Preparation of Functionalized Lignin Beads from Oak Wood Alkaline Lignin

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ABSTRACT: Alkaline lignin extracted from oak wood cooperage wastes was chemically modified to prepare beads by suspension polymerization on water without the use of organic solvents. These beads were macroporous and swelled in hydrophilic solvents. They were functionalized under microwaves to be used as scavenging agents in winery applications. The beads prepared by this approach have the advantage of being more acceptable by winemakers than synthetic polymer supports previously reported. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

INTRODUCTION

Sulfur dioxide, the most widely used additive in enology,[1] is indispensable for wine conservation thanks to its combination of antioxidant and antimicrobial properties. Sulfur dioxide, either in its free form or as carbonyl bisulfites, presents toxicity; this has led authorities to regulate its acceptable levels in wine. We recently developed a new methodology based on selective liquid–solid extraction to reduce the SO_2 binding power of wines.[2,3] Some of the carbonyl compounds responsible for SO_2 binding phenomena[4] were removed with phenylsulfonylhydrazine grafted on an insoluble support via a heterogeneous reaction to preserve the wine's organoleptic properties.[5] However, the insoluble supports used in these studies were modified with a commercially available sulfonic resin. Contaminant analysis indicated the presence of traces of undesirable compounds in the wine after treatment. Therefore, we decided to adapt the nature of the support to our purpose by synthesizing it from a raw material that was potentially more acceptable in the wine-making process.

The use of oak wood for making barrels is a practice that dates back to the antique Celtic world. Oak was chosen because of its tight-grained wood, which allows the production of leak-proof barrels. Indeed, in ancient times, barrels were multiple-purpose storage containers and were used for both solid and liquid cargo. Only much later did it occur to people that the (prolonged) storage of wine in such oak vessels contributed highly to the taste profile of the final wine. The polyphenolic components present in oak wood are primarily responsible for these taste modifications.[6] It has been well established that the best results are obtained when 100- to 150-year-old French oak trees (*Quercus petraea*) are used.[7] However, the very skilled process conducted from the timber to the final barrel occurs with a high loss (at least 70%) of valuable wood.

The overall composition of oak heartwood, whatever type it is, has similar values (40% cellulose, 20% hemicellulose, 25% lignin, and 10% tanins).[8] Oak wood lignins, like those of other angiosperms, are polymers formed principally by the copolymerization of two phenylpropenoic alcohols: hydroxyl-4-methoxy-3-cinnamic alcohol (guaiacyl structure, coniferyl alcohol) and hydroxyl-4-dimethoxy-3,5-cinnamic alcohol (syringyl structure, synapylic alcohol). Extensive ^{1}H-NMR and ^{13}C-NMR studies conducted on extractable lignin from oak wood suggest a specific structure presenting a syringyl/guaiacyl (S/G) structure (S/G > 1) with a large amount of β-O-4 and β–β linkages.[9]

In this study, we examined the synthesis and functionalization of an insoluble support in bead shape with lignin extracted

from oak wood cooperage wastes as the main raw material for specific applications during the winemaking process.

EXPERIMENTAL

Materials
Propylene oxide, acryloyl chloride, 1-methylimidazole (1-MIM), calcium chloride, ethylene glycol dimethacrylate (EGDMA), trimethylsilylchlorosulfonate, acacia gum, azobisisobutyronitrile (AIBN), and hydrazine hydrate were purchased from Aldrich and were used as supplied. The solvents were synthetic grade.

Chips of oak wood (*Q. petraea*) were collected in a cooperage plant (Tonnellerie Demptos, Bordeaux, France).

