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Scaling up production of colloidal lignin particles

Timo Leskinen, Matthew Smyth, Yao Xiao, Kalle Lintinen, Maija-Liisa Mattinen, Mauri A. Kostianen, Pekka Oinas, Monika Österberg

KEYWORDS: Colloidal lignin particles (CLP); Lignin nanoparticles (LNP); Tetrahydrofuran (THF); Emulsion; Nanoprecipitation; Fractionation; Upscaling; Thermal post-treatment

ABSTRACT: Fundamentals of nanoprecipitation process to form colloidal lignin particles (CLPs) from tetrahydrofuran (THF)-water solvent system were studied, and applied in establishment of a robust reactor design for scaled-up CLP production. Spherical lignin particles with an average diameter of 220 nm could be produced by the new reactor design. Evaporation was applied for removal of THF, concentration of the CLP dispersions, and finally for drying of the CLPs into flake like dry form. The dried CLPs could be re-dispersed in water to restore their colloidal form by applying short physical agitation. Salt triggered sedimentation of the particles was also investigated as a way for reducing the energy consumption related to water evaporation from the CLP dispersions. Aqueous thermal post-treatments were demonstrated to yield structural reinforcement of the CLP structure against solvation in various lignin solvents. In summary, the presented work pushes forward the conceptual design of large-scale CLP production, and addresses some of the foreseen technical challenges.

ADDRESSES OF THE AUTHORS: **Timo Leskinen** (timo.leskinen@aalto.fi), **Matthew Smyth** (matthew.smyth@aalto.fi), **Maija-Liisa Mattinen** (maijsa-liisa.mattinen@aalto.fi), **Monika Österberg** (monika.osterberg@aalto.fi), Bioproduct Chemistry, Department of Bioproducts and Biosystems (Bio2), School of Chemical Engineering, Aalto University, P.O. Box 16300, FI-00076 Aalto, Espoo, Finland, **Yao Xiao** (yao.xiao@aalto.fi), **Pekka Oinas** (pekka.oinas@aalto.fi), Plant Design, Department of Chemical and Metallurgical Engineering, Aalto University, P.O. Box 16100, FI-00076 Aalto, Espoo, Finland, **Kalle Lintinen** (kalle.lintinen@aalto.fi), **Mauri Kostianen** (mauri.kostianen@aalto.fi), Biohybrid Materials, Department of Bioproducts and Biosystems (Bio2), Aalto University, P.O. Box 16300, FI-00076 Aalto, Espoo, Finland

Corresponding author: Timo Leskinen

Introduction

Green technologies are being developed continuously to answer the global demand of energy and raw materials, and biopolymers are seen to have a pivotal role in this development. The recent trends in climate policies, such as the Paris agreement, and governmental interest in bioeconomy (Ministry of economic affairs and employment of Finland 2014) have amplified the biopolymer research based on lignocellulosic raw materials during the last years. Lignin biopolymer as part of woody biomass has been harvested and processed sustainably for decades by the existing biorefining

infrastructure of pulping and biochemical industries, while the end use has been mainly limited to energy production. (Ragauskas et al. 2014; Norgren, Edlund 2014; Gellerstedt 2015; Strassberger et al. 2014). Kraft lignin as a low refined by-product has very limited value and markets as a polymeric raw material, and only about 90 thousand tons is sold annually of the total 50 million tons that is processed by pulping industry (Strassberger et al. 2014).

To provide higher value to the producers than just its value as an energy source, upgrading of raw lignin into higher quality raw material for material applications has been studied actively for decades (Duval, Lawoko 2014; Kai et al. 2016). The general understanding of fundamental characteristics of lignin and pathways for modification its physical properties have gained good ground, but there are still several challenges to be overcome to harness profit from this biopolymer. The commercial breakthrough of lignin as polymeric raw material faces several challenges: 1) Requirement of low investment costs in terms of energy usage and infrastructure 2) Lack of competitive properties of low cost lignin products in comparison to existing products on the market. 3) Fulfillment of the aforementioned requirements by inexpensive green process solutions. The scarcity of existing commercial lignin products is a good indicator that such demands have not been met with the past technologies, and therefore new technologies for value addition are needed. Lignosulfonates form an exception with their estimated annual production of one million ton for applications such as dispersing agents in concrete (Strassberger et al. 2014). Conclusively, there is a great need for lignin upgrading technologies that could utilize lignin streams generated by kraft pulping or enzymatic saccharification of lignocellulosic biomass for biofuels, as these are or will be the major sources for low refined lignins.

Conversion of lignin into colloidal lignin particles (CLPs), or lignin nanoparticles (LNPs), shows great promise for refining the technical lignins into more widely utilizable raw materials, as these possess different properties in terms of colloidal behavior and surface chemistry (Duval, Lawoko 2014; Fortunati et al. 2016; Zhao et al. 2016, Ago et al. 2017; Beisl et al. 2017). Colloidal lignin particles can be produced by a variety of methods, as summarized by Beisl et al. in their recent review. Widely used method is the nanoprecipitation process, denoted also as solvent shifting (Beisl et al. 2017), where supersaturation of lignin polymer solution is caused by shifting into non-solvent rich medium in a controlled fashion to produce particles. Typically this done by adding water (a non-solvent) into lignin solution in organic solvent (a solvating solvent, such as THF). (Lievonen et al. 2016; Li et al. 2016a; Li et al. 2016b; Yearla, Padmasree 2016; Richter et al. 2016; Xiong et al. 2017a; Xiong et al. 2017b; Tian et al. 2017) The resulting particles commonly have spherical shape, diameter of above hundred nanometers, and negative surface charge, providing them

with stability in aqueous dispersions. Particle properties, such as morphology, particle size and surface charge are largely determined by the applied parameters of the formation process, such as solvent system, concentration and characteristics of the lignin polymer, and technique applied for implementation of the solvent shifting process. (Lievonon et al. 2016; Li et al. 2016a; Li et al. 2016b; Yearla, Padmasree 2016; Richter et al. 2016; Xiong et al. 2017a; Xiong et al. 2017b; Tian et al. 2017) Adsorption-based processes utilizing synthetic polymers (Lievonon et al. 2016; Richter et al. 2016) or proteins (Leskinen et al. 2016) can be used for further tailoring of the surface chemistry of the CLPs. Even magnetization of the particles has been introduced for high-end applications, such as drug delivery (Lintinen et al. 2016; Figueiredo et al. 2017). Such modifications to the chemical compatibility and functionality will widen the applicability window of CLPs for colloids, composites, and various healthcare products.

