Size-Exclusion Chromatographic Study of Humic-Like Substances from Oxidized Lignin

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1. INTRODUCTION

Biorefineries will receive and process massive amounts of lignin. For this reason, how lignin can be best used to support the economic health of the biorefinery must be defined. Different strategies for obtaining lignin-based value added products have been recently reviewed (1). Derivatization of lignin macromolecules have been proposed as one of the most promising approaches to obtain new products. Given that lignin is a cross-linked natural polymer composed of phenylpropanoid units (2), one of its promising transformations might be oxidation to humic-like substances (HLS). Humic substances are known to possess substantial biological activity (3). Hence, the HLS produced by lignin oxidation can start a new value chain of the lignin-based bioproducts. The important issue in establishing new products is developing quality control techniques. Size-exclusion chromatography (SEC) is a powerful technique traditionally used for quality control of HS (3-5). This technique was also applied to analyse native lignin (6).

The purpose of this study was to evaluate a role of non-exclusion effects in SEC analysis of the HLS obtained from lignin oxidation upon applying routine conditions used in HS analysis and to elaborate optimal conditions minimizing non-exclusion effects and maximizing column recovery.

2. MATERIALS AND METHODS

Humic and humic-like materials: Six samples of oxidized lignin were received from "Nobel" ltd (St. Petersburg, Russia). Two natural HS – humic and fulvic acids (HA and FA) isolated from leonardite and the IHSS standard fulvic acid (Suwannee River SRFA, USA) correspondingly – were used as comparison samples.

SEC: Liquid chromatographic system consisted of a solvent pump, a packed column and a UV-vis detector with variable wavelength was used as described (5). The UV-absorbance was measured at 254 nm. The SEC column was 15x250 mm packed with Toyopearl HW-55S ("Toso-Haas", Japan). Phosphate buffer (PB) with different pH and ionic strength was used as a mobile phase at a flow rate of 1 ml/min. The following phosphate buffer pH and concentration values were applied: 0,03M and pH 6,8 (the

standard conditions for HS analysis); 0,01M and pH 6,8; 0,03M and pH 8,0; 0,03M and pH 8,0 + 10% THF (vol.).

The column was calibrated using sodium polystyrenesulfonates (PSS): 4,48, 14,00, 20,70, 45,10 and 80,84 kDa ("Polymer Standard Service", Germany). Blue dextran (2000 kDa) served as a void volume probe (V_0), acetone – as a permeation volume probe (V_p).

RESULTS AND DISCUSSIONS

Given hydrophobic character of the HLS samples, the non-exclusion sorption effects were expected. To minimize these effects, three different modifications of the standard eluent were undertaken: a decrease in ionic strength, an increase in pH, and addition of hydrophobic solvent (THF). Typical chromatograms obtained are shown in Figure 1.
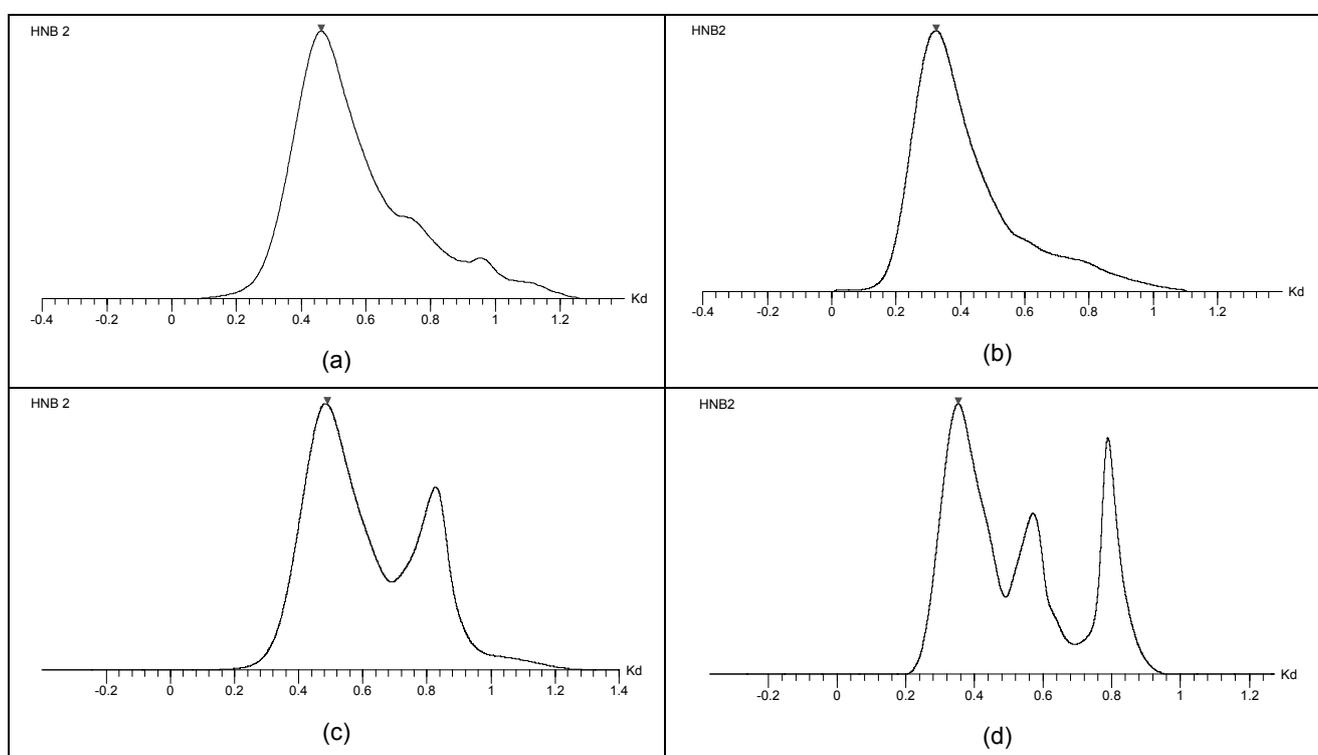

