

Micro/nanosized Lignin for Biomedical Application

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Abstract: Lignin, the second most abundant bio-renewable polymer in the world after cellulose, is widely used in industrial production. In recent years, nanoparticles have attracted increasing attention due to their excellent properties. Therefore, the preparation of lignin nanoparticles to obtain value-added products is an effective way to utilize their potential completely. This article describes the preparation methods of micro/nanosized lignin with different sizes and shapes, and provides a detailed introduction to their applications in biomedicine.

Keywords: lignin; nanoparticles; biomedical; application

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1 Introduction

Lignin is the most abundant aromatic organic polymer compound in nature. However, for a long time, it has been a by-product of cellulose production in pulp and paper industry. It has not been fully used to all its potential, and its added value is extremely low. In addition to the structural complexity imparted by biomass lignin, the currently applied pretreatment technologies in many cases further improve its complexity and heterogeneity ^[1]. Both of which add additional challenges to the downstream processing of lignin ^[2]. Lignin produced by different processes can be roughly divided into sulfate lignin, alkali lignin, liginosulfonate, organic soluble lignin, steam explosion lignin, and enzymatic lignin ^[3]. Each type of lignin is related to special changes in its chemical structure. However, lignin has several unique properties, such as resistance to corrosion and biological attack, UV absorption, high hardness, and resistance to oxidation. Therefore, it has the potential to be used as a large-volume raw material to produce high-value products ^[4-5].



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Most lignin can only be soluble in alkaline solution, which is the key limitation for its application on an industrial scale; however, in recent years, the potential of preparing aqueous lignin nanoparticles (LNPs) dispersions has been reported ^[6-7]. Therefore, the preparation of LNPs has attracted the interest of researchers. Nanostructured materials, especially in the range of 1–100 nm, provide unique performance owing to their increased surface area ^[8], and their chemical and physical interactions are controlled by surface properties. Different preparation methods of micro/nanoparticle lignin can produce LNPs with different geometric shapes. Nanomaterials can be divided into granular, layered, and fibrous materials based on the geometry ^[9-10]. In this review, we described a dimensional range of lignin from a few nanometers to a few micrometers, not only showing the applied production methods used and how these methods interact with different raw materials and affect the final products, but also discussing lignin-based materials in various forms for biomedical applications.

2 Preparation of micro/nanosized lignin

2.1 Solvent exchange

For the solvent exchange method, the raw materials are dissolved in a good solvent, and excess water is injected into the above solution for mixing. Nanoparticles are produced due to the loss of solubility of lignin. The precipitated phase obtained is solid or hollow spherical nanoparticles in this process. For cases of solid and hollow particle formation, a list of methods is presented in Table 1.

There are several process conditions that are used to produce solid spherical-like particles, and water is used as an anti-solvent. Lievonen et al ^[11] used kraft-treated softwood lignin as a raw material and dissolved it in tetrahydrofuran (THF). The dissolved solution was placed in a dialysis bag and immersed in excess deionized water for dialysis and precipitation. The spherical colloidal LNPs with an average diameter in the range of 200–500 nm was obtained.

In addition, Xiong et al ^[12] used acetic acid lignin as

raw material and dissolved it in THF. Subsequently, the lignin solution was added to a stirred vessel filled with water. The obtained suspension was centrifuged, repeatedly washed with deionized water, and lyophilized to yield dry nanoparticles. Overall, the higher the lignin solution concentration, the larger the particle size. Qian et al ^[13] acetylated wheat straw alkali lignin prior to the precipitation step. Acetylated lignin was dissolved in THF. After completing the colloidization process, excess water was added to the dispersion, and THF was removed via rotary evaporation. Moving forward a single step, Qian et al ^[14] designed three different sizes of nanospheres and microspheres, with raw materials that ranged from organosolv lignin (OL) extracted from pines and enzymatic hydrolysis lignin (EHL) extracted from corncobs, all of them were formed under non-acetylated and acetylated conditions. The three methods for obtaining nanoparticles in the size range used acetone/water with a volume ratio of 8 : 1 as solvent that was diluted with NaCl and deionized water. The large particles obtained were centrifuged, washed with water, and freeze-dried, while the small particles were rotary-evaporated and then freeze-dried to obtain a solid powder ^[14].

