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All-lignin approach to prepare cationic colloidal lignin particles: Stabilization of durable Pickering emulsions

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Surface modification of colloidal lignin particles (CLPs), which are obtained from renewable resources, is a plausible route towards novel biomaterials. Here we show that adsorption of cationic lignin onto spherical CLPs produces positively charged particles with tailored properties for the stabilization of Pickering emulsions. The threshold dosing of cationic lignin needed to achieve colloidal stable cationic dispersions was 4% relative to the dry weight of CLPs. Compared to irregular kraft lignin particles or regular CLPs, cationic CLPs stabilized a broader array of durable Pickering emulsions. This all-lignin adsorption process to prepare cationic CLPs is advantageous because it minimizes the consumption of synthetic polymers, and opens new application opportunities for structurally defined nano- and microscale lignin particles.

Results and discussion

Lignin characterization

Lignins are heterogeneous materials by nature, and industrial kraft pulping causes additionally process-dependent degradation of native interunit linkages, necessitating structural

Introduction

Lignins are natural methoxylated polyphenols, and untapped co-products of biorefineries and chemical pulping of wood. The amphiphilic nature of lignin is an attractive property for large-scale industrial applications requiring stabilization of interfacial systems. Lignosulfonates and kraft lignin, which are the most abundant technical lignins, have been studied previously especially in oil/water emulsions with hydrocarbons.5–8 Despite these studies, inconsistent or insufficient properties of the structurally heterogeneous lignins compared to synthetic surfactants have obstructed commercialization. Bulk chemical derivatization of lignin is a popular strategy to improve its surfactant properties,4,6,7 but such modifications should be minimized to reach sustainable and low-cost materials.

Nanoparticles and microscale lignin particles have arisen as structurally defined alternatives to regular lignin powders. Among the various production methods reviewed recently,5,9 the solvent exchange technology10 holds potential for up-scaled production of colloidal lignin particles (CLPs). Compared to colloidal unstable and interpenetrating solid lignins, spherical CLPs assemble more efficiently at the oil-water interface and stabilize so-called Pickering emulsions.11 As a good demonstration of interfacial compatibility of lignin, emulsions have been used to template spheres or microcapsule architectures by crosslinking of emulsified solid lignin particles.12,13 Surface activity of lignin is usually observed under neutral to alkaline conditions due to the dissociation of phenolic hydroxyl groups. From the perspective of sustainable chemistry, and to build ground for new applications, it would be important to develop CLP-stabilized surfactant-free Pickering emulsions in a broader pH range.

A plethora of non-covalent approaches exists for surface modification of CLPs to improve their interfacial functionality. Lignins are anionic polyelectrolytes that attract positively charged ions14 and polymers15 in aqueous media. Taking use of this electrostatically favoured adsorption could yield cationic CLPs for replacing e.g. synthetic cationic surfactants,16 biocides17–19 or polymer particles.20 However, prior literature contains strikingly few demonstrations of adsorption of cationic polymers21,22 or proteins23 on CLPs, despite the broad industrial importance of cationic chemicals, including cationic lignin polymers23 and oligomers.24 There is also a lack of literature on colloidal stability of surfactant-free Pickering emulsion formulations incorporating cationic CLPs.

In the present work, we report a straightforward way to prepare cationic CLPs by adsorption of cationic lignin (Catlig) onto CLPs. X-ray photoelectron spectroscopy (XPS) analysis delivered information on the optimized adsorption of Catlig on CLP surfaces. After selecting the suitable conditions for the fabrication of cationic CLPs, we determined their capability to stabilize Pickering emulsions. Finally, potential applications such as lipase-catalysed reactions utilizing the developed oil-in-water emulsions are discussed.
characterization. In addition to the analysis of softwood kraft lignin, it was necessary to investigate the cationic kraft lignin obtained from the reaction with glycidyl trimethyl ammonium chloride (GTMAC). The cationization reaction produced cationic lignin in two fractions after dialysis under neutral conditions. The neutral water-soluble fraction (Catlig) was obtained at a yield of 55 wt% when separated by centrifugation from the insoluble pellet that could be solubilized at pH 3. Aliphatic, phenolic and carboxylic hydroxyl groups of Catlig and softwood kraft lignin were quantified by $^{31}$P NMR spectroscopy. The spectrum of the starting kraft lignin exhibited a predominant guaiacyl (G) phenolic hydroxyl peak in addition to aliphatic OH and COOH functionalities (Fig. 1a). These features are typical to softwood lignin.

