Sign in

Patents   English   French

Find prior art   Discuss this application   ⚙ ▾

Novel process for producing value-added lignin-based chemicals from alkaline effluent of chemical treatment of lignocellulosic biomass

WO 2016123520 A2

ABSTRACT

The invention discloses a novel process to make hydrophobic interaction between lignin and ABA-block-copolymers, providing solubilization or encapsulation of the lignin in block copolymer micellar systems and utilizing the micellar systems to perform chemical reaction with non-polar or polar agents. The result is that solubilization and interaction of hydrophobic phenolic compounds with PEO/PPO copolymer micelles in water emulsions enables high value lignin derivatives from lignin pretreatment effluent in biorefineries to be produced.

DESCRIPTION (OCR text may contain errors)

NOVEL PROCESS FOR PRODUCING VALUE-ADDED LIGNIN-BASED CHEMICALS

FROM ALKALINE EFFLUENT OF CHEMICAL TREATMENT OF LIGNOCELLULOSIC BIOMASS

RELATED APPLICATION

[0001] The present application claims under 35 U.S.C. § 119, the priority benefit of U.S.

Provisional Application No. 62/109,525, filed January 29, 2015, entitled: "NOVEL PROCESS FOR PRODUCING VALUE-ADDED LIGNIN-BASED CHEMICALS FROM ALKALINE

EFFLUENT OF CHEMICAL TREATMENT OF LIGNOCELLULOSIC BIOMASS." The disclosure of the foregoing application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to a carrier micelle system having hydrophilic and hydrophobic portions configured to physically trap modified lignin, and more particularly to a carrier micelle system having hydrophilic and hydrophobic portions for producing a polymer-lignin micellar system in high pH aqueous solution in order to be used as a platform for conducting chemical reactions with a non-polar or polar agent.

Discussion of the Related Art

[0003] Developing valuable products from lignin can improve the economics of a biorefinery using lignocellulosic biomass. Lignin is present in significant amounts in plants cell walls, accounting for 15-25% (w/w) of herbaceous biomass. To date however, lignin has little high-value practical use. As a heteropolymeric natural aromatic compound, the lignin structure is composed of

CLAIMS (OCR text may contain errors)

We Claim:

1. A method of producing lignin-derived products comprising:

solubilizing/encapsulating lignin derivatives from at least one of spent liquor and effluent of alkaline chemical treatment of lignocellulosic biomass in a micellar emulsion, further comprising adding block copolymer agents to a resultant alkaline lignin solution to form a polymer micellar emulsion containing the lignin via the aid of π-π aromatic interaction; and

using the polymer micellar emulsion of lignin to perform a chemical reaction to modify the lignin compounds.

2. The method of claim 1, wherein the alkaline chemical treatment comprises at least one treatment selected from: a Kraft pulping, anozonolysis, a soaking aqueous ammonia pretreatment, and an alkaline pulping and pretreatmentprocesse.

3. The method of claim 1, wherein the at least one of spent liquor and effluent includes black liquor from pulping and effluent of alkaline pretreatment of lignocellulosic biomass by combination ozonization/soaking aqueous ammonia treatment.

4. The method of claim 1, wherein the lignocellulosic biomass comprises at least one of herbaceous, soft wood, hard wood, straw, and red algae.

5. The method of claim 1, wherein the block copolymers is a block copolymer selected from: diblock copolymers, triblock copolymers, and amphiphilic block copolymers.

chain. This configuration has many properties that could give rise to the possibility of using lignin for producing value-added products. However, the heterogeneity of the lignin structure makes it difficult to break down and isolate into a targeted product. Due to the complexity of the lignin structure and the effect of pretreatment processes on it, identifying and extracting chemicals from lignin requires extensive characterization to understand the lignin's polymeric properties, linkages, and properties of the functional groups connected to the aromatic ring. [0004] Background information for fabricating a mesoporous carbon composition from lignin is described in U. S. Patent Application No. 20140227325 Al, entitled, "LIGNIN-DERIVED

POROUS CARBON COMPOSITION, METHODS OF PREPARATION, AND USE THEREOF," to Naskar et al., including the following, "[a] method of fabricating a porous carbon composition, the method comprising subjecting a precursor composition to a thermal annealing step followed by a carbonization step, the precursor composition comprising: (i) a templating component comprised of a block copolymer and (ii) a lignin component, wherein said carbonization step comprises heating the precursor composition at a carbonizing temperature for sufficient time to convert the precursor composition to a carbon material comprising a carbon structure in which is included mesopores having a diameter within a range of 2 to 50 nm, wherein said porous carbon composition possesses a mesopore volume of at least 50% with respect to a total of mesopore and micropore volumes. Also described are the resulting mesoporous carbon composition, a composite of the mesoporous carbon material and at least one pharmaceutical agent, and the administration of the carbon-pharmaceutical dosage form to a subject." However, the process is not involved in any separation and purification of low molecular weight lignin-based chemicals in aqueous media.

[0005] Background information on a method that utilized water soluble, surface active, polymeric agents to the digest liquor prior to acidification so as to provide separable lignin coagulant after acidification, is described and claimed in, U.S. Patent No. 5,635,024, entitled, "PROCESS FOR SEPARATING LIGNINS AND DISSOLVED ORGANIC COMPOUNDS FROM KRAFT SPENT LIQUOR," issued June 03, 1997, to Shall et al., including the following, "[d]igestion liquor is used in the paper pulping process to dissolve lignins and free the cellulose fiber from raw wood chips. After pulping, spent digestion liquor is laden with lignins. Heretofore, it was known that lignins could be coagulated by acidification of the digestion liquor, but such method coagulated the lignins in a slimy gelatinous mass that was difficult to separate from the liquor. It has been discovered that the addition of water soluble, surface active, polymeric agents to the digestion liquor prior to acidification, results in an easily separable lignin coagulant after acidification." However, the above captioned application did not separate lignin-derived low molecular weight chemicals through polymer micellization systems and also did not use chemical reactions for separating lignin derivatives from polymer nanaostructures.

[0006] Thus, a need exists in the industry for a system and method to address the

aforementioned industrial concerns. The novel embodiments of the present invention is directed to such a need by providing for methods that utilizes a novel system disclosed herein wherein chemical reactions in micellar systems for separating, transforming, and preparing lignin-based value added products can be beneficially realized.

SUMMARY OF THE INVENTION

[0007] It is to be appreciated that the present example embodiments herein are directed to a method of producing lignin-derived products to include: solubilizing/encapsulating lignin derivatives from at least one of spent liquor and effluent of alkaline chemical treatment of lignocellulosic biomass in a micellar

6. The method of claim 1, wherein the polymer micellar emulsion containing the lignin includes at least one of: spherical micelles, rod shaped micelles, multilamellar vesicles, and unilamellar vesicles.

7. The method of claim 1, wherein the chemical reaction includes at least one of: oxidation, de-esterification, transesterification, reduction, solvolyses, enzymatic reactions, metalloenzyme reactions, C-C coupling reactions, methathesis reactions, C-C cleavage reactions, and ether cleavage.

8. The method of claim 1, wherein the step so as to modify the lignin compounds includes β-O-4, α-O-4, 5-5, β-5, 4-0-5, β-β, β-1, dibenzodioxocin cleavages.

9. The method of claim 1, wherein the step so as to modify the lignin compounds includes incorporating at least one of NIPH, TEMPO, AZADO cocatalyst so as improve the selectivity of the oxidation of lignin aliphatic secondary alcohol and simultaneously maintain the reactivity.

10. A polymeric micelle lignin carrier, comprising:

a lignin derivative physically trapped in a polymeric micelle carrier composed of a block copolymer, wherein the lignin derivative is trapped via the aid of lignin-block copolymer interactions.

11. The polymeric micelle carrier of claim 10, wherein the block copolymer is a block copolymer selected from: diblock copolymers, triblock copolymers, and amphiphilic block copolymers.

12. The polymeric micelle lignin carrier of claim 10, wherein the lignin-block copolymer interactions include at least one of: π-π aromatic interactions, hydrogen bonding interactions, hydrophilic interactions, hydrophobic interactions, and Van der Waals interactions.

13. The method of claim 10, wherein the polymeric micelle lignin carrier is formed from the alkaline chemical treatment comprising at least one treatment selected from: a Kraft pulping, an ozonolysis, a soaking aqueous ammonia pretreatment, and an alkaline pulping and pretreatment process.

14. The polymeric micelle lignin carrier of claim 10, wherein the polymeric micelle lignin carrier is formed from at least one of spent liquor and effluent including black liquor from pulping and effluent of alkaline pretreatment of lignocellulosic biomass by combination ozonization/soaking aqueous ammonia treatment.

