Lignin supracolloids synthesized from (W/O) microemulsions: use in the interfacial stabilization of Pickering systems and organic carriers for silver metal†

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Taking advantage of the aromatic and cross-linking tendency of lignin macromolecules extracted from plants, we present a novel method for their assembly into supracolloidal structures. Specifically, spherical particles with controllable size (~90 nm to 1 μm) were obtained from water-in-oil (W/O) microemulsions formulated with a mixture of nonionic surfactants and a colloidal dispersion of a low molecular weight alkali lignin. After spontaneous emulsification, the internal lignin-rich phase was cross-linked to produce the solid particles that could be easily separated by removal of the organic, continuous phase. The efficiency of the fractionated lignin particles to stabilize hexadecane-in-water Pickering emulsions was demonstrated and their properties were compared against those obtained by using traditional inorganic particles. The effect of the particle size of lignin on the emulsion structure is discussed. As a proof of concept we further introduce the use of related emulsions to enable in situ reduction of silver and loading of silver nanoparticles in lignin carriers.

Introduction

Lignin is a biopolymer constructed from monolignol units linked together forming a complex macromolecule. Lignin is readily available and is typically obtained as a byproduct in the chemical processing of wood to liberate cellulosic fibers, making it an attractive aromatic, highly branched macromolecule.1,2 Lignin is recognized as a “green” and easily modifiable molecule with potential use as an inexpensive precursor in the chemical and materials industries. Lignin in its native form plays important roles in plants, which also translates as functional features after its separation, making it an attractive environmentally friendly material for various technologies.3 In addition, it bears abundant functional groups that can be easily modified by methods such as carboxymethylation,4 propargylation,5 etc.

Currently lignin is industrially utilized as a fuel additive,6 strengthener in the concrete industry7 and as a precursor for chemicals.8 Previous research focused on understanding lignin biosynthesis, origins and the structure and chemical features in its native8 as well as isolated9 forms; however, lignin research efforts have shifted recently to highlight its potential for development of high value materials including polyurethanes,10 carbon fibers,11 and biomedical products.12

Recently, preparation of particles from lignin has gained interest given its benign nature, as an alternative to toxic nanoparticles,13 for stabilizing emulsions,14 and for delivery of hydrophobic molecules.15 Lignin particles are known to spontaneously occur when woody biomass is processed by thermomechanical treatments at temperatures above lignin’s glass transition. Heating above the glass transition temperature enables lignin to migrate from the cell wall and precipitate as spherical particles in the nano, submicron and micron size scales.16 Lignin particles can also be engineered by acid and solvent–antisolvent precipitation as well as emulsification.17 Condensation or emulsion polymerization can yield lignin particles that can be used in chemical scavenging18 or for ion-exchange resins.19 Although the cited approaches for lignin particle synthesis have been successful, they also hold a few drawbacks, including the lack of particle integrity and control of size and size distribution. For example, in emulsion polymerization the shear applied to stabilize the emulsion as well as other hydrodynamic effects can have a significant influence on the structure and size of the particles formed. In the case of traditional synthetic polymers, such effects are minimized by preparing polymer particles in the so-called microemulsion region of the surfactant, oil, water (SOW) system.20,21 A microemulsion approach
enables formation of nano- to sub-micron sized droplets dispersed in a continuous phase in the presence of a surfactant (a mixture of lipophilic and hydrophilic surfactants) and an alcohol as a co-surfactant. Microemulsions form spontaneously, and are transparent and thermodynamically stable. Hence, unlike emulsions, microemulsions do not need shear to maintain the dispersed structure.\(^\text{23}\) As stated, this suggests obvious benefits regarding the control of the formed particles. Furthermore, the droplet size in microemulsions can be controlled by tuning the SOW formulation and concentration.\(^\text{24}\) Here we propose a microemulsion system to generate lignin particles.