Fundamental research is still required to develop process-engineering solutions for feasible upscaling of the CLP technology from laboratory scale to commercial production. Further insight to the formation mechanisms of CLPs is needed in order to establish key parameters that need to be optimized for large-scale operations. Ultimately, the true value of CLP technology is determined by its suitability for high volume applications, such as composite materials (Jiang et al. 2013; Yang et al. 2015; Fortunati et al. 2016), or emulsifier systems (Nypelö et al. 2015; Ago et al. 2016). For commercial applicability, the formed CLP dispersions should be able to be converted into dry powder preparations, and physicochemical durability of the particles under various conditions should be guaranteed. Establishment of ways to do so is essential for paving the way for CLP technology from laboratories to industrial realm.

This article covers various aspects related to up scaling of CLP production into a feasible commercial operation. We have examined how the formation of lignin particles in THF-water based solvent system can be influenced by controlling several factors related to the used conditions, and propose a formation mechanism that controls the CLP production process. In addition, we demonstrate a technology for CLP production that can be readily scaled-up from few milliliters to several liters. The design of this process allows it to operate independently from the total volume of the reactor, making readily up-scalable. We demonstrate a scalable method for obtaining highly concentrated CLP dispersions and powdery re-dispersable lignin particles. As a post-treatment, CLPs were subjected to a thermal treatment in order to induce condensation of the lignin polymers to produce a more durable particle structure.

Materials and Methods

Materials

Solvents used were reagent grade from suppliers Sigma-Aldrich (Finland) or Fisher Scientific (Finland). De-ionized water was used for all sample preparations. Starting material for CLPs was LignoBoost™ softwood Kraft lignin from Valmet (Finland).

Preparation of aqueous CLP dispersion

Lignin was first dissolved in THF-water-mixture. A ratio of 3:1 of the solvents was used in all other experiments, except for the addition rate experiments where a 5:1 ratio was used. Lignin was let to dissolve into solvent mixture with applied magnetic stirring until full dissolution. In case of batches above 4 ml, the solution was filtered with Whatman GF/F filter (GE Healthcare, USA) prior to CLP formation. In majority of the experiments, water was added rapidly into the lignin solution either by pouring from a beaker or by syringe, depending on the scale of the experiment, while applying rapid magnetic stirring. Alternatively, lignin solution was injected into water by syringe. Flow rate of injection was controlled with different needles and syringe sizes. In the experiment with controlled pH, the required concentrations of sodium hydroxide between 0.1 and 6 mM were supplemented to added water in order to obtain different pH values of final dispersions.

Scaled-up CLP preparation was carried out in a cylindrical 10-liter glass vessel equipped with an overhead stirrer (presented in Fig 1). First 4.0 l of water was loaded into the reactor whereupon 2.0 l of 2 % lignin solution in THF-water (3:1) was added into the reactor from an addition funnel via a tube while maintaining rapid stirring. An increase of temperature by 5°C was recorded from the dispersion upon CLP formation.

THF was removed from the formed CLP dispersions, either by dialysis in (Spectra/Por® 1 Standard RC Dry Dialysis Tubing, 6–8 kDa, Spectrum Labs, USA), or by evaporation with pressure controlled rotary evaporator (Büchi Labortechnik AG, Switzerland) as described below. In the case of small-scale 4 ml analytical experiments, 1 ml samples were withdrawn right after CLP formation and diluted for particle size analysis. THF was not removed, as its concentration in the diluted samples was below 2 wt%.

Fractionation of lignin by THF-water

LignoBoost lignin was first dissolved in THF-water 3:1 solvent mixture to make a 15 wt% solution. After full dissolution of lignin into the initial solvent mixture containing 25 vol% water, more water was added slowly with rapid stirring until the water content reached 55 vol%. At this stage, a gel like precipitate was slowly formed, that was sedimented by centrifugation at 3500 RCF/4000 rpm for 20 min using SL 40 FR centrifuge with a TX-750 swinging bucket rotor (Thermo scientific, USA). The clear supernatant was recovered by pipetting and the solid product recovered by solvent evaporation. The gel like insoluble fraction could not be fully separated from the rest of the supernatant. In another experiment, the insoluble fraction was let to sediment by gravity, and the amount of soluble material was determined based on gravimetric solids content of the supernatant and the bottom layer of the solution that contained the sediment and part of the dissolved material.

Concentration, drying and re-dispersion of CLP

Evaporation was performed using a rotary evaporator. The majority of the THF was evaporated first at 40 °C under 80 mbar and water was evaporated at 45 °C under 30 mbar

pressure until desired concentration or completely dry material was obtained.

For re-dispersion, 0.7 g of dried CLPs were added to 70 ml of de-ionized water. Then the mixtures were agitated for 1, 3, 5, and 15 min with Ultra-Turrax T18 basic homogenizer (IKA, Germany) or Sonorex digitec ultrasonic bath (Bandelin, Germany). Longer agitation times of 15 min, 1 h and 24 h were used with magnetic stirring.

Sedimentation and re-dispersion of CLP by salt addition

Sodium chloride (1 mol/l) was added into CLP dispersion and mixed until fully dissolved. The dispersion was let to stand at RT for 4 days, with the majority of the sediment formed during the first 12 h, resulting in a sediment on the bottom of the bottle consisting of the majority of CLPs. The supernatant was recovered by pipetting from above the sediment. The CLP sediment was dispersed in 60 ml of water and added into ultrafiltration cell. The diafiltration was carried out using Amicon 8200 stirred cell (Merck Millipore, Germany) with UP150 P-polyethersulfone-coated polyethylene/polypropylene (PE/PP) 150 kDa cut-off membrane (Microdyn-Nadir, Germany). The filtration was done using 1 bar pressure. 20 ml portions were filtered out and replaced by fresh water in 6 consecutive cycles, diluting the salt concentration below 1 mM. The diafiltered product was diluted to 50 ml volume prior to analyses.