15. The polymeric micelle lignin carrier of claim 10, wherein the lignin employed in the polymeric micelle lignin carrier is derived from lignocellulosic biomass comprising at least one of herbaceous, soft wood, hard wood, straw, and red algae, green algae, wood chips, sawdust, flakes, fruit and nut waste.

alkaline lignin solution to form a polymer micellar emulsion containing the lignin; and using the polymer micellar emulsion of lignin to perform a chemical reaction to modify the lignin compounds.

[0008] Another beneficial aspect of the embodiments herein includes a polymeric micelle lignin carrier that includes a lignin derivative physically trapped in a polymeric micelle carrier composed of a block copolymer, wherein the lignin derivative is trapped via the aid of lignin- block copolymer interactions. Such lignin-block copolymer interactions include π-π aromatic interactions, hydrogen bonding interactions, hydrophilic interactions, hydrophobic interactions, or Van der Waals interactions

[0009] Accordingly, the polymeric nanostructures resultant from the methodologies herein operate as novel nanocontainers to solubilize lignin derived chemicals in micelles and can be used as a desirable platform to perform a chemical reaction. The amphiphilic block copolymers disclo sed herein have many potential applications such as, but not limited to, synthesis of nanoparticles, fabrication of mesoporous material, and in chemicals extraction. Therefore, the solubilization and interaction of hydrophobic phenolic compounds with PEO/PPO copolymer micelles in water emulsions, s disclosed herein, are beneficial in the context of separation and utilization of high value lignin derivatives from lignin pretreatment effluent produced in

biorefineries. The embodiments herein demonstrate that lignin feedstock obtained from alkaline effluent of pretreatment of, for example, wheat straw is a desirable source of providing for value added products, such as, but not limited to, biofuels and antioxidant chemicals to include guaiacol and 4-vinyl guaiacol. BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 shows an example general shows an example process flowchart of the methodology herein.

[0011] FIG. 2 shows a Transmittance versus Wavenumber (cm-1) ATR-FTIR spectroscopy plot of a pretreated wheat straw lignin sample.

[0012] FIG. 3 shows an Abundance versus Retention time (min) Pyrogram plot of pretreated wheat straw lignin.

[0013] FIG. 4 shows a second Abundance versus Retention time (min) pyrolysis plot indicating identities and relative molar mass of the released phenolic compounds.

[0014] FIG. 5 shows an Ultraviolet- Visible (UV-VIS) spectrum of lignin and commercial antioxidants ferulic acid, butylated hydroxytoluene (BHT), and Guaiacol.

[0015] FIG. 6 shows diffusion coefficient changes of Pluronic F-68 ABA block copolymer as a function of various concentrations of the block copolymer (0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12 mM, pH 11.4 and 7) and addition of guaiacol, dimer, and ferulic acid (0.04 and 0.12 mM, pH 11.4).

[0016] FIG. 7A shows a graph of chemical shift changes ($\Delta\delta/H\zeta$) for PEO-(CH_2-CH_2) and PO- (CH_3) of Pluronic F-68 block copolymer (0.04 mM, pH 11.4) as a function of various concentrations (C/mM) of guaiacol, phenol and eugenol.

[0017] FIG. 7B shows a graph of chemical shift changes ($\Delta\delta$) for PEO-(CH_2-CH_2) and PPO- (CH_3) of Pluronic F-68 block copolymer (0.04 mM, pH 11.4) as a function of various

concentrations (C/mM) of lignin, ferulic acid and dimer.

[0018] FIG. 8 shows H NMR stacked plots of guaiacol, ferulic acid, guaiacylglycerol-beta- guaiacyl ether (dimer) and lignin with concentrations 0.01-0.20 mM (from top line to bottom line) in Pluronic F68-D_2O solution with 0.04 mM concentration and a pH 11.4.

[0019] FIG. 9 shows reaction mechanisms formulated for the micellar embodiments herein, wherein solubilization of lignin in ABA block copolymer micelle with π- π aromatic stacking- hydrophobic interaction with the core of the micelle and lignin propanoid polar functional groups interaction with shell (palisade region) of the micelle: Organized host-guest structure. Also of note in step (d) is the reduction of molecular oxygen via four one-electron steps (Metal-free Fenton type reaction). DETAILED DESCRIPTION

[0020] In the description of the invention herein, it is understood that a word appearing in the singular encompasses its plural counterpart, and a word appearing in the plural encompasses its singular counterpart, unless implicitly or explicitly understood or stated otherwise. Furthermore, it is understood that for any given component or embodiment described herein, any of the possible candidates or alternatives listed for that component may generally be used individually or in combination with one another, unless implicitly or explicitly understood or stated otherwise. It is to be noted that as used herein, the term "adjacent" does not require immediate adjacency. Moreover, it is to be appreciated that the figures, as shown herein, are not necessarily drawn to scale, wherein some of the elements may be drawn merely for clarity of the invention. Also, reference numerals may be repeated among the various figures to show corresponding or analogous

Additionally, it will be understood that any list of such candidates or alternatives is merely illustrative, not limiting, unless implicitly or explicitly understood or stated otherwise.

[0021] In addition, unless otherwise indicated, numbers expressing quantities of ingredients, constituents, reaction conditions and so forth used in the specification and claims are to be understood as being modified by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the subject matter presented herein. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the subject matter presented herein are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical values, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

General Description

[0022] Alkaline aqueous treatments of lignocellulosic biomass produce two main products; delignified fibers and process effluent. The fibers utilized for making paper or bio-fuels in paper plant and biorefineries, respectively. The process effluent or spent liquor is laden of lignin-based phenolic compounds which derived from lignocellulosics cell walls. Heretofore, it is known that lignin can be isolated from the alkaline spent liquor by using acidic precipitation methods, filtration and centrifuge. However, such methods separated lignin macromolecules in sludge form along with a considerable high molecular weight; a factor leads to a significant reduction of lignin usage. A repulsion associated with lignin's negative surface charge governs the dispersion of lignin low molecular weight derivatives in alkaline effluent. Considering the repulsion phenomenon and the accessibility of suspended low molecular weight phenolic compounds in the alkaline effluent, the embodiments herein provide for the addition of block copolymers to the spent alkaline liquor so as to results in a solubilization and encapsulation of lignin-derived chemicals in polymer micellar systems. Such a solubilization methodology results in lignin derivatives becoming more concentrated within the micelle than in the surrounding water phase and leads to an acceleration of the reaction and causes selective effects. Polymer nanostructures operate as containers to provide a platform for conducting chemistry reactions. The polymer-lignin micellar system disclosed herein is used to perform chemical reaction(s) for converting lignin to value-added chemicals, such as, for example, value-added low molecular weight lignin-based chemicals.

Specific Description

[0023] The embodiments, as disclosed herein, are directed to a process to make a considerable hydrophobic (aromatic) and hydrophilic interaction between lignin and ABA-block-copolymers, providing solubilization or encapsulation of the lignin in block copolymer micellar systems and utilizing the micellar systems to perform chemical reaction(s) with non-polar or polar agents.

Solubilization/encapsulation, as referred herein, is an increase in the solubility of hydrophobic compounds in water through the influence of amphiphilic molecules. Since a micelle possesses regions of different polarity, both polar and non-polar reagents can be incorporated into the micelle.

[0024] Turning now the drawings, FIG. 1 shows an example process flowchart of the

methodology herein, wherein lignin is partially decomposed during pretreatment of lignocellulosic biomass ((a), reference character 12), and removed from the biomass composition as smaller, soluble fragments through the use of a water washing process which produces a black effluent ((bl), reference character 14). In most pulp mills the main lignin utilization pathway has been to concentrate the black effluent by evaporation and burn the residual sludgy lignin to supply energy to the pulping plant. Increasingly, however, lignin is being considered as a renewable source of value-added aromatic compounds and fuels. Significant economic barriers in the use of such feedstock arise from the cost of chemical plant equipment needed for various chemical processing steps, and also the cost of chemicals, particularly solvents. Considering the amphiphilic character of lignin ((b2), reference character 16), the embodiments herein are thus designed to use the solubilization ability of block copolymer micelles ((cl), reference character 18) toward lignin derivatives to concentrate lignin in the aqueous environment of pulping and pretreatment effluents, greatly reducing costs, and opening possibilities for selective chemistries. For this reason, characterization of block copolymer micelles is highly desirable ((c2), reference character 20). A particular but non-limiting choice of non-ionic ABA-block-polymers 22 to enable a block copolymer-lignin derivative nanostructure 26, is based on the novel use in diverse applications, such as delivery of drugs, as scavengers for pollutants, as chemical extraction aids in separation processes, and for micellar catalysis.

[0025] In general terms, more than one type of unimer is utilized for polymerization of block copolymers. Usually, the properties of the blocks within the copolymer are quite different from one another and this results in spontaneous self

predicts the formation of aggregates at the critical micelle concentration (CMC) in aqueous media. Such aggregates or micelles, as utilized herein, can have spherical, cylindrical, rod-shaped or lamellar (e.g., spherical micelles, multilamellar vesicles, and unilamellar vesicles shapes). In polar solvents such as water, the hydrophobic blocks are incorporated in the core and the hydrophilic blocks, along with water, form the shell. Due to the polarity gradient present in micelles, the aggregates are able to accommodate hydrophobic substances and thus enhance the solubility of these materials, which are normally relatively insoluble substances in aqueous solutions.

