Study of Extraction and Characterization of lignin from different Sources

V AshaRanjani*, G Tulja rani, M Pramod Reddy, MSthiya, G Kavya Reddy Malla Reddy Pharmacy College "*Maisammguda, Dhulapally, Medchal District, Hyderabad"

ABSTRACT: Plantcellwallscontain lignin, acomplexaromaticpolymerthatisvitalformaintaining structural integrity and resistanceto deterioration. In this work, lignin is extracted and characterized froma varietyofbiomasssources, such as wood and agricultural waste. Ligninisisolated using avariety of extraction techniques, including kraft pulping, organosolv procedures, and enzymatic treatments, with an emphasis on comprehending how these techniques affect yield and characteristics. The structural characteristics and functional groups of the extracted lignin are examined using characterization techniques such as Fourier-transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The findings show notable differences in the molecular weight, solubility, and chemical makeup of lignin obtained from various sources.

KEYWORDS: Nanoparticles, lignins, nanolignins, nanofilms, acid precipitation, organosolvents, antimicrobial, antioxidant

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I. INTRODUCTION:

Ten to twenty-five percent of lignocellulosic biomass is made up of lignin, the second the most abundantnaturalpolymeraftercellulose.Coniferyl,sinapyl,andP-coumarylalcoholsarethethree types of substituted phenols that make up this highly cross-linked, three-dimensional macromolecule. Enzymatic polymerization produces large number functional я of groups and connections.Numerousmaterials,suchasjute,hemp,cotton,andwoodpulp,containlignin.One of the most plent if uland renewable resources on the planet is lign in, an amorphous natural polymermade from phenylpropane. Because lignin is difficult to isolate, analyze compositionally, and characterize structurally, its definition has never been as simple as that of other natural polymers like cellulose. Instead of being a single molecule, lignin is a collection of naturally occurring phenolic polymers with a diverse variety of compositions and interunit connections. Lignin's intrinsic complexity and the fact that its composition and structure vary depending where it comesfrommakeitchallenging todefineprecisely. Oneimportantstep on intheconversion process involving lignocellulosic biomass is pretreatment, which aims to break down the lignocellulosic matrix. Three-dimensional, irregular, hydrophobic, non-toxic, and biodegradable, lignin is a macromolecule. Lignin has gained increased recognition as a low-cost byproduct of the pulp and paperindustry.AccordingtoArgopoulosandMenachin(1998),thereareanestimated300billion metric tonnes of lignin in total on Earth, of which 20 billion metric tonnes are created each year through biosynthesis. As a result, lignin is anticipated to be a key raw material in the worldwide bio-basedeconomy, particularly in the production of biofuels and bioproducts. Increased interest

in lign in inher baceous plants is mostly due to two factors: (a) their capacity for yearly regeneration and the second second

and(b)thefactthatherbaceousplantsaccumulatethemostbiomassannually.Growinginterestin lignocellulosic material as a source for energy, chemical, and material production has resulted from the persistent oil shortage. The main function of lignin is to bond fibers, giving them structural stiffness and shielding trees from physical and chemical dangers.

Historically, lignin was predominantly seen as a challenge to industries that sought to extract celluloseandhemicellulosefromplantbiomass.Itsrobustandcomplexstructure,interwoven

witharomaticringsandirregularlinkages,posedobstaclestofacileprocessing.Consequently, lignin was relegated to a secondary role, often incinerated or relegated to lower-value applications]. However, the paradigm surrounding lignin has been rapidly evolving, and researchers are now recognizing its immense potential to address environmental challenges.

Lignin'sresurgenceisunderscoredbyitsubiquity in natureand itspivotalroleincarbon cycling within ecosystems . As a fundamental component of plant cell walls, lignin contributes to the support and structural integrity of terrestrial vegetation . Its degradation, orchestrated by a consortium of microorganisms, facilitates the recycling of carbon back into the soil, per mechanical petuating the delicate balance of nutrient cycling. This inherent capacity of lignin to undergo biodegradation forms the basis for its potential as an environmentally benign material fordiverseapplications, includingenvironmentalremediation. Indeed, lignin's unique properties make it an attractive candidate for a variety of applications, particularly in the realm of environmental remediation. As a polyphenolic compound, lignin possesses a high density of functional groups that lend themselves to interactions with a broad spectrum of pollutants . The development of lignin nanoparticles, harnessing the power of nanotechnology, has further expanded its application potential. These nanoparticles, with their high surface area to volume ratio and enhanced reactivity, hold promise for revolutionizing the way we approach environmental cleanup .

Ligninisacomplexpolyphenolicmacromoleculethatispartofthelignocellulosicbiomass, and represents one of the most abundant aromatic biopolymers found on Earth. ¹It is primarily composed of three basic monomericunits, *p*-hydroxyphenyl(H), guaiacyl(G), and syringyl(S), which are interconnected by β -O-4 linkages, among others. ¹The proportion of both monomeric units and the type of linkages in the lignin structure can vary according to the biomass source, that is, whether the lignin is isolated from softwoods, hardwoods, or grasses, which ultimately affect the mechanical and physicochemical properties of the lignin polymer. Despite the high

abundance, only around 2% of the annually extracted lignin has been utilized, mainly as dispersants, additives, adhesives, while most of the lignin is directly combusted to generate and heatand electricity.Duringthepastyears,ligninhasgainedincreasedattentionfromtheresearch community and has shown tremendous potential for advanced applications due to its unique features, such as antioxidantand antimicrobialproperties, UV-blocking ability, biodegradability, and biocompatibility. However, the lignin valorization has been hampered by its complex and heterogeneous molecular structure, which is highly dependent on the source and extraction method. One way to overcome these limitations is to transform raw lignin into lignin nanoparticles (LNPs), enabling the typically water-insoluble lignin to form stable colloidal dispersions in water, and increasing the antioxidant activity due to the higher specific surface area. Furthermore, the LNP surface can be easilv chemically modified due to the large availabilityofdifferentfunctional groups, including a liphatic and phenolic hydroxy and carboxy groups.¹¹ Therefore, the development of LNPs allowed them to be employed in several high value-added applications, including drug delivery, antibacterials, and emulsion stabilizers, among others.

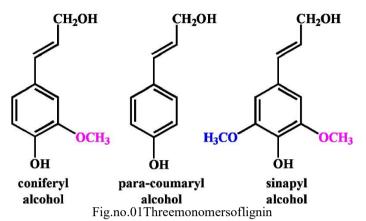
Lignin is the second-largest lignocellulosic biopolymer (after cellulose) on earth and one of the main components of theplantcellwall. Threephenylpropanemonomers makeup lignin's three- dimensional network structure. They are para-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol. These are linked together by carbon–carbon and ether linkages. A variety of functional groups differentiate thearomaticring thatmakes up thepolymericstructureof lignin. It contains aliphatic and methoxyl groups, carboxylic, carbonyl, and phenolic hydroxyl groups, as well as a propanoid chain. It functions as a physical barrier to stop the spread of diseases and their toxins becauseofitsheterogeneity,hydrophobicnature,andinsolubilityinaqueousenvironments. Asa result, efforts are being made in both research and industry to use lignin in the manufacturing of polymeric drug encapsulation and scaffold materials. The lignin's physical and chemical behavior will be different according to the plant source and extraction conditions, temperature, and residue/solvent ratio due to variations in the basic compound of lignin.