Lignin stabilization is the key factor in realizing economically feasible second-generation biological purification. Tian et al ^[15] designed a lignocellulosic biorefinery process chain that used poplar, *Lodgepole* pine, and corn stover to produce LNPs. The raw materials used in this method must be steam-pretreated ^[16] before enzymatic hydrolysis. The solid extracted from the enzymatic hydrolysis was obtained without drying, while dimethyl sulfoxide (DMSO) was in a very mild condition. The resultant lignin solution was isolated from the solid residue via filtration and then transferred directly to the dialysis bag. During the dialysis process, deionized water was replaced regularly until DMSO in the wastewater was removed. Open-mouthed hollow nanocapsules have received extensive attention in scientific research circles because of their high absorption capacity, diffusion, and

catalytic properties ^[17–19]. Besides, the potential value of selective encapsulation with controllable cavities and particles is also an advantage ^[20]. Xiong et al ^[21] prepared open-mouthed hollow nanocapsules by adding deionized water to the precipitate containing different concentrations of EHL/THF solution. It was observed that the opening diameter decreased and the shell thickness increased with the increasing the initial EHL concentration by the transmission electron microscope. During the precipitation process, THF/water was used as a solvent/anti-solvent, but with a lower initial lignin concentration and a higher addition rate, which led to the formation of hollow particles. Furthermore, Li et al ^[22] also produced open-mouthed hollow spheres with kraft lignin (KL) using THF/water as a solvent/anti-solvent. The results showed that there were two different-sized components in the smaller particles. Additionally, Li et al ^[23] investigated nanosphere formation of KL via self-assembly induced by adding water to a KL/dioxane solution. The checked result showed that the size of a nanomaterial can directly affect its performance. However, owing to

lignin heterogeneity (broad molecular weight distributions and extremely complex structures), the preparation of LNPs is still difficult to achieve, which makes it a challenging material for higher value application. Ma et al ^[24] reported a simple process to prepare LNPs with different particle sizes through the combination of nanotechnology and fractionation. The underlying mechanism is that different molecular weights and contents of functional groups of lignin affect the size of LNPs: the more hydrophobic the lignin, the smaller the size of the resultant LNPs. It is also worth noting that the size of LNPs from solid residue can be reduced to 21 nm, which could be the smallest size ever reported.

2.2 pH-shifting

The "standard methods" of LNPs preparation were designed by Frangville et al ^[6]. The lignin residues of *Arundo donax L.* after steam explosion and enzymatic hydrolysis were used as raw materials. A lignin/ethylene glycol suspension was produced. In the precipitation step, HCl was added to the reaction mixture. They eliminated insoluble components by

Table 1 Preparing micro/nanosized lignin by using solvent exchange method

Lignin	Reaction condition and methods	Morphology	Size/nm	Ref.
Softwood lignin from a kraft process	Dissolved in THF at various concentrations from 0.1 to 10 g/L and placed in a dialysis bag (6000–8000 Da) for 24 h	Solid particle	200–500	[11]
Lignin from an acetic acid organosolv process	Dissolved in THF with concentration ranged from 10 to 100 g/L, stirred vessel filled with water, centrifuged, washed with deionized water and lyophilized	—	151–161	[12]
Wheat straw alkali lignin	Acetylated lignin was dissolved in THF with concentration of 1 g/L, the water was added to the above solution, then THF was removed by rotary evaporation to obtain LNPs	—	110	[13]
Alkali lignin and hardwood lignin	Dissolved in acetone water solution with concentration of 10 g/L, then isolated by an acidic dioxane method	—	104±60	[17]
Organosolv lignin	Dissolved in acetone water solution with concentration of 5 g/L, then diluted with water and precipitated to obtain LNPs	—	80	[25]
Lignin from corn stove, hard wood poplar and softwood <i>Lodgepole</i> pine	Steam-pretreated before enzymatic hydrolysis, then the lignin was dissolved in DMSO and isolated from the solid residue via filtration and then transferred directly to the dialysis bag	—	218, 131 and 104	[19]
EHL	Dissolved in THF solution and precipitated EHL/THF solutions with the addition of water. The obtained suspension was centrifuged, repeatedly washed with deionized water, and lyophilized to yield dry nanoparticles	Hollow particle	419–566	[21]
KL	A solvent/anti-solvent system of THF/water, ultrapure water was added to the lignin/THF solution	—	145.5	[22]
	Ethanol as solvent for KL and water as anti-solvent	—	60–350	[26]

filtering the ethylene glycol solution before precipitation. As shown in Fig. 1, the first method is based on the precipitation of low-sulfonated lignin (Indulin® AT) from an ethylene glycol solution by using diluted acidic aqueous solutions, which yields LNPs that are stable over a wide range of pH value. The second approach is based on the acidic precipitation of lignin from a high-pH value aqueous solution which produces LNPs stable only at low pH value.