Catlig contained 35% of the initial amount of phenolic hydroxyl groups in softwood kraft lignin, indicating 65% conversion degree in the cationization reaction (Table 1). Elevated amount of condensed guaiacyl units of Catlig suggests that GTMAC reacted preferably with its sterically less hindered hydroxyl groups. The quantities of carboxylic hydroxyl groups remained unchanged in Catlig, explaining its observed pH-dependent (positive) net charge due to the quaternary amine groups as well as the remaining weak acid groups (Fig 1d).

Table 1 $^{31}$P NMR analysis of hydroxyl groups and condensation degree of softwood kraft lignin and corresponding cationized lignin (Catlig). Amounts are in mmol/g.

<table>
<thead>
<tr>
<th></th>
<th>Aliphatic</th>
<th>COOH</th>
<th>H</th>
<th>G</th>
<th>Tot. phenolic</th>
<th>Cond. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softwood kraft lignin</td>
<td>1.92</td>
<td>0.46</td>
<td>0.27</td>
<td>3.84</td>
<td>4.11</td>
<td>45.4</td>
</tr>
<tr>
<td>Catlig</td>
<td>2.37</td>
<td>0.44</td>
<td>0.09</td>
<td>1.33</td>
<td>1.42</td>
<td>50.3</td>
</tr>
</tbody>
</table>

$^a$: percentage of condensed guaiacyl units relative to total guaiacyl units. $^b$: Pooled percentage errors relative to the means (N=2) were 1% (Kraft lignin) and 4% (Catlig).

Degradation of lignin during kraft pulping was evident from the quantitative $^{13}$C NMR spectroscopy analysis. In native lignin, β-O-4 bonds comprise around 50% of inter-unit linkages, but only four β-O-4 bonds per 100 aromatic units were found in softwood kraft lignin. Details of the various moieties derived from $^{13}$C NMR spectra of acetylated and non-acetylated lignin (Fig 1b) are shown in the electronic annex (Table S1). A total of 81 methoxyl groups and 50 aliphatic OH groups were found per 100 aromatic units, further evidencing drastic sidechain cleavage and demethylation during kraft pulping. The quantity of phenolic OH groups was 3.73 mmol/g, compared to 4.11 mmol/g analyzed by $^{31}$P NMR spectroscopy. In summary, kraft lignin used in this work was highly enriched in carbon-carbon bonds, and these features expectedly also held for Catlig. The number of introduced quaternary ammonium groups exceeded the number of carboxylic acid functionalities, creating a good basis for the formation of a cationic net charge on coated CLPs below pH 8 (Fig 1d).

In addition to the chemical structure that influences the solubility and material properties of lignins, molecular weight distribution is an important property of polymers. Softwood kraft lignin exhibited a bimodal molecular weight distribution, with local peak maxima at 3100 and 3640 g/mol (Fig. 1c). Its number and weight average molecular weights are 1430 and 7350 g/mol, respectively. Despite its relatively large polydispersity index (Mw/Mn=5.1), relatively monodisperse CLP dispersion (PDI=0.2 determined by dynamic light scattering, DLS) was obtained from chemically unmodified softwood kraft lignin at 85 wt% yield.

The Catlig fraction that was soluble under neutral conditions was selected for coating of CLPs due to its broader pH-dependent aqueous solubility. Since the two fractions of cationic lignin contained approximately similar atomic-% of nitrogen (Table S2), it is postulated that Catlig (pH 7-soluble fraction) was of lower molecular weight, comprising mainly of the lower half of the bimodal molar mass distribution shown in...
Fig. 1c. Catlig exhibits a positive ζ-potential of +32 mV at pH 2, which declines as a function of increasing pH (Fig. 1d). First, the net positive charge decreases due to increasing amount of dissociated COOH-groups. At alkaline pH, also the residual phenolic groups dissociate, and finally the negatively charged groups dominate over the positively charged quaternary amine groups. CLPs exhibited a strengthening negative charge at pH 2-6, reaching a ζ-potential of -45 mV that remained constant until pH 10, which is expected from the charge properties predominated by carboxylic acids.