Heat treatment

CLP dispersion (100 ml; 3 mg/ml) was loaded into a 200 ml Parr 4755 general-purpose pressure vessel (Parr Instrument Company, USA). The reactor was submerged into a heated oil bath. The temperature inside the reactor was recorded using a Testo 175T3 data logger with a K-type thermocouple. Bath temperatures of 150, 180 and

200°C were used, where equilibrium temperatures of 144, 174 and 191 °C were recorded from inside the reactor. The warming of the reactor to equilibrium temperature took 30 min, and reaction at steady target temperature was continued for 30 min. After reaction, the reactor was cooled down rapidly in cold water.

Particle size and zeta potential analyses

A Nano-ZS90 Zetasizer (Malvern, UK) was used to determine average particle sizes and zeta potentials. Samples were withdrawn from the CLP preparations, diluted to below 0.3 mg/ml concentration, and 1 ml was added into a polystyrene cuvette. A dip cell probe was used for the electrophoretic mobility measurements, which were used to obtain the zeta potentials. Triplicate scans were recorded for zeta potential and duplicate for particle size measurement. Intensity based mean average particle sizes and zeta potential values of the CLP dispersions were collected.

Results and discussion

General scheme of scaled-up CLP production

This study focused on the unit operations that need to be established for the production of CLPs in industrial scale (see Fig 1). Experimentation was carried out around the particle formation by nanoprecipitation, recovery of the utilized solvent, drying of formed CLPs into solid form, and finally re-dispersing them into an aqueous dispersion. We demonstrate the first design of a robust bench scale reactor for the formation of particles, which holds potential to be scaled up further into pilot and commercial scale. Each of the following processes were also studied in order to gain insight about their feasibility for up scaling, and results are discussed in the following sections.

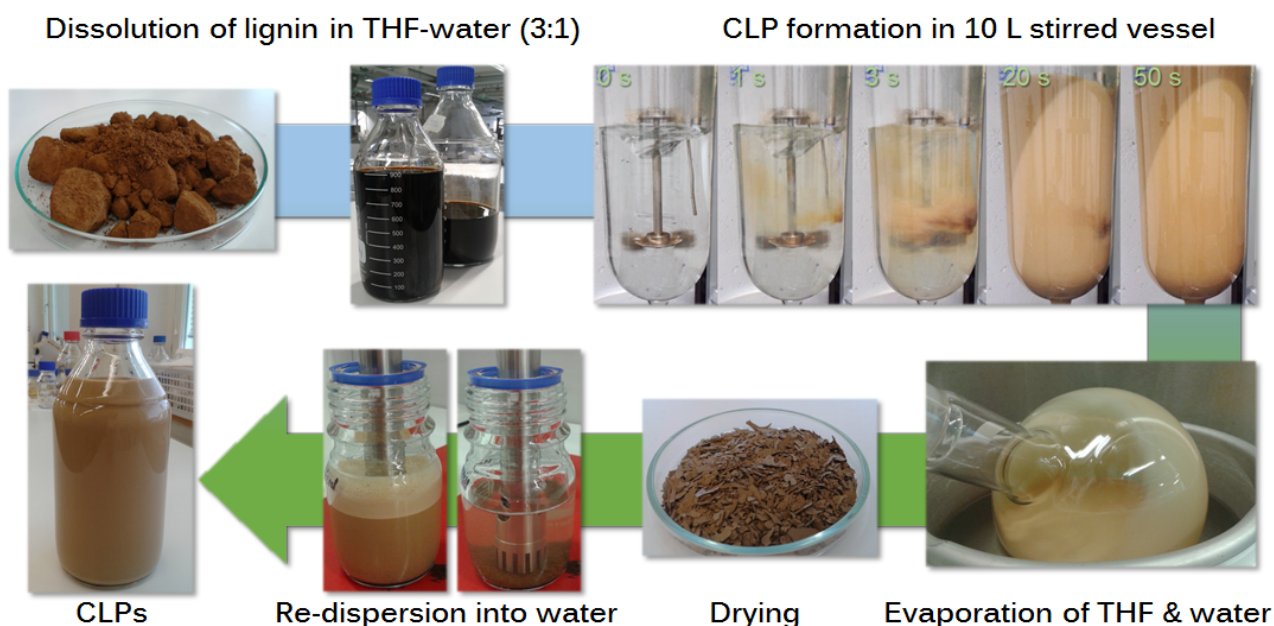


Fig 1 - Schematic illustration of the process for production of CLPs, including steps of drying and re-dispersion of the formed particles.

Formation of colloidal lignin particles by solvent shifting process

Method of solvent displacement process to form CLP:

Formation of lignin particles in the THF-water system is driven by large difference in the solubility of lignin in these solvents in their pure forms. THF can completely solvate and dissolve lignin, whereas the solubility in water is very low (< 5 m%). Anyhow, lignin is very well soluble into mixtures of these solvents when THF concentration is above 50%. Furthermore it is apparently the interplay between miscibility of these solvents and the solvated lignin polymer that plays a role in the formation spherical particles, as we will describe below.

