Synthesis of Lignosulfonate based Nanocarriers for the Delivery of Agrochemicals

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The study's objective is to develop lignosulfonate-based nanocarriers as a UV protectant for agrochemical delivery

Place and Duration of Study: Department of Nano Science & Technology, Tamil Nadu Agricultural University, Coimbatore. The research was carried out between March 2021 and January 2022.

Methodology: We demonstrate a straightforward approach for the solvent – anti-solvent conversion of lignosulfonate macromolecules from black liquor derived from the paper pulping industry to nanocarriers. Due to the amphiphilic nature of lignin, nanoparticles are generated by self-assembly. To create lignin nanoparticles, a drop-by-drop solvent exchange approach has been used. The lignosulfonate solution was prepared using solvents such as ethanol and tetrahydrofuran, and then water was added as an antisolvent, resulting in the creation of nanoparticles by self-assembly. The hydrophobic portion of lignin creates the particle's core, while the hydrophilic hydroxyl groups form the particle's shell. The size and stability of nanoparticles were determined using dynamic light scattering, and the form and size of the systems were imaged using scanning electron microscopy. The functional groups of the nanoparticles were determined using Fourier

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transform infrared spectroscopy.

Results: Solvent tetrahydrofuran generated uniform and spherical lignosulfonate nanoparticles than the solvent ethanol employed in the solvent exchange procedure. When ethanol and cetyl trimethyl ammonium bromide were used as solvent and surfactant, respectively, the size of lignosulfonate nanoparticles was smaller (270±31.9nm). However, the stability of nanocarrier systems was unaffected by the solvent used, with polydispersity index values of 0.435±0.003 and 0.401±0.028 for tetrahydrofuran and ethanol solvents, respectively. The existence of a distinctive peak at 526 and 609 cm^{-1} in the infrared spectrum corresponding to sulfonic stretching indicated the presence of lignosulfonate in the carrier systems.

Conclusion: Lignosulfonate-based nanocarrier systems were developed using the solvent exchange method. However, the nanocarrier systems are to be validated to assess the bioefficacy of active molecules.

1. INTRODUCTION

Lignin is the second most abundant biopolymer in plant biomass, following cellulose. Lignin is an aromatic polymer cross-linked by random phenylpropane units comprising guaiacyl, p-hydroxyphenyl, and syringyl groups coupled via covalent C-C and C-O linkages. The structural unit of plant biomass is lignin, which is found in the vascular and support tissues of middle lamellae, the secondary cell wall of xylem arteries, and sclereid cells. Lignin gives the plant cell wall its strength and rigidity. In plants, lignin covers the gaps between the cellulose, hemicellulose, and pectin polymers. Lignin concentration varies between 5% and 12% in monocots, between 25% and 35% in softwoods, and between 15% and 30% in hardwoods [1]. Currently, lignocellulosic biorefineries and the paper/pulp industry produce more than 80 million tons of industrial lignin [2], with 95 percent of the lignin produced being burned for heat recovery and the remaining 5% being isolated and used in value-added applications such as industrial dispersant and surfactant [3]. Thus, lignin is being investigated for various applications to increase the sustainability of the pulping industry. The lignosulfonate technique is commonly used in the paper pulping industry for cellulose extraction from woody biomass. The process begins with adding sulfurous acid or sulfite and bisulfite to the wood, which produces black liquor containing lignin and sulfur as a by-product. Lignin obtained by the sulfite paper pulping process is called lignosulfonates; this type of lignin accounts for approximately 90% of commercial lignin available in the market [4]. Globally, lignosulfonates are produced at around 1.8 million tons per year [5]. By definition, lignocellulosic components are in the nanoscale, which gives mechanical strength to the plant cell

wall [6]. Lignin's aromatic nature, increased reactive functional groups, high availability, biodegradability, increased thermal stability, UV resistance, and cost-effectiveness make it useful in various disciplines. Due to the exceptional characteristics of lignin at the nanoscale, lignin nanoparticles have acquired increased importance in recent years. Additionally, lignin nanoparticles are used as nanocarriers for active molecule delivery [7]. Numerous production approaches for lignin nanoparticles have been described, including nanoprecipitation [8,9], chemical modification [10], sonication [11], thermal carbonization [12], microwave-assisted acetylation [13], and self-assembly [14,15]. This study aims to synthesize lignin sulfonate nanoparticles from black liquor utilizing a self-assembly method using solvents such as tetrahydrofuran and ethanol. The solvent exchange approach produce highly organized lignin nanostructures without external force.