[0026] Solubilization phenomena in aqueous and nonaqueous solutions of conventional low molecular weight surfactants have been extensively investigated theoretically and experimentally. However, block copolymers as compared to conventional low molecular weight surfactants are beneficially utilized herein because of their micellization ability in a variety of solvents. Thus, block copolymer architecture (ABA, BAB, ABC, AB, BA), hydrophobic-lipophilic balance (HLB), and discrete block molecular weights can significantly influence their solubilization capacity. Many low molecular weight solubilizates (guest), including aromatic solvents benzene, toluene, naphthalene, pyrene, and anthracene, have been investigated in block copolymers. To harness the benefit of block copolymer solubilization phenomena, it is necessary to understand how the extent of solubilization can be affected by the structural properties of the solubilizate (guest) and the block copolymer. [0027] Hydrophobic and hydrophilic interactions between the solubilizate and the blocks that form the core and shell of micelles control the scope of solubilization. Using electron spin resonance spectroscopy (ESR) and nuclear magnetic resonance spectroscopy (NMR), the locations of antioxidant gallates in micelles can be be dependent on their hydrophobicity. Also, aromatic molecules are solubilized preferentially compared to aliphatic hydrocarbons in A-B-A type block copolymers. Furthermore, hydrocarbon solutes in polyethylene oxide (PEO)-polypropylene oxide (PPO) block copolymers demonstrate large solubilization capacities and high selectivity for aromatic over aliphatic solutes in block copolymer micelles, compared to conventional low molecular weight surfactant micelles. The molecular volume of the solutes can also influence the amount of

solubilization in micelles. Thus, solutes with larger size are solubilized in smaller number. However, given identical molecular volumes, the polarity of the compounds controls the selectivity of solubilization. For example, the solubility of benzene in polar and non-polar blocks of PEO/PPO is significantly larger compared to n-hexane in the entire composition of the block copolymer.

Beneficially, lignin, with its combination of aromatic and aliphatic hydrocarbons, having polar functional groups, aliphatic bridges and a variety of oxygenated moieties, shows a unique

solubilization pattern in aqueous dispersions of block copolymer micelles. Lignin is often described as a random polymer of three mono-lignols, p-coumaryl (H), coniferyl (guiacyl, G) and sinapyl (S) alcohols.

[0028] In an example non-limiting methodology herein, lignin is obtained from black effluent produced by the sequential ozone and soaking aqueous ammonia pretreatment of, for example, wheat straw, and was previously characterized (using chemical analytical instruments FT-IR, UV-Vis, Py- GC/MS, Py-TMAH-GC/MS, DPPH assay) as partially-depolymerized fragments of H-G-S with S/G ratio of 0.35 and average molecular weight of 750 (g/mol). Also, five phenolic aromatic model compounds, phenol, eugenol, guaiacol, ferulic acid and guaiacylglycerol-beto-guaiacyl ether (dimer) are employed in the methods herein. To illustrate the workings of the present embodiments herein, a commercially available non-ionic, amphiphilic tri-block copolymer, polyethylene oxide-block- polypropylene oxide-block- polyethylene oxide (under the trade name of Pluronic® F-68), was chosen for this invention, due to its known uses in solubilizing aromatic and aliphatic molecules in various applications. However, other example block co-polymers can also be employed where warranted. The solubilization behaviors and interactions between lignin or model compounds with block copolymer micelles in aqueous ammonia solutions (as found in the black effluent) can be studied using nuclear magnetic resonance spectroscopy (NMR) and diffusion ordered nuclear magnetic resonance spectroscopy (DOSY-NMR).

[0029] In particular, Nuclear magnetic resonance spectroscopy (NMR) can be used herein to characterize the structure, reactions and degradation processes of utilized woody biomass. Most lignin analyses by NMR are done by dissolving lignin from a particular source, treated or untreated, in deuterated dimethyl sulfoxide (d_6-DMSO) and performing a suite of experiments, including ID 1H and ^{13}C NMR experiments, 2-D correlation experiments, particularly HMQC, HSQC and HMBC, and/or by solid state NMR of extracted and dried lignin samples. Lignin' s low solubility in most solvents has been a major hindrance in the structural characterization of lignin polymers.

Furthermore, the randomly composed structure of lignin from its three major sub-unit classes (S, G and H subunits) provides no regular structure that can be fully characterized by any method. Such methods have thus been utilized herein in a beneficial manner to enable the processes herein by to investigating lignin structure and pathways of decomposition and reformation for many avenues of lignin modification, including processes of nature, e.g. degradation of wheat straw by Phanerochaete chrysosporium and hardwood lignin by the Western Poplar Clearwing Borer, Paranthrene robiniae (Hy. Edwards), as well as lignin digestion in every digestive organ of termites, and more recently, chemical processes, such as heterogeneous catalytic hydrodeoxygenation of biomass -derived lignin to aromatic hydrocarbons, aqueous ammonia

pretreatment of wheat straw, and physical processes including pyrolysis of corn stover and water-only flow-through pretreatment of Miscanthus.

[0030] A resultant beneficial process often but necessarily includes two steps: (1) solubilizing or encapsulating solute lignin derivatives in block copolymer micellar systems; and (2) utilizing polymer-lignin micellar system to conduct a chemical reaction. With respect to the step of solubilization of solute lignin derivatives, amphiphilic triblock copolymer Pluronic F-68, as an example, can be added to the lignin alkaline solution (e.g., having a pH 11.4). However, while amphiphilic triblock copolymer Pluronic F-68 is exemplary, it is also to be noted that other block copolymers, such as diblock copolymers, can be utilized herein. The liquid-state 1H NMR has confirmed the solubilization of lignin in polymer micellar system. The second step of chemical reactions provided for the lignin derivatives to be solubilized in the polymer micelles. Example chemical reactions include oxidation, de-esterification, transesterification, reduction, solvolyses, enzymatic reactions and metalloenzyme reactions, C-C coupling reactions, metathesis reactions, C-C cleavage reactions, and ether cleavage to modify the polymer-lignin nanostructure. The present invention will be more fully understood by reference to the following examples, which is intended to be illustrative of the present invention, but not limiting thereof.

EXAMPLE 1

STRUCTURAL CHARACTERIZATION OF LIGNIN

[0031] The structure of lignin obtained from the ozone and soaking aqueous ammonia

pretreatment of wheat straw was characterized utilizing chemical analytical methods in order to reveal its antioxidant characteristics, including attenuated total reflectance-Fourier transform infrared spectroscopy (ATRFTIR), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), pyrolysis/tetramethylammonium hydroxide-gas chromatography/mass spectrometry (Py/TMAH- GC/MS), gel permeation chromatography (GPC), ultra violet-visible spectroscopy (UV-vis), and 1,1- diphenyl-2-picrylhydrazyl (DPPH) antioxidant evaluation assay. The results demonstrated that the isolated lignin is a -hydroxyphenyl- guaiacylsyringyl(H-G-S)lignin, with S/G ratio of 0.35 and significant amounts of phenol 2-methoxy (guaiacol) and phenol 2-methoxy-4-vinyl (4-vinylguaiacol). The Py-GC/MS and Py/TMAH-GC/MS pyrograms indicated that the major units in this lignin are derived from hydroxycinnamic acids. The GPC results revealed the molecular weight of the lignin was considerably low and also the FTIR analysis showed that the lignin possessed hydroxyl and methoxy functional groups; the factors led to the extracted lignin having a comparable antioxidant activity to that of currently used commercial antioxidants. The UV-vis and DPPH antioxidant assay results suggested a percentage of inhibition ofthe DPPH radicals in the following order: guaiacol (103.6 + 1.36) > butylated hydroxytoluene (103.3 ± 1) > ferulic acid (102.6 + 0.79) > pretreated lignin (86.9 + 0.34).

Materials

[0032] The lignin sample was isolated from the ammonia effluent ofthe sequential ozone and soaking aqueous ammonia pretreatment of wheat straw by acetic acid precipitation method. The chemicals 2-methoxy phenol (guaiacol), trans-ferulic acid, I,l-diphenyl-2- picrylhydrazyl(DPPH),tetramethylammonium hydroxide (TMAH), acetic acid, and 2,6-di-tert-butyl- 4-methoxyl phenol (BHT) were purchased from Sigma-Aldrich Inc (Milwaukee, US).