Among the applications suggested for lignin particles, interfacial stabilization holds great promise, even for large volume utilization. For example, particle-stabilized emulsions or Pickering emulsions\(^\text{25-26}\) can be deployed in food, household products, cosmetics, medicine and others. Pickering emulsions are extremely stable due to the high particle adsorption energy at the interface.\(^\text{27}\) Emulsion type, drop size and stability are dependent on particle hydrophobicity, that is, the contact angle at the three-phase contact line between the particles and the continuous and dispersed phases. It has also been shown that increasing the particle size results in an increased droplet size.\(^\text{28}\) Lignin domains have been shown to be able to stabilize Pickering emulsions as demonstrated by Wei \textit{et al.}\(^\text{29}\) They prepared colloidal clusters of lignin by the addition of hydrochloric acid to adjust the solution pH to 3 and initiate precipitation of lignin into the particle clusters. This dispersion was used for stabilizing styrene-in-water emulsions followed by crosslinking of styrene to polystyrene. It was further shown that lignin particles can be used to form Pickering high-internal-phase-emulsions as a precursor for macroporous foams.\(^\text{30}\)

In this paper we present a method to transform aqueous colloidal dispersions of lignin into particles or supracolloidal structures via condensation. This is achieved by using microemulsification of SOW systems. We explore the lignin microemulsion system as well as investigate the fundamental synthesis factors affecting the structure of the formed particles. Furthermore we observe how the resulting particles assemble at O/W interface leading to the formation of Pickering emulsions. We take advantage of lignin fluorescence under UV\(^\text{31}\) to unveil, via confocal fluorescence microscopy, the location of the lignin particles at the emulsion interfaces.

We explore the effect of the particle size and concentration on stabilization efficiency and compare the efficiency of lignin particles against typical systems used in Pickering stabilization. Finally, reverse micelles containing lignin are proposed as a viable nanoreactor\(^\text{32}\) to prepare gold,\(^\text{33}\) silver,\(^\text{34}\) and magnetite\(^\text{35}\) particles. Therefore, in addition to neat lignin particles, with regular spherical shape and tunable size distribution, the lignin-based microemulsion droplets were used to prepare organic carriers of silver metal.

**Experimental**

**Materials**

Industrial Kraft lignin with low ash content (0.5%) was kindly donated by Domtar Inc. (Plymouth Pulp Mill, NC) and purified by centrifugation (Beckmann Centrifuge Model J-21C, 3000 rpm) by using milliQ-water (Millipore) to yield a solid residue containing 98.4 wt% of lignin. After the purification and prior to use, the lignin was freeze-dried. The lignin molecular weight was ca. 5500 g mol\(^{-1}\).

**Lignin particle preparation**

A surfactant mixture for microemulsification containing sorbitan monoleate (Span 80, Sigma-Aldrich), polyoxyethylene (20) sorbitan monooleate (Tween 80, Sigma-Aldrich) and 1-pentanol (99% pure, Acros Organics) were mixed at a \(2 : 1.2 : 0.9\) ratio (by volume) and dissolved in octane (reagent grade 98%, Sigma-Aldrich). This formulation was designed according to a target hydrophilic–lipophilic balance value, HLB, of \(\sim 7\). Lignin was dissolved in 1 M aqueous sodium hydroxide to a concentration of 20 wt%. For preparing the microemulsion, the lignin solution was added to octane containing the surfactant mixture. The system was mixed by turning the system gently upside-down until the internal phase was completely dispersed. Epichlorohydrin (1-chloro-2,3-epoxypropane, Sigma-Aldrich) was added to the emulsion under gentle stirring for 15 minutes. Epichlorohydrin-to-lignin weight ratios (dry basis) ranged from 0.1 : 1 to 5 : 1. The respective emulsion was then kept at 55 °C for 40 minutes under gentle stirring to initiate the crosslinking reaction between epichlorohydrin and lignin. The resulting particles were separated from the oil by adding excess water and mixing. This produced a turbid emulsion that was then subjected to centrifugation (9000 rpm, 20 min) to separate the oil (upper) phase from the bottom (aqueous) phase containing lignin particles. The clear oil phase was replaced with water and the procedure was repeated until all of the oil was removed. The dispersions were stored until use in a refrigerator at 8 °C.