2. MATERIALS AND METHODS

The black liquor was graciously provided by Tamil Nadu Newsprint and Papers Limited (TNPL), Kagithapuram, located in Tamil Nadu's Karur district. Purification of the black fluid was achieved using oxalic acid (Product No. 75688) obtained from Sigma-Aldrich. Tetrahydrofuran AR was obtained from Central Drug House Private Limited, New Delhi (Product No. 657836). Cetyl trimethyl ammonium chloride AR was purchased from LOBA Chemie Private Limited, Mumbai.

According to previous reports [16], black liquor was pretreated with oxalic acid. Oxalic acid at 1% was introduced dropwise from a burette to a beaker holding 20ml of black liqueur with pH of 13 held at 350 rpm at room temperature under

constant magnetic stirring, resulting in the precipitation of pure lignin. The precipitate was filtered out using WhatmanTM Grade 40 filter paper. The precipitate was dried in a laboratory oven at 60°C until consistent weights were observed in subsequent experiments. For the synthesis of nanoparticles, the lignin sulfonate sample was macerated with a mortar and pestle and stored at 4°C.

The lignosulfonate nanoparticles were produced utilizing a solvent exchange process in which ethanol and tetrahydrofuran were used as solvents. The approach for synthesizing lignosulfonate nanoparticles was adapted from [17] with minor modifications. Ten milliliters of lignosulfonate dispersed in water at concentrations of 10%, 30%, and 60% (w/v) were held at 250rpm in a magnetic stirrer, to which 10 mL of ethanol and water (3:2 ratio) containing 2% cetyl trimethyl ammonium chloride were added dropwise by syringe. The contents were then added to 10 mL of deionized water as an antisolvent to promote spontaneous self-assembly and the creation of lignosulfonate nanoparticles. Similarly, in another series of experiments, 15 mL of tetrahydrofuran was added dropwise to 10 mL of 20, 40%, and 60% lignosulfonate solution held on a magnetic stirrer at 500 rpm. To generate self-assembly of lignosulfonate nanoparticles, 10 mL of deionized water was added to the solution as an antisolvent (Fig. 1).

The solution containing lignosulfonate nanoparticles was characterized using a particle size analyzer to determine the particle size and polydispersity index. A scanning electron microscope and Fourier transform infrared spectroscopy were used to create surface topography and confirm the presence of functional groups in the nanoparticles. To determine the particle size and stability of ligosulfonate particles, a particle size analyzer (Nanopartica, HORIBA-SZ-100) was utilized. For consistent dispersion of nanoparticles, aliquots of 10µL were dispersed in 10 mL of deionized water held in a water bath sonicator for 24 minutes. The analysis was then performed using 3mL of dispersion in a glass cuvette. The surface morphology of particles was imaged using a scanning electron microscope (FEI, Quanta 250). On the stub, a drop of a sample containing nanoparticles was deposited and allowed to dry before imaging at a magnification of 20000X with a spot size of 3.5 and a voltage of 10KV. In Attenuation Total Reflectance mode, a Fourier Transform Infrared Spectrometer (JASCO International, FTIR – 6800) was scanned throughout the frequency range 400-4000cm^{-1} with a resolution of 4cm-1. The spectrum was acquired utilizing the Spectra Manager TM cross-platform, which allows for labeling spectra peaks with their associated wave number.