Lignin Extraction from Oak Wood Chips extracted lignin (EL)
Wood chips (100 g, size = 0.5–1 cm) were introduced into a 1-L stainless steel autoclave with a solution of concentrated NaOH (800 mL, ∼ 2.5 mol/L). The reactor was heated to 175°C (heating rate = 30°C/min) and maintained at that temperature for 1 h. After natural cooling, the black suspension was filtered off on a Buchner funnel, and the insoluble residue was discarded. The obtained black filtrate, containing mainly the lignin and mineral salts, was acidified to pH 1.5 with concentrated sulfuric acid to precipitate the lignin. The precipitate was dissolved in an aqueous solution of NaOH (5 wt %). The alkaline lignin solution was acidified to pH 3 with concentrated sulfuric acid to precipitate the lignin. The precipitate was filtered on a Buchner funnel *in vacuo*, copiously washed with water, and dried at 60°C until a constant weight was reached. A brown powder was collected (20 g). The phenolic group and total hydroxyl group contents were estimated according to a published procedure[10] to be 2.0 and 3.0 mmol/g, respectively. The same procedures were used for all other hydroxyl content determinations.

Synthesis of the Hydroxypropyl Lignin (HPL)
Alkaline lignin (20 g, 60 mequiv of OH) was dissolved in an NaOH aqueous solution (300 mL, 0.1 mol/L of NaOH). The dark brown solution was then introduced into a 500-mL reactor regulated at 30°C. A condenser cooled at −15°C was connected to the reactor. Propylene oxide (7 g, 0.12 mol) was added to the solution with a syringe pump (addition rate = 1 mL/h) under mechanical stirring. At the end of the addition, the viscous reaction mixture was acidified to pH 2 with sulfuric acid and was then centrifuged at 3000 rpm for 15 min to obtain the HPL precipitate. The resulting product was washed with hexane (3 × 50 mL) and dried *in vacuo* at 60°C until a constant was reached. HPL was collected as a brown powder (27 g). The total hydroxyl group content was estimated to be 3.0 mmol/g, and the total phenolic group content was estimated to be 0.2 mmol/g.

Synthesis of the Acrylated Hydroxypropyl Lignin (AHPL)
HPL (10 g, 30 mequiv of OH), acryloyl chloride (5.9 g, 65 mmol), and 1-MIM (0.2 mL, 0.206 g, 2.5 mmol) in solution in tetrahydrofuran (THF; 50 mL) were placed in a 100-mL flask and heated to 50°C under nitrogen with vigorous stirring.[11] The mixture was left to react overnight to ensure complete conversion. The solution was then poured into water (300 mL) and stirred for 30 min. The mixture was then centrifuged at 3000 rpm for 30 min. The pasty residue obtained was then washed with water (3 × 50 mL) to remove 1-MIM. AHPL was then dried *in vacuo* until a constant weight was reached and collected as a viscous black oil (9.5 g, yield = 81%). The total hydroxyl group content of AHPL was estimated to be 0.3 mmol/g, and the total phenolic group content was estimated to be 0.2 mmol/g. The total acrylic group content was estimated with a quantitative IR method to be 2.9 mmol/g.[12]

Suspension Polymerization [of Lignin Beads (LBs)]
Finely ground calcium chloride (1 g) was added to AHPL (22.5 g), and the viscous mixture was treated with ultrasound for 15 min at room temperature to ensure complete homogenization. A radical initiator (AIBN, 100 mg) and a crosslinking agent (EGDMA; 7 g, 35 mmol) were then added to the solution. A specially designed, three-necked, 1-L, parallel-side flanged suspension reactor equipped with a double-bladed mechanical stirrer was charged with the continuous phase, which was prepared by the mixture of the stabilizing agent (acacia gum, 7 g) with distilled water (500 mL). The AHPL phase was then added to the reactor at 50°C, and the two-phase system was stirred for 24 h. The stirring speed was adjusted to about 300 rpm to obtain beads of satisfactory size. After it was cooled to room temperature, the reaction product was collected on a sieve (80 mesh, 0.18 mm) and washed thoroughly with tap water. Spherical brown beads were collected (27 g, yield = 98%).