Firstly, the method used to induce solvent shifting and supersaturate the lignin solution is a very influential factor for the characteristics of the formed particles. In the majority of existing studies this has been done via dilution of lignin polymer solution by addition of water (Li et al. 2016b; Richter et al. 2016; Xiong et al. 2017) or dialysis against water (Lievonen et al. 2016), but alternatively the lignin solution can also be injected into an excessive volume of water (Yearla, Padmasree 2016). It is this modification to the nanoprecipitation process that makes the CLP formation process to take place independently from the total volume of the reactor or mixing system, as the injected THF rich solution faces a great excess of water in the surrounding medium. This is not happening when THF solution is diluted by water addition. In Fig 2 the effect of these two methods on average CLP size is compared. When water was added to the lignin solution to form the particles, the rate of addition greatly influenced the obtained particle size distribution (Fig 2A, 2B). Slow particle formation process via dropwise addition of water resulted in particles that were not truly colloidal due to average size above 700 nm, while fast mixing of water into lignin solution resulted in colloidal stable particles of ca. 200 nm average size (Fig 2A). Conversely, injection of lignin solution into excess of water at various rates resulted in a similar average particle size, independently of the applied addition rate. Thus, it is evident that to produce small, uniform-sized particles, the water concentration

within the system needs to increase rapidly. This is easily achieved when lignin solution is added to an excess of water.

Mechanistically the observed phenomena may be explained by a particle formation mechanism related to a spontaneous microemulsification process, also denoted as the “ouzo effect”, that is known to occur in nanoprecipitation systems (Aubry et al. 2009; Schubert et al. 2011; Lepeltier et al. 2014).

In the case of lignin systems, the existing studies that have observed the CLP formation by microscopy (Xiong et al. 2017) support a mechanism where the supersaturation and emulsion formation within the nanoprecipitation system may lead to a metastable droplet like state (see Fig 3). Such droplet formation in nanoprecipitation is in general kinetically controlled (Lepeltier et al. 2014), rather than being a thermodynamically driven process, such as the formation of polymersomes (Discher, Eisenberg 2002). Structurally, and even mechanistically, the metastable state seems to have some resemblance to Pickering emulsions (Aveyard et al. 2003). During the rapid injection of lignin solution into an excess of water, the lignin polymers and potentially already existing small agglomerates concentrate within the separating THF rich droplets. Lignin coils located at the interface of forming droplets are expectedly taking a solid packed form due to non-solvating water rich environment surrounding the droplet interface. Solidification of the droplet shell may stabilize the forming spherical structure of the metastable droplet, similarly to Pickering emulsions. In turn, when diluting lignin solution by water addition, the forming lignin droplets therein retain the soft metastable state for longer periods of time, thus having a higher probability for coalescence via Oswald ripening mechanism (Lepeltier et al. 2014). This is reflected in the observed inverse correlation between the addition rate and average particle size when adding water into lignin solution (Fig 2A-B). The mechanism involving an emulsion like intermediate stage could also better explain the reported formation of hollow lignin spheres (Li et al. 2016a, Xiong et al. 2017a) when low concentration of lignin is used.

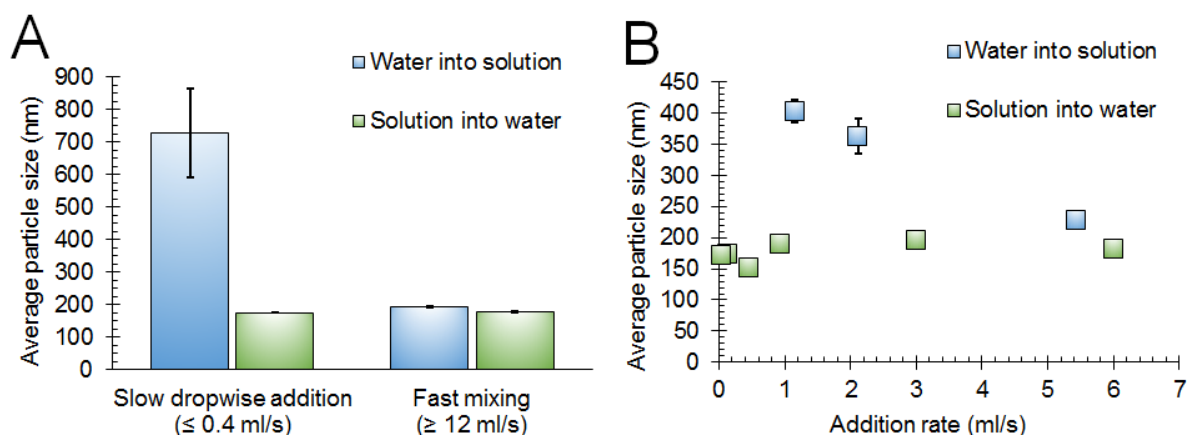


Fig 2 - Influence of solvent shifting technique on average particle size of CLPs. Solution used in the experiment was 2 wt% lignin dissolved in 5:1 THF-water mixture, yielding 0.3 wt% dispersions upon mixing with water. A) Difference between slow dropwise addition and fast pouring of the whole added volume. B) Influence of the injection rate in injection type addition.

