1 Solubilization of lignin in copolymer micelles in aqueous solution

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5 **ABSTRACT:** The solubilization of lignin in self-aggregating triblock amphiphilic poly(ethylene
6 oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) in aqueous solution has
7 been studied utilizing liquid-state proton nuclear magnetic resonance spectroscopy (¹H NMR)
8 and diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY NMR). Our hypothesis
9 for copolymer micelle incorporation of lignaceous species includes a "hydrophilic-aromatic
10 interaction model", in which lignin derivatives with amphiphilic characteristics, known to be
11 abundant in the effluent from pulp mill and pretreatment of various lignocellulosic materials,
12 have an affinity for these micelles, and tend to form relatively organized structures within them.
13 Here we show how the chemical/structural features of lignin units and small molecule lignin
14 models determine their solubilization behavior in the micelles. It was found that a buildup of
15 concentration series of lignin and lignin-based model compounds guaiacol, eugenol, phenol,
16 guaiacylglycerol-beta-guaiacyl ether, ferulic acid in Pluronic[®] F68-deuterium oxide solution
17 results in significant upfield chemical shifts of PEO-(CH₂-CH₂) and PPO-(CH₃) proton
18 resonances, and at a critical guest concentration (CGC), dramatic upfield shifts due to gross
19 structural transitions in the micelles. We present evidence that copolymer micelle-lignin
20 interactions depend on both chemical functional group characteristics of solute (i.e., polarity, H-
21 bonding ability) and π - π interactions between aromatic/conjugated groups. Our results
22 demonstrate how the loci of incorporated solute in the block copolymer micelles are affected by
23 these features.

Keywords: Lignin; Block copolymer; Micelle; Solubilization

1. Introduction

Block copolymers comprise more than one type of block. Usually, the properties of the blocks within the copolymer are quite different from one another and this results in spontaneous self assembly in aqueous solution [1]. One of the key determinants of block copolymers, hydrophilic-lipophilic balance (HLB), predicts the formation of aggregates at the critical micelle concentration (CMC) in aqueous media. Such aggregates or micelles may have spherical, cylindrical, rod-shaped or lamellar shapes [2]. 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 [3, 4].

Solubilization phenomena in aqueous and nonaqueous solutions of conventional low molecular weight surfactants have been extensively investigated theoretically [1, 5] and experimentally [6, 7]. However, block copolymers have shown remarkable potential compared to conventional low molecular weight surfactants because of their micellization ability in a variety of solvents [8]. Block copolymers are utilized in diverse applications, such as delivery of drugs, as scavengers for pollutants, as chemical extraction aids in separation processes, and for micellar catalysis [9-11]. Block copolymer architecture (ABA, BAB, ABC, AB, BA), hydrophobic-lipophilic balance (HLB), and discrete block molecular weights can significantly influence their solubilization capacity [12]. Many low molecular weight solubilizates (guest/solute), including aromatic solvents benzene, toluene, naphthalene, pyrene, and anthracene, have been investigated in block copolymers [7, 13]. 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 and the block copolymer.

Hydrophobic and hydrophilic interactions between the solubilizate and the blocks that form the core and shell of micelles control the scope of solubilization. For instance, the solubilization of gallates in polymeric micelles has been studied by Heins and coworkers [14]. Using electron spin resonance spectroscopy (ESR) and nuclear magnetic resonance spectroscopy (NMR), the authors reported that the locations of antioxidant gallates in micelles are dependent on their hydrophobicity. Also, aromatic molecules were found to be solubilized preferentially compared to aliphatic hydrocarbons in A-B-A type block copolymers [5]. Furthermore, experimental results for hydrocarbon solutes in polyethylene oxide (PEO)-polypropylene oxide (PPO) block copolymers demonstrated large solubilization capacities and high selectivity for aromatic over aliphatic solutes in block copolymer micelles, compared to conventional low molecular weight surfactant micelles [13]. The molecular volume of the solutes can also influence the amount of solubilization in micelles. It has been observed that solutes with larger size are solubilized in smaller number [15]. However, given identical molecular volumes, the polarity of the compounds controls the selectivity of solubilization [7, 16]. It was noted that the solubility of benzene in polar and non-polar blocks of PEO/PPO was significantly larger compared to n-hexane in the entire composition of the block copolymer [7]. Lignin, with its combination of aromatic and aliphatic hydrocarbons, having polar functional groups, aliphatic bridges and a variety of oxygenated moieties, can show a unique solubilization pattern in aqueous dispersions of block copolymer micelles. Lignin is often described as a random polymer of three monolignols, *p*-coumaryl (H), coniferyl (guaiacyl, G) and sinapyl (S) alcohols [17].