Bead Functionalization
Sulfonation. The LBs (3 g) were suspended in a dichloromethane solution (60 mL) of trimethylsilylchlorosulfonate (4.2 mL, 27 mmol). The suspension was gently stirred at room temperature for 24 h. The beads were then filtered, washed copiously with water, and then poured into a sulfuric acid solution (1 mol/L, 20 mL) and left for 48 h. The beads were then filtered, copiously washed with water again, and then dried *in vacuo* at 50°C for 24 h. Elemental analysis (S) was used for loading determination. The SO_3H loading was 1.3 mmol/g.

Microwave Hydrazination. A Start Synth single-mode microwave synthesizer (Milestone, Inc., Rockford, IL) was used. This apparatus allowed us to simultaneously control the temperature, power, and stirring. The sulfonated LBs (3 g, 3.9 mmol of SO_3H) were suspended in a hydrazine hydrate solution (8 mmol, 10 mL) in a closed polytetrafluoroethylene (PTFE) flask. The flask was irradiated at 50°C under 250 W for 30 min under continuous stirring. After they were cooled to room temperature, the beads were filtered and washed with water until they reached pH 5. After they were dried *in vacuo*, elemental analysis (N) was used for loading determination. The SO_3NHNH_2 loading was 0.7 mmol/g.

Solvent Bead Swelling. Solvent swelling of the materials was measured with either dry THF or an ethanol/water mixture (12/88 v/v) adjusted to pH 3.5 with tartaric acid (4 g/L). The dry sulfonated LBs (1 g) were placed in a 25-mL fritted graduated burette, and the dry bead volume (V_0) was estimated. The swelling solution (5 mL) was then added, and the beads were gently stirred with a spatula and allowed to sit at room temperature for 24 h. The swollen bead volume (V_S) was then estimated. The $V_S - V_0$ value represents the swelling level (mL/g).
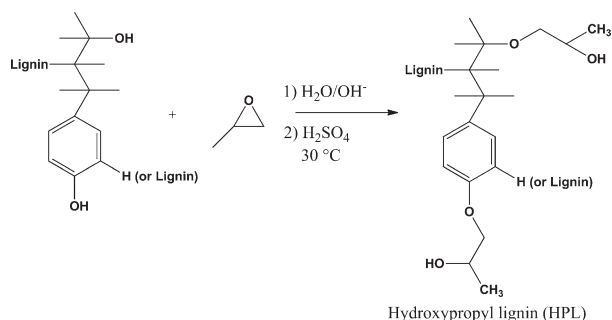
Figure 1. Preparation of HPL.

Figure 2. Acrylation of HPL (AHPL).

Characterization of the Beads

Porosity Determination. The effective porosity and the connection size distribution of the beads were determined by mercury intrusion porosimetry with a Micromeritics Norcross, GA Autopore IV 9500 porosimeter.

Specific Surface Area and Mesoporosity Determination. The specific surface area was determined by N_2 adsorption measurements performed on a Micromeritics ASAP 2010. The collected data were subjected to Brunauer–Emmet–Teller (BET) analysis.[13]

Electron Microscopy Investigations. The morphology of the beads was observed by scanning electron microscopy (SEM) on a Hitachi Tokyo, Japan TM-1000 microscope. Photographs were taken at several different magnifications between 500 and 2000×. The beads were mounted on a carbon tab, which ensured a good conductivity. A thin layer of gold–palladium was sputtered onto the beads before analysis. The average pore diameter was estimated for some samples from the SEM micrographs after image processing with ImageJ freeware (NIH). The mean and standard deviation were determined by manual measurements of the diameters of a population of at least 50 pores. To get a better estimation of the real pore diameter, a statistical correction was introduced by multiplication of the observed average value by a factor $K = 2/(3^{1/2})$.[14]