II. PHYTOCHEMICALSTRUCTUREOF LNP'S:

Lignin's three-dimensional network structure is made up of three phenylpropane monomers (paracoumarylalcohol,sinapylalcohol,andconiferylalcohol,Figure1)connectedbycarboncarbonandetherbonds (1).Numerousactivefunctionalgroups,includinghydroxyl,aromatic, and aliphatic groups, are present in it.

The different types, extraction techniques, purifying procedures, and sources of lignin are the

primaryelementsthatcontributetoitscomplexityanddiversity(2).Asaresultofvariouspulping and extraction techniques, the structures and properties of ligninin fluence its future advances and applications.



The chemical structures of lignin are complex consist of various functional groups within the molecules including phenolic, methoxy, and analiphatic hydroxyl group. The pure form of natural ligniniscolorless, however, its color changest obrown or dark brown after acidoral kalitreatment. Theproperties of ligninrely onthesefunctionalgroups andthemost abundanthydroxylgroup in ligninisthealiphatichydroxylgroup.Differentsourcesoflignincanhavedifferentproportionsof hydroxyl groups.²¹Lignin can be classified as native lignin and technical lignin. Original lignin structure without any iscallednativeligninwhereaslignin modification extracted fromindustrial byproductisknownastechnicallignin.Technicallignincanbeofdifferenttypesdependingupon the sources and extraction methods. Lignin acts as a binder to assemble the plant cell walls by associating with the cellulose and hemicellulose in the plant cell. This phenomenon provides mechanical strength to the plant cell wall and also regulates the water conduction as well as protecting other components from enzymatic degradation. Therefore, lignin can be termed as a "building element" in the design of biomaterials (eg lignin-based materials, epoxy, or polyurethane). It has resistant to most of the biological attacks in comparison to hemicelluloses/cellulose. Moreover, the biomedical properties of the lignin-based materials also depend on their structure, type of active functional groups, molecular weight, type of lignin obtained from the plant sources, isolation methods, etc.

III. SYNTHESISMETHODSOFLNP's:

Lignocellulose's physical and chemical properties, such as its antibacterial, antioxidative, corrosionresistant, and non-toxic properties, are receiving increasing attention (3,4). The creation of nanoparticles using cheap and abundant lignin raw materials is essential to increasing their high-value-added application. These days, self-assembly, progressive addition, anti-solvent precipitation, and mechanical procedures are the main ways employed to manufacture nano-lignin. The numerous sizes and shapes of lignin nanoparticles that can be created with these different methods and conditions would be highly advantageous for their potential use in a variety of industries in the future.

3.1 Anti-SolventPrecipitation

Although lignin is soluble in typical organic solvents such as THF and DMF, it is frequently insoluble in acidic or aqueous solutions(5). In the process of making lignin nanoparticles, THF and DMF are commonly used as organic solvents to dissolve lignin due to their excellent performance and incapacity to change the structure of lignin.

3.1.1 WaterasAnti-Solvent

Because lignin is not as soluble in water as it is in organic solvents, adding water reduces the solubility of the nanoparticles, which leads to their precipitation out of the solution.

Li and colleagues [14] used self-assembly to produce spherical hollow nanocapsules with a diameterofapproximately63nmbyincorporatingwaterintoasimplekraftlignin/ethanolsolution mixture. The diameter of the nanocapsules increased with increasing lignin concentration and decreasing water addition rate.

Using an ethylene glycol solution, Richter et al.(6)produced kraft lignin nanoparticles (45–250 nm) using HNO₃ flash-precipitation. Applying a cationic polyelectrolyte to the nanoparticles' surface changed their surface properties and increased their stability in high pH conditions.

Chen et al. (7) obtained quasi-spherical lignin nanoparticles around 100 nm by introducing deionized water into lignin dispersed aqueous sodium *p*-toluenesulfonate (pTsONa) solution. Various types of lignin (kraft lignin, sulfonate lignin and alkaline lignin) could be completely dissolved in the pTsONa solution at room temperature. The nanoparticle diameter could be controlled by varying the pH of the solution. The size of nanoparticle decreased as the pH value increased, which could be explained by the synergistic dissociation of pTsONa and the phenolic OHandCOOHfunctionalgroupsofligninnanoparticles. Thismethodavoidssomelimitationsof the solubility of lignin species and the use of organic solvents. Nevertheless, the revealed irregularityofnanoparticle morphology andthe instability indifferentpHsolutionsaredrawbacks.

Camargos et al. (7)andYearla et al.(8) utilized a solution of acetone/water to dissolve lignin extracted from corn biomass, hardwood lignin and softwood alkali lignin, respectively. The spherical lignin nanoparticles around 100 nm were obtained by controlling the solution pH and dropping double-distilled water rapidly.

3.1.2 AcidSolutionasAnti-Solvent

Themethodusedtoproduceligninnanoparticles,acidprecipitation,issimilartousingwaterasan antisolventbecauselignin isinsolubleinbothorganicsolventsandacidsolutions.Moreover,the electricaldouble layer theory claims that theprecipitation of lignin nanoparticles is facilitated by the high H+ concentration in the acid solution (9)(10)

Richter et al.(11) obtained kraft lignin nanoparticles (45–250 nm) through HNO_3 flash- precipitation from ethylene glycol solution. The surface of the nanoparticles was coated with a cationic polyelectrolyte, which made their surface properties adjustable and increased their stability in high pH system.

Beisl et al.(12,13) designed three different precipitation setups (batch, T-fitting, static mixer) with different mixing speeds to generate lignin nanoparticles, introducing H_2SO_4/H_2O solution intoethanolaqueousmixture. The smallest nanoparticles (almost 100nm) could be produced by static mixer setup with the highest mixing speed. The molecular weight and chemical structure of lignin nanoparticles did not change during the precipitation process.

3.1.3 SupercriticalCO2asAnti-Solvent

Because of the many distinct physical and chemical properties that occur, supercritical flow technology has fabrication materials(12)(13). been widelv used in the of nanoparticle Supercriticalantisolventprecipitationmethodisbasedontheideathatlignindissolves more readilyin asolventthanin asupercritical fluid Lignin becomes less soluble in the originalsolventwhenthesupercriticalfluiddissolvesintothesolution. (14)Highsaturationlasts only a short time until high-purity lignin nanoparticles precipitate. Because of the supercritical fluid's low viscosity and minimal surface tension, the produced nano-lignin has a narrow distribution and small particle size, which is one of the benefits of the supercritical process. The commonly used CO2 is also inexpensive and non-toxic.