**Development of procedures for the fabrication of cationic CLPs**

Neutralization and overcompensation of the negative charge of carboxylic acid-rich CLP surfaces by adsorbed Catlig was apparent from the positive ζ-potential of cationic CLPs (c-CLPs) below pH 7 (Fig. 1d). The cationic charge decreases sharply between pH 6.5 and 10, showing an isolectric point at approximately pH 7.1. Dosing of Catlig at increasing dry weight ratios relative to CLPs revealed that ca. 20 mg/g is required for full neutralization of the surface charge (Fig. 2a). At this threshold dosing, particle diameter measured by DLS expectedly increased sharply due to aggregation of non-charged particles (Fig. 2b). However, with increasing Catlig dosages the particle size of c-CLPs decreased to 274±5.3 nm, which is close to the original size (259±4 nm) of CLPs. This process gave colloidal stable particles carrying relatively thin cationic coronas. The cationic CLP dispersions with > 40 mg/g Catlig dosing had a ζ-potential of +20 mV and were stable as confirmed by re-measurements after 140 h and by visual observation in the course of several weeks (data not shown). The dose-dependent response of ζ-potential of c-CLPs was strikingly similar compared to the adsorption profile determined previously with poly(diallyldimethylammonium chloride). This is a promising result from the green chemistry perspective, as in this process cationized lignin could substitute the synthetic polymer entirely derived from fossil sources.

**Figure 2.** Optimization of cationic CLP production: effect of addition of Catlig on (a) ζ-potential and (b) average particle sizes of CLPs immediately (black circles) after addition of Catlig into CLP dispersion and after 140 h holding of the mixture at room temperature (white circles). (c) Structural example of Catlig based on a published softwood kraft lignin structure.26 (d) Schematic model of coating CLPs with Catlig showing the adsorption of cationic water soluble lignin (items in the middle) to anionic CLPs (spheres to the left) and leading either to charge neutralization and aggregation (upper scheme) or stable dispersion of cationic CLPs (lower scheme). Note that the scheme is not drawn to scale. Error bars in (a) and (b) indicate one standard deviation (N=3) from the mean values.

Alternative methods to produce cationic CLPs were assessed besides the coating approach. As the cationization significantly reduced solubility of lignin in organic solvents, aqueous dispersions of cationic lignin particles could not be formed
directly from the cationic lignin using the same approach as for the regular CLPs due to the negligible solubility of Catlig in organic solvents (THF, DMSO, etc.) and high solubility in aqueous media. However, an inverse solvent exchange method could be carried out, utilizing water solutions of Catlig injected into an excess of organic solvent (Fig. S1). The same solvents that can be used to form CLPs from unmodified kraft lignin seem to work as non-solvents for Catlig, and particularly 1-propanol produced colloidal particles by the inverse solvent exchange. While such particles may be useful in organic solvents or oils, drawbacks of such system are anyhow the weak colloidal stability and dissolution once water content of the dispersion increases beyond approximated 30 % v/v.

It was also found that cationic particles form once the pH 7-insoluble fraction of cationic lignin is dissolved with unmodified lignin at low pH, and subsequently injected into alkaline water as a non-solvent to produce a dispersion above pH 3. As a result, ζ-potential of these composite particles remains around +9 mV with cationic lignin proportion of 150 mg/g of unmodified lignin. This is a lower charge in comparison to what was obtained by coating pre-formed CLPs with a similar or even much lower amount of Catlig. It may be that such “composite” approach drives cationic lignin into the bulk structure of the particles. Hence, the adsorption method was more favourable, as in this “all lignin” approach the ratio of modified Catlig to CLPs can be minimized since the modified Catlig is located on the particle surfaces only.