In this study, lignin is obtained from black effluent produced by the sequential ozone and soaking aqueous ammonia pretreatment of wheat straw, and was previously characterized as partially-depolymerized fragments of H-G-S with S/G ratio of 0.35 and average molecular weight of 750 (g/mol) [18]. Lignin is partially decomposed during pretreatment (Scheme1.a), and removed from the biomass composition as smaller, soluble fragments through the use of a water washing process which produces a black effluent (Scheme1.b1) [18]. To date, 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 and biorefinery. Increasingly, however, lignin is being considered as a renewable source of value-added aromatic compounds and fuels [19, 20]. Significant economic barriers to use of this 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 (Scheme1.b2), we propose to use the solubilization ability of a commercially available non-ionic, amphiphilic tri-block copolymer, polyethylene oxide- polypropylene oxide- polyethylene oxide (under the trade name of Pluronic[®] F-68) (Scheme1.c1) toward lignin derivatives to concentrate lignin in the aqueous environment of pulping and pretreatment effluents, greatly reducing costs, and opening possibilities for selective chemistries [21]. Also, five phenolic aromatic model compounds, phenol, eugenol, guaiacol, ferulic acid and guaiacylglycerol-*beta*-guaiacyl ether (dimer) are employed in order to assist us in studying the solubilization behavior of lignin in micellar system. The uses of block copolymers (i.e., Pluronic[®] F-68) in solubilizing aromatic and aliphatic molecules in various applications were reported previously; however, the solubilization of lignin in the block copolymer has not yet been studied.

In the present work, we have studied the solubilization behaviors and interactions of lignin and model compounds with Pluronic[®] F-68 in aqueous ammonia solutions (black effluent) using nuclear magnetic resonance spectroscopy (NMR) and diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY-NMR).

Place of Scheme 1

Nuclear magnetic resonance spectroscopy (NMR) has been used extensively to characterize the structure, reactions and degradation processes of woody biomass [22]. Most lignin analyses by NMR are done by dissolving lignin from a particular source, treated or untreated, in deuterated dimethyl sulfoxide (d₆-DMSO) and performing a suite of experiments, including 1D ¹H and ¹³C NMR experiments, 2-D correlation experiments, particularly HMQC, HSQC and HMBC, and/or by solid state NMR of extracted and dried lignin samples [23]. 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. Much effort has been made to refine NMR methods of structural analysis for lignins as a class of compounds because of the great interest in these natural substances by plant biochemists, agronomists, materials scientists, lignin producers, end users for various lignin-derived products, and the sustainable biofuels industry [24]. We have used these methods extensively to investigate lignin structure and pathways of decomposition and reformation for many avenues of lignin modification, including processes of nature [25], e.g. degradation of wheat straw by *Phanerochaete chrysosporium* [26] and hardwood lignin by the Western Poplar Clearwing Borer, *Paranthrene robiniae* (Hy. Edwards), [27] as well as lignin digestion in every digestive organ of termites, [28, 29] and more recently, chemical processes, such as heterogeneous catalytic

hydrodeoxygenation of biomass-derived lignin to aromatic hydrocarbons, [30] aqueous ammonia pretreatment of wheat straw, [31] and physical processes including pyrolysis of corn stover [32] and water-only flow-through pretreatment of Miscanthus [33].

The knowledge obtained on the solubilization of the lignin in the copolymer micelle will aid to establish processes to convert the lignin into value-added products such as the antioxidant guaiacol, aroma and flavor compound eugenol, or to aromatic hydrocarbons and cycloaliphatics for jet-fuel and provide economics benefit for biorefinery.

2. Materials and methods

2.1. Materials

PEO-PPO-PEO block copolymer (Pluronic[®] F-68) was purchased from Sigma-Aldrich (Scheme1-c1). 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 (dimer) (>97%) were purchased from Sigma-Aldrich (Milwaukee, USA) and TCI America (Portland Oregon USA), respectively (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 (D₂O, 99.9%) from Cambridge Isotope Laboratories, Inc.

2.2. Lignin preparation procedure

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. The pretreatment process has been described in our previous studies [18, 31]. 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 disclosed in our previous study [18] 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 (Scheme1.b1, b2).