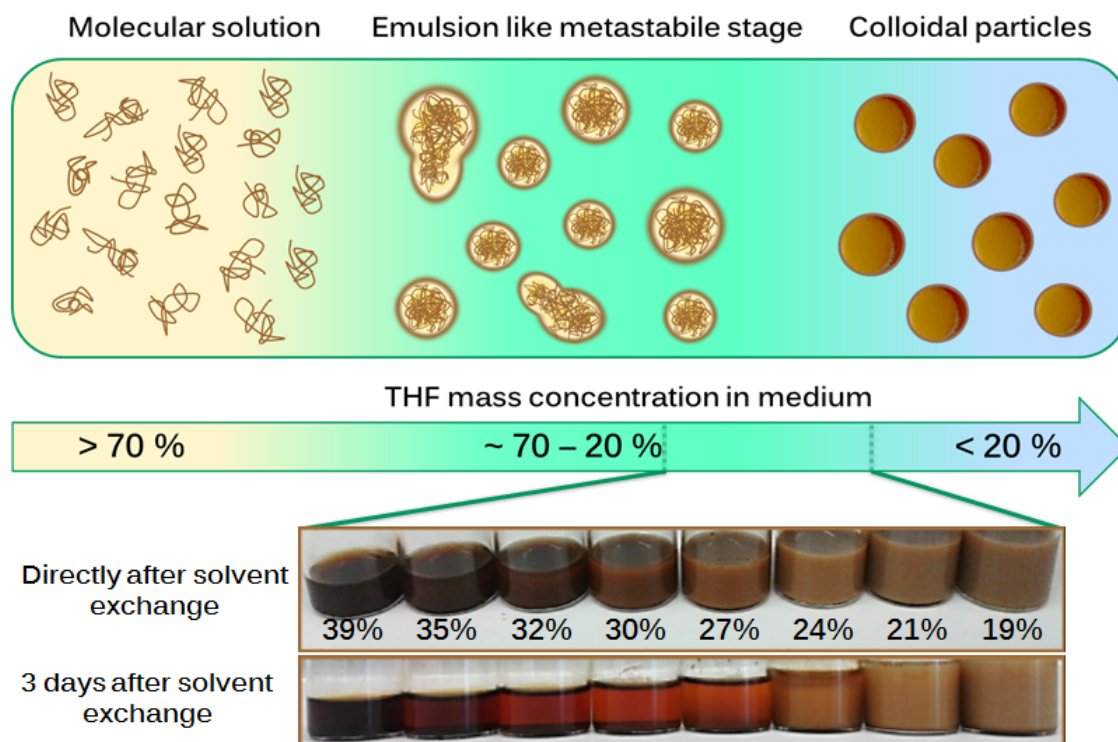


Fig 3 - Illustration of proposed CLP formation mechanism via emulsion like soft intermediate stage where formed structures remain stable only for a limited time before coagulation and phase separation. THF solution was injected into excess of water to result final THF mass concentration.

With respect to general literature around CLP formation, the use of addition rates in association with the solvent shifting process is rather arbitrary, as this does not express well the actual rate of dilution in various sized experimental setups. Rate of dilution in respect to the system size would rationalize the observations more accurately. Effective rate of dilution is extremely high in the case of adding solution into water, regardless of the addition rate, and thus can rationalize the negligible observed effects of addition rate when lignin solution is injected into water.

Composition of solvent system and concentration of lignin: Use of THF as a solvent system for lignin dissolution prior to the CLP formation has been found efficient in previous studies (Lievonen et al. 2016; Li et al. 2016a; Xiong et al. 2017a; Xiong et al. 2017b), while there are also alternative solvent systems for CLP formation, such as acetone (*Appendix 1*). Unlike in the studies where pure THF solvent was used, we have applied THF-water (3:1) mixture as solvent for lignin. This is efficient for lignin dissolution, but most importantly it is relevant for large-scale operations where the lignin feedstocks are expectedly in form of an aqueous slurry or at least contain significant amount of moisture. Presence of water as a component of the solvent system is thus practically unavoidable, and also beneficial in terms of solvent recovery and recycling, as the THF solvent can be readily evaporated from the dilute CLP dispersions as an azeotropic mixture containing water.

In general, the boundaries for solute concentrations that can be used for nanoprecipitation are in range of few mass percentages, depending on the system (Aubry et al. 2009;

Schubert et al. 2011). Lignin concentrations between 0.05 to 1 wt% have been demonstrated so far to produce CLPs from THF solutions (Lievonen et al. 2016; Xiong et al. 2017a; Xiong et al. 2017b), with low concentrations below 0.2 wt% producing hollow sphere structures (Li et al. 2016a, Xiong et al. 2017a). We have mapped the influence of lignin concentration to the characteristics of formed particles (*Fig 4*) in THF-water system, and our results agree with existing literature (Lepeltier et al. 2014; Lievonen et al. 2016; Xiong et al. 2017b) that the particle size grows with increasing polymer concentration of the feed solution.

Lignin is a mixture of polymers with heterogeneous functional groups and high polydispersity. Thus it is expected that supersaturation of a THF-lignin-solution, and consecutive solidification of lignin into CLPs, does not take place distinctly. From *Fig 3*, we can observe a THF concentration region where metastable CLPs are formed (around of 30 wt% THF concentration), but eventually at this solvent composition the majority of CLPs will agglomerate and form a separate oily phase. This Ostwald ripening driven phenomenon (Lepeltier et al. 2014) is also influenced by the lignin concentration (*Fig 4*¹).

¹) Small sized CLPs were formed upon sampling and dilution of the formed dispersion for analysis, while a major fraction of the lignin remained dissolved or dispersed in the actual sample. The 53 wt% of water added result in the formation of agglomerates that remain dispersed by a fraction of the dissolved lignin.