LNPs prepared by the above two methods have significantly different properties, which were caused by the fact that low-sulfonated lignin is difficult to be dissolved in water under neutral and acidic conditions^[27]. In contrast, it has obvious solubility in ethylene glycol solution with pH value higher than 10. Based on this property, two methods for the preparation of LNPs were developed by adding HCl aqueous solution to the solvent of ethylene glycol, followed by selective crosslinking and water dialysis. The second method was to adjust the pH value to 12 with water, using NaOH as a solvent, and HNO₃ was used to lower the pH value to precipitate lignin^[6].

Yang et al^[28] performed filtration after precipitation and obtained well-proportioned LNPs with higher yields. Moreover, Li et al^[29] reported that nanoparticles with a size distribution ranging from 50 to 280 nm

could be prepared by dissolving purified lignin in an alkaline solution, using NaOH, KOH, and ammonia as a base. Nanoparticles with excellent separability and adsorption properties can be obtained via precipitation with sulfuric, nitric, or hydrochloric acid which followed by freeze-drying. Wei et al^[30] dissolved alkali lignin and the aqueous dispersions of lignin with different solid contents were prepared by adding different amounts of lignin powder to a fixed amount of deionized water, the pH value of the lignin dispersion was adjusted by adding concentrated ammonia solution (37 wt%), until the lignin was completely dissolved. The pH value was approximately 11 at this point. Then the pH value of the lignin solution was adjusted to around 3 by adding HCl (1 mol/L). Lignin particles were formed, and after 10 min of standing it was observed that particles coagulated. Using this method, pH-responsive Pickering emulsions with an average particle diameter of 182 nm were obtained^[6].

To simplify the pretreatment processes of raw lignin, Ma et al^[31] used industrial black liquor as the raw material to prepare LNPs. They controlled the size of LNPs by tuning pH value. Owing to the small amount of hemicellulose and abundant ionic groups in the system, the LNPs showed extremely long-term stability. As shown in Table 2, the above pH-shifting

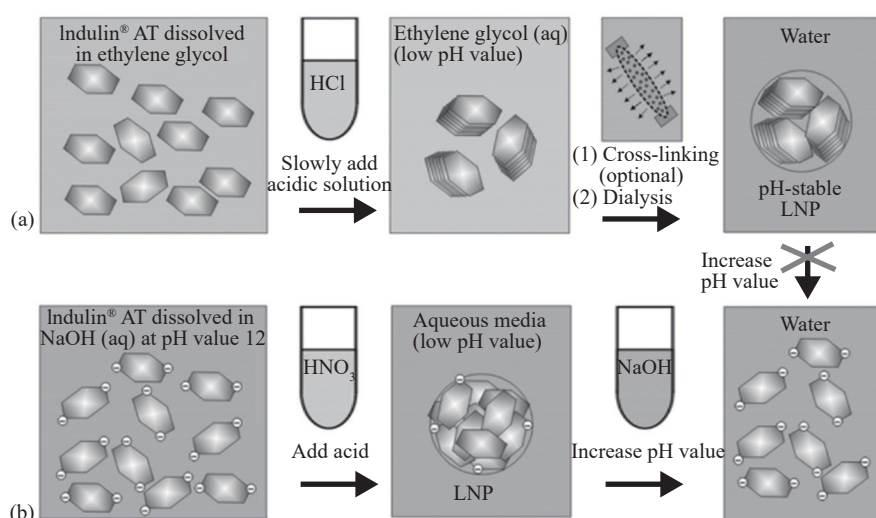


Fig. 1 The "standard methods" of LNPs preparation: (a) Low-sulfonated lignin (Indulin® AT, lot MB05, IAT) was supplied by MeadWestVaco Corporation (Richmond, VA, USA) is precipitated from ethylene glycol with HCl aqueous solution, and subsequent cross-linking and dialysis. (b) IAT was precipitated from a high pH value aqueous solution to a low pH value aqueous solution.