3. RESULTS AND DISCUSSION

As indicated in the experimental section, lignosulfonate nanoparticles were collected in colloidal form. SEM and dynamic light scattering were used to characterize the surface morphological characteristics, suspension particle size, and stability. When tetrahydrofuran was utilized as a solvent, the size of the lignosulfonate nanoparticles varied from 275 to 411 nm. In another experiment, using ethanol as the solvent, the size of lignosulfonate nanoparticles was measured to be between 234 and 294 nm. Similarly, the concentration of lignosulfonate used in the procedure affects the size of the nanoparticles formed. The polydispersion index of nanoparticles was less than one, suggesting that they were more stable and regular under the varied lignosulfonate concentrations and solvents utilized in the study (Table 1, Fig 2 and Fig 3). Scanning electron micrographs of nanoparticles revealed a spherical shape (Fig 4 and 5). However, regular spherical nanoparticles were detected when tetrahydrofuran was employed as the solvent. When ethanol is used as a solvent to create nanoparticles, the particles are smaller than when tetrahydrofuran is used. The solvent–water interaction, the solvent's velocity of diffusion into the water, the solvent's viscosity, and the supersaturation level all contribute to the size of lignin nanoparticles. Solvents having a higher diffusion rate into the water, a more significant number of hydrogen bonds with lignin and a higher supersaturation level in lignin's aqueous solution result in smaller particles. In the experiment, ethanol may have a quicker and more uniform diffusion rate than tetrahydrofuran in an aqueous lignin solution, resulting in a faster supersaturation rate, which may lead to smaller particles [18]. Due to its increased hydrophobicity, lignin with a greater molecular weight contributes to a quicker nucleation rate during self-assembly [19].
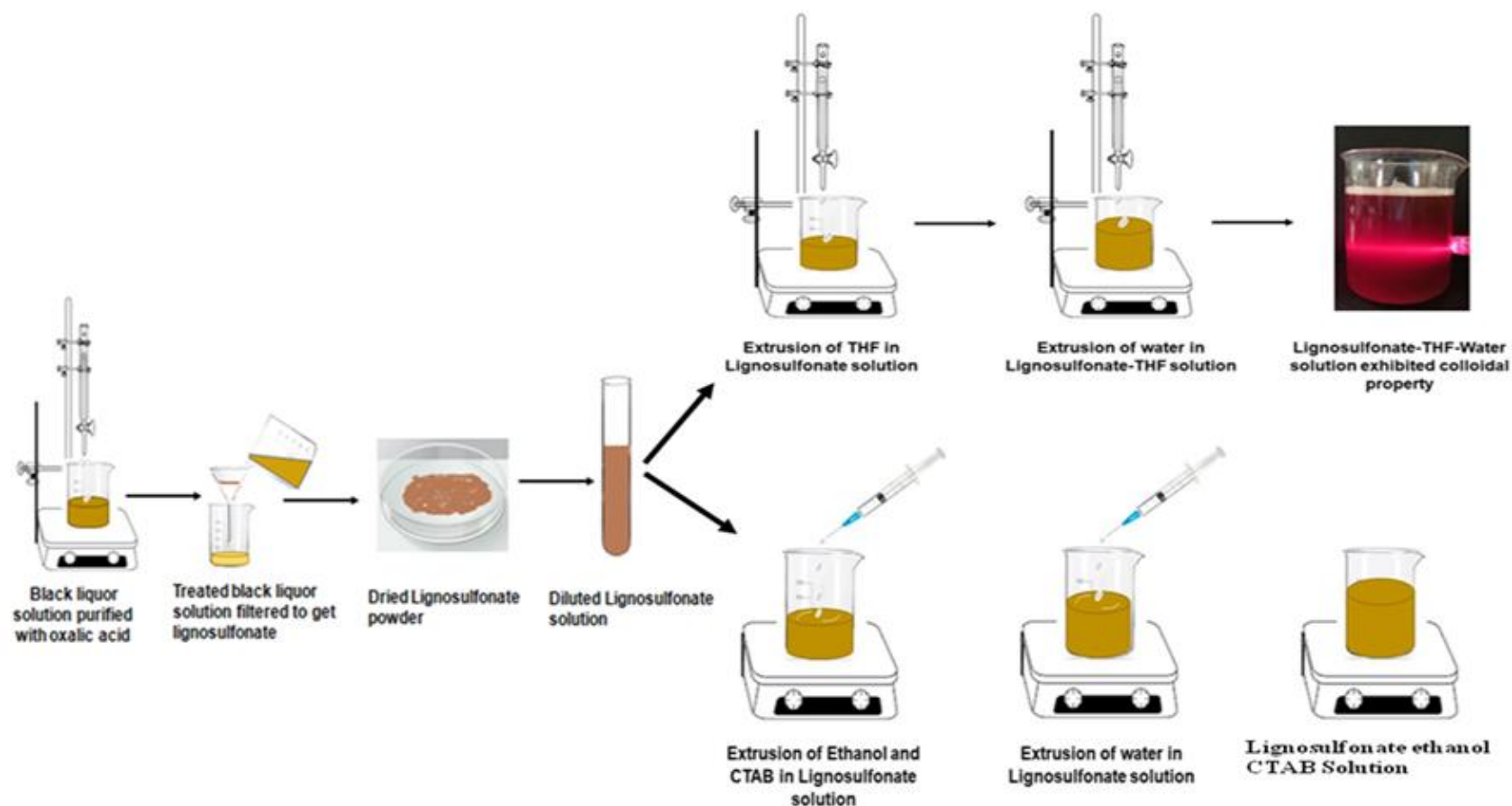
Fig. 1. Sequence of Processes involved in the purification of black liquor from the paper pulping industry and synthesis of lignosulfonate nanoparticles utilizing tetrahydrofuran and ethanol as solvents and water as anti-solvent