Myint et al.(15)successfully prepared the quasi-spherical lignin nanoparticles (38 nm) through introducingcompressedCO₂intokraftlignin/DMFsolution,whichexhibitedhighmonodispersity

anduniformsize. Thesize of nanoparticles increased as the temperature increased and the pressure decreased. Furthermore, the influence of two different solution flow rates (0.03 and 0.06 kg/h) on the size was discussed: lower flow rates showing an increasing trend on nanoparticle diameter. These effects on the nanoparticles formation were attributed to the change in solubility between DMF and CO₂. Moreover, the nanoparticles exhibited excellent properties, for instance, favorable thermal degradation, outstanding dispersion stability, excellent UV absorption and non- cytotoxicity.

A similar process with supercritical CO_2 was used by Lu et al.(18)to prepare spherical nanoparticles around 144 nm in size. Lignin was dissolved in an acetone solution and CO_2 was added at 35 °C and 30 MPa. No change in the amorphous chemical structure was confirmed by FTIR analysis and XRD analysis in the supercritical antisolvent process. The nanoparticles possessed significantly improved solubility in water and antioxidant activity as a result of the enhanced specific surface area, compared with original lignin.

Theabovethree anti-solventprecipitation methods areoften usedto prepareligninnanoparticles, but there are some disadvantages. For example, lignin cannot be completely uniformly dispersed inthesolvent, and agglomeration may occur insome cases. In the subsequent process, the solvent has to be removed by rotary evaporation or freeze-drying, which is unfavorable for precise control of the size and morphology of the nanoparticles.

3.2 PhysicochemicalMethod

Byavoiding the use of harsh chemical sororganics olvents, the physicochemical creation of lignin nanoparticles may contribute to less environmental harm. In order to effectively avoid complicated aftertreatment, the methodology mainly entails preparing nano-ligninus ingmechanical force or

ultrasound, which includes the high shear homogenization method, ultrasonication, and a variety of mechanical methods.(16)

3.2.1. Ultrasonication

Even though high size dispersion has its disadvantages, the ultrasonication technique and other mechanical treatments are still commonly employed to reduce the particle size to the nanoscale scale due to their convenience and ease of usage.

Nanoparticles are already synthesized via ultrasonication from biopolymers like chitosan and cellulose(17). Because ultrasonic cavitation generates high temperatures and pressures, it can lead to polymer breakdown, chemical bond breaking, and the generation of free radicals(18). To breakthemolecularbondsinlignin, the ultrasonic methodem ploysultrasonic radiation(19). The macromolecular lignin disintegrates into small molecular lignin, resulting in lignin particles that are smaller in size. The ultrasonic approach produces more stable and less sedimenting lignin nanoparticles.

Solidandhollowspherical lignincolloids were synthesized through ultrasonics pray-freezing (1.7) and the second synthesized through ultrasonics pray-freezing (1.7) and t

MHz)ofanalkalilignin/DMSOsolutionon liquidnitrogencooled copperplatebyMishra et al. . Thisprocessconsistedofdropletandparticleformations,whichweredeterminedbythefrequency of ultrasonic nebulizer and the diffusional nature of solution. The hollow particles and solid ones were formed through peripheral or volumetric precipitation, respectively. The enhanced UV absorption ability because of layer by layer deposition was obviously observed.

Tortora et al.developed ultrasound irradiation driven assembly of kraft lignin into spherical microcapsules (300–1100 nm) at the water/oil interface. The mechanism of microcapsules formation was explained by emulsification and cross-linking during the ultrasound treatment. Specifically, this was because ultrasound irradiation induced the formation of -OH radicals and the combination of OHradicals and the combination of and cross-linking during the ultrasound treatment.

cytotoxicityofligninmicrocapsuleswereexhibitedbyencapsulationandfurtherdelivery of the hydrophobic molecule Coumarin-6 in vitro.

The alkali lignin nanoparticle dispersion (200 nm) and nanocomposite hydrogels were generated

byChenetal.[80]viaultrasonicationusinganultrasonicHomogenizorat25kHzfrequencyfor1

h.Althoughtherearenoseparatednanoparticlesandnoresearchonstabilityindifferentmedium, the simple operation and environmental protection are obvious advantages.

3.2.2. Homogenization

Theligninparticlesinasuspensionoremulsionsolutionaredispersedandhomogenizedusingthe high shear homogenization method. In contrast to the ultrasonic technique, a strong shear force breaks the lignin's chemical connections. Nair et al.(20)used a high shear homogenization process at 15,000 rpm for 4 hours to mechanically prepare the kraft lignin nanoparticles in deionized water.

Rao et al.(21)produced the colloidal spherical lignin nanoparticles by homogenizing the organosolv lignin residues/ethanol/water mixtures for 45 minutes. The interaction of the hydrophilic moieties of organosolv lignin residues and ethanol may account for the more homogeneous size of the colloidal spheres in solutions with higher ethanol contents.

3.3. AcidPrecipitation Method

Frangville et al. (22) developed a new method for preparing acids to make lignin nanoparticles. Thework presented two alternative approaches.

Inthefirstway, ligninnanoparticles were formed as precipitates that we restable across a widep H range by adding hydrochloric acid to the lignin's ethylene glycol solution, followed by cross-linking and water dialysis. Toproduceligninnanoparticles, the second technique included rapidly adding HNO₃ solutions to high pH aqueous

lignin solutions, which were only stable at low pH. Gupta et al.(23)used an acidprecipitationapproachtocreateligninnanoparticles, which were then examined by TEM and DLS in this France illustrate Additionally Denoted (24) and here the provide the france ligning ligning the product of the pr

DLS, justlike Frangville et al. Additionally, Pangetal. (24) produced nanoparticles from alkaline lignin using the acid precipitation method.

3.4. Solventexchange/solventshifting

Another technique for creating high-quality LNPs with diameters ranging from 80 to 360 nm is solvent exchange/solvent shifting, which has outstanding stability(25). The use of costly and hazardous chemicals is required for this In addition to other biomass. Jiang al. process. et (26)producedLNPsfromcorncob,pine,birch,pubescens,andvinasse.Theseeffluentswereconverted into LNPs via a solvent shifting technique, producing spherical, evenly sized nanoparticles. The experimental results indicate that arange of industrial applications can make use of nanoparticles with diameters ranging from 300 nm to 4 um. Even though the solvent exchange/shifting technology is easier to produce, it also makes dialysis membranes more expensive, which ultimately increases the cost of the operation.

3.5. Polymerization Method

Early in 2006, Barakat et al.(27)reported employing the polymerization technique to turn the syntheticligninpolymerarabinoxylan-dehydrogenationpolymerintonanoparticles.Inthe presenceofheteroxylans,coniferylandsinapylalcoholswerepolymerizedtoproducethearabinoxylan-dehydrogenationpolymernanoparticles.Inordertodescribetheshapeoftheproduced nanoparticles, TEM and multidetected size exclusion chromatography were employed. Usingatomtransferradicalpolymerization(ATRP), Qianet al.(28)developedanovelmethod forproducinglignin-basednanoparticlesbygrafting2-(diethyl-amino)ethylmethacrylate (DEAEMA)onalkali lignin.The resultingnanoparticleswere usedassurfactantsforCO₂/N₂- switchablePickeringemulsions,whichwasacreativewaytodevelopahigh-valueusageoflignin products.