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Table 2 Preparing micro/nano-sized lignin by using pH-shifting method

Lignin	Reaction conditions and methods	Advantage	Size/nm	Ref.
Low-sulfonated lignin	Ethylene glycol was used as solvent and precipitation occurred by adding HCl aqueous to the solution, then an optional crosslinking step occurred by dialyzing with water	Low-sulfonated lignin is insoluble in water under neutral and acidic conditions	100	[6]
	NaOH aqueous as solvent for lignin and precipitation was achieved by decreasing the pH value with HNO ₃		71	[6]
Alkali lignin	Dissolved in the ethylene glycol and precipitated using aqueous HCl, dialyzed with water	Excellent storage stability with minor changes in particle size after three weeks	125	[32]
	Dissolved in an alkaline solution and the LNP precipitates were produced by acid freeze-drying	Good dispensability and absorption properties	50–280	[29]
	Dissolved in ammonia solution until pH value was 11, then adding HCl until the pH value of lignin solution was 3 to form lignin nanoparticles	The pH-dependent stability was used for the fabrication of pH-responsive Pickering emulsions	182	[30]
Lignin residue	Lignin/ethylene glycol suspension with a concentration of 4 wt%, HCl was added to lignin solution with a rate of 2 drops/min	Higher yields (approximately 10%) and more homogeneous LNPs	48.85±16.3	[28]

methods for preparing micro/nano-sized lignin is summarized.

2.3 Cross-linking/polymerization

2.3.1 Solid structures

Nypelo et al.^[33] used micro-emulsification of the surfactant-oil-water (SOW) system to convert the aqueous colloidal lignin dispersion into a granular or super colloidal structure through condensation. The composition of the emulsion formulation and internal phase, concentration of surfactants, and cross-linking agents were used to control the size and integrity of the resulting particles. In addition, the microemulsion containing lignin can effectively synthesize carriers for AgNPs.

Lignin was separated by alkaline treatment of wheatgrass, three types of commercially available alkali lignin were used by Popa et al.^[34] for the synthesis of nanoparticles by hydroxy methylation and epoxidation. The product was recovered by lowering pH value, precipitation, and centrifugation. Epichlorohydrin was added to the alkaline lignin solution for epoxidation and then centrifuged to obtain the final LNPs. Popa and Gilca^[35] repeated the above-mentioned epoxidation under optimum conditions and obtained nanoparticles with average particle diameters between 70 and 200 nm.

Reversible addition-fragmentation chain transfer

(RAFT) allows the high-precision synthesis of polymer-grafted nanoparticles, and it can improve the properties of composite materials^[36]. Hence, Gupta et al.^[37] paid more attention to nanoparticles, and Silmore et al.^[38] paid more attention to the behavior of nanoparticles in emulsions. The synthesis consists of two parts: preparation of macroinitiator and grafting.

The assumed conformation of the polymer-grafted lignin is shown in Fig. 2, where it is presumed that the polymer graft adopts an extended conformation at the air-water interface and a collapsed conformation at the hexane-water interface. Ellipsometric measurements of the coverage of the air-water and hexane-water interfaces can offer insight into the differences in the fillers at the lignin-based surfactant interface. The results showed that the average diameter of the nanoparticles is between ~15 nm and ~367 nm, which is caused by the degree of polymerization and grafting, and the type of monomer^[39–40].

2.3.2 Hollow structures

Microemulsion or miniemulsion polymerization/crosslinking methods are used to synthesize microcapsules and nanocapsules at the oil-water interface, which realizes the encapsulation of hydrophilic and hydrophobic ingredients in amphiphilic lignin^[41–42].