Table 1. Particle size and polydispersity index of lignosulfonate nanoparticles prepared through tetrahydrofuran and ethanol through solvent exchange method
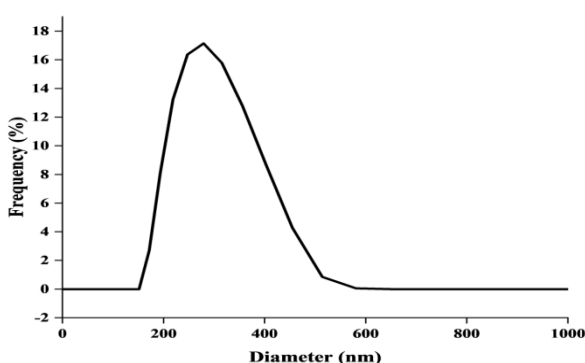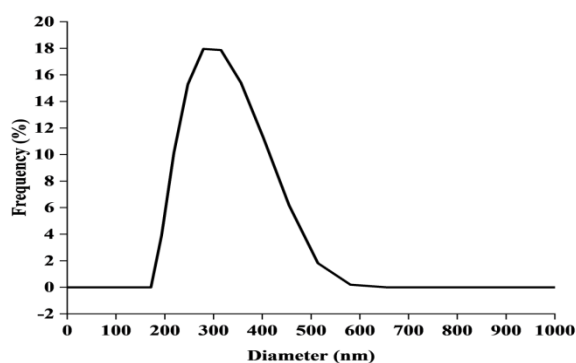
Solvents used in solvent exchange method	Particle size (nm)*			Poly Dispersity Index*		
	Concentration of lignosulfonate (%)					
	20	40	60	20	40	60
Tetrahydrofuran	306±10.2	275±11.9	411±14.4	0.434±0.017	0.439±0.011	0.430±0.012
	Concentration of lignosulfonate (%)					
Ethanol	10	30	60	10	30	60
	234±3.3	284±5.9	294±5.6	0.420±0.009	0.370±0.004	0.428±0.018

* mean of triplicates

Self-assembly of nanoparticles refers to the production of an ordered structure from randomly oriented units due to non-covalent internal interactions. Lignin is a supramolecular structure isolated from plant biomass using acid, alkali, lime, and hydrogen peroxide as pretreatments. The cleavage of the β-O-4 linkage in lignin during pretreatment results in forming a positive carbon center in the aliphatic chain of the lignin backbone, which forms a C-C covalent bond with the electron-rich carbon of the guaiacyl unit. This mechanism increases the stability and recalcitrance of the lignin molecule. Weak forces such as π-π staking, van der Waals forces, and hydrophobic contacts all contribute to the self-assembly of lignin sulfonate molecules in the solvent – anti-solvent approach. In pulping industries, sulfonation results in decreased surface free energy for lignin molecules, promoting weak interactions and self-assembly of lignin molecules [20].