2.3. Critical micelle concentration (CMC) evaluation by DOSY-NMR

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 D₂O 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. This leads to two dimensions; the first dimension ($F2$) accounts for the conventional chemical shift and the second one ($F1$) for diffusion coefficient (D) [34]. DOSY NMR measurements were performed on a Varian VNMRS 600 (liquid) NMR spectrometer operating at 600 MHz, equipped with z-axis pulsed field gradients. A Varian pulse sequence DgcsteSL_cc (gradient compensated stimulated echo with spin lock and convection compensation) was employed, incorporating both convection compensation and eddy current

compensation. Samples were temperature equilibrated at 25°C for at least 20 minutes prior to acquisition. An array of 15 gradient power level settings was used with a fixed gradient length of 5ms and diffusion delay of 50ms. Sweep width, acquisition time and PRESAT parameters were the same as for the 1D experiments, except that a purge cycle for water suppression was excluded to avoid RF heating of the sample, and the relaxation delay was decreased to 5s. 8192 complex data points were collected. Processing of the data involved application of line broadening (2Hz) prior to Fourier transform and baseline correction, followed by a multi-component fit (number of components = 4-6) of the arrayed frequency spectra to a modified Stejskal-Tanner equation (Eq. 1) employing a polynomial correction factor for non-uniform gradients.

$$S(G) = S(0) \exp \left[-\sum_{n=1}^5 \eta_n G^{2n} \right] \quad (1)$$

Where, $\eta = D\gamma^2\delta^2 \Delta$ and G is the nominal gradient amplitude.

2.4. Solubilization study by liquid-state 1H NMR

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 solute 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 μ l 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.

^1H NMR spectra were obtained at 25°C in D_2O (Isotech, 99.8%D) solution using a Varian/Agilent 600MHz NMR spectrometer equipped with a DD2 console and One Probe multinuclear probe and z-axis pulsed field gradients. Water suppression (PRESAT/purge, 2-step) was used due to residual water arising from use of aqueous ammonium hydroxide to adjust pH to 11.4. All spectra were taken using a calibrated 90 degree pulse and relaxation delay of 15 seconds. A minimum of 16 scans were required at the lowest concentrations of model compounds, and up to 128 scans were collected in some cases to reach acceptable signal-to-noise ratios. Throughout the study a sweep width of 6313.1Hz was used, acquisition time 1.3 seconds, and 16384 data points. ^{13}C -decoupling was not employed. Line broadening of 1.5Hz was used prior to Fourier transform of each data set. All resonances were referenced to the residual water peak at 4.65 ppm. Chemical shifts resulting from interactions of the A-B-A-block-co-polymer with model compounds and lignin were measured in hertz relative to the first concentration data point. In most cases, a blank polymer spectrum (no added model compound) was not recorded.

3. Result and discussion

Although our end goal is to show that hydrophilic and aromatic interactions between lignin and block copolymer can form a type of micelle structure in which diverse reaction chemistry, some previously unavailable in aqueous solution, can be performed efficiently on lignin to produce value-added products, we decided that it was of paramount importance to first study the formation of micelles in our proposed system, as well as their ability to solubilize guest molecules, including partially decomposed lignin from process streams, and to elucidate the

interactions that occur between host micelles and guest model compounds representing lignin structural features, and lignin itself.

3.1. Critical micelle concentration (CMC) determination

Amphiphilic molecules spontaneously self-assemble, at or above a critical concentration in aqueous solutions, to form aggregates of various morphologies [35, 36]. 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 [37]. Techniques which monitor formation of micelles, rather than changes in aggregation number, are preferable for the CMC determination of block copolymer micelles [38]. Many techniques have been used to determine CMC values, including fluorescence [39], light scattering [40, 41], osmometry [42], viscosity [43] and DOSY NMR [44, 45]. In this study, the CMC of the PEO-PPO-PEO block copolymer (Pluronic[®] F68) solution in deuterium oxide was estimated 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 critical micelle concentration of this polymer in aqueous solution is not altered at high pH.

A further study of the effect of added model compounds on the critical micelle concentration of the block copolymer 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 Figure 1, the CMC was not significantly altered by addition of selected guest model compounds at a guest concentration of 0.04mM. At a three-fold higher

loading of guests (0.12mM), however, the diffusion coefficient of the Pluronic F-68 PEO block generally decreased with the addition of these model compounds, consistent with additional loading of guests and/or reordering of the micelle structure. Based on the above results, a concentration of 0.04 mM Pluronic[®] F-68 was used throughout the solubilization study.

Place of Fig.1.