Similar microemulsion systems with total 20 wt% lignin dispersed in an oil-continuous phase were prepared in the presence of silver nitrate (AgNO\(_3\), 99% pure Sigma-Aldrich) and sodium borohydrate (NaBH\(_4\), 99% Acros). AgNO\(_3\) and NaBH\(_4\) were each incorporated into two separate microemulsions at a concentration in the precursor lignin solutions of 0.1 M. Reduction to silver occurred by combining the lignin microemulsions carrying the silver nitrate and sodium borohydrate and the obtained lignin-based droplets were cross-linked with epichlorohydrin, yielding silver-loaded lignin particles.

**Preparation of Pickering emulsions with lignin particles**

The synthesized lignin particles were used to prepare Pickering emulsions produced by mixing hexadecane and an aqueous phase containing the lignin particles (forming stable hexadecane-in-water macro-emulsions). Emulsification of the system was performed by sonication (1 min) with a Branson S-450D microtip sonicator. The 1 min sonication consisted of cycles of 3 s sonication and 3 s pause times, using a 10% amplitude. Additionally a photocurable product, Norlan optical adhesive 81, was used to prepare samples of the particle-stabilized emulsions for SEM observation. The adhesive was added to the aqueous solution containing the lignin particles, the system was sonicated similarly to produce hexadecane-in-water emulsions.
emulsions and exposed to UV light for 30 s. Finally a drop of the system was placed on a silicon surface and dried at 40 °C.

Characterization methods
A Malvern Zetasizer NanoS90 was used to measure the size of the lignin particles and a Zetasizer ZS to determine the zeta potential. A Scanning Electron Microscope FEI Quanta 3D FEG was used for imaging the lignin particle dispersions dried on silicon surfaces. A Perkin Elmer Fourier Transmission Infrared (FTIR) spectrometer equipped with an Attenuated Total Reflectance (ATR) unit was used for analyzing the dried lignin dispersions. A dynamic contact angle analyzer PHX300 from Surface Electro Optics (SEO) equipped with a FireDragon camera from Teli Digital was used for analyzing wetting of lignin films. The lignin films were prepared by dissolving 0.5 wt% of lignin into 1,4-dioxane and using a spin coater (WS-400A-6NPP, Laurell Technologies Company) at 3000 rpm to apply it on a gold substrate. The Pickering emulsions were investigated by fluorescence microscopy using a Zeiss LSM 710 confocal unit attached to a Zeiss Axio Observer Z1 microscope. The objective used was LD C-Apochromat 40 ×/1.1 NA Water Immersion (from Zeiss). Prior to droplet imaging, the emulsion was diluted by adding excess of water. Then a drop of the diluted sample was placed on a microscope glass slide. A cover glass was placed on top of the drop while keeping the two cover glasses apart with a spacer, to avoid compression of the emulsion droplets.

Results and discussion
We first present a novel route for the formation of lignin-based supracolloidal structures from microemulsion precursors. The usefulness of the synthesized particles, which can be obtained in a range of sizes, is then demonstrated in the stabilization of oil-in-water Pickering emulsions. Finally, the synthesis of lignin particles carrying silver metal is reported. For this purpose two lignin-containing microemulsions, one carrying silver nitrate and the other with a reducing agent, are reacted to trigger silver nanoparticle formation.

Microemulsions to prepare lignin particles
Microemulsions were used as precursor systems to generate lignin particles after crosslinking the dispersed (internal) lignin solution in the continuous oil phase. The mixed surfactant system used for the microemulsion formation consisted of nonionic surfactants and a co-surfactant (pentanol) that resulted in a hydrophilic–lipophilic balance (HLB) of ~7. This formulation enabled formation of translucent water-in-oil (W/O) microemulsions that formed spontaneously, as reported in the case of other systems stabilized by surface-active molecules.21 In the context of the present lignin particle synthesis, the combination of lipophilic and hydrophilic surfactants as well as the co-surfactant allowed the formation of the lignin-containing microemulsion.