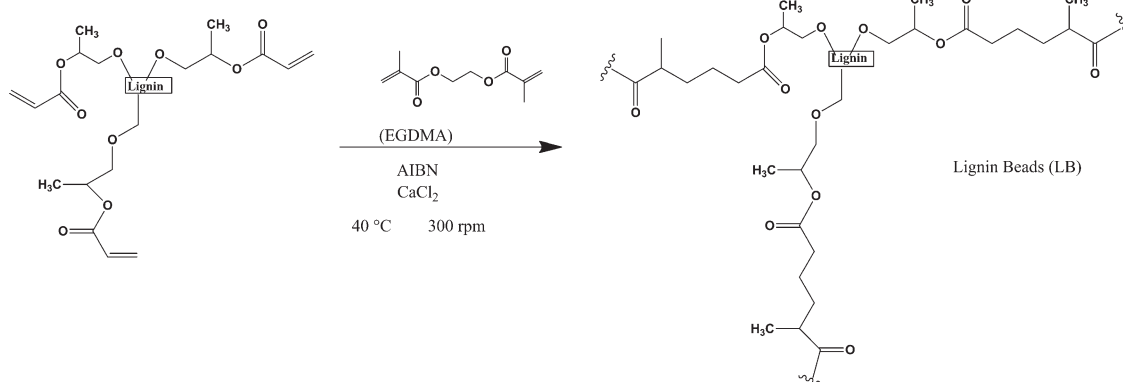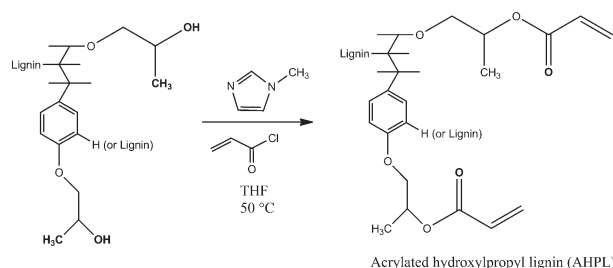**Elemental Analysis.** Elemental analyses were performed by the Service Central d'Analyses (Vernaison, France).

RESULTS AND DISCUSSION

For lignin to be used as starting material in chemistry, it must be removed from the plant. Lignin recovery processes that extract lignin from wood change its chemical and functional group composition and make this material extremely heterogeneous. The lignin used in this work was extracted from oak wood chips with an alkaline process. This treatment was based on the industrial Kraft process with the omission of sodium sulfite for safety reasons.[15] During this treatment, the insoluble network of native lignin covalently linked with hemicellulose, and cellulose was converted into lignin fragments dissolved in the alkaline solution.[16] Precipitation by acidification allowed the recovery of an extracted lignin that was essentially free from polysaccharides.[17] After drying, the obtained powder was only soluble in alkaline media and polar hydrophilic solvents, such as dioxane or dimethylformamide.

In a previous article,[18] we reported a method for preparing LBs from unmodified Kraft lignin. This approach, although it is rather straightforward, presents the drawback of generating nonporous material. Furthermore, the use of a rather large amount of a chlorinated solvent as a suspension medium limits its development. For better sustainability, it would be more desirable to use water as a continuous suspension medium. Lignin extracted from oak wood comes out as a powder. To convert this raw material into beads of satisfactory shape and size with suspension polymerization in water, its good water dispersibility has to be ensured. Numerous chemical modification routes have been proposed to conduct this so-called liquefaction.[19] It can be achieved via etherification with ethylene, propylene, or butylene oxide and gives access to a variety of hydroxyalkyl lignins.[20]

Figure 3. Preparation of the beads by the polymerization of AHPL.
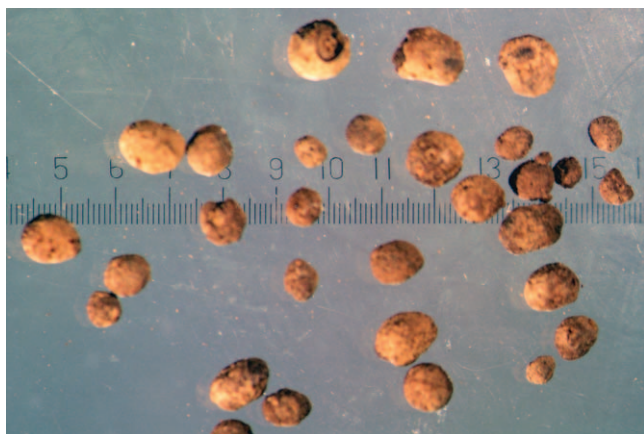
Figure 4. Optical photography of the LBs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

HPL is usually prepared in the dry state under harsh conditions.[21] Here, HPL was prepared by reacting the lignin extracted from oak with propylene oxide in aqueous alkaline media at low temperature (Figure 1).[22] Hydroxyl group content (total and phenolic) determination indicates a high level of chemical modification of the extracted lignin.