3.2. Solubilization of solute in micellar aqueous solution: Effect of π - π aromatic stacking and hydrophilic interactions on the micelle-lignin structure

NMR studies of solubilization of model phenolic compounds and lignin in PEO-PPO-PEO block copolymer micelles revealed that all the models tested in this study were able to solubilize into these micelles, presumably due to the hydrophobic interaction between the core of the micelles and aromatic rings of the solute. As seen in Fig. 2 (a-b), 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-CH₂-CH₂ resonances, and for methyl resonances of the B-block (micellar core, PPO-CH₃) of Pluronic[®] F-68 block copolymer as a function of concentration of the tested model compounds, similar to the method described by Kim, Im and Oh for sodium dodecylsulfate micelles [46].

Place of Figs.2. (a&b)

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

As can be seen in Fig. 2(a), at low model concentrations there is a gradual upfield shift in the PEO-CH₂-CH₂ resonances with increasing concentration of model, which is mirrored by a chemical shift change in the PPO-CH₃ 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-CH₂-CH₂ resonances and 195.2Hz for PPO-CH₃ resonances of the polymer; for phenol 233.4Hz and 150Hz, respectively; and for guaiacol 273.3Hz and 173.6Hz, respectively. 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. 3, photos). These emulsions remain stable in solution for weeks, and do not further aggregate or release their payloads. The upper limit of micellar loading for each model compound has not yet been determined. The graphs in Fig. 2(a) clearly show that above the CGC, the chemical shift differences ($\Delta\delta$) of polymer CH₃ resonances versus CH₂-CH₂ resonances differ by as much as 100Hz, indicating a differential effect of the guest on 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.

Further insight into the morphological transitions of micelles incorporating aromatic solute can be gleaned from the literature. 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 [38]. It has been stated that dehydration of the PEO shell causes a type of shape change in micelles [48, 49]. Kadam [47] described the effect of added salt on Tetronic T904 aggregation behavior in aqueous solution. The authors reported that the presence of salt dehydrates the PEO shell, and consequently the spherical shape of the micelles is changed to prolate ellipsoidal. The influence of salicylic acid on the aggregation characteristics of Pluronic[®] P85 has been studied in the aqueous medium by Parekh and coworkers [48]. The authors stated that 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. Dey and coworkers [49] 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-1-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-CH₂-CH₂ and PPO-CH₃ regions of the NMR spectrum (as well as in the aromatic signals for the solute) were accompanied with clouding of the samples (Fig. 3, photos). It was found that the phenolic model compounds, phenol, guaiacol and eugenol predominantly affected the palisade layer of the micelles, and PEO-CH₂-CH₂ resonances shifted upfield significantly (Fig. 2a). 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 solute 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. 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 (~ 0.05 mM), 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.

3.2.2. Solubilization of model phenolic compounds ferulic acid, guaiacylglycerol-beta-guaiacyl ether, and partially decomposed lignin (Group B) in PEO-PPO-PEO block copolymer micelles

Patterns of solubilization behavior for ferulic acid, lignin, and guaiacylglycerol-beta-guaiacyl ether in the micellar system are shown in Fig. 2(b). 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-CH₂-CH₂ resonances and 46.9Hz for PPO-CH₃; for lignin, 35.5Hz and 13.82Hz for PPO-CH₃ and PEO-CH₂-CH₂ signals, respectively; and for guaiacylglycerol-beta-guaiacyl ether were only 6.94Hz for both PEO-CH₂-CH₂ and PPO-CH₃ 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. 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-CH₂-CH₂ vs. PPO-CH₃ for eugenol. 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. Thus, we conclude 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.

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 [50]. It has also been shown that the water solubility of

polymer aggregates increases with the presence of polar substituents on solute, and this helps the compound dissolve not only in the most hydrophobic part of the micelle but also in the less hydrophobic palisade region [51]. The same observation was reported by Tehrani-Bagha and Holmberg [52] 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 [3, 4]. This highly ordered solubilization results in selective effects [21], which lend themselves well to micellar catalysis and acceleration of the rate of reactions [53, 54], a primary goal of our research group.