Ozone and soaking aqueous ammonia (OSAA) pretreatment

[0033] The wheat straw was first soaked in water to bring the moisture content of the mass up to 45% (w/w) water, and then subjected to ozone pretreatment. The ozonation reaction was performed with 5.3% ozone concentration (5.3%, w/w) at oxygen flow rate of 2 L/min for 10 min. Following the ozone pretreatment, the wheat straw was flushed to soaking aqueous ammonia pretreatment, where 20% ammonia solution was used to treat the biomass for 4 h at a 1:4 biomass to aqueous ammonia loading, including heating time. The ammonia solution was recovered through the use of a two step washing process with E-pure water, and the obtained effluent was gathered for lignin precipitation step.

Precipitation and purification of lignin

[0034] The presence of non-lignin components could influence antiradical activity of lignin. Hydrogen bonding between carbohydrate admixture polar groups and lignin phenolic groups could be formed and results in a reduction of antioxidant activity. An acetic acid precipitation method which does not contain sulfur and uses an environmentally-friendly reagent for precipitating lignin in effluent was employed. Before precipitation, the ammonium in effluent needs to be removed as much as possible. In order to evaporate the ammonium hydroxide, the measured effluent was put in a vented oven for 1 h at 75 °C. The precipitation of lignin performed by adding 20 ml acetic acid:water (9: 1, v/v) into effluent (50 ml). Next, the blend was placed in a warm water bath for 10 min at 80 °C, and then the suspension was centrifuged for 5 min at 3500 rpm (MSE, Centaur 2 Sanyo). The precipitate was water washed in 400 ml E-pure water. This suspension was centrifuged for 5 min with speed of 3500 rpm and the liquid- solid phases was separated several times. The final separation tube was put in a warm water bath for 30 min at 75 °C. Then, a centrifuge step for 10 min was performed also at 3500 rpm. The liquid phase was separated and the residual lignin was put in a drying oven at 50 °C overnight. This resulted in pure solid lignin

Characterization of lignin structure

[0035] The physicochemical structural of the lignin by utilizing the outcome of the analytical instruments was assessed. Attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR) was utilized wherein FTIR analysis was performed for analysis of functional groups in the lignin macromolecules. The FTIR spectra were obtained on an ATR-FTIR spectrophotometer (Shimadzu, Japan) with 64 scans. The ATR supplement works by measuring the changes that occur in a totally internally reflected infrared beam when the beam contacts with a sample. Finely powdered lignin were pressed on the crystal surface of the ATR probe. Samples were analyzed from 4000 to 800 cm-1 at a resolution of 4 cm-1. [0036] FIG. 2 shows the ATR-FTIR spectroscopy of the pretreated wheat straw lignin sample and the corresponding assignments of bands are listed in Table 1 below. In particular, FIG. 2 itself thus presents the view of the wavelength region from 600 to 3900 cm-1 with bands of interest identified by their wave number (cm-1).

Table 1: Assignment of ATR-FTIR absorption bands of pretreated wheat straw lignin.

assignment lignin λ (cm^{-1}) T (%)

-OH stretching vibration of polymer 3200 98. 95

-CH stretching vibration of polymer (-CH$_3$ and -CH$_2$-) 2921 98. 38

2851 98. 82

C=0 stretching vibration-/) substituted aryl ketone 1653 96. 55

(1660-1675 guaiacyl)

aromatic skeletal vibration 1555 91. 16

aromatic skeletal vibration 1411 90. 31

G ring breathing with CO stretching 1269 94. 94

aromatic C-H in plane deformation (S) 1127 95. 73

aromatic C-H in plane deformation (G>S) 1044 94. 41

aromatic C-H out-of plane deformation (S+H) 887 97. 72

[0037] FIG. 2 along with Table 1 shows the observation of a strong hydrogen band (-OH) stretching at 3200 cm-1 and C-H stretching at 2851, 2921 cm-1 in the lignin sample. A strong wide band between 3500 and 3100 cm-1 which is assigned to -OH stretching vibration is an observable characteristic for lignin IR spectra. The antioxidant activity of phenolic compounds is found to depend on the number of the hydroxyl groups linked to the aromatic ring. The assigned bands around 2850 and 2925 cm-1 are related to the C-H (-CH3 or -CH2-) stretching vibrations of the methyl and methoxy groups. The presence of alcoholic and phenolic hydroxyl groups can also be seen from the strong band in this region. The position of the methoxy group on the phenolic ring can also have positive effect on radical scavenging activity of lignin. Observations indicate that methoxy substitution in the ortho position increases the radical scavenging efficiency of guaiacol as compared to phenol.

[0038] As known to those skilled in the art, the ortho substitution with the electron donor methoxy group is also a factor in increasing the stability of the phenoxy radical and therefore increases its antioxidative efficiency. The effect of the presence of another methoxy group on the ring as di-methoxy compounds on their antioxidant activity has also been studied. The studies reported that the 2,6-dimethoxy phenol has higher antiradical activity than 2, methoxy phenol. It has been reported that there are no absorption bands that are lignin derived between 2800 and 1800 cm-1 wave number range. Some previous work stated that a band around 1720 cm-1 is the characteristic band for ester linkage absorption. However, it was observed that the ester linkage absorption in the lignin samples disappears. The loss of the 1720 cm-1 band absorbance and reduction in the 1653 cm-1 band absorbance in the lignin indicates that significant amounts of ester-linked subunits were released during the pretreatment. It has been found that an alkaline pretreatment selectively cleaves the ester linkages in lignin structure. A shoulder was observed at 1664 cm-1, which is the characteristic band for the carbonyl stretching group in guaiacyl units. This is related to the ester linkages in lignin structure and originates from the conjugated carbonyl stretches, possibly indicating the occurrence of hydroxycinnamic acid derivatives in the lignin structure. It has been reported that the hydroxycinnamic acids have higher antioxidant activity in compared with hydroxybenzoic acids. Other work has shown that the vibration of aromatic ring is assigned to bands around 1600-1400 cm-1. The absorption bands at 1555, 1411 cm-1 (T%, 91.16/90.31) lignin indicate the aromatic skeletal vibration of the lignin, respectively. Monteil- Rivera et al. found that band at 1265 cm-1 indicates characteristic of guaiacyl units in wheat straw lignin. The band at 1269 cm-1 indicates the G-ring breathing in lignin samples. A week band at 1127 cm-1 is

spectrum indicates that the content of the guaiacyl units are higher than syringyl units in the lignin. The band at 1044 cm-1 corresponds to aromatic C-H plane stretching of G units.

Pyrolysis-gas chromato graph v/mass spectroscopy (Py-GC/MS)

[0039] Pyrolysis of lignin samples was performed with using a CDS Pyroprobe 5000 connected to an Agilent GC/MS system (7890A GC, 5975 C mass selective detector). The lignin samples were loaded into a quartz tube and gently packed with quartz wool prior to pyrolysis. The pyrolysis was performed at 610 °C (1 min). The inlet temperature was maintained at 250 °C, and the resulting pyrolysis vapors were separated by a (5%-phenyl)- methyl polysiloxane hydrophobic column with a 30 m x 25 mm inner diameter. Helium was the carrier gas (1 ml min-1). The gas flow rate was 1 ml min-1, and the oven was retained at 280 °C for 10 min to prevent any residuals from remaining in the chamber. This gas was then analyzed with a mass spectrometer (InertXL MSD, Agilent Technologies), and C02 was used as the internal standard. The compounds were identified by comparing their mass spectra with those of the Wiley and the National Institute of Standards and Technology (NIST) electronic libraries. The pyrolysis of lignin, released phenolic compounds, which were observed to be derived from G, S, and H lignin units. The approximate abundance of phenolic-derived compounds was 65.09%, 23.36%, 11.5% for G, S, and H lignin units, respectively.

[0040] The pyrogram as shown in FIG. 3 reveals that the sample is a-hydroxyphenyl- guaiacylsyringyl (H-G-S) lignin and the identities and relative molar mass of the released phenolic compounds are listed in Table 2, as shown immediately below.

