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SHORT COMMUNICATION

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Aluminium ions at polyelectrolyte interfaces. II. Role in the surface-area-exclusion chromatography of humic acid

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Abstract Surface-area-exclusion chromatography using Whatman glass microfibre filters modified by adsorption of aluminium ions as a stationary phase was used to evidence the different interfacial behaviour of aluminium ion complexed (tritium labelled) humic acids. Histograms with adsorption peaks, plateaux and humps on the stationary phase showed the various adsorption characteristics of the modified humic acid. The absence of adsorption for those constituents which were recovered in the effluent showed the very low degrees of complexation by aluminium ions to be responsible for the absence of interaction of the components with the stationary phase.

Key words Surface-area-exclusion chromatography · Adsorption of humic acid · Humic acid complexation · Humic acid heterogeneity

Introduction

Surface-area-exclusion chromatography (SAEC) was recently shown to evidence adsorption phenomena of complex systems, such as diblock copolymers, whose solutions are composed of unimers and micelles, or interfacial exchange of macromolecules [1, 2]. As expected from numerical simulation studies, chromatograms of copolymers show the peak appearing at the column inlet to be representative of the surface-area exclusion of micelles owing to the presence of already adsorbed unimers [3]. Chromatograms of polyelectrolytes show no peak and evidence the interfacial exchange of polymers of different molecular weight [4]. The present investigation constitutes the first tentative attempt to apply SAEC to natural organic molecules in order to obtain some surface and solution characteristics.

Adsorption studies of natural substances on oxides and clays constitute a huge domain of investigation and are the key to understanding the fate of organic and inorganic constituents of the environment [5, 6, 7, 8, 9, 10]. Understanding adsorption phenomena is complicated by complex formation phenomena, which are common for charged systems [11, 12], and by interfacial selectivity effects, which are common for macromolecular systems. Modifications with time of the adsorption of humic substances on γ-Al_2O_3 particles were attributed to the preferential adsorption of large molecules, which slowly displace the formerly adsorbed small macromolecules [13]. Relatively fast interfacial exchange involving humic and fulvic acids was evidenced by Vermeer [14], who observed no change in the total adsorption over 25 h. For synthetic polyelectrolytes exerting only electrostatic interactions with the charged adsorbent, exchanges were found to be fast [4], but the transfer was slowed greatly when hydrophobic and/or hydrophilic interactions contributed to retain the macromolecules in the adsorbed state [15, 16]. With natural substances, selectivity phenomena in adsorption are more complicated than with synthetic macromolecules differing only in molecular weight. Fractions of natural substances with different molecular weights may differ in chemical composition, structure and conformation [17]. It has been established that hydrophobic and hydrophilic fractions isolated from a compost leachate contribute differently to adsorption on aluminium oxide as a function of the concentration in solution [18]. The phenomenon was attributed to the easier interaction of the carboxylate groups of the hydrophilic fraction with alumina. The complexity of the previous situation is enhanced when complex-forming ions are present in the system. As a result, the spatial distribution of positive, negative or neutral chain segments or of hydrophobic and hydrophilic moieties may influence the interaction forces exerted by the different components with the adsorbent [19, 20, 21, 22]. Obviously, in the natural soil or aquatic environment, such complex systems are highly abundant and it is accepted that all these phenomena must finally be taken into account to interpret the global behaviour of organic and inorganic matter in the environment.

In the present study, SAEC experiments were performed under conditions for which the distribution of hydrophobic and hydrophilic domains is slightly changed, and owing to the short period of the experiments, selectivity due to polydisperisty in molecular weight is expected not to control selectivity in adsorption.

Materials and methods

Humic acids

The peat-derived humic substance was supplied as sodium humate by Aldrich Chimie and some characteristics are given by Ochs et al. [13]. Extraction of the soluble phase from the product immersed in water at pH 1.5 indicated the presence of fulvic acid at a concentration of 8%. This mixture of fulvic and humic acids is called humic acid. The humate powder dissolved in water gave an alkaline solution of pH 8 and the nonsoluble constituents were centrifuged. Stock solutions at a concentration of 1 g/l in 10^{-3} mol/l aqueous KCl solutions at pH 5.0 appeared coloured. Acid–base titration of the humic acids showed the equivalent concentration of acid groups to be 0.003 mol/g. The titration curve showed the presence of at least two different weak acidic groups with different acid strengths [23, 24]. Turbidity measurements at 500 nm on solutions with varying polymer concentrations showed the organic substance to be soluble in aqueous solution at pH 5 and 25 °C in the presence of aluminium ions below a threshold concentration of 2×10^{-4} mol/l $AlCl_3$.