3.6. CrosslinkingMethod

Yiamsawas et al. (29) first synthesized the biodegradable hollow nanocontainers with a hydrophilic core from sodium lignosulfonate and alkali lignin. For the preparation of lignosulfonatenanocontainers, sodium lignosulfonate was first dissolved in aqueous NaCl solutions to generate the dispersed phase, which was subsequently mixed with cyclohexane containing thebiocompatiblesurfactantPGPR (polyglycerolpolyricinoleate). emulsionwasthenultrasonicatedsoastoformastablemini-Theobtained preemulsion. The polyaddition reaction occurred at the interface of the mini-emulsion droplets, which was initiated by dropwise adding toluene diisocyanate (TDI)/cyclohexane solutions into the mini-emulsion. After keeping at room temperature overnight, the lignosulfonatenanocapsule dispersions were successfully formed, which could still remain stable when being transferred into aqueous dispersions because of the presence of sulfonic groups. The preparation process of alkali lignin nanocapsules is basically the same, but sodium dodecyl sulfate should be added if the nanocapsules are required to redispersein water. Theseobtained lignin nanocontainers had aparticlesize in therangeof 150-200 nm and could keep stable in aqueous or organic dispersions over a long period (several weeksorevenmonths).Tortoraet al.(30)createda novelsynthesisofkraft lignin microcapsules byfirstpreparingoilinwateremulsions followedby ultrasound-assistedcrosslinkingofligninat the oil/water interface. Taking the ultrasound preparations of lignin microcapsules in the presence of H₂O₂ as an example, the specific process was described as follows. Firstly, olive oil and H₂O₂ were added into lignin alkali solutions, and then the whole mixing system was sonicated. Next, lignin microcapsules were obtained by centrifuging and washing. The finally formed lignin microcapsules had an average particle size of 0.3-1.1 µm with a spherical configuration. The formation mechanism was also revealed by means of GPC and NMR measurements.

3.7. Self-assembly

It is a process in which an ordered or organized structure is generated due to some specific intermolecular noncovalent interactions such as hydrophobic, electrostatic, hydrogen-bonding and Van der Waals interactions directions. frequently-used absence of any external This in is а methodtopreparenanoparticles, which we would put emphasison in this section. Qianetal. (31) used the self-assembly method to produce the uniform lignin-based colloidal spheres. After acetylation, the alkali lignin (AL) was transformed into acetylated lignin (ACL) and then dissolved into THF. With the gradual addition of water into the ACL/THF solutions, the ACL molecules started to associate to form colloidal spheres through the hydrophobic interaction.

After rotary evaporation to remove THF, colloidal spheres with a hydrodynamic radius of 110

nmweresuccessfullyobtained. Thisstudygivesimportantenlightenmentonhowtoconvertthe irregular lignin-based polymers into the ordered colloidal spheres. Qian et al.also reported a novel approach to fabricate lignin reverse micelles (LRMs) via self-assembly. In this method, LRMs were formed by adding cyclohexane into the alkali lignin/dioxane solutions. With the increasing amount of cyclohexane, LRMs were separated from the solutions in the form of precipitation. Deng et al.proposed a simple and feasible method in the formation of hollow

ligninazocolloids.Theyfirstmodifiedalkalilignin(AL)intothelignin-basedazopolymer(AL- azo-H). Then, water was gradually added dropwise into the AL-azo-H/THF solutions. With the further addition of water above 53 vol%, AL-azo-H colloidal dispersions were obtained, and the average particle size of the formed spheres was approximately 170 nm. Li et al.prepared lignin hollow microspheres fromthe esterifiedorganosolvligninmodified with maleic anhydrideusing theself-assembly method in themixed solventof THFand water. Richter etal.provided asimple self-assembly method for the synthesis of biodegradable lignin nanoparticles using organosolv ligninasarawmaterial.Specifically,organosolvligninnanoparticleswereobtainedbygradually dropwiseaddingwater intoorganosolv lignin/acetonesolutions.Nanoparticlesobtainedfromthe above-mentioned preparation process showed a spherical shape and relatively uniform size (except organosolv lignin nanoparticles produced by Richter et al. (2016) due to the broad molecular weight distribution of organosolv lignin and inhomogeneous mixing, etc). However, there also are some limitations. They all utilized hazardous and expensive chemical reagents, such as acetyl bromide, cyclohexane, dioxane, NaNO₂, maleic anhydride, THF, and acetone, or involved complicated chemical modification reactions. Li et al. (32) in their work, presented a simple, green, and low-cost preparation of nanocapsules through self-assembly from kraft lignin (KL) without any chemical reactions. During the process, water was added dropwise into the KL/ethanol solution by the peristaltic pump until the water content reached 90 vol%, at which the formation of KL nanocapsules was completed. The particle sizes of KL nanocapsules could be easily adjusted by changing the dropping speed of the water.

OtherPreparations

There are some disadvantages in the preparation of lignin nanoparticles by organics olvent-based self-assembly. For example, the additional separation steps and the treatment of residual organic solvents are required. The morphology and size of lignin nanoparticles are affected by the amount of organic solvents. In addition to the above-mentioned relatively mature methods for preparing nano-lignin, some new methods have been explored based on the characteristics of the raw materials selected and the specific applications of nano-lignin (33).

IceSegregation-InducedSelf-Assembly

Spenderetal. presented anice-segregation-induced self-assembly process to produce kraftlign in the segregation of the segrega

nanofibers(diameter<100nm)inaqueoussolution.Specifically,theligninsolutionwasdispersed on a steel surface tempered with liquid nitrogen to 77 K through syringe. The nanofibrous morphology and the phenomenon of priority alignment on the frozen front were obviously observedbySEM.Thehighanduniformfreezingratesandlowsolutionconcentrationsplayedan

extremelyimportantroleinformingmuchsmallerandmoreuniformnanofibers.Furthermore,the carbon nanofiber network structurewas generated after carbonization of lignin fibers at1000 °C. Thisreproducible methodologycouldbeemployedforpreparationofcarbonnanofibersfromother water-soluble polymers.

Aerosol-FlowSynthesis

Ago et al. (34) developed an aerosol-flow method to synthesize the spherical particles (30 nm-2 µm)withdifferent ligninsfrom the respective H₂OorDMF solution via insitu size fractionation. The lignin droplets were transported to the laminar flow reactor by a nitrogen stream, and then dried to form solid particles. Although the fractional yields were affected by the solution concentrations and types of lignin, the total yield could reach more than 60%. The increase insize and narrower distribution of the lignin particle could be observed as the concentration of lignin solution increased. In addition, the lignin particles could be uniformly and stably re-dispersed in different oil/water media and polar/non-polar solutions under shear or heating, even in Pickering emulsions. Thisnovelandhighyieldmethodthroughaerosolflowreactorwillbenefitfromlarge- scale production of lignin nanoparticles with controllable size and morphology.