Among the questions that we sought to answer next was whether or not the shape of the CLPs and the stability of the dispersion is maintained after adsorption of cationic lignin onto the particles. To avoid agglomeration of CLPs (schematically shown in Fig. 2d), which occurs with 8-40 mg/g Catlig to CLP ratios, a dosing ratio of 50 mg/g (Catlig/CLPs) was used to prepare cationic CLPs (c-CLPs). AFM images recorded from the c-CLP dispersion deposited and air-dried on a freshly cleaved mica did not show irregular precipitates, and confirmed that the coated particles remained spherical (Fig. 3a1-4). c-CLPs exhibited a tendency to aggregate upon drying, as is typical due to capillary forces during drying from aqueous dispersion. The particles looked very similar to uncoated CLPs (Fig 3a5-6).

The fact that a part of the silicon wafer surfaces remained uncovered when deposited with c-CLPs allowed us to assess by XPS analysis whether the cationic lignin adsorbed predominantly onto the CLP surfaces, instead of precipitating freely from the solution. If there were an excess of Catlig in the solution, then it would also be found on the silicon surface. Softwood kraft lignin as such or in particle form yielded O/C atomic ratios (0.26–0.32) and proportions of C-C linked carbon (52%–59%), which correlate well with theoretical and experimental values reported for kraft lignin.27,28 Nitrogen turned out to be a good marker for the quaternary ammonium group of cationic lignin: its atomic concentration in the non-modified of softwood kraft lignin film was below the detection limit of 0.1% (Fig. 3b and Table S2), while the cationized lignin films had both clear nitrogen signals: surface nitrogen content was 1.5±0.3 at% in Catlig and 1.3±0.1 at% in the pH 7 insoluble cationic lignin fraction (1.3±0.1 atomic %). The cationic CLP dispersion exhibited a nitrogen concentration of only 0.5±0.1 at%. However, in this case the XPS background analysis29,30 revealed that silicon substrate was partially uncovered. When only particles were taken into account (by subtracting the contribution of silicon substrate, 46% of the area analysed) this equals 0.85±0.1 at%. This value allows the conclusion that the majority of Catlig adsorbed onto the CLP surfaces, rendering their net charge positive, as already discussed.
Emulsion formation and stability

We selected toluene, olive oil, and silicone oil as the water-immiscible phases to assess suitability of cationic CLPs as emulsifiers. The prepared emulsions were held at room temperature without stirring and photographed as such during one week to assess their tendency to undergo phase separation. Toluene-in-water emulsions were formed with CLPs, c-CLPs, and Catlig separately. While increasing the CLP concentration in the emulsion mixture from 2.5 to 7.5 g/L improved the emulsion stability, no such trend was observed with c-CLPs. This indicates that there was a sufficient amount of emulsifier present even at the lowest 2.5 g/L concentration of c-CLPs that stabilized smaller and homogeneously dispersed droplets compared to agglomerated toluene droplets after one week in CLP-containing emulsions (Fig. 4a).

In general, the emulsion droplet size decreases as the surfactant concentration increases, and hence we can estimate that the critical emulsion forming concentration in the presence of c-CLPs is <0.5% (w/w lignin/oil). This value is approximately one tenth of the concentration of non-spherical kraft lignin particles at the break point of surface tension. In accordance with the preferential properties noted above, c-CLPs produced smaller emulsion droplets than softwood kraft lignin when compared at a fixed concentration of 5 g/L at pH 4 (Fig. 4b). Previously, Ago et al. presented emulsions with 6 g/L CLP concentration in kerosene-in-water 1:1 v/v emulsions, and noted that the particle size influences stability of the emulsions to withstand a 2 min centrifugation at 2000 rpm. Additionally, CLPs with a diameter of 356 nm gave more stable emulsions compared to those with CLPs at Ø =1019 nm. The average particle size of our CLPs was smaller, 259 nm, which probably correlates to a better stability of the Pickering emulsions.