The introduction of a vinyl function by acrylation allows the hydroxyalkyl lignin to yield a highly crosslinked, glassy insoluble network by copolymerization with a difunctional comonomer.[23] Therefore, in a second step, dry HPL was reacted with acryloyl chloride in the presence of 1-MIM to give an AHPL (Figure 2). The hydroxyl group content (total and phenolic) and acrylic function determination indicated an almost complete substitution of alcoholic groups by acrylic groups.

In both the HPL and EL IR spectra, bands at 1592, 1509, and about 800 cm^{-1} were attributed to aromatic skeletal vibrations. The band at 1460 cm^{-1} was attributed to aliphatic CH bonds. The broad band around 3410 cm^{-1} was characteristic of the presence of hydroxyl groups. Therefore, it was difficult to clearly prove the presence of hydroxypropyl moieties in HPL from the IR data. The analysis of the AHPL spectrum clearly showed the
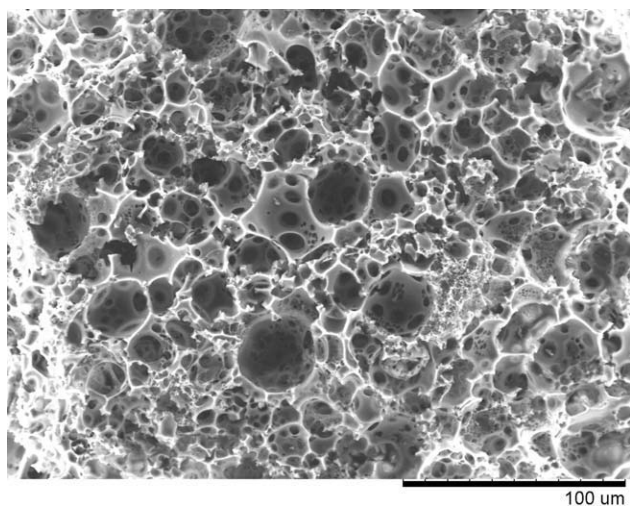
disappearance of the v_{OH} band at 3409 cm^{-1} and the appearance of a broad band at 1721 cm^{-1}, which corresponded to the $v_{C=O}$ vibration of the introduced acrylic function.

The best way to obtain polymer beads of the desired size (100–800 μm) was through suspension polymerization.[24] To achieve this, AHPL had to be suspended as droplets in an aqueous solution containing a stabilizer (Figure 3). The chosen stabilizer was acacia gum, which has already been successfully employed.[25]

To generate macroporosity inside the lignin network, Langer's methodology was used.[26] Therefore, calcium chloride particles were added to the AHPL and were dispersed with ultrasound. These particles, dissolved by the remaining water present in lignin, behaved as a soft template and resulted in the generation of macropores. They were eliminated during the aqueous washing of the final beads. An optical photograph of the obtained beads is shown in Figure 4. An SEM microphotograph of their surface is shown in Figure 5.

Qualitative observation of Figures 4 and 5 clearly indicated that (1) the LBs appeared to be spherical in shape, with a rather narrow diameter dispersion and (2) the morphology of the bead surface was constituted of large macroporous spherical voids (or cells) connected by smaller pores. Quantitative analysis of the morphology of the LBs was performed. Mercury penetrometry allowed us to determine the connection pore size, whereas SEM micrograph analysis gave us indications about the diameters of the voids. The results obtained are reported in Table I. They confirmed the high total porosity of the beads and the presence of voids of large diameter (30 μm) connected by numerous micrometer-sized pores.