Fourier Transform Infrared spectra of lignosulfonate revealed many hydroxyl groups in lignin nanoparticles through the solvent exchange method (Fig 6 and 7). The peak at 3804 cm^{-1} corresponds with the stretching band –OH group, while the broadening of the hydroxyl peak in the self-assembly nanoparticles explains the exposure of more aliphatic and phenolic hydroxyl groups during self-assembly. The peak represented carboxyl groups at 3015 and 2865 cm^{-1} in the bulk black liquor samples. The smoothening of such in the lignosulfonate nanoparticles exhibits the involvement of cross-linking of carboxyl groups [21]. The peak at 1739 cm^{-1} represents the presence of carbonyl groups in the lignosulfonates, the appearance of the peak at 526cm^{-1} confirmed the presence of the sulfonate group in the black liquor sample, while the peak for sulfonic group was weak in the nanoparticles which explain the involvement of the sulfonic group in hydrogen bonding [22]. The smoothening and weakening of corresponding peaks to hydroxyl, carbonyl and sulfonic groups in the nanoparticles confirmed the self-assembly of nanoparticles. Sulfonic groups were also found at 553 cm^{-1} in the nanoparticles prepared with ethanol as a solvent. Peak found at 1634 cm^{-1} shows the presence of C=C Stretching of an aromatic ring. Ring deformation mode was also found at 1045 cm^{-1}.

Fig. 2, 3. Particle size of lignosulfonate nanoparticles using ethanol (left) and tetrahydrofuran (right) as a solvent during solvent exchange method
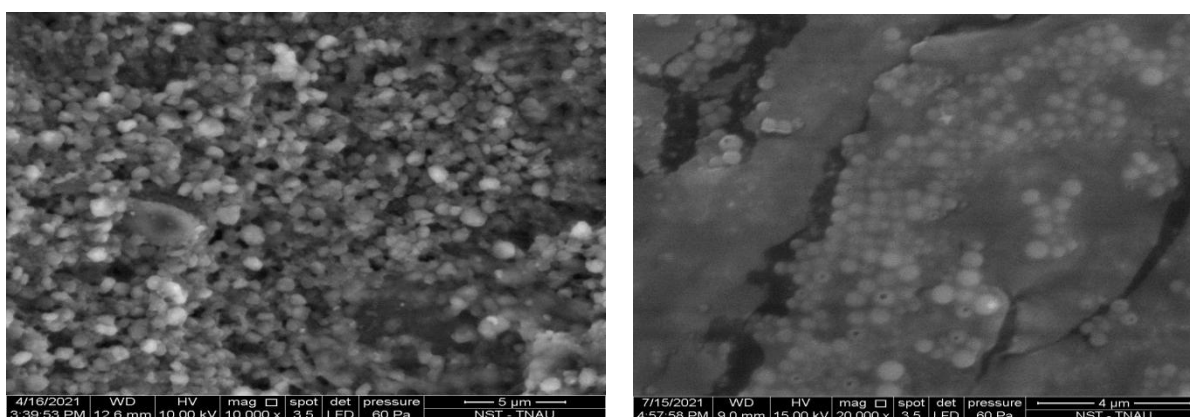
Fig. 4, 5. Electron microscope image of (spherical morphology) of lignosulfonate nanoparticles using ethanol (left) and tetrahydrofuran (right) as a solvent during solvent exchange method
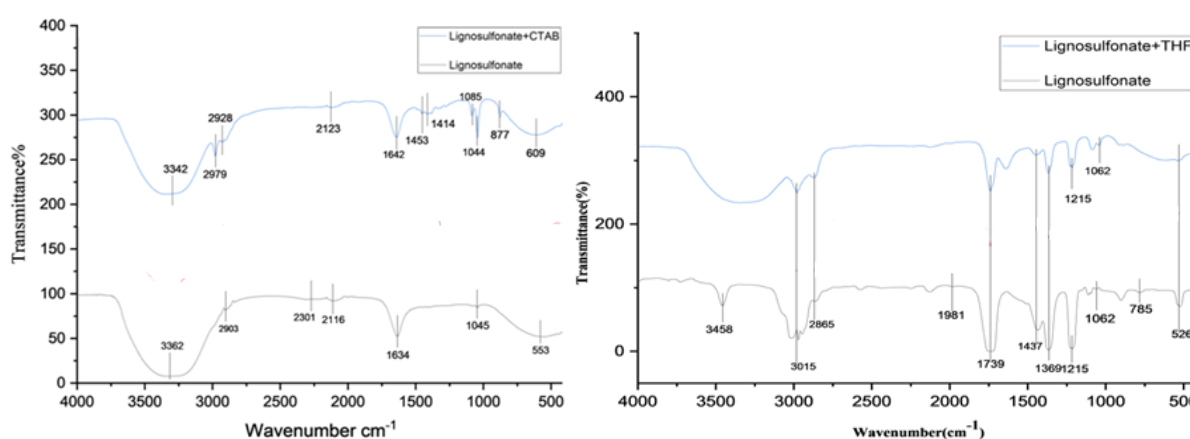

