhamnolipids have a similar disorganized hydrophilic/hydrophobic character to PGLNs. It is interesting to note that despite having greater hydrophilic character, the surface activity of PGLNs is primarily due to the hydrophobicity of the lignin core. The properties of the hydrophobic lignin core are responsible for the decrease in aggregation as a result of salt effects, which is why interfacial tension lowering and improved emulsion stability were observed. However, it is important to note that kraft lignin without polymer grafting does not exhibit these properties. We suggest that polymer grafting is essential for leveraging the intrinsic interfacial

activities of lignin by minimizing aggregation but not abolishing the tendency to orient at interfaces.

We have demonstrated that polymer grafting significantly enhances these abilities and alters colloidal and emulsion properties in a controllable and predictable fashion. By altering properties such as graft density and salinity, one is able to directly influence PGLN intermolecular forces and consequently create Pickering emulsions with a wide range of properties. Such “tunable” behavior [9,22] could find numerous applications in the formation of emulsions and dispersions, especially considering the availability of lignin and the relatively small amount needed to stabilize emulsions. Despite the complexity of lignin chemistry, polymer grafting allows the design of advanced colloidal materials from renewable resources.

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Graphical Abstract