ROLE OF NON-EXCLUSION EFFECTS IN GEL-PERMEATION FRACTIONATION OF HUMIC SUBSTANCES

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Humic substances are ubiquitous in the environment. They comprise a large class of natural refractory organic compounds. By the chemical nature, they represent randomized polymers of polycarboxy- polyhydroxy- aromatic acids. This provides for polyelectrolytic nature of humic molecules. Large molecular polydispersity is a fundamental property of humic substances that hinders quantitative evaluation of their molecular weights. Size-exclusion chromatography (SEC) is a powerful technique for determination of the molecular weight distribution (MWD) of synthetic and natural polymers. It has been widely used for analysis of HS. However, due to mentioned polyelectrolytic properties, 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 analyte 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.

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. Also the influence of pH on size-exclusion fractionation of HS was investigated.

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.

This research was supported by ISTC (project KR-964) and Interdisciplinary Program of the Lomonosov Moscow State University (project MNP-04, 2007)