Tortora et al.^[43] prepared oil-filled KL microcapsules

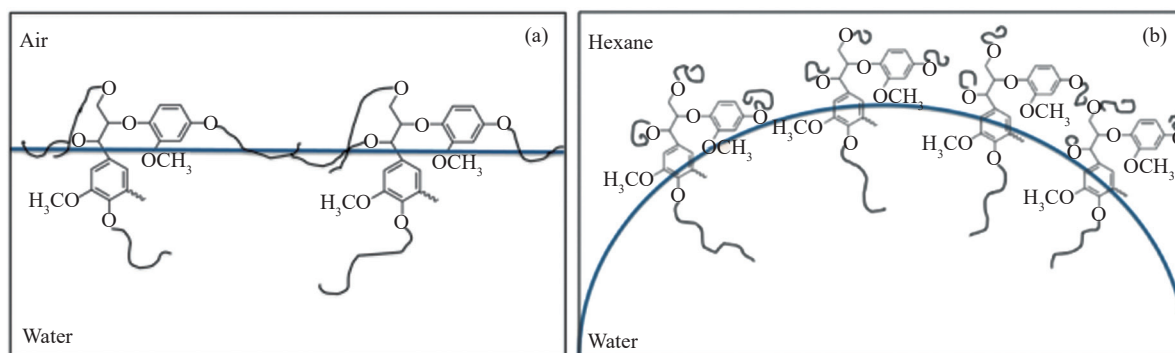


Fig. 2 Diagram of polymer-grafted lignin at the air-water interface (left) and the hexane-water interface (right). Reprinted with permission from [39]. Copyright 2014 American Chemical Society.

by preparing oil-in-water emulsions and applying high-intensity ultrasound at the water-oil interface to assist in the cross-linkage of lignin (Fig. 3). In this study, three different methods were used to prepare microcapsules: (1) no cross-linking agents during the preparation of the capsules, (2) preparation of capsules in the presence of H_2O_2 , and (3) use of polyethylene glycol diglycidyl ether for the preparation of the capsules. The three methods involve the use of an aqueous lignin solution to form an emulsion with olive oil and the use of a high-intensity ultrasound to ensure cross-linking. All the methods successfully yielded spherical micro/nanocapsules with an average diameter ranging from 0.3 μm to 1.1 μm .

The pH-responsive lignin-based nanocapsules that controlled the release of hydrophobic molecules were

prepared by Chen et al.^[44] First, liginosulfonate was grafted with allyl groups by etherification, and the modified liginosulfonate was further dispersed in the oil-in-water miniemulsion under ultrasonic treatment. At the interface of the miniemulsion droplets, the allyl group was functionalized. The lignin and the thiol-based cross-linking agent undergone a thiol-ene radical reaction to form a shell (Fig. 4). By controlling the process parameters, the particle size of the nanocapsules could be adjusted within 100–400 nm.

2.4 Other preparation methods

2.4.1 Mechanical treatment

Mechanical treatment is the most primitive and simplest method to reduce the particle size to nanometer levels, but the size distribution of

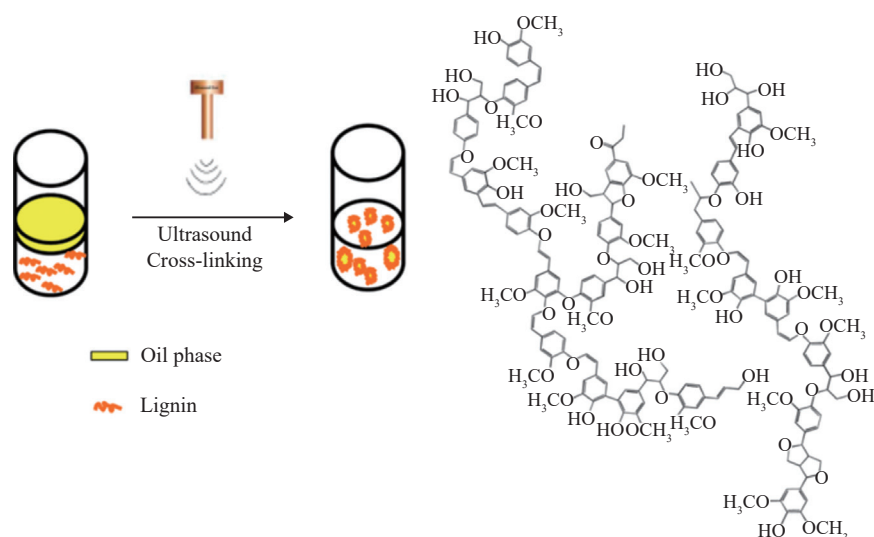


Fig. 3 Diagram of the cross-linking routes for oil-filled KL microcapsules. Reprinted with permission from [43]. Copyright 2014 American Chemical Society.

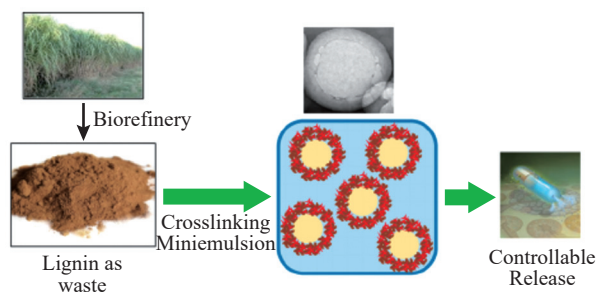


Fig. 4 The production of lignin nanocapsules by cross-linking reaction of interfacial microemulsion. Reprinted with permission from [44]. Copyright 2014 American Chemical Society.

nanoparticles obtained by this method is non-uniform and has a wide range^[45].