Figure 1. Typical chromatograms of HLS-2 studied: a) 0,03M PB, pH 6,8; b) 0,01M PB, pH 6,8; c) 0,03M PB, pH 8,0; d) 0,03M PB, pH 8,0 + 10% (vol.) THF.

The appearance of second peak at pH 8 (Fig. 1 c, d) may be caused by high HPO_4^{2-} ions concentration. Due to repelling forces of the column, these ions form the additional concentration front that carries along a portion of HLS. Appearance of the third peak in case of buffer modified with THF (Fig. 1 d) could be caused by the portion of HLS carried along with the organic solvent. As it can be seen from Figure 1, both a reduction in ionic strength and addition of organic solvent bring about lower elution volumes of HLS. In case of reduction in ionic strength (Fig. 1 b), this can be caused by an increase in electrostatic

repulsion that results in shorter discharge time of HLS through the column. In the case of PB modification with THF (Fig. 1 d), the observed effect can be caused by a decrease in sorption. The molecular weight characteristics and column recovery values of the HLS and HS analyzed are shown in Table 1.

Table 1. The MW characteristics and column recovery values of the HLS and HS studied

Sample	Mpeak, kDa	Mn, kDa	Mw, kDa	Mz, kDa	**Mw/Mn**	Recovery, %
0,03M PB pH 6,8						
HLS1	3.6	0.6	3.2	5.9	5.5	12
HLS2	3.7	1.1	3.5	6.4	3.3	9
HLS3	3.4	0.7	2.9	5.3	4.0	10
HLS4	4.0	1.6	3.9	6.1	2.4	24
HLS5	4.5	1.3	4.0	6.6	3.1	9
HLS6	4.5	1.2	4.1	7.0	3.3	12
Coal HA	8.1	2.1	10.8	30.7	5.3	68
River FA	6.2	3.4	6.6	9.1	1.9	80
0,01M PB pH 6,8						
HLS1	17.3	32.1	15.7	2.6	6.0	12
HLS2	16.4	24.4	13.2	1.5	8.9	12
HLS3	16.7	23.6	13.2	1.7	7.7	14
HLS4	17.9	29.5	15.3	1.6	9.6	29
HLS5	17.1	26.1	13.7	1.3	10.5	15
HLS6	17.7	35.7	16.4	2.4	6.9	14
Coal HA	22.9	50.1	25.2	4.2	6.0	38
River FA	20.7	28.3	20.1	7.8	2.6	83
0,03M PB pH 8,0						
HLS1	5.5	1.4	4.8	9.1	3.3	21
HLS2	4.6	1.0	4.1	7.2	4.3	20
HLS3	4.5	1.0	3.8	6.3	3.9	18
HLS4	4.6	1.3	4.1	6.5	3.3	40
HLS5	5.1	1.2	4.7	8.2	3.8	21
HLS6	5.4	1.7	4.9	8.4	2.9	23
Coal HA	8.7	3.8	8.1	11.5	2.2	66
River FA	8.2	5.6	8.8	11.8	1.6	100
0,03M PB pH 8,0 + 10% (vol.) THF						
HLS1	6.1	0.7	4.6	8.4	6.9	24
HLS2	6.1	0.8	4.8	8.0	6.0	25
HLS3	6.6	1.0	5.3	9.4	5.3	29
HLS4	6.8	1.0	5.7	9.6	5.8	46
HLS5	6.8	0.6	5.6	10.4	9.0	39
HLS6	6.7	0.8	5.2	9.4	6.2	24
Coal HA	8.6	2.5	7.6	11.0	3.0	81
River FA	9.1	6.1	9.5	12.4	1.6	100

The molecular weights were calculated on the basis of the first major peak observed on the obtained chromatograms. It can be seen that sorption effects (recovery values) decreased with an increase in pH and upon adding THF. At the same time, the measured increase in molecular weights could be indicative of the fact that relatively high molecular weight fractions of the samples were preferentially sorbed on the gel under the standard elution conditions. Electrostatic repulsion in the case of the reduced ionic strength leads to the observed increase in molecular weights.

4. CONCLUSIONS

The decrease in non exclusion effects (sorption on column gel) was achieved under conditions of using phosphate buffer with higher pH (8.0) and its modification with THF.

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