The tritium-labelled sample was obtained after reacting a very small number of the -CHO groups of humic acid with KB^3H_4 in aqueous solution. The products were purified by extensive dialysis and finally the humic acid was recovered by freeze-drying. The specific radioactivity was determined by the liquid scintillation method using the Tricarb counter equipped with the colour calibration kit (Packard) to be 236,000 dpm/mg of dry humic acid. Determination of the amount of humic acid adsorbed on each individual filter was determined by putting the filter in a glass vial containing the liquid scintillator and determining the sample radioactivity as a function of time. We determined that the humic acid adsorbed slowly desorbs with time in the organic medium and from the radioactivity determined at the plateau (after immersion for 1 week) the true value of the polymer adsorbed can be derived. Counting of the filter taken from the vial and rapidly rinsed indicated no residual radioactivity in all situations.

Humic acids in the presence of Al ions

In the present context, the important parameter of the system is the degree of complexation, α_{comp}, which is defined by Eq. (1) and may be derived from the change in the H^+ concentration $[H^+]$ resulting from mixing the constituents as indicated in Eq. (1) [19]:

$$\alpha_{comp} = \frac{[(AH{-}COO)_3Al]}{3[AH{-}COOH]} = \frac{3}{n^+ - 3_\alpha} \frac{[H^+]}{[AH{-}COOH]} \ . \qquad (1)$$

$[(AH{-}COO)_3Al]$ is the concentration of complexed carboxylic groups and $[AH{-}COOH]$ is the total concentration of humic acid. α is the usual degree of dissociation of the acidic group (which was estimated to be close to 0.1) and n^+ is the mean charge of Al ions in the solution at the given aluminium ion concentration.

Surface-area-exclusion chromatography

In SAEC, separation is based on the interfacial characteristics of macromolecules of complex structure or the polydispersity in molecular weight and the technique is well adapted to investigate such effects [2]. The solute was injected into a chromatography column whose stationary phase was constituted of Whatman glass microfibre filters modified by adsorption of aluminium ions. The Whatman filter sheet was first immersed in an aqueous solution containing Al at a concentration of 0.1 mol/l at pH 5.0, then thoroughly rinsed with a solution at $3–10 \times 10^{-5}$ mol/l and dried. To obtain information on the modifications of the glass fibre surface induced by adsorption of aluminium ions and the subsequent adsorption of humic acids, we measured the electrophoretic mobility of the different materials, which provided values of the ζ potential close to −52 mV for the bare glass fibre and 16 mV for the modified glass fibre. This potential is very close to that of modified kaolinite [19].

Actually, the column was composed of a calibrated glass tube of 5-mm diameter and stoppers, which were modified to be fitted with input and output apertures of 0.5 mm, allowing the eluant or the humic acid solution to be injected at a controlled rate with the aid of an automatically driven syringe at one point and recovered at the other one. The stationary phase was constituted of stacked circular glass microfibre filters of 5-mm diameter. The surface area developed by the individual filter has been calculated previously on the basis of its polymer adsorption capacity and is estimated to be 1 dm^2 [4]. After injection of the humic acid samples, the stationary phase was eluted with the aqueous solution of (10^{-3} mol/l KCl, $3 - 10 \times 10^{-5}$ mol/l $AlCl_3$ and 10^{-5} mol/l HCl).

Two nonadsorbing discs of large porosity were clamped close to the input piston inside the column to immediately establish a homogeneous distribution of the injected solution through the first and successive glass microfibre filters. These two filters were, however, measured for radioactivity content and the counting appears in the histograms on $i = 1$ and 2. The accidental high radioactivity of filter 2 was attributed to a certain pollution originating from the presence of entangled glass microfibres apparently belonging to glass filter 3.