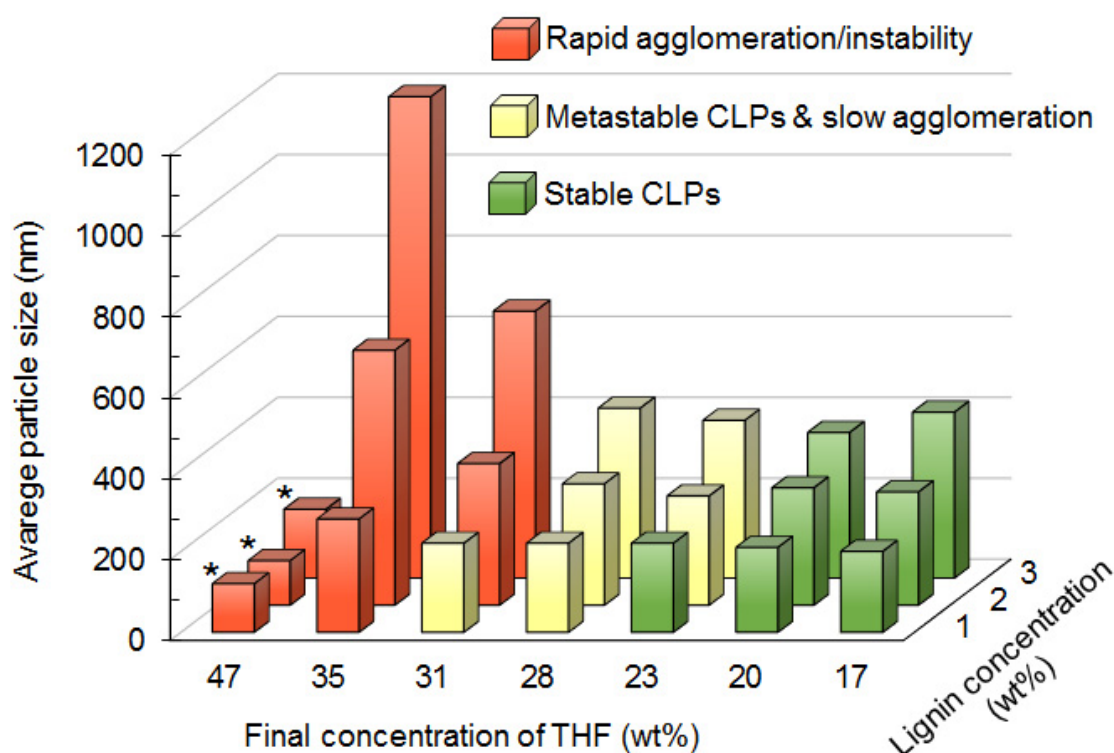


Fig 4 - Influence of lignin concentration in 3:1 THF solution and THF concentration in the final dispersion to formation and stability of CLPs. Addition process was done by injecting solution into water.

As a summary regarding the THF-water (3:1) solvent system, two important factors were found to be important for production of fully stable CLP dispersions: 1) Maximum lignin loading of 2 to 3 wt% in the feed solution and 2) Final THF concentration below 25 to 20 vol% in the formed dispersion prior to removal of THF solvent, depending on the initial lignin loading. The yield of converting lignin into colloidal particles is around 85%, while some small sized components remain dissolved in the water rich medium of the formed dispersion and can be removed via ultrafiltration or dialysis.

Alternative solvent systems for lignin were also tested, such as acetone-water (9:1) and ethanol-water (3:1). Results from these experiments are reported in the Appendix 1. In light of CLP formation through an emulsion-based mechanism, the solvent choice is expected to influence greatly on the particle formation, as evidenced by these studied solvent systems.

Strategies to improve CLP formation at higher lignin concentrations in THF-water system: The CLP are stabilized by their negative surface charge, mainly produced by the carboxylic acid functionalities present in lignin. (Lievonon et al. 2016; Li et al. 2016b; Richter et al. 2016) Accordingly, the carboxylic acid protonation has been reported to influence on the average size the formed CLP in dialysis system utilizing DMSO solvent (Tian et al. 2017). Fig 5 illustrates the effect of base supplementation into THF system in order to drive carboxylic acid deprotonation and enhance the double layer repulsion to stabilize CLPs during their formation process. In our experiment, CLPs were formed from a 4 wt% lignin

solution by adding aqueous NaOH solutions (0.1 to 6 mM) as non-solvent. The trend seen in Fig 5 clearly shows how the particle size of CLPs decreases from approximately 440 nm at pH 3.5 to 325 nm at pH above 5, where practically all of the acid groups in lignin are in their dissociated form. The reduction in the average size by base supplementation to the system can be attributed to reduced agglomeration of the metastable CLP intermediates by increased ionic double layer repulsion, and possibly to influence on the surface energy of the droplet interface towards more hydrophilic direction. Due to the high 4 wt% lignin loading applied in the experiment a portion of the CLPs showed instability and sedimentation, especially without any base supplementation, and such process was significantly reduced at elevated pH. In conclusion, the base supplementation shows promise for reaching higher concentrations in CLP production.

The chemical properties of different lignin types are known to influence particle formation (Lievonon et al. 2016). For this reason, fractionation of the lignin starting material was evaluated as a way of influencing the agglomeration behavior and stability of the CLPs. An attractive feature of the method is that the operation can be carried out with a similar solvent system as is used for CLP formation, practically as a preconditioning step of the lignin solution. We have fractionated the starting material lignin into two fractions utilizing the same solvent used for CLP formation, just by adjusting the water content to 55 vol%. This results in the precipitation and sedimentation of approximately 20 wt% of the starting material.

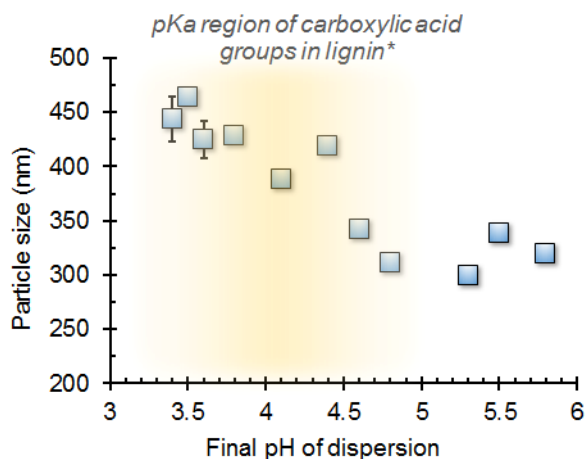


Fig 5 - Particle size of CLPs as a function of pH of the formed dispersion, after applying NaOH into the water non-solvent as supplemented base. Particles were formed by injecting NaOH solutions into 4 wt% lignin solution. The pH region where typical carboxylic acid groups in lignin have their pKa is highlighted in the figure (Ragnar et al. 2000).

When the fraction remaining dissolved in this solvent composition (approx. 80 wt%) was used to form CLPs from a 4 wt% feed solution, the average particle size of formed particles reduced to 330 nm, compared to 440 nm for unfractionated lignin. Only minor changes were observed in the zeta potential. The CLPs formed from the fractionated material also showed significantly reduced tendency for agglomeration and sediment formation in comparison to the particles from unfractionated lignin. Thus, it seems that the fraction of the lignin with lowest solubility may contribute the most to the instability of the formed CLPs.

Besides changes in the particle size, fractionation of lignin also influences the physicochemical properties of the resulting CLPs. Alteration in the cohesion energy characteristics of the particles, that can be expected to follow from the fractionation process, can potentially be used to influence CLP performance in e.g. emulsion or composite applications. Fractionation of lignin without conversion into colloidal particles knowingly influences the compatibility in melt blending (Sadeghifar 2016), where application of CLP form of lignin seems promising with regard to improved dispersion into polymer matrix.