Table 2: Identities and relative molar abundances of the phenolic compounds released

from pretreated wheat straw lignin after Py-GC/MS.

label compound MW Origin [a]

1 phenol 94 H

2 phenol, 2-methyl- 108 H

3 phenol, 2-methoxy- (guaiacol) 124 G

4 phenol, 2-methoxy-4-[l-propenyl] 138 G

5 phenol, 4-ethyl 2-methoxy 152 G

6 phenol, 2-methoxy- 4-vinyl 150 G

7 phenol,2,6-dimethoxy- (syringol) 154 S

8 phenol, 2-methoxy-4-[l-propenyl] (trans- isoeugenol) 164 G

9 phenol,2-methoxy-4- [1 -propenyl] 164 G

10 5-tert-butylpyrogallol 182 S/H

11 2-propanon, l-[4-hydroxy-3-methoxy phenyl] 180 G

12 2H- 1 -benzopyran-3 ,4-diol, 316 H

13 phenol, 2,6-dimethoxy-4-(2-propenyl)- 194 S

14 phenol, 2,6-dimethoxy-4-(2-propenyl)- 194 S 15 phenol, 2,6-dimethoxy-4-(2-propenyl)- 194 S

16 ethanone, 1 - (4-hydroxy- 3 , 5 -dimethoxyphi 196 S

17 estra-l,3,5(10)-trien-17a-ol 256 H

[0041] The predominant lignin derivatives included phenol 2-methoxy, phenol 2-methoxy-4- vinyl, and 2, 6-dimethoxy phenol. The identified phenolic compounds showed that the abundance of G-units was higher than S-lignin units, with a S/G ratio of 0.35. Moreover, high levels of phenol

2-methoxy-4-vinyl (25.83% of total area of phenolic-derived compounds) was released from the lignin. This evidence demonstrates the presence of hydroxycinnamic acids derivatives in solid lignin. It is in agreement with the loss of the 1720 cm-1 band absorbance and reduction in the 1653 cm-1 band absorbance in the ATR-FTIR spectra; indicates significant amounts of ester- linked lignin units were cleaved. It has been reported that herbaceous crops possess hydroxycinnamic acids, ferulic and -coumaric acids, which make linkages between lignin and hemicellulose. The presence of the 4-vinylphenol and 4-vinylguaiacol released from Abaca fibers after Py-GC/MC was likely due to the presence of

During pyrolysis, decarboxylation of the hydroxycinnamic acids results in those compounds. In order to assess, the predominant presence of phenol 2-methoxyl, phenol 2-methoxy-4-vinyl, and 2,

6-dimethoxy phenol, tetramethylammonium hydroxide (TMAH) was used as a methylating agent.

[0042] The pyrolysis in presence of the TMAH helps to prevent decarboxylation and causes methylation of the phenolic and carboxyl groups. The pyrogram is shown in FIG. 4, and the identities and relative molar mass of the released phenolic compounds are listed in Table 3 shown below. Py/TMAH-GC/MS of lignin demonstrated considerable levels of phenol 2-methoxy-4-vinyl and phenol 2-methoxy confirming the predominant appearance of G-lignin units in solid lignin.

Table 3: Identities and relative molar abundances of the phenolic compounds released from

pretreated wheat straw lignin after Py/TMAH-GC/MS.

label compound MW Origin

1 phenol 94 ÏÍ

2 phenol, 2,5-dimethyl- 122 H

3 phenol, 3 -methyl- 108 H 4 phenol, 2-methoxy (guaiacol) 124 G

5 phenol, 2-methoxy-4-methyl- 138 G

6 3,4-dimethoxytoluene 152 G/H

7 phenol, 4-ethyl-2-methoxy 152 G

8 2-methoxy-4-vinylphenol 150 G

9 phenol, 2,6-dimethoxy (syringol) 154 S

10 phenol, 2-methoxy-4-(l-propenyl)-, (E) (isoeugenol) 164 G

11 2-propanone, l-(4-hydroxy-3-methoxyphenyl) 180 G

12 3-tert-butyl-4-hydroxyanisole 180 G/H

13 ethanone, l-(3,4,5-trimethoxyphenyl) 210 S Gel permeation chromato raphy (GPC)

[0043] The molecular weight of extracted lignin was determined by utilizing gel permeation chromatography (GPC). The molecular weight of the lignin was taken from our previous study: Isolated lignin was acetylated in pyridine/acetic anhydride (1: 1, v/v) mixture prior to GPC analysis to improve their solubility in THF solvent. Each 10 mg lignin was suspended in 2 ml of

pyridine/acetic anhydride mixture for 72 h in dark. The supernatant was added into 48 ml of cold water and stirred at room temperature for 1 hour. The precipitate was water washed, ether washed and freeze dried. After that, 2 mg of sample was dissolved in 1 ml THF and store at 4 °C before analysis. Gel permeation chromatography of the isolated lignin was performed on 1260 infinity HPLC system (Agilent technology) with refractive index detector (RI-G1362A). The column was PL gel mixed-C (300 x 7.5 mm, 5 m) and tetrahydrofuran (THF) was used as eluent. The molecular weight of isolated lignin was recorded at 25 °C. The molecular weight of the lignin were 772 (g/mol) and 3735 (g/mol) and the molecular weight of milled wheat straw lignin were 2026 (g/mol) and 3857 (g/mol) for the number- average (Mn) and weight- average (Mw) molecular weight, respectively. Lignin with low molecular weight is more reactive than those with high molecular weight molecules. There have been reports by those of ordinary skill in the art that molecular weight data does provide some useful guide. Such reports are based on the premise that small molecular weights are more desirable for further chemical or catalytic conversion. High molecular weight increases heterogeneity and polydispersity which are factors that decrease radical scavenging activity.

[0044] It has been reported that Kraft lignin with a higher molecular weight has a lower antiradical activity when compared with the methanol fraction of ent lignin with a lower molecular weight. The molecular weight of lignin is also able to affect its functional groups distribution.

[0045] Others have stated that alkaline fraction of wheat straw lignin with lower molecular weight showed higher contents phenolic hydroxyl group and methoxy functional group; the factor led to the extracted lignin with the lowest molecular weight (Mn: 1500) having the highest antioxidant activity as compared to the fraction with higher molecular weight (Mn: 3000). Low molecular weight results from extensive depolymerization of lignin, particularly cleavage of ether linkages, which lead to the formation of new aromatic hydroxyl groups.

[0046] In the development of the embodiments herein, it was observed that the amount of G- lignin units is significant when compared to other lignin units. According to the presence of free positions in C5 of the aromatic ring in G-lignin units, these units can also form carbon-carbon bond in comparison to S-lignin units. Accordingly, the low number- average molecular weight of this lignin was essentially due to the cleavage of ester bonds, which is the predominant reaction that occurs during the alkaline pretreatment of lignocellulosic biomass that can include Kraft pulping, ozonoly sis, a soaking aqueous ammonia pretreatment, and an alkaline pulping and pretreatment process. However, the carbon-carbon linkages in G-units were not broken during lignin treatment because of their high stability. Because carbon-carbon bonds are sensibly stable during alkaline conditions, the amount of the weight-average molecular weight of the lignin could be related to the presence of inter-molecular carbon-carbon and ether bonds.

Lignin antioxidant activity

[0047] The antioxidant activity of the lignin in terms of the percentage of inhibition of 1,1- diphenyl-2-picrylhyrazyl (DPPH) radicals was determined. As part of this determination, the commercial antioxidants guaiacol, ferulic acid, and butylated hydroxytoluene (BHT) were utilized as controls for comparison. An antioxidant activity was employed with some modifications as needed for application to the lignin sample. A sample of each test material (0.1 mg) was mixed with a 4 ml methanolic solution of DPPH (2.5 mg/100 ml methanol) and after 30 min. The absorbance at 515 nm of the mixture was measured using a UV-vis spectrophotometer. The antiradical activity of the test materials was determined as the decrease in absorbance of the DPPH expressed as a percentage of the absorbance of a control DPPH solution without test compounds using a UV-vis spectrophotometer (Shimadzu, Japan).

[0048] During the reaction between antioxidants and the DPPH, reduction of the DPPH radicals occurred followed by decreasing in its absorbance at a characteristic wavelength (515 nm). The absorption disappears by reduction effect of antioxidant or a radical species. The percentage of inhibition of the DPPH radicals was calculated using Equation 1:

(1) Inhibition % = $A_{\infty\eta\bar{u}0I}$ - A_{samp}ie/ A_{con}troi x 100,

where A control is the absorbance in blank probe (antioxidant was omitted) and A sample is the absorbance in the sample.

[0049] The results were presented as mean + standard error (SE). All were assessed by two- factor analysis of variance. All experiments were run triplicate. The results of the antioxidant activity test in terms of the percentage of inhibition of the DPPH radicals were 86.9 + 0.34, 103.3 + 1, 102.6 + 0.79, 103.6 + 1.36 for the pretreated wheat straw lignin, and commercial antioxidants butylated hydroxytoluene (BHT), ferulic acid, guaiacol, respectively. The higher the value, the higher the radical scavenging activity ofthe compounds tested.

[0050] As exemplified in FIG. 5, the UV-vis spectrum indicated that the high peak (at 515 nm) of DPPH solution was completely removed by adding the lignin in solution. This showed that the DPPH radicals have been trapped by the lignin phenolic compounds. The lignin as an alkali lignin showed that it contains hydroxycinnamic acids-derived units, particularly phenol 2-methoxy-4- vinyl, and guaiacyl derivatives. It has been reported that ferulic and -coumaric acids are a main fraction of free phenolic monomers in alkali lignins and likely to consider for the high radical scavenging activity. The number of hydroxyl groups on the aromatic ring and ortho substitutions with the electron donating methoxy group which increase the stability of phenoxy radical, determine the antioxidant activity of hydroxycinnamic acids.