Electrospinningof Solutions

Electrospinning is a process of forming a jetstream of lignin solution under high-voltage electrostatic stretching and spraying from the spinneret hole to form polymer nanofibers. During electrospinning, the positive electrode is placed on the syringe containing the lignin solution and thenegativeelectrodeisconnectedtoacollectionplate. When there is no external voltage applied, the lignin solution fluid formsdropletssuspended at theneedle mouthunder the action of gravity and surface tension. When turning on high voltage, the droplets spray a fine stream from the needle under the action of electric field force. As the solvent evaporates, the fine stream solidifies on the collection device to form lignin fibers.

Ruiz-Rosasetal.employedtheelectrospinningofAlcelllignin/ethanolsolutionstogeneratelignin

submicronfibers(400nm–1 μ m).A76% yield of ligninfibers after the stabilization treatment and the 40% yield of carbon fiber after the carbonization treatment could be obtained, respectively. Weight-lossowing to the release of H₂O and CO₂ could be illustrated by the cleavage of hydroxyl and carboxyl groups in the lignin degradation. The smooth surface without any defects in lignin submicron fibers was characterized by SEM and TEM images. The microporous structure of the carbon fibers was confirmed by N₂ adsorption-desorption isotherms at -196°C. Furthermore, the carbon fibers possessed the favorable stability in air at low temperature and excellent oxidation resistance.

The similar electrospinning method was utilized by Ago et al. to prepare lignin-based nanofibers reinforcedwithcellulosenanocrystals. The effect of electrical conductivity and surface tension of solutions on the electrospinnability and morphology of lignin-based nanofibers was investigated by SEM images. Additionally, then anofiber composites possessed the enhanced thermal stability due to hydrogen bonding interaction between lignin and cellulose nanocrystals.

IV. EXTRACTIONPROCESS:

4.1 Formicacid/aceticacidtreatment

Inordertoextractligninfromdifferenttypesofbiomass,thefirststepwaspulping,whichentailed slicing the biomass into tiny pieces and placing them in а conical flask. In order to bring the biomassintheflasktoaboilonahotplatefortwohours, it was combined with85%organicacid (theformicacid/aceticacidratiowas70:30byvolume)atafibertoliquidratioof1:8.Twohours were spent for the flask and its contents to cool to room temperature. The fibers were filtered through a Buchner funnel and then cleaned with 80% formic acid and hot distilled water.

4.2 Peroxyformicacid/peroxyaceticacid(PFA/PAA)treatment

Pulpstreated with FA/AA were further delignified after pulping by exposing them to a PFA/PAA

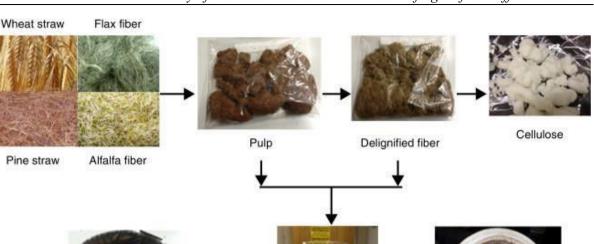
solutionmixturefortwohoursinahotwaterbaththatwassetat80°C.ForthePFA/PAAsolution, an85%formic acid/aceticacid mixturewas mixed with8ccof 35%H2O2... Toseparate cellulose fromcookingliquor,amixtureofligninandhemicellulosewithformicacid,thedelignifiedfibers were filtered and then rinsed with hot water.

4.3 Bleaching

A hot water bath set at 8 °C for two hours was used to bleach the delignified fibers using 14 milliliters of a 35% H_2O_2 solution (pH 11–12). Finally, distilled water was used to rinse the pulp and remove any final bits of lignin. This process was repeated to remove the lignin completely.

4.4 Isolationof lignin

To isolate lignin, the procedure suggested by Nuruddin et al. and shown in Fig. 2(35)was employed. The spent liquor was heated to 105 °C after the pulping and delignification process. Using distilled water five times the volume of concentrated liquor, the lignin was dissolved in formic acid, and the precipitate was filtered using a Buchner funnel. The precipitated lignin was washed with distilled water and vacuum-dried on P2O5.



Solid residue

Concentrated liquor

Lignin

Fig.no.02Isolationoflignin

V. CHARACTERIZATIONOFLNP's:

5.1 Size and morphology

Usingscanningelectronmicroscopy(SEM)andtransmissionelectronmicroscopy(TEM), the size and shape of LNPs were examined.

A spin coater was used to spin a drop of LNP onto a glass slide for 10 seconds at 4000 rpm in ordertoconductSEMexaminations.TheLNP-containingslideunderwentathree-minutegold sputter coating to provide electron beam conductivity. By applying high-tension voltage (3–5 kV)toaCarlZeiss Ultra55apparatus witha30µm aperture,SEM micrographs werecreated.

ForTEMstudies,adropofnanosuspension(DLNP/ALNP)wasspin-coatedontoacoppergrid mesh substratecoated with carbon. This substratewas immediately used for TEM analysis ata 200 kV accelerating voltage without any additional processing.

ImageJ software (version 1.45) was used to calculate the size of LNP from TEM and SEM micrographs.Atleast150nanoparticlesonaveragewererecordedfromthreetofourdifferent experiments (either triplicates or duplicates) carried out on different days.

5.2 Structural Analysis (FT-IR)

Chemical functional groups were examined in the zinc selenide attenuated total refraction process (ATR) cell of an infrared spectrometer using a spectrum FT-IR Prestige-21 spectrophotometer(Shimadzu,Chiyodaku,Tokyo,Japan).Before FT-IR testing,10 mmby10 mmfilms weremade, trimmed, and oven-dried at60 °C for 24 hours. Wemeasured thespectra

 $in the transmittance mode, recording the wavenumber of each film between 400 and 4000 cm^{-1}.$

5.3 UVSpectroscopy

UV spectroscopy is absorption spectroscopy in the UV range (200–400 nm). In practical use, a spectrophotometercontraststheintensityoflighttravelingthroughthesample(I)withtheintensity oflightpreceding itspassage(Io). Theformulafor transmittance,oftenknownastheI/Ioratio, is (%T). To calculate absorbance (A), transmittance (A) is utilized. Transmittance is measured by placing samples in a UV-Vis spectrophotometer.

Asolventistypicallyusedtodissolveligninbecausethemajorityofsamplesareliquids.Because of its ease of use, UV spectrophotometric analyses are widely employed to analyze lignin. The spectra maybealteredbysolvent effects, socaution mustbe used while selecting theappropriate solvent. Potential solvents include pyridine, water, ethanol, 2-methoxyethanol, dioxane, dimethylsulfoxide (DMSO), dichloroethanecellosolve,

hexafluoropropanal, and dimethylformamide.AccordingtoWegeneretal.(1983),thepolysaccharidebreakdownproducts have no effect on the solvent's strong UV transmittance properties. The significant absorption of lignin in the UV spectrum is due to its aromatic nature.

VI. APPLICATIONS:

Growinginteresthasbeenshowninligninnanoparticlesbecauseoftheirrenewable,abundant,and environmentally friendly characteristics as well as their biodegradability, biocompatibility, and antioxidant, antibacterial, or UV absorption properties(36). In many applications, such as food packaging, biomedicine, adsorbent materials, drug release and control(37), environmental restoration, and nanocarriers(38), they are excellent substitutes for partially hazardous nanomaterials. As per the development principles of green chemistry, this not only enhances the value-added uses of lignin nanoparticles but also tackles the potential safety hazards of conventional nanomaterials from their source.