Unexpectedly, our work indicates that compared to toluene-in-water emulsions, less stable emulsions were formed with olive oil, which is a substantially more polar fluid and has the ability to form hydrogen bonds with lignin. On the other hand, triglycerides are bulkier and more viscous compared to the rigid and rather planar toluene molecule. It can be anticipated that toluene stacks better with aromatic rings of lignin, while the two might also exhibit several attractive forces such as π-π and cation-π interactions. Compared to regular CLPs, c-CLPs performed better in general, but rather counterintuitively, increasing concentration led to visually less stable olive oil-in-water emulsions (Fig. 4a). In the case of silicone oil, water-in-oil emulsions were formed, with c-CLPs giving the most stable systems; likewise to the case with toluene, no clear concentration effect was observed. The observed formation of w/o emulsions with polymeric silicone oil is preferred due to its higher molecular weight and viscosity compared to toluene.
Figure 4. Photographs of emulsions at various time intervals: (a) One week stability of emulsions formed in the presence of CLPs, cationic CLPs (c-CLPs), and neutral water-soluble fraction of cationic lignin (Catlig). Concentrations relative to the total volume of the emulsion mixture were 2.5, 5, 7.5 g/L for CLPs and c-CLPs, and correspond to 0.6%, 1.2%, and 1.7% of lignin relative to the weight of toluene. (b) Comparison of toluene-in-water emulsions at pH 4 after 1, 24, and 183 h storage times. Emulsions were stabilized by CLPs, Catlig, c-CLPs, or homogenized insoluble softwood kraft lignin at indicated initial aqueous phase concentrations. Oil phases were stained with Sudan Black prior to emulsification with lignin dispersions or Catlig solution at a 1:1 volume ratio.

Pickering emulsions are promising media for facilitating aqueous-organic interfacial catalytic reactions in general, and also those targeting chemical or enzymatic modification of lignin. We demonstrated that compared to emulsions formed with commercial nonionic or cationic surfactants, CLP- or c-CLP-stabilized Pickering emulsions are equally good systems for carrying out lipase-catalyzed hydrolysis of olive oil (Fig. S2). Our ongoing work focuses on biocatalytic synthetic reactions in biphasic media such as hydrocarbon:water systems in which CLPs show several benefits. Apart from enzymatic reactions, toluene is a common solvent in versatile biphasic and emulsion-phase reactions. Non-colloidal lignin particles from acid precipitation process have been used to stabilize toluene in water emulsions in order to fabricate polymer microparticles or macroporous foams, but CLPs and c-CLPs provide significantly better colloidal stability in aqueous media and, as a consequence, their ability to stabilize emulsions is also better.

To build basis for future applications, we determined abilities of surfactant-free toluene-in-water Pickering emulsions to withstand centrifugation at pH 2–10 (Fig. 5). The procedure involved centrifugation of emulsions at 500g for 30 min, and weighing the fraction of toluene separated from the creamy layer. Among the compared lignins, non-colloidal kraft lignin produced weakest emulsions, exhibiting an upward parabolic trend with a valley point at pH 5. Regular CLPs did not provide a clear improvement of emulsion stability as compared to dispersed kraft lignin, with a majority of toluene migrating off from the emulsion regardless of the pH. c-CLPs and dissolved Catlig formed durable emulsions stable in the acidic pH region, but a steep decline in stability was observed between pH 5 and 6. Thereafter, cationic emulsions were less stable compared to those stabilized by CLPs or kraft lignin particles (Fig. 5). This emulsion behaviour appears to show some correlation with the pH-dependent surface charge of c-CLPs, with the major charge neutralization observed between pH 6 and 7 (Fig. 1d).

Figure 5. Effect of pH on the stability of toluene-in-water emulsions to withstand 30 min centrifugation at 500g. Emulsion stability was determined as the percentage of toluene remaining in the emulsion phase after centrifugation. Emulsions were formed by mixing an aqueous phase of lignin with toluene in a 1:1 volume ratio. The final concentrations in the total volume of the emulsion mixture were 5 g/L for softwood kraft lignin, CLPs and c-CLPs, and 0.25 g/L for Catlig.
Interfacial behavior of Catlig and c-CLPs differed notably from the surface activity of alkali lignins, which mainly prevails in non-acidic conditions due to the dissociation of carboxylic and phenolic hydroxyl groups. Intrigued by this, we measured water contact angles (θ) of softwood kraft lignin and Catlig on silicon wafer surfaces. Values at 10 s were selected for comparison. Softwood kraft lignin exhibited θ of 60º, while Catlig was markedly more hydrophilic with a water contact angle of 22º (Fig. 6). The value of softwood kraft lignin is comparable to prior literature, but no water contact angle data of cationic lignin was found for comparison. These contrasting surface properties of softwood kraft lignin and Catlig help explain the observed emulsion stabilization properties of Catlig and c-CLPs (Fig. 4). Compared to unmodified lignin or regular CLPs, Catlig and c-CLPs are more amphiphilic and may exhibit additional intramolecular as well as intermolecular stabilization by electrostatic and cation-π interactions. These traits contribute to stabilization of inter-particle adhesion at oil-water interfaces.