[0051] The pretreated wheat straw lignin showed a significant antioxidant activity when compared with other commercial antioxidants tested. The antioxidants trapped DPPH radicals from the solution, and this reaction was confirmed by the color change from deep violet, DPPH solution or blank probe, to pale yellow color or colorless, DPPH solution plus antioxidants, which is a typical of radical scavenging activity assay. Similarly, the lignin also showed positive antioxidant activity assay results. The analyses of the analytical data of FTIR, Py-GC/MS, Py/TMAH-GC/MS, GPC, UV-vis, and DPPH assay led us to greater understanding of the antioxidant characteristics of derived lignin from wheat straw by ozone and soaking aqueous ammonia pretreatment.

[0052] The strong hydrogen band (-OH) stretching at 3200 cm-1 and C-H stretching at 2851, 2921 cm-1 and the C-H stretching bands at 2850 and 2925 cm-1 (-CH3 or -CH2-) pertinent to the vibrations of the methyl and methoxy groups confirmed the presence of phenolic compounds and their methoxy functional groups in the lignin. It was observed that the content of guaiacyl units (1265 cm-1) were higher than syringyl units (1127 cm-1) with a S/G ratio of 0.35. Results also indicate that the ester linkages in the lignin structure were selectively broken (1720 and 1653 cm-1) during the pretreatment. This possibly due to the occurrence of hydroxycinnamic acids derivatives in the lignin. Likewise, high levels of phenol 2-methoxy-4- vinyl (25.83%) from the pyrograms were identified. Also, it has been found that the presence of lignin-derived compounds with low molecular weight (772 g/mol) induced lignin' s antioxidant activity (86.9 + 0.34).

EXAMPLE 2

SELF- ASSEMBLED NANOSTRUCTURES OF LIGNIN WITH A PEO-PPO-PEO BLOCK COPOLYMER

[0053] The interaction of partially-depolymerized wheat straw lignin and a polyethylene oxide- Wocfc-polypropylene oxide-Wocfc-polyethylene oxide (PEO-b-PPO-b-PEO) in aqueous media was investigated using liquid-state 1H NMR and DOSY NMR in order to probe the influence of hydrophilic and aromatic interactions on the formation of desirable nanostructural self-assembly of copolymer micelle-lignin for up-converting lignin to value-added materials such as antioxidant guaiacol, aroma and flavor compound eugenol.

[0054] The hypothesis herein for copolymer micelle incorporation of lignaceous species includes a "hydrophilic-aromatic interaction model", in which lignin derivatives with amphiphilic characteristics, known to be abundant in the effluent from pretreatment of various lignocellulosic materials, have an affinity for these micelles, and tend to form relatively organized structures within them. Here we show how the chemical/structural features of lignin units and small molecule lignin models determine their solubilization (guest) behavior in nanoscopic micelles (host).

[0055] Using proton NMR, a buildup of concentration of lignin and lignin-based model compounds guaiacol, eugenol, phenol, guaiacylglycerol-beta-guaiacyl ether, ferulic acid in

Pluronic® F68-deuterium oxide solution results in significant upfield chemical shifts of micellar PEO-(CH_2-CH_2) and PPO-(CH_3) proton resonances, and at a critical concentration of aromatic guest, dramatic upfield shifts due to gross structural transitions in the micelles.

[0056] As evidenced herein, the copolymer micelle-guest interactions depend on both chemical functional group characteristics of guests (i.e., polarity, H-bonding ability) and π-π interactions between aromatic/conjugated groups. Results herein demonstrate how the loci of incorporated guests in A-B-A block-co-polymer micelles are affected by such features.

Materials

[0057] PEO-PPO-PEO block copolymer (Pluronic® F-68) was purchased from Sigma-Aldrich (Schemel-cl). Lignin was obtained from the spent ammonia liquor produced by sequential ozone and soaking aqueous ammonia pretreatment of wheat straw. Phenolic model compounds included phenol (>99%), guaiacol (98%), eugenol (>98%), trans-ferulic acid (99%), and guaiacylglycerol- beta-guaiacyl ether (>97%) were purchased from Sigma-Aldrich (Milwaukee, USA) and TCI America (Portland Oregon USA), respectively (see FIG. 2). Chemicals were used without further purification. NMR sample tubes, 5 mm N51A 600MHz, were obtained from Kimble Chase, USA. Solutions for solubilization and pH adjustment (ammonium hydroxide solution 28-30 % from Sigma-Aldrich, Milwaukee, USA) were prepared in deuterium oxide (D_20, 99.9%) from

Cambridge Isotope Laboratories, Inc.

Lignin preparation procedure

[0058] The spent ammonia liquor produced by the soaking aqueous ammonia pretreatment of wheat straw was recovered through the use of a two-step washing process with E-pure water, and the obtained effluent was gathered for lignin precipitation and purification steps. In order to eliminate any interfering effect of non-lignin components in pretreatment effluent on lignin solubilization behavior in the micellar system, a precipitation-purification method was employed with some modifications as needed for application to this study. In general, the precipitation of lignin was performed by adding 20 ml acetic acid : water (9: 1, v/v) into effluent (50 ml). Then, the precipitated lignin was stored at room temperature (25 °C) for further solubilization study (FIG. 1, bl, b2).

Critical micelle concentration (CMC) evaluation by DOSY-NMR

[0059] The critical micelle concentration (CMC) of the PEO-PPO-PEO block copolymer (Pluronic® F-68) was evaluated by diffusion ordered nuclear magnetic resonance spectroscopy (DOSY-NMR). The micelle copolymer solutions were prepared by direct dissolution of the copolymer in D_20 at different concentrations (mM), and values for the diffusion coefficients D were determined for each concentration. The CMC was determined from the inflection point of a plot of diffusion coefficient vs. polymer concentration. DOSY-NMR is a two dimensional NMR technique, usually employing a magnetic field gradient along the z-axis of the sample, in which the NMR signal decays exponentially with increasing gradient power, according to the diffusion behavior of individual molecules.

[0060] This leads to two dimensions; the first dimension (F2) accounts for the conventional chemical shift and the second one (Fl) for diffusion coefficient (D). DOSY NMR measurements were performed on a Varian VNMRS 600 (liquid) NMR spectrometer operating at 600 MHz, equipped with z-axis pulsed field gradients. Amphiphilic molecules spontaneously self-assemble, at or above a critical concentration in aqueous solutions, to form aggregates of various morphologies. The resulting micelles are stabilized in water by the favorable interactions between hydrophilic groups and water molecules, forming a stable hydrated shell between the hydrophobic core and the bulk water. For ionic surfactants, the shell

surrounding the core is a charged layer, or stern layer, while for non-ionic surfactants it is referred to as a hydrated palisade layer.

[0061] Techniques which monitor formation of micelles, rather than changes in aggregation number, are preferable for the CMC determination of block copolymer micelles. Many techniques have been used to determine CMC values, including fluorescence, light scattering, osmometry, viscosity and DOSY NMR. In this study, the CMC of the PEO-PPO-PEO block copolymer

(Pluronic[®] F68) solution in deuterium oxide was determined by diffusion ordered nuclear magnetic resonance spectroscopy (DOSY-NMR). A plot of concentration of polymer vs. diffusion coefficient (D) reveals an inflection at 0.04 mM (CMC = 0.04mM) at both pH 7.5 and 11.4. This demonstrates that the micellar structure of this polymer in aqueous solution is not compromised at high pH. A further study of the effect of added model compounds on the physical structure of the polymer micelles was carried out by DOSY NMR. Diffusion coefficients of copolymer solutions at pH 11.4- after addition of the model compounds-were obtained at the CMC of the polymer (0.04mM). As shown in FIG. 6, the diffusion coefficient of the Pluronic F-68 PEO block generally decreased with the addition of model compounds, indicating a slower self-diffusion coefficient of the PEO block comprising the palisade region of the micelle. The results show that the micelle structure was not disrupted by addition of model compounds. Based on the above results, a concentration of 0.04 mM Pluronic[®] F-68 was used throughout the solubilization study. Solubilization study by liquid-state 1H NMR

[0062] A Stock solution of the PEO-PPO-PEO tri-block copolymer (Pluronic[®] F-68) was prepared by dissolving the appropriate amount of the block copolymer in deuterium oxide (99%) under gentle agitation, such that the resulting stock solution (50 ml) had a concentration of 0.04 mM (at the CMC). To make the concentration series for each model compound (guest), known volumetric amounts of the guests were added to aliquots of stock solution (1 ml) of the tri-block copolymer. Lignin was weighed as a solid to make weight per volume dilutions. This resulted in preparation of six samples with different concentrations (0.01, 0.04, 0.08, 0.12, 0.16, 0.2 mM) for each tested material. The pH of all samples was adjusted to 11.4 using ammonium hydroxide. Preparation of solutions and the solubilization process took place at room temperature (25 °C) without heating or mechanical stirring. The solutions were then stored for 24 hour to equilibrate before assessment of solubilization by NMR. 700 µĩ of each sample solution was injected into a 5 mm NMR sample tube with a syringe, and the NMR tube was sealed with a plastic tube cap.