Demonstration of a robust liter scale CLP reactor design

Injection of lignin solution into an excess of water provides a robust way for CLP formation, with great potential for scaling the process up for industrial production. As a demonstration about the up-scalability of the method, we used a 10 l stirred vessel to form 6 l of CLP dispersion in a single batch (see Fig 1). Injection of 2 wt% lignin solution into excess of water loaded into reactor, with rate

of 40 ml/s, resulted in particles with an average diameter of 220 nm and -36 mV zeta potential.

Processing of CLP dispersions into products ready to be marketed

Solvent recovery, drying and re-dispersion of CLP: The majority of the THF could be recovered from the produced dispersion by rotary evaporation at 40 °C and 80 mbar pressure, resulting 80 % solvent concentration based on the density and refractive index. The residual solvent remaining in the remaining water rich dispersion was completely removed when the particles were converted to dried powders as described below.

Besides solvent recovery and concentration of the obtained dispersions by evaporation, another key challenge for commercial breakthrough for CLP products is the conversion of colloidal material into powdery dry products for transportation and storage. In our study the drying of the particles was successfully carried out by rotary evaporation at 45 °C and 30 mbar pressure, resulting in a flaky dry product in the flask (see Fig 1). The dried product could be re-dispersed in water with the aid of physical agitation, either by ultrasonication, high-speed homogenization, or simply by magnetic stirring. As expected, the time requirement was proportional to the energy input, and ultrasonication or homogenization could re-disperse the CLPs within minutes, with close to original particle size distribution after 15 min re-dispersion (Fig 6A). Magnetic stirring was not as efficient, although the majority of the dried product could be recovered into its original colloidal form. Only a minor fraction of the dried material could not be re-dispersed seemingly due to irreversible aggregation.

The energy consumption associated with the removal of water from the original dispersion and physical agitation required for the CLP re-dispersion are still to be evaluated. In the meantime, these initial demonstrations of drying and re-dispersion of CLP are most encouraging concerning their commercialization.

Salt-triggered sedimentation for concentration and drying of CLPs: It has been proposed that salt could be utilized for the concentration, purification and size based fractionation of nanomaterials in large-scale processes (Wang et al. 2010; Missoum et al. 2012). CLPs are also known to lose their colloidal stability, agglomerate, and sediment upon salt addition (Lievonon et al. 2016). Utilization of salt-triggered sedimentation may provide a feasible technical solution for dealing with excessive amount of water required for the CLP production. Furthermore, salt has been found to prevent the irreversible aggregation of nanocellulose upon drying (Missoum et al. 2012). Besides evaporation or ultrafiltration of dilute particle dispersions, controlled agglomeration and sedimentation of colloidal particles provides an alternative for production of concentrated or dried CLPs, as the majority of the solvent can be recover via low energy decanting process in this case.

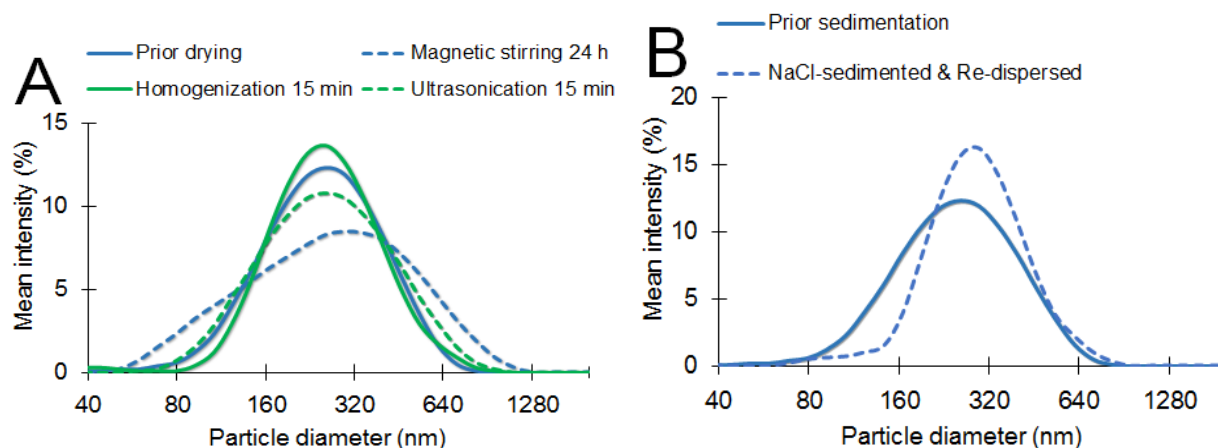


Fig 6 - Particle size distribution of CLPs after A) Drying and re-dispersion B) Salt-triggered sedimentation and diafiltration.

As an aqueous dispersion, CLPs show long-term colloidal stability if particle size is below ca. 500 nm, while larger particles or agglomerates start to sediment in a matter of hours. High centrifugal force is needed in order to sediment smaller particles, and such process can even be used for fractionation of CLPs according to their particle size (Appendix 2). As the typical CLPs have diameter of only few hundred nanometers, salt needs to be added in order to form agglomerates above 500 nm diameter that can be sedimented. Introduction of NaCl up to concentration of 1 M was found to result in the agglomeration and sedimentation of CLPs by gravity in less than 12 h. After removal of the saline supernatant, consisting only of minor fraction of colloids and some dissolved material, the sedimented CLPs could be re-dispersed without agitation by simple mixing with water and residual salts could be then removed by diafiltration. The resulting particle size distribution was similar to the original dispersion (Fig 6 B), while showing reduced concentration of small particles, which have remained dispersed in the saline supernatant.

The salt triggered sedimentation technology may solve some of the observed challenges related to the concentration and drying of the CLPs. Regardless of some associated technical challenges, this method may have benefits in terms of associated energy consumption, removal of water-soluble impurities, narrowed particle size distribution, and improved re-dispersion of CLPs in comparison to ones dried directly by evaporation.