6.1 AntibacterialEffect

Lignin's phenol units and benzene ring structure provide it with antibacterial properties. The inhibitory impact of the phenolic unit on bacterial growth has been extensively studied by earlier researchers (39)(40). They found that different methods of extracting lignin showed different bactericidal properties, mainly because of the diversity of phenolic chemicals. Nano-lignin's increased specific surface area and phenolic side chain count provide it superior antibacterial qualities over regular lignin.

Richter et al. developed lignin-based-Ag⁺ ions nanocomposites coated with a cationic polyelectrolyte in aqueous solution. The antibacterial experiments showed that the nanocomposites could kill common Grampositive and Gram-negative pathogenic bacteria and quaternary amine-resistant bacteria. Furthermore, the nanoparticles amount of silver used in the nanocomposites was 10 times less than that used in traditional methods. This was attributed to the dual bactericidal properties of lignin nanoparticles and silver ions. However, the reduction of the silver ion content in the nanocomposite resulted in a decrease in the antibacterial effect. It was really necessary to solve the problem of preparationofbiodegradablenanoligninrelease from lignin nanoparticles. The silver ions silverionsnanocomposites will enlight entheemergence of more nanomaterials loaded with various metal ions. The lignin nanoparticles are promising high-value biological additives for polymer nanocomposite films, which can be used in the field of advanced food packaging. The nanocomposite films of lignin nanoparticles (LNP), chitosan (CH)andpolyvinylalcohol(PVA)weresuccessfullypreparedbyYangetal.(41)throughsolvent casting. Two bacterial plant pathogens, Xanthomonasarboricolapv. pruni and Pectobacteriumcarotovorum subsp. odoriferum, were used to evaluate the antibacterial properties of the nanocomposite films. The results indicated that binary (PVA/LNP and CH/LNP) and ternary (PVA/CH/LNP) nanocomposite films could significantly inhibit the growth plant of bacterial pathogens.Moreimportantly,theternarycompositesystemexhibited higher antibacterial activity than others.

Although the addition of lignin nanoparticles reduced the transparency of nanocomposite films, it obviously increased thermal stability.

6.2 ReinforcingMaterials

Since lignin nanoparticles are inexpensive, low in density, renewable, degradable, and exhibit surface-active qualities, they have drawn a lot of interest as nanofillers. Jiang et al. reported the impactofresidualligninonthestructure, content, and characteristics of cellulose films and fibrils

(42). It was discovered that lignin enhanced the hydrophobicity and thermal stability of the film made of cellulose fibrils.

Lignin'shighcarboncontentmakesitsuitableforusageasaprecursorintheproductionofcarbon nanofiber composites. Following the carbonization and thermal stabilization of lignin, carbon nanoparticles were created and used as reinforcing materials(43).

The composites films of lignin nanoparticles and polylactide (PLA) were prepared by Lintinen et al. The ligninnanoparticles could be uniformly and stably dispersed in PLA matrix and no changes insize and zetapotential were observed. Moreover, the tensile tests confirmed that the composite films possessed enhanced mechanical properties due to the loading of nano-lignin. Yang et al. compared the tensile properties of nanolignin/PLA bionano composites synthesized by melt extrusion and solvent casting. The results indicated that the tensile strength and modulus of melt- extruded films were higher than that of solvent-cast films. When the addition of nano-lignin was increased from 1 wt.% to 3 wt.%, the elongation at break of melt-extruded films enhanced while the tensile strength and modulus decreased.

nanocomposites in the poly(trimethylene terephthalate) (PTT) matrix by Gupta et al.. Compared with pure PTT matrix, the tensile flexibility and impact performance of nanocomposites significantly improved due to the addition of 1.5wt.% nano-lignin. Additionally, the impact strength of the ternary hybrid nanocomposites was higher than that of nanolignin/PTT nanocomposites.

In order to improve the thermal stability and mechanical properties of natural rubber and styrene butadiene rubber, Jiang et al.(44) introduced nano-lignin into the rubber matrix to prepare the nanocomposites. The nanocomposites of natural rubber and poly(diallyldimethylammonium chloride)modifiedcolloidalligninweresuccessfullyproduced. The significantlyimproved tensile strength and shear strength were observed as the content of nano-lignin in the rubber matrix increased. Compared with pure natural rubber, the increased decomposition temperature of the nanocomposites was attributed to the interaction of nano-lignin-based nanosheets were used to reinforce styrene-butadienerubber. The glass transition temperature and storage modulus of the nanocomposites obviously increased due to the the compatibility of nano-lignin and rubber matrix. Therefore, lignin nanoparticles are a renewable alternative to carbon black for preparing various rubber products.

6.3 Anti-Ultraviolet Effect

Functionalgroupslikephenolandketoneinligninhaveexceptionalantioxidantandantiultraviolet properties because they effectively absorb a range of UV light and reduce UV radiation(45). In several applications, nanolignincan beemployed in place of some inorganic nanoparticles due to its renewable nature, environmental friendliness, and degradability(46).

6.4 FoodPackaging Films

Free radical polymerization and masterbatch techniques were combined for the first time to produce lignin nanoparticle and poly(methyl methacrylate) (PMMA) nanocomposites(47). The higher UV resistance of the nanocomposite films over pure films was due to the homogenous dispersion in the polymer matrix and the ultraviolet absorption capabilities of nanosized lignin. This masterbatch method demonstrated exceptional processability for the production of nanolignin-based composites, even though the addition of nanolignin caused the composite films to lose a significant amount of their deformation.

6.5 SunscreensandCosmetics

A lotof activemolecules arecreated and oxidativestress mechanisms areinitiated when theskin isexposedtoUVlightforalongtime.Oxidativedamageandproblemswithcellmetabolismresult

fromthis(48). The use of chemical subscreen stop revent cellaging is becoming more and more common. Lignin is appropriate for use in cosmetic sunscreen compositions due to its exceptional antioxidant and UV-absorbing qualities. The natural polyphenol extract from lignin not only effectivelyblocksUVraysbutalsorepairsdamagedDNA(49).Qianetal.(50)reportedthat lignin-based broadspectrum sunscreens were made by mixing lignin colloidal spheres with pure skin cream.

The spherical lignin nanoparticles were isolated from elephant grass by antisolvent precipitation and then added to an eutral cream to produce lignin-based sunscreens. Compared with commercial sunscreens. the creams with 10% lignin nanoparticles exhibited lower light transmittance. Lee et al.mixed light-colored lignin nanoparticles from rice husks into a moisturizing cream to produce broad-spectrum sunscreens. The higher sun protection factor was demonstrated comparing with nonnanoparticles.Inaddition,thesynergisticeffectofligninnanoparticlesandorganicUV-filter sunscreens enhanced the UV resistance performance. The applications of lignin nanoparticles in cosmetic sunscreens play an important role in the development of high-value lignin.