Solubilization of guests in micellar aqueous solution: Effect of π-π aromatic stacking and polar interactions on the micelle-lignin structure K

[0063] NMR studies of solubilization of model phenolic compounds and lignin in PEO-PPO- PEO block copolymer micelles revealed that all the models tested were able to solubilize into these micelles, presumably due to the hydrophobic interaction between the core of the micelles and aromatic rings of the guests.

[0064] As seen in FIG. 7A and FIG. 7B, Group A model compounds primarily interact with the oxyethylene blocks (polyethylene oxide) of the copolymer, compared to Group B model compounds which mainly affected the polypropylene mid-block of the copolymer. Patterns of solubilization-interaction behavior of model phenolic compounds (Groups A and B) and partially decomposed lignin with copolymer micelles was investigated by monitoring 1H NMR chemical shift changes for PEO-CH_2-CH_2 resonances, and for methyl resonances of the B -block (micellar core, PPO-CH_3) of Pluronic[®] F-68 block copolymer as a function of concentration of the tested model compounds.

Solubilization of model phenolic compounds phenol, guaiacoh eugenol (group A) in PEO-PPO- PEO block copolymer micelles

[0065] As can be seen in FIG. 7A, at low model concentrations, there is a gradual up field shift in the PEO-CH_2-CH_2 resonances with increasing concentration of model, which is mirrored by a chemical shift change in the PPO-CH_3 resonances. The small, almost linear change in chemical shifts proceeds until a "critical guest concentration" (CGC) is reached, whereupon a large chemical shift is observed, indicating a reordering of the structure of the micelles. The greatest chemical shifts observed at a guest concentration of 0.20mM of each, eugenol, phenol, and guaiacol were, for eugenol, 282.5Hz for PEO-CH_2-CH_2 resonances and 195.2Hz for PPO-CH_3 resonances of the polymer; for phenol 233.4Hz and 150Hz, respectively; and for guaiacol 273.3Hz and 173.6Hz, respectively.

[0066] The changes in chemical shift are concentration dependent and are non-uniform, particularly at the concentrations 0.04 mM in eugenol, 0.12 mM in phenol and 0.16 mM in guaiacol, where an inflection occurs. The dramatic chemical shift changes are accompanied by clouding of the solution, as a macroscopic emulsion forms (FIG. 8, photos). These emulsions remain stable in solution for weeks, and do not further aggregate or release their pay loads. The upper limit of micellar loading for each model compound has not yet been determined.

[0067] The graphs in FIG. 7 A clearly show that above the CGC, the chemical shift differences (Δδ) of polymer CH_3

polymer PEO regions over PPO regions. In general, for group A model compounds, proton chemical shift changes ($\Delta\delta$) for PEO methylene resonances (palisade region of micelles) was greater than for the PPO methyl (core) resonances. The greater magnitude of chemical shift change for the PEO block indicates that the guest is preferentially oriented within the hydrated palisade layer of the micelle, but also extends at least partially into the core.

[0068] With respect to the morphological transitions of micelles incorporating aromatic guests, in nonionic PEO-PPO-PEO block copolymer micelles, the hydrophilic palisade layer comprising the hydrated PEO blocks controls the structural properties of the micelles (micellar shape), maintaining a hydrophobic core and hydrophilic shell in aqueous solution. In addition, the dehydration of the PEO shell causes a type of shape change in micelles. Moreover, the effect of added salt on Tetronic T904 aggregation behavior in aqueous solution has been described. The description provides that the presence of salt dehydrates the PEO shell, and consequently the spherical shape of the micelles is changed to prolate ellipsoidal.

[0069] The influence of salicylic acid on the aggregation characteristics of Pluronic® P85 has also been studied in an aqueous medium by those skilled in the art. As such, the salicylic acid resides in the micellar shell and consequently dehydrates the corona region, resulting in a sphere- to-rod shape transition. Dehydration of core and shell regions of micelles is associated with restructuring and growth processes of micelles. Others reported that corona specific micellar dehydration by NaCl induces inter-micellar interaction and consequent formation of micellar clusters. On the other hand, it also was stated that core specific micellar dehydration by butan-l-ol brings about sphere-to-rod micellar shape transition on approaching the cloud point of copolymer solutions. Similarly, in the model compounds Group A, the structural transitions made apparent in both PEO-CH$_2$-CH$_2$ and PPO-CH$_3$ regions of the NMR spectrum (as well as in the aromatic signals for the guests) were accompanied with clouding of the samples (see FIG. 8 photos).

[0070] It was found that the phenolic model compounds, phenol, guaiacol and eugenol predominantly affected the palisade layer of the micelles, and PEO-CH$_2$-CH$_2$ resonances shifted upfield significantly (FIG. 7A). The observed π-π aromatic interaction of Group A model compounds is indicated in the aromatic region of the NMR spectrum as well, especially in eugenol and guaiacol, as individual guest molecules stack in an alternating orientation, such that methoxy groups occupy an opposite configuration on each alternating stack member, relieving steric interactions between the methoxys. The reordering of guests in the micelles by π-π interactions may allow more efficient loading of guest within micelle, and could also result in the dehydration of the palisade region and a gross structural transition in the micelle. The changes in morphology of the micelles for Group A model compounds is thought to arise from π-π interactions between guest molecules, which have the opportunity to achieve increased order or stacking within the scaffold provided by the polymer chains of the A-B-A block copolymer micellar structure.

[0071] In contrast, π-π interactions of model compounds in free solution is not observable by NMR. For a given model compound, it is apparent that the buildup of π-π interactions is dependent on the chemical structure of a given model, such that eugenol has the lowest CGC (~0.05mM), followed by phenol, then guaiacol. It is apparent that the methoxy group plays a role in promoting π-π interactions in these systems, but it is also clear that more than one effect determines the extent and form of π-π interactions, the CGC for a given model, and the loci of the models within their host micelles. Future studies will expand the concentration range studied and the diversity of lignin model compounds, and will attempt to determine the shape and size of the micelles obtained.

Solubilization of model phenolic compounds ferulic acid, guaiacylglycerol-beta-guaiacyl ether, and partially decomposed lignin (Group B) in PEO-PPO-PEO block copolymer micelles [0072] Patterns of solubilization behavior for ferulic acid, lignin, and guaiacylglycerol-beta- guaiacyl ether in the micellar system are shown in FIG. 7B. For Group B models, the magnitude of $\Delta\delta$ is much smaller, in general, than for Group A. The greatest chemical shifts observed for ferulic acid were 45.2Hz for PEO-CH$_2$-CH$_2$ resonances and 46.9Hz for PPO-CH$_3$; for lignin, 35.5Hz and 13.82Hz for PPO-CH$_3$ and PEO-CH$_2$-CH$_2$ signals, respectively; and for guaiacylglycerol-beta- guaiacyl ether were only 6.94Hz for both PEO-CH$_2$-CH$_2$ and PPO-CH$_3$ resonances. After addition of Group B model compounds to the block copolymer solution, the magnitude of chemical shifts of both PPO methyl protons and PEO oxyethylene protons followed the order of ferulic acid > lignin > guaiacylglycerol-beta-guaiacyl ether.

[0073] Having the highest rate of chemical shift change at lower concentrations, ferulic acid stands out, and can be compared qualitatively with eugenol, in that both models have a propenyl side chain, but ferulic acid has a carboxylic acid group at the terminus of its propyl chain. Ferulic acid shows an earlier onset of interaction with polymer, but addition of eugenol results in a much greater chemical shift effect, and greater differentiation between core and shell regions of the micelle, as evidenced by the difference in magnitude of chemical shift change between PEO-CH$_2$- CH$_2$ vs. PPO-CH$_3$ for eugenol.

[0074] In the case of ferulic acid, PEO methylene and PPO methyl signals move upfield at virtually the same rate, and level off together, suggesting there is little differentiation between core and shell in the distribution of ferulic acid in the

micelle. The result is that monolignol-like model compounds having polar, hydrogen bonding substituents interact with both core and shell regions in micelles of PEO-PPO-PEO block co-polymers roughly equally.

[0075] There is a third factor at work in the reordering of micelles incorporating guest molecules modeled on lignin. The polyoxyethylene chains of PEO-PPO-PEO block copolymers that cover the micelle core are hydrated. It has been reported that each oxyethylene unit binds around three water molecules (in the unloaded polymer), and that the bound water is relatively equally distributed through the head group layer. It has also been shown that the water solubility of polymer aggregates increases with the presence of polar substituents on guests, and this helps the compound dissolve not only in the most hydrophobic part of the micelle but also in the less hydrophobic palisade region. The same observation was reported by others that the dyes, acid orange 20 and basic yellow 2, were able to dissolve in the palisade layer of the nonionic surfactant. The polarity gradient present between hydrophilic shell and hydrophobic core regions of the micelle provides that both nonpolar and polar reagents-or amphiphiles-can be solubilized with preferential orientation within the micelle. This highly ordered solubilization results in selective effects, which lend themselves well to micellar catalysis and acceleration of the rate of reactions, a primary goal of our research group. O

[0076] One effect of polar groups is to dampen the effect of π-π interactions within the micelles. The carboxylic acid moiety of ferulic acid, with respect to eugenol, causes an apparent drastic reduction in chemical shift due to interactions with polymer chains, and causes the relative Δδ between PEO and PPO resonances to greatly decrease in magnitude, and virtually eliminates the differentiation of ferulic acid between core and shell regions. The existence of π-π interactions in this system is by no means ruled out, but the effect of hydrogen bonding from the carboxylic acid seems to dominate the overall interaction.