Our results with c-CLPs extend the applicable pH range in Pickering emulsions, and offer an easy way to binding enzymes, nucleic acids, or other negatively charged molecules. Besides the lipophilic-hydrophilic ratio, particle size of CLPs is another property that influences emulsion stability. Ago et al. found that among thermally formed spherical lignin micro- and nanoparticles, the ones obtained from kraft lignin functioned better in stabilization of Pickering emulsions compared to those prepared from a more hydrophobic organosolv lignin. However, these authors did not quantify the pH-stability of the Pickering emulsions. If not subjected to external centrifugal forces, either CLPs or c-CLPs stabilize emulsions in a broad pH range. However, in applications in which prolonged stability is required, this surface modification approach widens the applicability of lignin for emulsion stabilization. Non-modified CLPs function well in neutral to alkaline pH, while c-CLPs perform markedly better under acidic conditions.

To compare regular and cationic lignin particles further, turbidity measurements of Pickering emulsions stabilized by CLPs and c-CLPs at pH 5 were made under ambient conditions. Fig. 7a and 7b show that the lower aqueous phase became less turbid during the first 2 h, with slow changes thereafter. Unlike with regular CLPs, a clear aqueous phase formed with c-CLPs, indicating a complete partitioning of cationic particles into the emulsion phase due to their affinity towards toluene/water interfaces. Optical light microscopic images indicate that the toluene droplet size was indeed smaller in the emulsion containing c-CLP compared to the emulsion stabilized with unmodified CLPs (Fig. 7c). In accordance with optical microscopy, confocal microscopy images also showed that c-CLP-stabilized emulsions contained smaller droplets compared to the case with regular CLPs (Fig. 7d). Overlaid confocal images showed that in the presence of c-CLPs the toluene was dispersed to 50–100 µm size droplets dispersed in a surrounding matrix of smaller 5–10 µm droplets.

Another observed difference is the contrastive drying behavior of emulsion droplets; prior to complete drying the droplets stabilized with c-CLPs revealed smooth-surfaced intermediate structures resembling coffee beans while the droplets with regular CLPs collapsed into crumpled spheroids (Fig. S3). Shells consisting of c-CLPs maintained their circular structure even after complete evaporation of toluene (Fig. 7c), suggesting that cross-linking could achieve cationic lignin microcapsules, which have not been demonstrated yet. These microscopic observations underline the high affinity of c-CLPs to assemble onto the droplet surface, greatly exceeding the unmodified lignin or regular CLPs in such attribute.
**Figure 7.** Toluene-in-water Pickering emulsions stabilized by kraft lignin, CLPs, Catlig, and Catlig-coated CLPs. Turbidity scans at 0, 10, 20, 30, 420 min (curves from left to right) of emulsions stabilized at pH 5 by (a) CLPs and (b) c-CLPs. Optical (c) and (d) CLSM autofluorescence microscopic images of the CLPs (c1-c2 and d1-d4) and c-CLPs (c3-c5 and d5-d7, d5 with Nile Red staining) stabilized emulsions at pH 5. The image d6 overlays two images recorded at a fixed x,y position, but at different focus heights (the one on the top with false colour), revealing presence of 5-10 µm emulsion droplets around the larger 50-100 µm droplets.