6.6 Nanocarriers

Amongthemanyfunctionalgroupsfoundintheligninstructurearehydroxyl,carboxyl,methoxy, andbenzenerings (51).Itsrangeofapplicationsisgreatlyexpandedbythefactthatlignincanbe

chemicallymodifiedbyavarietyofsubstancesduetothesefunctionalgroups.Duetotheirdiverse spectrum of shapes and high level of biocompatibility, lignin nanoparticles are interesting drug delivery systems.

The effect of three different morphologies ligninnano carriers on the release capacity for UV-active cargo 2propylpyridine was illustrated by Yiamsawas et al. [91]. The release efficiency of solid nanoparticles, porous structures and hollow nanocapsules reached 10%, 25% and 40%, respectively. In addition, a significantly increased release efficiency was observed due to the degradation of nanolignin in the presence of enzyme laccase. The drug delivery in biomedicine will benefit from the lignin-based nanocarriers with morphologycontrolled release ability.

| Three | other | drugs | were | used | to c | letermine | the | loading | and | release | abilities | of | the | lignin |
|---------|-----------|--------|----------|----------|--------|------------|---------|------------|---------|------------|-------------|------|-------|----------|
| nanopa | rticles(L | NPs)by | Figueir | edoetal. | .Thepo | oorly | | water-solu | ubleson | rafenib(Sl | FN)and | | cy | rtotoxic |
| benzazi | ulene(B | ZL)cou | ldbeeffi | ciently | loaded | lonnanopai | rticles | ,whereash | ydrop | hiliccaped | citabine (C | CAP) | could | not be |
| loaded. | Th | e re | elease | effic | iency | of | SFN | -LNPs | and | BZL- | LNPs | both | r | reached |

about100%atpHof7.4throughtheenzymaticdegradationofLNPs.ItturnedoutthatBZL-LNPs had an increased antiproliferation effect on cancer cells comparing with the free BZL.

Sipponen et al. fabricated renewable biocatalysts of hydrolases-cationic lignin nanosphere compositesforbutylbutyratesynthesisin aqueous medium. Firstly,theligninnanoparticleswere coated with hydrolytic enzymes (cutinase and lipase). Afterwards, the biocatalysts were synthesized through entrapment of hydrolases-coated nanospheres by calcium alginate hydrogel beads. It turned out that butyl butyrate in the water/hexane mixture under enzyme catalysis was effectively synthesized. The lignin-based biocatalysts withoutchemical modification willbenefit aqueous ester synthesis.

The high specific surface area and porous structure of lignin nanoparticles make them excellent carriers for loading metal ions. The potential applications of lignin-based metal ion nanocomposites, such as biocataly standnano-adsorbents, will be further developed and utilized.

Thestructural diversity of metal-organic nanocomposites formed by ligninnanoparticles and iron isopropoxide in THF solution was investigated. The different morphology, such as network structure, solid nanoparticles, uniform spheres and hollow nanospheres, was prepared under different hydrolysis reaction conditions. Moreover, the obviously higher magnetization of Fe(OiPr)₃-lignin nanocomposites was exhibited, compared with Fe(OiPr)₂-lignin nanoparticles.

Metalalkoxidesnanocompositeshavebeenwidelyusedinrubbercompositematerials,directional polymerization catalysts, ceramics and metal oxide films . Ni nanoparticles were supported on lignin-based carbon nanofibers as catalysts to depolymerize lignin in supercritical ethanol/water. Theresultsshowedthatthecatalystsaccelerated thedepolymerizationof lignin andincreased the yields of phenols and lignin fragments. The loading of Ni nanoparticles on nanofibers did not change significantly after three cycles of catalysis.

6.7 HybridNanocomposites

Lignin nanoparticles are often blended with polymers as reinforcing agents, which make the obtained copolymers exhibit better mechanical, thermal, and biocompatible properties than the original polymers. Chung et al. transformed lignin into a lignin-g-poly(lactic acid) (PLA) copolymer by grafting lactide onto lignin using triazabicyclodecene (TBD) as a catalyst so as to improve its miscibility with other bioplastics. The chain length of PLA could be adjusted by preacetylation treatments or changing the ratio of lignin and lactide. When 10% lignin-g-PLA copolymers were added, both the UV absorbance and mechanical properties of PLA composites improved. Jiang et al.prepared nano-lignin from lignin and poly (diallyldimethylammonium chloride) (LPCs) (PDADMAC) complexes through self-assembly. The obtained lignin particles nano-lignin hadanaveragesizeoflessthan100nmandcouldstablydispersein aqueoussolutions.Whenthe was blended with natural rubber (NR) latex, it could be homogeneously dispersed in the NR latex at the nanoscale, which resulted the improved thermal stability mechanical in and propertiesofLPCs/NRcomposites.Nairetal.presentedanovelmethodtoconvertlargemicrontonanosizedkraftligninparticlestonanoligninparticlesusingasimplehighshearhomogenizer, whichwouldnotcauseany chemical composition changes. When these nanoligning articles were blended with polyvinyl alcohol (PVA), the thermal stability of obtained nanolignin/PVA composites increased in comparison with the original lignin/PVA composites. Qian et al. (52) introduced the formed lignin reverse micelles (LRMs) into high-density polyethylene (HDPE). Taking the LRM addition of 5 wt % as an example, it was observed that the elongation at break increased to 1030% from 671% and Young's modulus increased to 2104 MPa from 1066 MPa, respectively. This indicated that the incorporation of LRMs could cause an obvious improvement in the mechanical properties of HDPE (elongation at break and Young's modulus).

6.8 Antioxidant Agents

The functional groups such as methoxy and phenolic hydroxyl groups in lignin are able to cause the termination of oxidative propagation reactions through hydrogen donation. Therefore, lignin nanoparticles could be introduced into various materials to produce antioxidant products with different applications. Lu et al. developed nanoscale lignin through the supercritical antisolvent (SAS) method employing supercritical carbon dioxide as the antisolvent and acetone as the solvent. Due to the increased solubility in water, the resulting nanoscale lignin had a higher antioxidant activity, which exhibited enhanced superoxide radical scavenging activity, DPPH radical scavenging activity, and reducing power. In another study performed by Ge et al., nanolignin was prepared through the alkaline solution precipitation method. Free Radical Scavenging(FRS)activity analysisdisplayedthatnanoligninshoweda3.3-foldhigheractivityin contrast to the control sample. According to the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay, the IC50 value increased to 2.70 ± 0.17 mg/mL for nanoscale lignin from