Fig. 6, 7. Fourier Transform Infrared spectroscopy graph of lignosulfonate nanoparticles using ethanol (left) and tetrahydrofuran (right) as a solvent during solvent exchange method

The peak at 2903 cm^{-1} corresponds to the presence of C-H stretching groups in the sample [23]. Hydroxyl groups of phenolic and carboxylate groups were found at 3362 cm^{-1}. Peaks at 3351 cm^{-1}, 1452 cm^{-1} and 1383 cm^{-1} showed asymmetric C-H scissoring vibrations of CH_3-N^+ moieties and CH^2 scissoring mode, indicating the presence of a long aliphatic chain cetyl trimethyl ammonium bromide structure [24]. The peak at 2918 cm^{-1} shows asymmetric (V_as (CH_2, d$^-$) stretching, while the spectral peak at 2899 cm^{-1}, 2928 cm^{-1} and 2977 cm^{-1} showed the presence of (C-H) stretching groups in the sample. Peak found at 609 cm^{-1} shows the presence of sulfonic groups (S-O stretching vibration) in the lignosulfonate nanoparticles (Fig 6). The self-assembly spectral peaks in the self-assembly showed the characteristic weakening of peaks during the involvement hydrogen bonding between solvent and lignin interactions or between lignin molecules [25].

4. CONCLUSION

The paper pulping industry produces 400 liters of black liquor for every ton of wood biomass processed. Black liquor's by-products should be efficiently exploited to enhance value and make the business more sustainable. However, lignin is underused in heat-generating applications. The research was conducted to develop lignosulfonate-based nanoparticles for efficient agrochemical delivery in this context. The nanoparticles were synthesized utilizing the solvent exchange approach using tetrahydrofurans and ethanol. The use of ethanol as a solvent lead in smaller particles, whereas tetrahydrofuran results in particles with a standard spherical form. To stimulate self-assembly of ligno nanoparticles, water was utilized as an antisolvent. However, nanoparticle recovery is reduced, necessitating optimizing the solvent-antisolvent ratio and pH conditions. The

intense UV resistance of lignin nanoparticles protects active molecules entrapped in lignin particles from photolysis and oxidation.