As a reference, ternary phase diagrams of lignin-free systems were obtained by using formulation scans that indicated the maximum amount of the aqueous solution (dispersed phase) that can be incorporated into the oil (continuous phase) while displaying the characteristics of a microemulsion. The highest surfactant concentration used was 15 vol% and the phase behavior with surfactant concentrations below this limit can be observed in Fig. 1 that includes a ternary phase diagram. Here, a 1 M NaOH solution was used as the internal phase (no lignin added). The solid black line in Fig. 1 of the ternary phase scans represents the composition limits between neighboring single-phase (1) or two-phase (2) zones.

Conventional microemulsions can hold charged polymers (polyelectrolytes) in the dispersed phase provided their size (defined mainly by the molecular mass) is small enough compared to that of the droplets.24 The molecular mass of the lignin utilized here is in fact small enough for effective incorporation into stable, translucent microemulsions (see the photo in Fig. 1). No phase separation, precipitation, flaking or creaming was observed with time.

In addition to the molecular mass, incorporation of macromolecules into microemulsions depends on the interactions with the surfactant stabilizing the dispersed droplets. Electrostatic repulsions between a surfactant and the given macromolecule can lead to a reduced single-phase microemulsion zone of the ternary diagram.25 However, in the case of lignin and nonionic surfactants, such interactions may not be relevant.

Compared to the case of lignin-free aqueous solution (solid line in Fig. 1), the presence of lignin in the internal phase facilitated microemulsion formation as can be noted by the higher capacity to hold the internal phase. In other words, the single-phase zone of the ternary diagram is expanded with lignin addition (note the dashed line in the ternary diagram of Fig. 1 and the proportion of aqueous phase W). This effect was noticeable in the case of the 10 vol% surfactant concentration, which allowed an increase (by 5 vol%) of the internal phase. In the case of the 15 vol% surfactant system, incorporation of 5 vol% internal phase resulted in partial precipitation. Similarly, with the 5 vol% surfactant system only 1 to 2 vol% of the internal phase could be added without precipitation. Indeed, it has been previously shown that incorporation of polyelectrolytes into microemulsion droplets can expand the inverse micellar region, that is, increase the amount of the internal phase.35 This is due to the increased stability of the droplets resulting from polyelectrolyte–surfactant affinity. Lignin and nonionic surfactants are known to interact in an aqueous environment via a number of interactions;36,37 hence, lignin can increase the amount of internal phase that can be incorporated, especially in the case of 10 vol% surfactant systems.

Supracolloidal structures were prepared from microemulsions that contained lignin in the internal (aqueous) phase and with octane as the continuous phase. This was carried out by the addition of epichlorohydrin to the microemulsion and heating (55 °C) for 40 min. The resulting structures shown as solid particles were separated from the synthesis medium by centrifugation. Compared to traditional emulsification techniques, the benefit of microemulsions as the precursor system of such supracolloids lies in the fact that their particle size can be controlled by the choice of the surfactant and composition.24
Additionally, shear is not required to maintain the stability. The emulsification conditions chosen for generating lignin particles are designated with letters ("a" to "h"), in the ternary phase diagram of Fig. 1. As indicated, the dashed line in the ternary diagram divides the conditions for producing single- and two-phase lignin-based emulsions. Experimental points within both conditions were chosen. Using surfactant concentrations of 5, 10 and 15 vol% led to the formation of mainly lignin spherical particles from the microemulsions (SEM images a–h in Fig. 1) and these findings are discussed below in detail.

By using 5 vol% surfactant and lignin concentrations in the dispersed phase of 1 and 2.5 vol% (Fig. 1a and b) spherical supracolloids were obtained as solid, integral particles. A further increase in the amount of the dispersed phase resulted in fused structures (Fig. 1c). This latter observation agrees with the stability of the microemulsion system (see the dashed line in the ternary phase diagram) since at internal phase concentrations higher than 2.5% the system is no longer a single-phase microemulsion. Increasing the surfactant concentration to 10 vol% allows the occurrence of a single-phase zone, even at high internal phase content, yielding spherical particles (Fig. 1d–f). The diameter of the particles depended clearly on the amount of the dispersed phase and correlated with the increase in internal phase concentration. The clustering/fusing of the particles seen in some of the SEM images may be an artifact from sample preparation (drying, etc.).