Nair et al^[46] used a high-shear mechanical homogenizer to process softwood KL. The results showed that the particle size distribution after the treatment narrowed with the extension of the shear time, and the chemical structure and molecular weight of KL remained unchanged. In addition, Gilca et al^[47] used ultrasound to treat the lignin of wheatgrass, and the molecular weight was decreased. However, Tortora et al^[48] used the same method (ultrasound treatment), which led to an increase in molecular weight due to cross-linking.

2.4.2 Ice segregation-induced self-assembly (ISISA)

ISISA technology refers to dissolve or suspend a material in water and then freeze it. When the solution was frozen, the growing ice crystals would shift, and the polymeric substance would be phase-separated in an orderly manner, essentially surrounding the ice crystals to form a polymer template. After freezing for a specific period of time, substances such as low- or high-molecular-weight precursors and colloidal systems were thawed to form aqueous solutions, suspensions, or hydrogels^[49].

2.4.3 Template-based synthesis

As the requirements for nanomaterials continue to increase, monodisperse nanorods and nanotubes of almost any geometric shape have been prepared, and the "template-based synthesis" method has spontaneously emerged. By using various porous "templates", nanostructures are formed in the pores. These nanostructures can stay in the voids, or they can

be released and aggregated as a group of free nanoparticles, or they can protrude from the surface like the bristles of a brush. The nanostructures formed in this way are ordered and diverse in morphology and have a wide range of potential applications. They have been used to prepare nanotubes and nanofibers composed of conductive polymers, metals, semiconductors, carbon, and other materials^[50].

2.4.4 Aerosol processing

The raw materials are transformed into nanomaterials by driving aerosols to high temperatures in flames, plasmas, and other reactors. Depending on the desired product performance, the particles are immediately collected from the gas phase or processed through aerosol coating and/or functionalization steps^[51].

This is a simple and effective one-step continuous process that can directly produce particles with consistent and controllable properties within the ideal particle size range. Using this method, drug nanoparticles are synthesized, the drug solution is atomized to produce droplets, which are then suspended in the carrier gas and passed through a heated tubular laminar flow reactor, and finally, the particles are collected. The temperature is adjusted so that the solvent evaporates, and the particle formation is completed in the flow reactor^[52].

2.4.5 Electrospinning

Electrospinning is a very versatile method for processing solutions or melts using an electric field to prepare continuous fibers with diameters ranging from a few nanometers to a few microns. This technique is suitable for almost soluble or meltable polymers^[53].

Ago et al^[54] prepared flawless electrospun fibers using lignin, polyvinyl alcohol (PVA), and cellulose nanocrystals (CNCs) as aqueous dispersions, and used CNCs to reinforce nanoparticles. The research showed that embedding CNCs into lignin-based electrospun fibers and spinning coatings improved their thermomechanical properties.

2.4.6 CO₂ anti-solvent

The solute (polymer) precipitation that occurs when the CO₂ fluid contacts with the organic liquid phase

containing the solute is the basis of the anti-solvent process. The mutual diffusion of the organic solvent and the CO₂ fluid provides conditions for the supersaturation of the solute in the organic phase, because the newly formed CO₂ solvent mixture has a lower solubilizing ability than pure solvents. For the process to be successful, CO₂ must be completely miscible with the solvent, and the solute must be insoluble in CO₂ [55].

Myint et al [56] prepared hierarchically porous carbon nanoparticles using commercial KL as a renewable feedstock. The method involved a two-step process: (1) LNPs were prepared by the compressed liquid CO₂ anti-solvent method as described above, and (2) LNPs were thermally stabilized and carbonized under specific conditions, demonstrating the effect of LNP precursors with different sizes and morphologies on the formation of carbon nanoparticles.

3 Biomedical applications

Currently, the concept of a circular bioeconomy is ever present in the public view, which has drawn the attention of the scientific community to lignin and its extensive utilization. As a natural polymer compound for which ecotoxicity is not worth nothing in its utilization, lignin has the potential to enhance biosecurity, and its medical applications have expanded markedly in recent years [57]. We described some recent developments related to the biomedical applications of lignin and related compounds.