 32.21 ± 0.1 mg/mL for microscale lignin, suggesting a higher antioxidant activity of nanoscale lignin. Yang et al. (53) introduced the obtained lignin nanoparticles (LNP) via acid precipitation into a poly(vinyl alcohol) (PVA)/chitosan (CH) mixture. DPPH radical scavenging activity measurement results showed that LNP exhibited a synergic effect with CH in antioxidant responses of the resulting PVA/CH/LNP nanocomposites. Together with the antimicrobial activity, PVA/CH/LNP nanocomposites could be potential candidates for use in various biomedical applications such as tissue engineering, drug delivery, and wound healing. Yearla et al. fabricated two different kinds of alkali lignin nanoparticles (ALNP) and dioxane lignin nanoparticles (DLNP). Compared to the original alkali lignin and dioxane lignin polymers, both ALNPandDLNPshowedhigherantioxidantactivityaccordingtotheradicalscavengingactivity analysis. Additionally, DLNP could provide more pronounced protection for *Escherichia coli* against UV than ALNP. On account of these good antioxidant and UV protection properties, DLNP can be therefore further applied in pharmaceutical, food, and cosmetic industries. Tian et al.prepared two types of LNP via self-assembly from ethanol-organosolv and deep eutectic solvent(DES)extracted lignins, respectively. When these ligninnanoparticles wereincorporated intoPVA, the resulting copolymerobtained additional antioxidant functionalities. Heetal. firstly produced LNP through an acid precipitation method, which was then esterified and etherified by citric acid to form modified LNP (MLNP). When LNP and MLNP were also separately blended with PVA through the solvent casting approach, the antioxidant property of the obtained MLNP based nanocomposite films was superior to that of LNP based nanocomposite films, which provided potential for these materials as environmental friendly antioxidantadditives in the food and packaging industry.

6.9 Biocompatible Applications

In the domains of in vivo biology and medicine, nanotechnology has been gaining more interest due to its numerous potential applications(54) .Nanolignin'sphenylpropane skeleton contains polar phenolic and aliphatic hvdroxvl groups, which make it suitable for usage in the production of micellesor capsules. Furthermore, the biomedical and pharmaceutical industries stand to be nefit greatly from the remarkable qualities of biocompatibility, adsorption of biological macromolecules, and absence of cytotoxicity (55).

6.10 Drugdelivery

The resultant lignin-based nanomaterials can be used as high-value-added materials for potential applications in delivery biomedical domains. particularly in drug/gene and various tissue engineeringduetosynergeticinteractionsfrombothorganicligninandinorganicnanocomposites. The binding ability of lignin with nanomaterials or other multivalent metal ions involves active functional groups (phenol, hydroxyl, and carboxyl groups), which act both chelating as and reducingagentstothemetalcenter.(56)Thechelatingabilityoflignindependsonthetypeoflignin anditschemicalstructure.Ligninisusedaswoodadhesivestoimprovethemechanicalproperties ofthermoplasticpolymercomposites. For example, the phenolinphenol-formal dehyderes inwas

replaced with lignintoim proveits compatibility and mechanical properties. (57) Recently, it has

beenreported that the ligninnan oparticles that dispersible in the water can be used to

stabilizePickeringemulsionsandalsousedasasilverionscarrierinantimicrobialapplications. They have also been utilized successfully in drug delivery and tissue engineering applications.

6.11 LigninNanoparticlesinCancer DrugDelivery

The alarming increase in the mortality incurred due to cancer urged the researchers all over the worldtodeveloprobusttherapeuticapproachestocombatthecancer.Itiswellknownthatsurgery and radiotherapy are the conventional therapeutic ways to fight against tumours. In recent times. nanomedicinehasrevolutionisedthefieldofcancertherapy.Inthiscase.NPshaveemergedasan intriguingcarriertodeliverdrugsspecificallytotumorcells,owingtotheirsmallsize, highsurface area, controlled drug release and low toxicity. Although, a wide range of NP systems have been thoroughly investigated for cancer treatment.Nano-sized lignin particles are fundamentally amphiphilic NPs, having its affinity towards both and hydrophobic drugs. hvdrophilic In particularstudy, Siddiquiandhisteam fabricated blank LNPs using kraftlignin. When LNPs were administered intravenously, the percent haemolysis was $0.08\pm0.03\%$ for 100μ g/mL dose which was increased to $1.56\pm0.33\%$ for the dosage of 1000 µg/mL. Furthermore, NPs failed to exert any toxic effects over normal cells (HEK-293), indicates which its role as safe drug carriers. Secondly, significant cytotoxicity was observed with folate receptor (FR)+vecellline, i.e., MCF-7, which could be useful in targeting FR +ve tumors and improve the effectiveness as an antineoplastic agent. Moreover, NPs were loaded with irinotecan to assess the drug-carrying potential of LNPs. Drug-loaded NPs displayed a mean size of 163.87 ± 2.81 nm and entrapment efficiency of $67.65 \pm 1.95\%$. Interestingly, sustained drug release was

observed from NPs, releasing about $86.72 \pm 7.05\%$ of the drug over 72 h, while the cytotoxicity studies showed that NPs were three times more potent than the drug solution

OtherApplications

Inaddition to the above, lignin-based nanoparticles have also been exploited for other industrial applications, such as antibacterial agents, adsorbents to remove heavy metal ions, and dyes as well as anticorrosive nanofillers.

The presence of phenolic components endows an antimicrobial characteristic to lignin, which therefore could be utilized as an antimicrobial agent. Kim et al.(58) developed nanoparticles combiningchitosanandlignosulfonatesforthefirsttime.Whenlignosulfonatewasincorporated into chitosan nanoparticles, the obtained composite nanoparticles showed greater antimicrobial activity in comparison with chitosan nanoparticles alone.

Additionally,lignincould alsoactasanadsorbentto removeheavymetalionsanddyes.Lietal. utilized the obtained porous lignosulfonate spheres (PLS) through a feasible gelation- solidification method, which possessed a total pore volume of 0.416 cm³/g and high porosity of 87.66%, to adsorb lead ions. The adsorption experiments showed that the adsorption efficiency of PLS for lead ions reached up to about 95.6% at the initial concentration of 25.0 mg/L, suggesting an excellent adsorption capacity of PLS. Therefore, PLS could be applied for the continuous treatment of industrial wastewater rich in heavy metals. Azimvand et al.studied the removal of Safranin-O from aqueous solutions using lignin nanoparticles and lignin

nanoparticle-g-polyacrylic acid as adsorbents, respectively. In this work, lignin nanoparticles (LN) were first dissolving the alkali lignin (AL) in polyethylene fabricated bv glycol and then slowlyaddinghydrochloricacidintoAL/polyethyleneglycolsolutions, and ligninnanoparticleacid g-polyacrylic prepared through (LN-g-PAA) was the copolymerization reactions between polyacrylicacidandligninnanoparticleusingpotassiumpersulfateasaradicalinitiator.Results indicated the adsorption capacity of LN-g-PAA copolymer increased nearly 1.4-fold in LN. Therefore, LN-g-PAA was considered to be proper for removing Safranin-O dye from wastewater.

Ultimately,Rahman etal.reported theuseoflignin nanoparticles as anticorrosivenanofillers.In this research, they evaluated the anticorrosive behavior of three kinds of lignin nanoparticles

(LNP)viaacidprecipitationindifferentmediums(castoroil,ethyleneglycol,andwater).Results showed that all three kinds of lignin nanoparticles could be dispersed in the epoxy matrix- forming nanocomposite coatings to protect the carbon steel (CS) from corrosion in a highly salineenvironment,Moreover,the LNP- dispersedepoxycoatingscouldprovidebetterprotection compared to the bare epoxy coating.

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