For a surfactant concentration of 15 vol% a reduced system stability was observed for relatively high internal phase concentrations (5 vol%). The 1 and 2.5 vol% lignin internal phase could be incorporated into the oil continuous phase. However, by using the 1 vol% internal phase concentration no particles were detected. It should be noted that upon heating, this emulsion tended to collapse resulting in phase separation. This is probably due to the high surfactant concentration compared to that of the internal phase. When the internal phase concentration was further increased the resulting particles fused in a lignin-rich film (Fig. 1g). When the composition of the system was outside the single-phase region (for example, at 5% internal phase concentration), large particle clusters were formed (Fig. 1h). This is expected, since this condition favors the coalescence of the precursor droplets, which serve as particle templates.

The droplet size and structure of typical microemulsions are affected by the surfactant formulation and concentration. In general, the microemulsion droplet size decreases as the surfactant concentration increases. Similar correlation should...
exist when the microemulsion droplets are collapsed into solid particles. This occurs in fact in the present system: note the change in the particle size for particles produced following the compositions corresponding to the solid black line in the ternary diagram (Fig. 1 and the corresponding SEM particle images in a, e and h). A similar trend in particle size can be observed for particles prepared using 1 and 2.5 vol% lignin internal phase with varying surfactant concentrations: the diameter of the particles decreases with surfactant concentration, within the single-phase region (note the trend in particle size in Fig. 1, between a and d as well as e and g). This effect does not apply for particles obtained with emulsions formulated outside the single-phase zone.

According to the SEM observations, the optimal conditions for preparing spherical, well-defined supracolloid particles include 5 and 10 vol% surfactant concentrations and an addition of 2.5 vol% of the dispersed, internal phase. These conditions resulted in particles with approximately 150 nm SEM diameter. Table 1 presents light scattering data confirming the particle sizes. In the case of the well-defined particles shown in Fig. 1b and e the light scattering particle size was also ~150 nm. In the case of the large, fused particles observed in Fig. 1c, f and h the increased standard deviation reflects the observed size irregularity. Light scattering analysis gives supporting evidence to the fact that the smallest particles observed by SEM imaging (Fig. 1d) aggregated into large clusters.

Crosslinking mechanism

The lignin particles presented in Fig. 1 were generated by (epichlorohydrin) crosslinking of the lignin-containing droplets dispersed in the oil-continuous phase. Crosslinking occurs by ring opening and attachment to the phenolic hydroxyl group of lignin (see Scheme S1a in the ESI†), dehydrochlorination and reaction of another phenoxy ion with the ring (Scheme S1b and c, ESI†), finally leading to the cross-linked structure (Scheme S1d, ESI†). Crosslinking is affected mainly by the epichlorohydrin-to-lignin ratio as well as the reaction temperature. According to Saidane et al. the epoxidation reaction rate (Scheme S1b, ESI†) is higher than that of crosslinking with the oxirane ring (Scheme S1c, ESI†), and hence, some of the requisites to favor crosslinking over epoxidation include a high lignin concentration in solution, high alkali concentration to enable dissolution and generation of phenoxy ions and low epichlorohydrin-to-lignin ratio to minimize the formation of epoxidized (Scheme S1e, ESI†) over cross-linked (Scheme S1d, ESI†) lignin. Temperatures above 70 °C have been reported to result in the epoxidized form whereas lower temperatures favor the crosslinking reaction.