DISCLAIMER

The products used for this research are commonly and predominantly used in our research area and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but the advancement of knowledge. Also, the research was not funded by the producing company; instead, it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pinkert A, Goeke DF, Marsh KN, Pang S. Extracting wood lignin without dissolving or degrading cellulose: investigations on the use of food additive-derived ionic liquids. Green Chemistry. 2011;13(11):3124-36.
2. Sharma S, Sharma A, Mulla SI, Pant D, Sharma T, Kumar A. Lignin as potent industrial biopolymer: an introduction. InLignin 2020 (pp. 1-15). Springer, Cham.
3. Lievonen M, Valle-Delgado JJ, Mattinen ML, Hult EL, Lintinen K, Kostiainen MA, Paananen A, Szilvay GR, Setälä H, Österberg M. A simple process for lignin nanoparticle preparation. Green Chemistry. 2016;18(5):1416-22.
4. Kim Y, Suhr J, Seo HW, Sun H, Kim S, Park IK, Kim SH, Lee Y, Kim KJ, Nam JD. All biomass and UV protective composite composed of compatibilized lignin and poly (lactic-acid). Scientific Reports. 2017; 7(1):1-1.
5. Stern T, Schwarzbauer P. Wood-based lignosulfonate versus synthetic polycarboxylate in concrete admixture systems: The perspective of a traditional pulping by-product competing with an oil-based substitute in a business-to-business market in central Europe. Forest products Journal. 2008 Jan 1;58.
6. Sahoo S, Misra M, Mohanty AK. Enhanced properties of lignin-based biodegradable polymer composites using injection moulding process. Composites Part A: Applied Science and Manufacturing. 2011; 42(11):1710-8.
7. Qiao Y, Wang B, Ji Y, Xu F, Zong P, Zhang J, Tian Y. Thermal decomposition of castor oil, corn starch, soy protein, lignin, xylan, and cellulose during fast pyrolysis. Bioresource technology. 2019;278:287-95.
8. Frangville C, Rutkevičius M, Richter AP, Velev OD, Stoyanov SD, Paunov VN. Fabrication of environmentally bio-degradable lignin nanoparticles. Chem Phys Chem. 2012;13(18):4235-43.
9. Yearla SR, Padmasree K. Preparation and characterisation of lignin nanoparticles: evaluation of their potential as antioxidants and UV protectants. Journal of Experimental Nanoscience. 2016;11(4): 289-302.
10. Gilca IA, Ghitescu RE, Puitel AC, Popa VI. Preparation of lignin nanoparticles by chemical modification. Iranian Polymer Journal. 2014;23(5):355-63.
11. Gilca IA, Popa VI, Crestini C. Obtaining lignin nanoparticles by sonication. Ultrasonics sonochemistry. 2015 Mar 1;23:369-75.
12. Gonugunta P, Vivekanandhan S, Mohanty AK, Misra M. A study on synthesis and characterization of biobased carbon nanoparticles from lignin. World Journal of Nano Science and Engineering. 2012; 2(03):148.
13. Wang D, Li G, Zhang C, Wang Z, Li X. Nickel nanoparticles inlaid in lignin-derived carbon as high effective catalyst for lignin depolymerization. Bioresource Technology 2019;289:121629.
14. Stanisz M, Smułek W, Popielski K, Klapiszewski Ł, Kaczorek E, Jesionowski T. Sustainable design of lignin-based spherical particles with the use of green surfactants and its application as sorbents in wastewater treatment. Chemical Engineering Research and Design. 2021; 172:34-42.
15. Lievonen M, Valle-Delgado JJ, Mattinen ML, Hult EL, Lintinen K, Kostiainen MA, Paananen A, Szilvay GR, Setälä H, Österberg M. A simple process for lignin nanoparticle preparation. Green Chemistry 2016;18(5):1416-22.
16. Liu Z, Luo XG, Li Y, Li L, Huang Y. Extraction of lignin from pulping black liquor by organic acid. InMaterials Science Forum. Trans Tech Publications Ltd; 2009;620:571-574.

17. Lievonen M, Valle-Delgado JJ, Mattinen ML, Hult EL, Lintinen K, Kostiainen MA, Paananen A, Szilvay GR, Setälä H, Österberg M. A simple process for lignin nanoparticle preparation. Green Chemistry 2016;18(5):1416-22.

18. Zou T, Sipponen MH, Henn A, Österberg M. Solvent-resistant lignin-epoxy hybrid nanoparticles for covalent surface modification and high-strength particulate adhesives. ACS nano. 2021;15(3):4811-23.

19. Mishra PK, Ekielski A. The self-assembly of lignin and its application in nanoparticle synthesis: A short review. Nanomaterials. 2019;9(2):243.

20. Shen Q, Zhang T, Zhu MF. A comparison of the surface properties of lignin and sulfonated lignins by FTIR spectroscopy and wicking technique. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2008;320(1-3):57-60.

21. Constant S, Wienk HL, Frissen AE, de Peinder P, Boelens R, Van Es DS, Grisel RJ, Weckhuysen BM, Huijgen WJ, Gosselink RJ, Bruijnincx PC. New insights into the structure and composition of technical lignins: A comparative characterisation study. Green Chemistry. 2016; 18(9):2651-65.

22. Fiţigău IF, Peter F, Boeriu CG. Oxidative polymerization of lignins by laccase in water-acetone mixture. Acta Biochimica Polonica. 2013;60(4).

23. Boeriu CG, Bravo D, Gosselink RJ, van Dam JE. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. Industrial Crops and Products. 2004;20(2):205-18.

24. Rogers LA, Campbell MM. The genetic control of lignin deposition during plant growth and development. New phytologist. 2004;164(1):17-30.

25. Feng Y, Yu J, Sun D, Ren W, Shao C, Sun R. Solvent-induced in-situ self-assembly lignin nanoparticles to reinforce conductive nanocomposite organogels as anti-freezing and anti-dehydration flexible strain sensors Chemical Engineering Journal. 2022;433: 133202.