**Conclusions**

Positively charged colloidal lignin particles can be formed by adsorption of cationized lignin on spherical lignin particles. Compared to alternative fabrication methods tested, the particles produced by the facile adsorption process were more stable and exhibited a higher cationic net charge, while the required proportion of chemically modified cationic lignin was several orders of magnitude lower. These colloidal cationic lignin particles outperform irregular solid lignin particles or unmodified CLPs in their capability to stabilize Pickering emulsions at pH 2 to 6. This is a suitable pH area for many important applications, such as interfacial biocatalytic esterification reactions, which are under investigation in our laboratory. Cationic CLPs are also foreseen to show affinity to various biologically and chemically important anionic interfaces. Overall, the demonstration of these “all lignin” cationic lignin particles is a step forward in sustainable use of renewable resources. This inexpensive and green surface functionalization strategy is expected to be applicable also in other material applications beyond the emulsion systems.

**Experimental details**

**Materials**

Kraft lignin used in this work was isolated from softwood using the LignoBoost® technology at Domtar’s Plymouth plant (NC, USA). All analytical grade solvents and chemicals were purchased from Sigma-Aldrich and VWR, and were used as received.

**Gel permeation chromatography**

Molar mass distribution, weight average molar mass (M̅w), number average molar mass (M̅n) and polydispersity index (PDI) were determined with gel permeation chromatography (GPC). Lignin was acetylated in pyridine:acetic anhydride 1:1 v/v for 24 h at room temperature, fully dissolved in THF, and chromatographed using a system equipped with a Phenogel pre-column (50 mm, inner diameter 7.8 mm and particle size 5 µm) and 50Å and 1000Å Phenogel chromatography columns (5 µm 300 x 7,8 mm) eluted with THF at a flow rate of 1 mL/min. The column effluent was connected to a UV detector operated...
at 280 nm. Calibration was constructed using polystyrene standards and smaller aromatic and phenolic compounds in the molecular weight range of 76600–92 g/mol.

**NMR spectroscopy**

A Bruker Avance III 400 MHz spectrometer was used to record quantitative $^{13}$C spectra of acetylated and non-acetylated softwood Kraft lignin (190 mg) in 0.55 mL of DMSO-d$_6$ containing 0.015 M Cr(acac)$_3$ as a relaxation agent and 1,3,5-trioxane (36.4 g/L) as an internal standard. A standard Bruker inverse-gated pulse sequence zgig with a 90° pulse angle was used to collect 20000 scans at a sweep width of 282 ppm. Acquisition time was 1.1 s and relaxation delay 2.5 s. To record quantitative $^{31}$P NMR spectra, 30 mg of lignin dissolved in 0.8 mL of a solvent mixture of DMF:pyridine:chloroform-d$_6$ 0.4:0.6:1 v/v/v containing 0.6 g/L chromium(III) acetylacetonate was phosphorylated with 0.15 ml of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Sigma-Aldrich). N-hydroxy-5-norbornene-2,3-dicarboxylic acid imine (10 μmol) was used as an internal standard. The pulse sequence zgig with a 90° pulse angle, 1 s acquisition time, and 25 s relaxation delay was used to record 128 scans.

**Preparation of cationic lignin**

The cationization reaction was adapted from the literature. Briefly, 2.50 g of lignin (dry basis) was dissolved in 0.2 M sodium hydroxide and glycidyl trimethyl ammonium chloride (5.0 g) was added dropwise to start the 1 h reaction 70 °C. Reaction products were purified at pH 7 by dialysis in a Spectra/Por® 7 tubing with a MWCO of 1 kDa against deionized water for 4 days. The final yields of pH 7 soluble and insoluble cationic lignin fractions were 55% and 29% (based on initial lignin dry weight).

**Preparation of colloidal lignin particles**

Colloidal lignin particles (CLPs) were prepared by dissolving lignin (2.18 g) in 108.9 g of THF:water 5:1, v/v over 3 hours and then precipitating by rapid pouring of the solution into 380 g of vigorously stirred deionized water. The stable colloidal dispersion was purified by dialysis in a Spectra/Por® 1 tubing with a MWCO of 6–8 kDa against deionized water for 4 days.