The particles presented in Fig. 1 were generated utilizing an epichlorohydrin-to-lignin ratio of 5 : 1 (by weight). For practical considerations as well as to favor the crosslinking reaction over epoxidation, a lower epichlorohydrin concentration can be used. Hence, the synthesis of particles utilizing 10 vol% and 15 vol% surfactant concentrations was repeated using 1 : 1 and 0.1 : 1 epichlorohydrin-to-lignin ratios (by weight). The microemulsions containing 1 or 2.5 vol% lignin using the reduced epichlorohydrin addition resulted in a low yield (larger removal of lignin during the centrifugation). However, the addition of 5 vol% lignin phase to the emulsion and decreasing the epichlorohydrin-to-lignin ratio from 5 : 1 to 1 : 1 resulted in the formation of spherical particles both with the 10 and 15 vol% surfactant systems, see Fig. 2a and b, respectively. The structure of these particles was significantly enhanced (better defined particles and more uniform size distribution) compared to their counterparts produced using the epichlorohydrin-to-lignin ratio of 5 : 1. The latter are presented in Fig. 1f and h, respectively. The light scattering particle sizes from the 1 : 1 crosslinker-to-lignin ratio were 467 ± 24 nm and 316 ± 8 nm for the 10 and 15 vol% surfactant systems, respectively. When the crosslinker-to-lignin ratio was further decreased to 0.1 : 1 the structure of the particles drastically changed towards particle clusters (Fig. 2c and d). Clearly this epichlorohydrin concentration was not appropriate to generate individual particles but soft particle clusters that formed after drying on the solid support used for SEM imaging. Hence, excess of epichlorohydrin leads to particle clustering (see Fig. 1h, for example) while a

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Fig. 2. Lignin-based particles obtained from microemulsions with an epichlorohydrin-to-lignin ratio of 1 : 1 (a and b) and 0.1 : 1 (c and d). The precursor system included lignin dispersed in water (5 vol%) and 10 (a and c) or 15 (b and d) vol% surfactant.
low dosage results in incomplete crosslinking (leading to fused particles as shown in Fig. 2d). An optimum addition level leads to generation of more integral particles (see Fig. 2a, for example). All in all, besides the surfactant formulation, composition and internal phase concentration the crosslinker-to-lignin ratio is an additional, critical parameter for generation of the particles.

The particles presented in Fig. 2a were tested for their integrity in alkaline conditions. The pH of the aqueous dispersion was set to 8.3 and the obtained particles were monitored during 24 h (light scattering and SEM imaging) before and after adjusting the pH to a value of 13. The particle size after the alkaline treatment was $479 \pm 15 \text{ nm}$ similar to that measured before increasing the pH ($467 \pm 24 \text{ nm}$). SEM observations further confirmed the integrity of the particles (ESI, Fig. S1†).

Crosslinking of lignin by epichlorohydrin was further verified by FTIR: see Fig. S2 of the ESI,† which contains detailed FTIR analysis of lignin particles, functionalization and cross-linking rate. As a conclusion, when the crosslinker concentration increases, more epoxidized lignin particles are obtained whereas lower epichlorohydrin dosages yield both epoxidized and cross-linked structures.

**Assembly of lignin particles at the O/W emulsion interface**

Selected grades of the lignin particles presented in Fig. 1 and 2 (particle size of $\sim 90$, 320 and 600 nm) were investigated with regards to their ability to assemble at an emulsion interface, that is, to stabilize Pickering emulsions. As such, the lignin particles were incorporated into a hexadecane-in-water system and emulsified. The assembly and the emulsion structure are mainly affected by the interaction of the particles with the oil and water phases. Evidently, for a stable emulsion to form the particles need to adsorb at the interface to prevent droplet coalescence. For spherical particles used as stabilizers the settling of the particles in an emulsion can be predicted by the contact angle at the interface. Moreover the particle packing at the interface affects the curvature. Typically a net hydrophilic layer will curve towards the oil and hence results in the formation of O/W emulsions. The water contact angle of a film consisting of the Kraft lignin was $ca. 50^\circ$, indicating a fairly hydrophilic system. Consequently, the Pickering emulsions stabilized by the lignin particles were expected to be oil-in-water (O/W).

