Compension of Non-Exclusion Effects During Gel-Permeation Fractionation of Humic Substances

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1. INTRODUCTION

Size-exclusion chromatography (SEC) is a powerful technique for determination of the molecular weight (MW) distribution of humic substances (HS). However, SEC of HS in aqueous phase is likely to give rise to artifacts. These arise from non-size exclusion effects, which include "ionic exclusion" and "specific adsorption". Ionic exclusion is a non-size exclusion effect arising from repulsive interactions between the charged analyte and the partially charged gel matrix. Specific adsorption occurs as a results of ion exchange adsorption and of hydrophobic or hydrogen bondings that take place between the anlyte and gel-matrix. To compensate for ionic exclusion, modification of eluent by changing of ionic strength is needed. To compensate for specific absorption modification of eluent by addition of organic solvents is needed. The goal of this research was to define fractionation conditions providing for compensation of non-size exclusion effects upon SEC-analysis of HS.

2. MATERIALS AND METHODS

Humic materials used were humic and fulvic acids isolated from soil, peat, and coal. Properties of humic materials are given in the Table 1.

Solutions of humic materials were prepared by dissolving solid samples in 0.1M NaOH and diluting with phosphate buffer to concentration of 50 mg/l. SEC analysis was performed under conditions similar to described by (1). SEC system (Abimed, Gilson, France) consisted of HPLC pump, autosampler, glass

column, and was equipped with UV detector. The column 25 mm × 20 cm packed with Toyopearl TSK HW-55S gel (Tosoh Bioscience, Japan) was used for separation. The phosphate buffer (pH 6.8) with different ionic strength and with addition of organic solvents was used as a mobile phase. The flow rate was set at 1 ml min^{-1}. The absorbance of eluate was detected at 254 nm. The void volume and total permeation volume of the column were determined using Blue Dextran 2000000 and acetone, respectively. Sodium salts of polystyrenesulfonic acids of molecular weight of 2.29, 4.48, 14.00, 20.70, 45.10, and 80.84 kDa (Polymer Standard Service, Germany) were used as markers for molecular weight calculations. Model low molecular weight compounds used were pyromellitic, phthalic, homophthalic, syringic, benzoic, 4-OH benzoic, phenylacetic acids. Data obtained were treated as described in (2).

Table 1: Properties of humic materials used in this study.

Sample	O/C	Carboxylic acidity, mmol/g	Total acidity, mmol/g	C_{Ar}, % C
CHA-Pow	0.38	2.9±0.1	5.8±0.5	46.7
CHA-GL-02	0.44	3.9±0.2	6.7±0.1	44.4
CFA-GL-02	0.68	6.3±0.1	9.3±0.1	30.6
SHA-Pw-96	0.46	3.0±0.3	3.3±0.2	32.0
SFA-Pw-96	0.6	4.2±0.1	9.6±0.1	22.0
SHA-Cm-98	0.39	5.8±0.4	6.7±0.3	51.5

3. RESULTS AND DISCUSSION

To evaluate the role of ionic exclusion and specific adsorption in SEC analysis of HS, SEC-fractionation of HS was conducted under increasing ionic strength and decreasing polarity of mobile phase. The first factor was to compensate for ionic exclusion, whereas the second was to prevent specific adsorption onto column. The ionic strength varied from 0.01 up to 0.13 M (phosphate salts). Organic solvents, such as methanol, acetonitrile and tetrahydrofurane (THF), were used as organic eluent modifier.

The obtained data were plotted as a relationship of distribution coefficient versus ionic strength. For humic materials tested, the given relationship reached plateau at 0.06 M (Fig. 1). However, for polystyrenesulfonates used as

calibration standards, strong absorption was observed at this ionic strength (0.06 M) that makes them non-applicable for molecular weight calculation (Fig. 2).
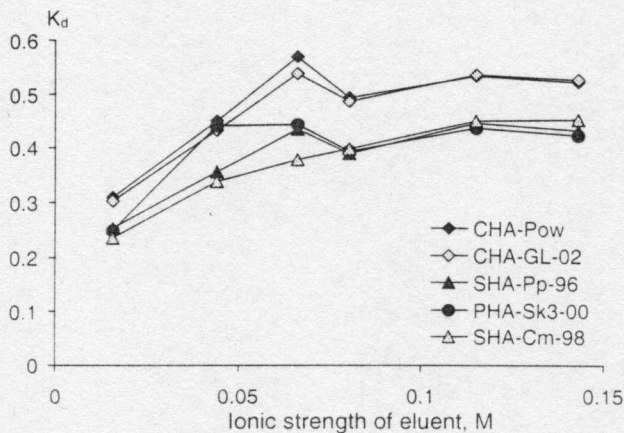

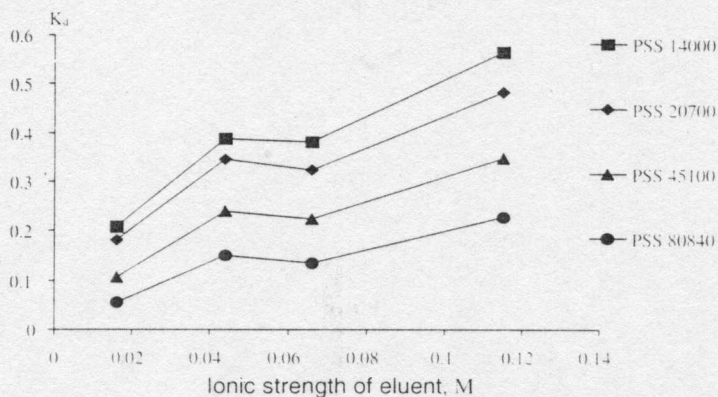Figure 1: Influence of ionic strength on K_d of HS

Figure 2: Influence of ionic strength on K_d of PSS

The recovery of HS from the columns was determined using by-pass measurements. It was about 80% for fulvic acids and did not exceed 20-50% for humic acids. The minimum value was observed for coal HA. This is indicative of specific sorption of HS onto the gel taking place at higher ionic strength. Hence, further experiments were conducted to suppress HS sorption on the gel.

Methanol, acetonitrile and THF were used in varying concentrations for modification of 0.1 M phosphate buffer. It was found that the most efficient organic modifier among the studied solvents was THF. An increase in its concentration in the mobile phase up to 20% caused increase in recovery of all

humic materials up to 80-100%, except for most hydrophobic humic acids from coal, which has increased up to 60%. Hence, the possibility of compensation of specific adsorption was shown. Further investigation are needed to optimise conditions of SEC analysis of coal HA.

4. CONCLUSIONS

The possibility for compensation for non-exclusion effects of SEC-analysis of humic substances was demonstrated using modification of eluent by increasing ionic strength and addition of organic solvents. Recommendations on the optimum SEC fractionation conditions for humics analysis are given.

ACKNOWLEDGEMENTS

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