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 $\Delta\delta$ 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. 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. 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. It can be seen from Fig. 2(b), 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 $\Delta\delta$ 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. We are actively seeking to determine the size and morphology of the polymer

micelles, and further elaborate 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 ($\Delta\delta \approx 7\text{-}8\text{Hz}$) 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 $\pi\text{-}\pi$ interactions. Aggregation of solubilized lignin was first observed by Benko in 1964 [55]. Two dominant views of the driving force behind lignin aggregation have been proposed. Lindstrom [56] described the role of hydrogen bonding between carboxylic acid groups of lignin and hydroxyls and other oxygen functions in lignin structures, while Sarkanen and Glasser [57, 58] proposed that $\pi\text{-}\pi$ interactions between aromatic rings in lignin were responsible for the aggregation phenomenon, and showed that acetylation of soluble lignin had no effect on aggregation, thus diminishing the role of hydrogen bonding interactions. The inherent ordering of lignin in solution due to “ π -stacking” has recently been more thoroughly studied by Deng [59] who demonstrated that the $\pi\text{-}\pi$ interactions could be disrupted by formation of charge-transfer complexes between iodine and the stacked aromatic rings, which resulted in dis-aggregation of lignin aggregates.

Place of Fig.3.

The current study sheds some light on that argument. At the inflection point (or CGC) of the curve for lignin in Fig. 2(b) the difference in $\Delta\delta$ 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, hydrophilic and π - π interactions.

4. Conclusion

In this work, ^1H NMR diffusion ordered spectroscopy (DOSY) was used to confirm the critical micelle concentration for a selected, commercially available ABA block copolymer (trade name Pluronic[®] F-68) at neutral and high pH. Results show that the incorporation of guest molecules did not disrupt the integrity of these micelles. Using ^1H NMR spectroscopy, we found that increasing the concentration of guaiacol, eugenol, or phenol (Group A model compounds) resulted in a type of macroscopic restructuring of the micelle. A dramatic upfield chemical shift of PEO-(CH₂-CH₂)- and PPO-(CH₃) proton resonances occurred at a critical guest concentration (CGC), accompanied by gross structural transitions in the micelle, clouding of the solution, and formation of highly stable macroscopic emulsions. Although lignin and the model compounds ferulic acid and guaiacylglycerol- β -guaiacyl ether (Group B model compounds) also showed concentration-dependent chemical shifts, large structural transitions seen in the Group A models did not produce visible changes in micellar solutions of Group B compounds. Nonetheless, liquid state ^1H NMR revealed complex behavior for these model-polymer interactions driven by both π - π interactions and hydrogen bonding.

Given these results, we infer that two primary driving forces govern the solubilization behavior and structural properties of micelle-solute assemblies: (1) the interaction of oxygenated functional groups of lignin, especially in the propanoid chain, with polyoxyethylene A-blocks of the copolymer, and (2) π - π interactions between lignin (or model) aromatic rings. We also infer that the hydration/dehydration of the palisade layer likely plays a role in the structural transitions

of micelle-solute structures, and is controlled by the relative levels of polar, hydrogen bonding substituents in a particular guest. Changes in the microscopic structure of the palisade layer due to guest incorporation are probably balanced by changes in hydration of the oxyethylene chains in the palisade region of the micelle, which can easily be hydrated or dehydrated in response to the influence of a particular guest or solute. We conclude that the overall effect for partially decomposed lignin from process effluent streams is solubilization of lignin with a preferred orientation in the micelle, guided by these distinct influences.

Acknowledgment

This material is based upon work supported by the National Science Foundation under Grant No. 1231085 and Washington State University. The authors would like to acknowledge the Center for NMR spectroscopy at WSU. The WSU NMR Center equipment was supported by NIH grants RR0631401 and RR12948, NSF grants CHE-9115282 and DBI-9604689, the Murdock Charitable Trust, and a generous donation by Don and Marianna Matteson. Also, the authors would like to acknowledge Jonathan Lomber, Analytical Lab Manager, of the Department of Biological Systems Engineering, Washington State University.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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Fig. 1.

Fig. 2. (a)

Fig. 2. (b)

Fig. 3.

Solubilization of lignin in copolymer micelles in aqueous solution

Figure caption

Scheme1. Process flowchart

Fig. 1. 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, guaiacylglycerol-beta-guaiacyl ether (dimer), and ferulic acid guests (each 0.04mM) at the CMC. The final data set on the right shows only the effect of addition of guaiacol, dimer, and ferulic acid (each 0.12mM) to the polymer solution at its CMC (0.04mM) at pH 11.4.

Fig. 2. (a) Graph of chemical shift changes ($\Delta\delta/\text{Hz}$) for PEO-(CH₂-CH₂) and PO-(CH₃) 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.

Fig. 2. (b) Graph of chemical shift changes ($\Delta\delta$) for PEO-(CH₂-CH₂) and PPO-(CH₃) 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.

Fig. 3. ¹H 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-D₂O solution with 0.04 mM concentration and pH 11.4 (See the supplementary data).