LB Functionalization

To graft the required phenylsyulfonylhydrazine scavenger groups, the AHPL beads had to be sulfonated. The usual way to sulfonate polymer beads is with concentrated sulfuric acid or chlorosulfonic acid.[27] In this case, both reagents contributed to the destruction of the lignin network. Therefore, sulfonation was carried out under milder conditions with trimethylsilylchlorosulfonate, according to a reported method.[28] The degree of sulfonation, as determined by titration, was about 1.3 mmol of SO_3H/g.

Solvent Swelling

To be useful, these beads must present a certain swelling in solvents, so their swelling behaviors in an organic solvent and in a

Figure 5. SEM microphotography of an LB surface.

Table I. Influence of the Formulation Parameters on the LB Morphology

Entry	LB
Average bead diameter (μm)	800 ± 100
Porosity (%)[a]	60 ± 5
Average apparent density (g/cm^3)[a]	0.52 ± 0.01
Average skeletal density (g/cm^3)[b]	1.29 ± 0.01
Specific surface area (m^2/g)[c]	96 ± 10
Average macropores diameter (μm)[d]	30 ± 5
Average connection diameter (μm)[a]	2 ± 0.5

[a]Estimated by mercury penetrometry, [b]Estimated by helium pycnometry, [c]Estimated by nitrogen adsorption with the BET model, [d]Estimated from the image analysis of the SEM micrographs.
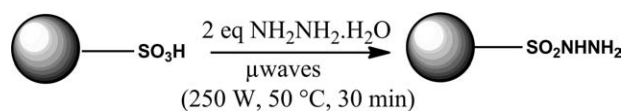
Figure 6. Preparation of the sulfonylhydrazine-grafted beads.

hydroalcoholic solution were estimated. The swelling levels of the sulfonated beads in THF and EtOH/water (12% v/v) were estimated to be 0.4 and 1.2 mL/g, respectively. These values appeared to be situated between those of gel-type, 2% cross-linked beads (3.9 mL/g) and macroreticular, 8% crosslinked polystyrene–divinylbenzene beads (0.7 mL/g).

Polymer-bound sulfonylhydrazine is an efficient scavenging agent for the removal of carbonyl compounds in organic media[26] and for protein extraction in aqueous media.[29]

Polymer-supported phenylsulfonylhydrazine is usually obtained from sulfonic resins by chloration with thionyl chloride, followed by hydrazine hydrate treatment.[30] However, this route presents the drawback of the necessity of using toxic reagents.

Microwave heating represents a major breakthrough in synthetic chemistry, as it can be considerably more efficient than conventional heating methods.[31]

Microwaves have been recently reported to efficiently convert several carboxylic acids into their hydrazide derivatives $(R—CONHNH_2)$.[32] Therefore, the efficiency of microwave irradiation in performing the direct conversion of sulfonic acid into sulfonylhydrazine (Figure 6) was evaluated. The reaction was performed smoothly with only a slight excess of hydrazine hydrate as a reagent in an aqueous solution. The loading of the LBs in (SO_2NHNH_2) was about 0.7 mmol/g.

CONCLUSIONS

Porous beads were prepared with alkaline lignin extracted from oak wood cooperage wastes. These beads contained more than 80% lignin. They were prepared after a rather straightforward chemical modification with an aqueous suspension polymerization medium. High yields and narrow size distributions were obtained once the reaction conditions were optimized. These beads were macroporous because of the addition of hydrated calcium chloride particles as a soft template in the dispersed organic phase. Sulfonation led to cationic ion-exchange sulfonyl resins that reacted efficiently with aqueous hydrazine hydrate under microwave conditions to give polymer-supported sulfonylhydrazine moieties. This solid-phase scavenger could be used for the selective extraction of carbonyl compounds in wines. This application is currently under development in our group, and the results will be reported in due course.

ACKNOWLEDGMENTS

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