Thermal post-treatment to reinforce spherical CLP structure: Unmodified CLPs will dissolve at pH above 12 or in some organic solvents, just like the original lignin. For the particles to retain their shape in harsh conditions, reinforcement of their spherical structure is required. Stability of the CLPs against dissolution is important e.g. for chemical surface functionalization, as the potential reactions typically require the presence of organic solvents or high pH of an aqueous medium. Thermal softening and agglomeration during e.g. melt blending of CLPs with other polymers for composite applications may be another potential challenge with unmodified CLPs.

Lignin is a naturally thermosetting polymer (Cui et al. 2013), i.e. once formed, it can't be re-shaped by heating.

As a part of plant cell walls, lignin has been found to undergo condensation during high temperature treatments in aqueous medium. (Li et al. 2007) Analogously, the spherical structure of CLPs in an aqueous dispersion could potentially be reinforced by a thermal post-treatment that induces interpolymer crosslinking. Aqueous-based thermal treatments for biomass are already in use in the current lignocellulose refining industry. (Larsen et al. 2012)

Thermal post-treatments of CLP dispersions were optimized to produce a sufficient degree of crosslinking between lignin polymers within CLPs to reinforce the spherical structure against dissolution in known solvent systems. 0.3 wt% CLP dispersions were heated in a PARR reactor at temperatures between 144 and 191 °C. The resulting dispersions retained well their colloidal properties as seen from the particle size and zeta potential analyses shown in Table 1. The treated particles showed reduced solubility in lignin solvent systems, such as THF and methyl ethyl ketone (MEK)-water, as a function of increasing treatment temperature, reaching a negligible dissolution level after 191 °C treatment. In the MEK-water biphasic system, the particles were transferred from the aqueous phase into the organic phase, where they remained dispersed. The unmodified CLPs dissolved fully in these solvent systems. An unwanted side effect of the thermal treatment was that the color of the dispersions got darker (Fig 7), with an introduction of malodorous odors of similar to the odors of sulfur compounds of the kraft pulping process. The odor, likely caused by the sulfur residues in kraft lignin, can potentially be eliminated by utilization of sulfur free lignins.

Table 1 - Colloidal properties of the aqueous CLP dispersions after thermal treatments at 144 to 191 °C

Treatment temperature (°C)	Average particle size (nm)	Zeta potential (mV)
Untreated	190	-41
144	180	-40
174	178	-35
191	174	-37

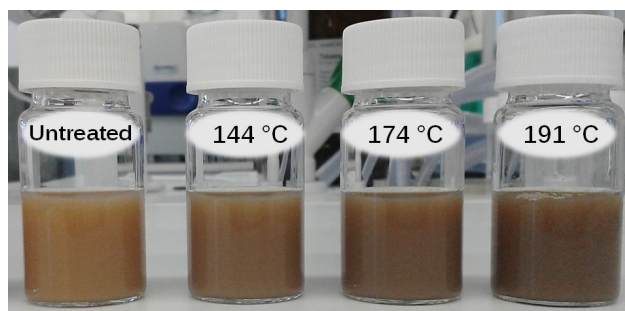


Fig 7 - Appearance of the aqueous CLP dispersions after thermal treatments at 144 to 191 °C

Conclusions

In this work, we have introduced developments regarding a robust up-scalable technology for the production of colloidal lignin particles. Based on a synthesis of the current literature and our results, we propose a mechanism where CLPs are formed via an emulsion-like metastable intermediate stage. In association to the CLP formation mechanism, we found that parameters such as the pH of the process and the fractionation of the lignin starting material have an influence on the CLP formation. Alteration of these parameters affected positively the stability of the formed particles, thus enabling the formation of CLPs at higher concentrations.

It was demonstrated that the THF:water mediated nanoprecipitation of CLPs could be scaled up into 6 liter volume without any negative influence on the particle size, charge, or dispersion stability. The utilized THF:water solvent mixture could be recovered by evaporation and applied for lignin dissolution. The produced dispersion of colloidal particles could be evaporated and dried into flake form, and most importantly, consecutively re-dispersed in water with aid of mild physical agitation.

Salt-triggered sedimentation of CLPs was also introduced as an alternative to energy intensive concentration, drying, and re-dispersion processes. Reinforcement of the spherical structure of the CLP was demonstrated by aqueous thermal treatments, providing particles with negligible solubility in organic solvents.

Due to the straightforward up-scalability, the demonstrated process for CLP formation shows a large potential for the valorization of the low-grade lignins from continuously expanding biorefining operations. The CLPs as low cost nanomaterials can be used as building blocks in environmentally benign consumer products, such as short life cycle compostable biocomposites for packing materials, or to replace oil based phenolic components in construction materials.

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Appendix 1 – Formation of CLPs in various solvent systems

Table A1 - Results from CLP formation with alternative lignin solvent systems

Solvent system (volume ratio)	Results – Diam. (nm)
Acetone-water 9:1	CLPs – 110 nm
Ethanol-water (3:1)*	CLPs – 190 nm
Methyl ethyl ketone(MEK)-water 11:1	Non-colloidal particles

*Incomplete solubility of Lignoboost©

Appendix 2 – Fractionation of CLPs by centrifugal sedimentation

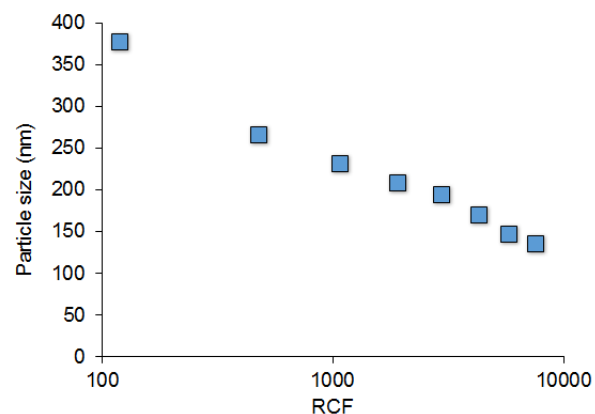


Fig A1 - Average particle size determined from a supernatant of CLP dispersion after centrifuging at various speeds.