Lignin particles of $ca. 320 \text{ nm}$ were used at 0.6 and 0.2 wt% concentrations based on the total emulsion. In both cases lignin particles surrounding the droplets were observed by fluorescence imaging (Fig. 3a and b). The diameters of the oil droplets were approximately $7.7 \pm 1.6$ and $22.3 \pm 3.6 \mu\text{m}$ for the 0.6 and 0.2 wt% particle concentrations, respectively. Clearly the lower stabilizing particle concentration led to larger droplets. Moreover, as observed in Fig. 3a and b, the coverage of the droplets by the lignin particles increased as the particle concentration increased. This implies that the particles have a high affinity to the interface compared to the bulk, continuous phase. Similarly, particles of $ca. 600 \text{ nm}$ led to encapsulated oil droplets (Fig. 3c). Evidently, the layer formed by the larger particles was thicker than that for the smaller, $320 \text{ nm}$ ones. This is explained by the fact that the large particles are less effective to form a dense, packed layer. The average diameter of the oil droplets in Fig. 3c was $35.4 \pm 2.2 \mu\text{m}$, that is, larger than the droplets stabilized by the small lignin particles.

![Fig. 3](Image)

Fig. 3  Fluorescence imaging of hexadecane-in-water (oil volume fraction, 0.3) Pickering emulsions containing 0.6 (a) and 0.2 wt% (b) lignin particles (diameter, 320 nm). Included is also the case of emulsions with 0.2 wt% of lignin particles of larger (600 nm) (c) and smaller (90 nm) sizes (d). SEM images of lignin particles on a surface of oil-in-water emulsion containing a photocured polymer (Norlan optical adhesive 81) as the oil phase and 0.4 wt% of the lignin particles in the aqueous phase are shown in (e) and (f), respectively. The scale bar in (a–d) is 10 \mu\text{m}.

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Although the lignin particles with a diameter of ca. 90 nm adsorbed at the interface between hexadecane and water (Fig. 3d), the emulsions retained their structure only for a few hours. This is in contrast to the results from the larger particles and is explained by the fact that the particles adsorbed at the interface reduce the contact area between the two immiscible liquids. The contact angle of the particle at the interface as well as the size of the particles determines the extent of the area lost. This area together with the tension of the interface determines the energy required to remove the particle from the interface. The energy of desorption increases with increasing particle size until gravitational forces dominate. Small particles can behave similar to surfactants at the interface and adsorb and desorb fast.

The emulsions presented in Fig. 3a–c containing lignin particles of ca. 320 and 600 nm were stable over an observation period of one week (see light microscopy images in the ESI, Fig. S3†). The sizes of the oil droplets corresponding to the emulsions in Fig. 3a–c after one week were 9.3 ± 1.6, 21.9 ± 3.6 and 34.9 ± 5.7 μm, respectively. Hence, no apparent changes in the droplet size were observed. In Pickering emulsions coalescence of the droplets is inhibited by the formation of a particle network at the interface. This stabilization is further improved if the particles can keep a large enough distance between the droplets in order to minimize their interactions. The most efficient way to do this is by favoring conditions for the formation of a 3-dimensional structure in the continuous phase. In the present case the larger particles were effective in this regard.

To further examine how the lignin particles assemble at the oil/water interface we prepared an O/W emulsion using a UV-curable adhesive as the oil phase. The concentration of lignin particles (150 nm diameter) was approximately 0.4 wt% of the total volume (Fig. 1e). The emulsions resembled those prepared with hexadecane as the oil phase. The optical adhesive could be cured by exposure to UV light and it solidified into large spherical droplets. SEM images of these droplets (Fig. 3e) revealed that the lignin particles settled at the interface (Fig. 3f) but did not fully cover the surface. As a result, the solid (adhesive) droplets merged together forming a connected network. It is clear from these observations and from Fig. 3a and b that high particle coverage is needed for an efficient barrier against droplet coalescence. However, quite interestingly, the structure shown in Fig. 3e suggests that such a moderate coverage could be used for preparing porous structures by allowing the droplets to connect upon crosslinking.