3.1 Drug delivery

In recent years, research related to nanomedicines has developed rapidly and has been applied to the treatment of clinical diseases. The most critical part of nanomedicines is the nano-drug delivery system/nano-drug carrier, which is usually composed of natural polymers (polysaccharides, proteins, etc.) or chemically synthesized materials (phospholipids, polymers, porous silicones, etc.) [58–59]. Nano-drug carriers usually have no bioregulatory function of their own, and their primary function is to load biologically active substances, deliver, and release them to target

tissues and cells. In this way, drugs can be enriched and can act on the target tissues efficiently and accurately, enhancing their therapeutic effect, while reducing the impact on normal tissues and mitigating the side effects [60].

For the past few years, many researchers have been interested in the development of LNPs and their application in drug delivery systems [61–63]. There have been reports on the preparation of drug-loaded LNPs by precipitation, dialysis, solvent exchange, and ultrasonic radiation. Frangville et al [6] prepared LNPs by precipitation, and the formed nanoparticles contained tightly packed lignin domains. Even in a high-pH environment, more stable nanoparticles can be obtained. Moreover, owing to the highly porous structure and smaller lignin domains of these nanoparticles, a higher loading capacity could be achieved through hydrophilic active substances. Drug molecules can be introduced into or on LNPs by typical methods, including entrapment, encapsulation, surface physical adsorption, and chemical bonding on the particle surface [64]. Dai et al [65] fabricated trans-resveratrol (trans-RSV) and Fe₃O₄ nanoparticle-embedded LNPs (AL/RSV/Fe₃O₄ LNPs), which were insoluble anticancer drugs, to achieve passive targeting, the magnetron target of trans-RSV, and reinforced bio-dispersion and treatment effects. Experimental results showed that AL/RSV LNPs and AL/RSV/Fe₃O₄ LNPs inhibited tumor growth and enhanced survival rates. The excellent curative effect of the AL/RSV/Fe₃O₄ LNPs was due to their stable sustained release of trans-RSV, suitable particle size, good magnetic properties, remarkable biocompatibility, dispersibility, and anti-ultraviolet capability.

Lignin is composed of an aromatic structure of the hydrophobic skeleton and hydroxyl amphiphilicity, it is considered a promising shell-core micro-nanocapsule for drug delivery. Li et al [66] used KL to self-assemble nanocapsules in a water/ethanol solution. The hollow core structure was retained by a high percentage of ethanol in the nanocapsules. It was not only the biocompatibility and biodegradability, but also the

stability and release characteristics of lignin capsules, which had attracted great interest for pharmaceutical and biomedical applications. Zongo et al.^[67] explored the stability of softwood lignosulfonate microcapsules (SLS-MCs) under different storage conditions. They found that SLS-MCs showed perfect long-term stability at neutral pH value and in a salt-free aqueous environment, as well as good stability in a bovine serum albumin solution. Furthermore, under standard sterilization test conditions, the SLS-MCs sustained a complete appearance, but at -20°C and atmospheric pressure, the shape of SLS-MCs changed significantly owing to the formation of ice crystals and the detrimental effect of internal microscopic oil phase crystals.

Pickering emulsions are stabilized by nanoparticles. This emulsion not only retains the common characteristics of traditional emulsions but also has high coalescence resistance, which allows the replacement of traditional emulsions in cosmetics and pharmaceutical applications. Surfactants usually exhibit adverse effects, such as irritation and hemolytic behavior in these cosmetics^[68]. The amphiphilicity of lignin is an important property for the development of special emulsifiers. Wei et al.^[30] first showed that colloid particles of alkaline lignin could stabilize styrene-in-water as Pickering emulsions. The LNPs were self-assembled from lignin under acidic conditions, which automatically surround styrene droplets through strong vibrations from Pickering emulsions. The advantages of Pickering emulsions make them attractive for biomedical applications. However, there are few studies on functional Pickering emulsions for the delivery and storage of photosensitive drugs. Dai et al.^[69] designed a thermo-responsive and UV-blocking Pickering emulsion system to stabilize the photosensitive drug trans-RSV that was maintained in palm oil droplets in water by the poly(*N*-isopropylacrylamide) (PNIPAM)-grafted LNPs (AL-g-PNIPAM LNPs). The dual-functional features were based on the UV blocking ability of the graft chain of PNIPAM and the UV photochromic group in lignin.