**Preparation of cationic lignin particles**

In the coating method, regular CLPs were coated with the soluble fraction of cationic lignin at varying weight proportions. Dialysis against deionized water for the duration of four days was used to purify coated particles from any residual non-adsorbed cationic lignin. In the bulk precipitation method, colloidal dispersion was obtained by adding water-soluble fraction of cationic lignin (1 volume) into an organic solvent (4 volumes). Alternatively, the water-insoluble fraction of cationic lignin and unmodified lignin were co-precipitated into CLPs by mixing these in THF-water 3:1 mixture and adjusting pH with hydrochloric acid or acetic acid. Upon acidification, also the Catlig dissolved and mixture was precipitated into particles analogously to preparation of regular CLPs, and readjusted to pH above 3 by aqueous sodium hydroxide.

**XPS spectroscopy**

AXIS Ultra electron spectrometer (Kratos Analytical) was used in XPS surface characterisation of cationic CLPs as well as non-cationized and cationized lignins. Samples on silicon wafers were mounted on sample holder with UHV compatible carbon tape and pre-evacuated overnight together with a fresh in situ reference of 100% cellulose (Whatman ash free filter paper). XPS spectra were recorded at 100 W and under neutralisation and the analysis was performed with CasaXPS software. Surface elemental composition was determined from wide scans, while surface chemistry was further studied from high resolution data from C 1s, O 1s and N 1s regions. Analysis area is less than 1 mm, XPS analysis depth is less than 1 nm, and each sample was analysed three times.

**Atomic Force Microscopy**

High-resolution AFM images of CLPs were recorded with a MultiMode 8 atomic force microscope equipped with a NanoScope V controller (Bruker Corporation, Billerica, MA). The images were obtained in tapping mode in air using NCHV-A probes (Bruker) with a tip radius below 10 nm. Research NanoScope 8.15 software (Bruker) was used for image analysis, processing and correction.

**Contact angle measurements**

Static contact angles of water (14 μL sessile drop) were measured on thin films of softwood Kraft lignin and Catlig on silicon wafers. A video based instrument KSV CAM 200 (KSV Instruments Ltd., Finland) was used to capture 50 images at one second intervals. Curve fitting and calculation of contact angles followed published procedures.

**Particle size analysis**

Particle sizes and ζ-potentials of the CLP dispersions were measured with a Malvern Zetasizer Nano-ZS90 Instrument. A dip cell probe was used for determination of the zeta potentials. All experiments were performed with 3 samples, with generally 10-50 scans recorded for zeta potential and particle size measurement. Intensity based mean average particle sizes and zeta potential values were used in analysis and reporting of the data.

**Emulsion formation and stability**

Emulsions were formed using variations of oily phase and emulsifier. Toluene, olive oil, and silicone oil were used as oil phases and CLPs, cationic lignin, c-CLPs, and softwood Kraft lignin were used as emulsifiers. Cationic CLPs were prepared by adding cationic lignin solution into CLP suspension at a dry weight ratio of 50 mg/g, and mixing with vortex for 30 seconds. The final concentration of insoluble lignins relative to the total volume of the emulsion mixture was varied at 2.5, 5, 7.5 g/L. Emulsions with Catlig only as the emulsifier contained 0.25 g/L
concentration of Catlig in the final emulsion mixture. Emulsions were prepared by adding the oil phase to the water phase at a 1:1 volume ratio and mixing with vortex on high speed for 60 seconds. Emulsions for visual observation at fixed pH 4 or pH 5 (turbidity scans) or pH-dependent quantification of emulsion stability contained 5 g/L concentration of insoluble lignins and 0.25 g/L of Catlig in the final emulsion mixture. Emulsion stability was determined as the weight fraction of toluene not phase separated from the emulsion layer after centrifugation of the freshly formed emulsions at 500 g (1670 rpm) in an Eppendorf 5804 centrifuge equipped with a type A-4-44 swing bucket rotor.

Confocal and optical microscopy imaging of emulsions

Stable toluene-in-water Pickering emulsions at pH 5 were imaged with and without staining with Nile red and imaged by Sony ExwaveHAD DSP video camera on a confocal laser scanning microscope (Leica DMRXE), using a HC PL APO 20x/0.70 CS objective. Optical microscopy images were recorded on a Leica DC 300 digital camera connected to a Leica DMLAM microscope with an N PLAN 20x/0.40 BD objective. Compression of the emulsions sample was avoided by using a spacer between the microscope slide and the cover slide.

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Notes and references