[0077] There is no bulk transition in morphology of micelles in the case of ferulic acid (and no clouding observed). Interestingly, for the guaiacylglycerol-beta-guaiacyl ether (dimer), the effect of primary and benzylic alcohol functions on the propyl bridge of the guaiacylglycerol-beta-guaiacyl ether is to shunt the magnitude of polymer-model interactions further, at least at the concentrations studied. The guaiacylglycerol-beta-guaiacyl ether, in fact, follows the same pattern observed for guaiacol throughout the concentration range studied. Whereas guaiacol shows a late transition in reordering of micellar structure, accompanied by differentiation between core and shell regions, the guaiacylglycerol-beta-guaiacyl ether shows only a small, incremental chemical shift progression, with no significant differentiation at the concentrations studied.

[0078] However, the guaiacylglycerol-beta-guaiacyl ether did show tangible evidence of π-π interactions, based on the coalescence of chemical shifts in the aromatic region. This is further evidence that both π-π interactions and hydrogen bonding play important roles in the solubilization behavior of lignin in these micellar systems. Partially decomposed lignin showed the most interesting and unexpected results in this concentration study of Group B model compounds.

Having many possible distributions of S, G and H monolignol units, lignin has a full range of possible electronic and steric effects that can influence the electronic environment of polymer chains, and their corresponding NMR signals, upon incorporation into the micelle. One might expect the solution structure of lignin to be homogeneous, such that all of its possible groups and molecular organizations exist randomly and equally in solution. [0079] It can be seen from FIG. 7B, however, that there is a differentiation of lignin between core and shell which is immediate, and which increases with concentration. Even more striking is that this differentiation of lignin between core and shell regions is opposite all of the small molecule models studied in which π-π interactions and/or bulk reorganizations were observed, in that lignin interactions with core PPO methyls dominate over PEO methylenes in the shell. There is a discernible inflection of both PPO and PEO curves at around 0.14mM lignin, which results in a dramatically greater Δδ with respect to earlier concentrations, leading to the conclusion that there is at least a moderate π-π interaction effect with increasing lignin concentration in the micelle, and possibly some change in morphology of the micelles themselves. However, no clouding was observed in the aqueous sample at the upper concentrations.

[0080] The present embodiments includes seeking to determine the size and morphology of the polymer micelles, and elaboration on any changes in morphology with increasing concentration of lignin. The chemical shift difference between polymer PEO block methylenes and PPO methyls is immediately apparent at low concentrations of solubilized lignin in the micelles, and remains relatively constant (Δδ =~7-8Hz) up to 0.12mM lignin. It has been a longstanding belief that partially decomposed lignin in certain pulp process effluent solutions has some inherent and ordered aggregation due to π-π interactions.

[0081] The present embodiments sheds indicates that at the inflection point (or CGC) of the curve for lignin in FIG. 7B, the difference in Δδ between PEO methylenes and PPO methyls roughly triples, indicating a reordering of the guest model-host polymer system. This is a weaker transition than for Group A model compounds, however, we postulate that the observed chemical shift data represents the net effect of two competing forces, hydrogen bonding and π-π interactions. Here, it must be concluded that both π-π orbital stacking and polar interactions (i.e., H-bonding ability, polarity) influence the nanostructural properties of micelle-guest assemblies.

MICELLAR-CATALYTIC VOLARIZATION OF LIGNIN FOR PRODUCTION OF

VALUABLE CHEMICALS: Reaction Mechanisms in Micelle-Lignin Nanostructure

[0082] The embodiments herein are directed to the addition of block copolymers to the spent black liquor produced form treatment of lignocellulosic materials so as to enable a solubilization and encapsulation of lignin derivatives in a polymer micellar system. This solubilization results in lignin derivatives becoming more concentrated within the micelles than in the surrounding water phase, and this opens possibilities for selective chemistries. Such chemistries allow for obtained micelle-lignin nanostructures having high potential to be employed as highly tunable nanoreactors. In order to cleave beta-aryl ether linkage of lignin trapped in micelle, reaction mechanisms have been formulated for the created micellar system.

[0083] The reaction mechanism comprises a series of selective oxidation reactions a, b, c, d, e as shown in FIG. 9 and also nucleophilic attack/substitution f, g, h, as also shown in FIG. 9, which result in conversion of lignin propanoid alcohols (a) to carbonyl group (b) and concomitant elimination of the beta-aroxy substituent and producing some simpler structures such as diols and guaiacol (h). The chemical reactions are utilized to produce aliphatic carbonyl group (non- phenolic) in lignin structure (b) as a starting point for a nuclephilic substitution (f) with

nucleuphiles, known to exist in alkaline black effluent, leading to cleavage of beta-aryl ether bond in lignin structure.

[0084] Such a method herein produces value-added low molecular weight lignin-based chemicals. In an alkaline medium, oxygen acts as free radical and attacks electron-rich sites on the substrate. Oxygen is reduced in a consecutive series of reactions (d) and the formation these species is favored at pH of 11-11.5. The most crucial problem, as addressed herein, is how to control its selectivity and enhance its selectivity. The objective provided herein is to control the reactivity of oxygen-derived radicals and enhance its selectivity. Also, how to improve the selectivity of the oxygen-based hydroxyl radical reactions and maintain its reactivity at the same time.

[0085] Oxygen produces hydroxyl radicals in alkaline condition (our system) via four-one electron transfer steps (d) in ambient temperature and diffuse some distance and react with lignin aliphatic hydroxyl groups relative to phenolic hydroxyl group in micelle-lignin

nano structure. NIPH, TEMPO, AZADO can be used as cocatalyst (c) (i.e., as effective oxidation catalysts) in the system disclosed herein. They improve the selectivity of the oxidation of lignin aliphatic secondary alcohol and maintain the reactivity at the same time. The stability of the radical in cocatalyst (e.g. TEMPO·) can be indicated by the weakness of O-H bond in the hydrogenated derivative (e.g. TEMPO-H). The produced O-H bond in cocatalyst is about 30% weaker (bond dissociation energy is about 70 kcal/mol in TEMPO-H) than typical -OH bond. Thus,

the regeneration and activation of cocatalysts can be obtained via the presence of hydroxyl radicals (e) produced in the system ($\cdot$OH + catalyst-O-H$\rightarrow$ -¾0 + catalyst-O$\cdot$); A nucleophilic

substitution (f) changes the produced carbonyl group (b) in non-phenolic structures to alkoxide anion (g) through the use of nucleophiles, known to exist in the spent black liquor (i.e., hydroxide ion OH-). A nucleophilic attack on the beta-carbon, through a neighboring groups participation reaction (g), results in an oxirane-type intermediate with concomitant elimination of the beta-aroxy substituent (release of guaiacol). Also, subsequent attack by the nucleophiles break the epoxide- type ring, producing some simpler structures (g, h).

[0086] The process as illustrated in FIG. 9 thus starts with the addition of a block copolymer to the alkaline lignin solution (bulk water). This station results in a formation of micelle-lignin nano structure. Then, the reaction mechanisms start with production of hydroxyl radicals in bulk water, regeneration of cocatalyts such as, for example, NIPH or TEMPO (in palisade layer of micelle or in micelle-water interface) which results in oxidation of lignin aliphatic alcohols (production of carbonyl group in palisade layer of micelle), and then a nucleophilic substitution with nucleophiles from bulk water leading to cleavage of beta-aryl ether linkage (in palisade layer of micelle or micelle- water interface) and producing simpler lignin-based structure along with the protection of aromatic ring of lignin against (destruction/modification) reaction agents (phenolic aromatic part of lignin is incorporated in core of the micelle (hydrophobic region).

[0087] It is to be understood that features described with regard to the various embodiments herein may be mixed and matched in any combination without departing from the spirit and scope of the invention. Although different selected embodiments have been illustrated and described in detail, it is to be appreciated that they are exemplary, and that a variety of substitutions and alterations are possible without departing from the spirit and scope of the present invention.

CLASSIFICATIONS

International Classification	C08L97/02
Cooperative Classification	C08L97/005, C08H6/00

Date	Code	Event	Description
Sep 21, 2016	121	Ep: the epo has been informed by wipo that ep was designated in this application	**Ref document number:** 16744203 **Country of ref document:** EP **Kind code of ref document:** A2