AL-g-PNIPAM LNPs remarkably improved the stability of trans-RSV and regulated the drug release behavior by adjusting the temperature.

3.2 Bio-imaging

Although the study of quantum (carbon) dots has been developed for more than a decade, using renewable resources as a starting material for environmental and economic sustainability to produce quantum (carbon) dots remains a challenge. Lignin which has a high carbon content and rich aromatic structure, is an ideal renewable candidate for fabricating bulk aromatic chemicals and high value-added carbon materials.

So far, there are some studies that have been reported doping heteroatoms to improve fluorescence intensity and stability of quantum (carbon) dots in lignin. Si et al.^[70] proved that using rice stalks as raw materials, carbon dots were synthesized using a microwave one-pot method in an acid-catalyzed ethanol-water co-solvent system. In the process of microwave irradiation, N-doped carbon might be doped with nitrogen from protein. Thus, obtaining 46% quantum yield and strong purple fluorescence.

In addition to the carbon dots, using lignin capped copper nanocolorants for cell imaging was reported by Pillai et al.^[71]. Copper nanoparticles have fluorescence characteristics to some extent, but their application in biological imaging is hindered by their low fluorescence intensity and poor dispersion^[72]. Lignin-encapsulated copper nanoparticles can support cell division without causing any physical damage to A549 cells, improving the stability of cell imaging fluorescence intensity^[71]. The exact formation mechanism of lignin-derived fluorescence quantum dots is still unclear, but quantum (carbon) dots with desirable photoluminescence and great biocompatibility can be used as a fluorescent nanomaterial for living cell imaging and other applications.

4 Challenges and opportunities

Bio-renewable polymers has emerged as an attractive alternative to conventional metallic and organic

materials for various application, because of their biodegradability, biocompatibility and low cost of preparation ^[73].

Lignin is a new type of green polymer material with great potential because of its important properties such as antioxidation, antibacterial property, and stability. However, the complexity and heterogeneity of its own structure, as well as the resulting molecular characteristics, biomass source variability, and pretreatment process are all important challenges. Various academic studies have been focused on the preparation and modification of industrial lignin in the form of nanoparticles, showing that these challenges can be solved by applying lignin in the form of colloidal particles. These particles are very stable in a wide range of pH value, and can be easily dispersed in organic solvents after being stabilized by cross-linking. The negative hydroxyl groups on the surface of colloidal particles can undergo various enzymatic and chemical modifications, such as polymerization and surface coating with positive polymers.

Lignin has the characteristics of biodegradability, biocompatibility, and anti-oxidation properties with no cytotoxicity, and can improve soil nutrients through microbial degradation. It is an ideal precursor for the development of environmentally friendly nanomaterials. Moreover, it is provided as waste in the form of industrial/agricultural biomass and is a low-cost biomaterial and the primary cost involves transporting it to processing facilities. The production of LNPs and its numerous high-tech applications are ideal ways to reduce agricultural and industrial biological waste ^[74]. Several methods of synthesizing LNPs involve hazardous materials (solvents such as THF) which reduce or even eliminate their applications, therefore replacing them with more environmentally friendly solvents is crucial, especially in industrial production. It is necessary to develop innovative methods to produce well-organized, inexpensive, time-saving LNPs possessing ideal structures, properties, and shapes suitable for applications under conditions that are conducive to the

ecology, especially for the high-value nano- and biomedical fields ^[75].

Despite the large-scale production of lignin, its use in high-value applications remains a major challenge. Extensive exploration of lignin-derived composite materials has confirmed their potential in scaffold materials and/or drug release research. In academic research, LNPs demonstrated their potential use in composite materials, UV absorbers, and antioxidants; further, the possibility of using LNPs in high-value medical applications has achieved promising results. The intrinsic biological activity of lignin derivatives will become the driving force for their wide application in the medical field.

5 Status and outlook

The size and shape of lignin particles, as well as their stability, depend on the type of lignin, preparation process, and production conditions. Lignin has broad application prospects in various biomedical fields, such as drug delivery and cancer treatment. The development of micro/nanosized lignin and their composites has aroused increasing attention because they can aid in the production of lignin-based value-added products. The application potential of lignin as micro/nanomaterials is increasing, and it is expected to become an important research field in the future.

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