Fig. 4 presents fluorescence microscopy images of the Pickering emulsions with increasing oil volume fraction, from 0.2, 0.3 and 0.5 and stabilized by lignin particles (Fig. 4a–c). The amount of the particles with respect to the total volume of the emulsions was kept constant. It can be observed that as the amount of the oil in the emulsion increases the size of the hexadecane droplets increased from 9.1 ± 2.2 to 11.0 ± 1.9 and to 15.2 ± 2.6 μm, respectively. Larger amounts of the dispersed oil phase were not explored but it has been shown that colloidal clusters can be used as a stabilizer for high internal phase emulsions.15

It is clear from the fluorescence microscopy images in Fig. 3 and 4 that lignin particles adsorbed at the oil/water interface enabling the stabilization of the Pickering emulsions. The difference in the appearance and structure of the Pickering layer as a function of particle size (case of the hexadecane-in-water emulsions) can be observed in the microscopy images presented in Fig. 3. The small lignin particles formed a thin and uniform layer whereas the larger particles formed a bulkier one. These Pickering emulsions followed the behavior of traditional systems:20 As the size of the stabilizing particles increases the amount needed for efficient stabilization decreases. The size of the stabilized oil droplets increased as the particle size increased whereas the stability in general decreased as the stabilizing particle size decreased.

Reaction in microemulsion media to synthesize functional lignin carriers

Microemulsion can be used as a template for the synthesis of inorganic particles.40 The well-defined droplet dimensions enable a confined volume for effective chemical reactions. The synthesis constituents are typically brought into contact by mixing two identical microemulsions containing, for example, a metal precursor salt and an initiator. Collision and coalescence of the droplets bring the reactants together enabling a close interaction and particle generation.41 Furthermore, by incorporating a polymer into the dispersed phase it is possible to obtain inorganic particles or to facilitate their modification.42
Silver reduction in W/O microemulsion has been reported by Barnickel et al. who used microemulsions containing AgNO₃ and NaBH₄. Following similar approaches, organic particles were functionalized with silver from lignin-containing microemulsion droplets. Here, the lignin droplet was expected to act as a nanoreactor for silver reduction. We prepared two microemulsions, one containing silver nitrate and the other with sodium borohydride. The dispersed phase consisted of 1 M NaOH (Aq) or 1 M NaOH (Aq) with 20 wt% lignin. Epichlorohydrin was used to crosslink the particles. In all cases 2.5 vol% of the internal phase was added and the oil phase contained 10 vol% of the surfactant mixture. These parameters correspond to the lignin particles presented in condition “e” of Fig. 1.

In the absence of lignin and by mixing the two microemulsions, one containing 0.1 M AgNO₃ and the other with 0.1 M NaBH₄, large clusters that precipitated immediately were size determined by light scattering was 227 ± 3 nm, larger than that of the corresponding neat lignin particles (153 ± 4 nm, Table 1). Bright spots were observed in SEM images within the lignin particles, which corresponded to the nucleated silver (Fig. 5b). However, it is expected that most of the silver synthesized resides inside the lignin matrix. The zeta potential of the silver-functionalized lignin particles was −43 ± 1 mV, slightly less negative than that of the neat lignin particles (−51 ± 1 mV).

From the FTIR results regarding the crosslinking of the particles it is concluded that the incorporation of silver interfered with crosslinking (Fig. S2, ES†). It is interesting to note that in the absence of epichlorohydrin the appearance of the dispersion observed prior to or after heat treatment consisting of the two microemulsions combined together was similar to those containing epichlorohydrin. However, during separation (via centrifugation) of the particles from the oil phase, the lignin phase remained in the supernatant and was removed, leaving only a small amount of a dark residue as a pellet. According to SEM, this residue contained aggregates rich in silver (Fig. 5c). Clearly the lignin cannot be kept as a solid particle without crosslinking. In general, it is demonstrated that lignin-containing microemulsions can be used as nanoreactor media to incorporate active substances or a cargo into biodegradable carriers.

Conclusions

In this work we have demonstrated that lignin can form sub-micron supracolloidal particles from microemulsion precursors. Emulsion formulation and composition of the internal phase, surfactant and crosslinker concentrations were used to control the size and integrity of the resulting particles. Moreover, lignin-containing microemulsions were effective to synthesize carriers of silver nanoparticles. The well-defined, lignin-based particles assembled at O/W Pickering emulsions. The effect of size of the lignin-based particles on the emulsion structure and stability followed the behavior observed in traditional Pickering emulsions: a larger particle size resulted in an increased emulsion drop size and stability.

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