Results and discussion

Solution characteristics of humic acid in the presence of aluminium ions

As developed in Ref. [19], complex formation of humic acid and aluminium ions in aqueous solution of 10^{-3} mol/l KCl and 10^{-5} mol/l HCl and various humic acid and $AlCl_3$ concentrations induces fast a pH drop, the amplitude of which serves to determine the degree of humic acid complexation. The maximum drop corresponds to the maximum humic acid complexation

(0.28 ± 0.02). The humic acid characteristics corresponding to the following experimental conditions are given in Table 1 for experiments a–d. It is worth noting that SAEC experiments were carried out under conditions ensuring plateau adsorption of carboxylic acids complexed at different degrees.

Fractionation effects in humic acid adsorption

Systematic SAEC experiments were carried out on freshly modified glass filters and freshly prepared humic acid solutions since we determined the histograms to be modified when aged solutions (2 days) were employed. Solutions of complexed ^{3}H–labelled humic acid in aqueous (10^{-3} mol/l KCl at pH 5.0, the $AlCl_3$ concentration being the parameter which was varied) were injected into the column and the effluent was sampled in aliquots of 0.5 ml and analysed for radioactivity content. Additionally, at the end of the experiment the successive filters of the stationary phase were taken out of the column with care and individually measured for radioactivity content. These two complementary analyses provide detailed information on the humic acid interfacial characteristics.

Experiment a

Figure 1 represents the amount of humic acid adsorbed on the filter as a function of the filter number *i* and the amount of humic acid recovered in the effluent for elution with an aqueous solution of humic acid at a concentration of 64 mg/l (7560 dpm/aliquot) containing 10^{-4} mol/l $AlCl_3$ as a function of the sample number *i*. The relative variation with *i* of the humic acid content in the effluent displays unexpected intermediary plateau values. The amount of humic acid adsorbed on the filter displays an initial peak of high adsorption to which is followed by different plateaux characterised by smaller adsorption amounts. It is shown that the humic acid content in the effluent increases almost immediately after injection despite the fact that the radioactivity determined on filters of *i* values greater than 22 cannot be distinguished from the background counting. Additionally, reinjection of the humic acid solution sampled at the column outlet (the nonadsorbed fraction) in a freshly prepared chromatography column does not induce perceptible adsorption, so one may conclude that the constituents of the humic acids, complexed at a mean degree of 0.20, display very different adsorption abilities.

Experiments b–d

The effects of the aluminium ion concentration in the solution on the humic acid adsorption characteristics are shown on the histograms giving the adsorption as a function of the filter number *i* (Fig. 2) and on the amount of humic acid in the effluent as a function of the aliquot number *i* (Fig. 3). SAEC provides different histograms for injection of identical amounts of complexed humic acid at a concentration of 32 mg/l (3780 dpm/aliquot). In the presence of 3×10^{-5} mol/l $AlCl_3$ (experiment b) the degree of complexation of

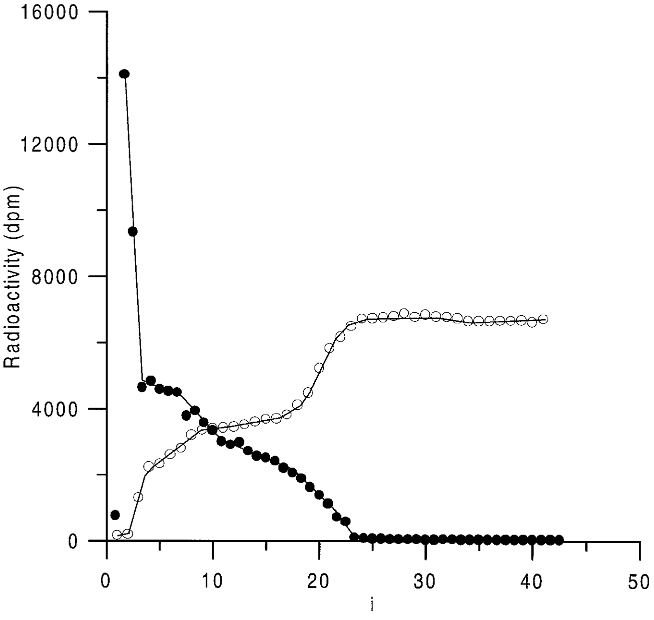

Fig. 1 Chromatograms of the elution of complexed humic acids at a concentration of 2×10^{-4} mol/l in an aqueous solution of 10^{-4} mol/ $AlCl_3$ and 10^{-3} mol/l KCl at pH 5.0 on a column constituted of stacked glass fibre filters modified by adsorption of aluminium ions. ● shows the radioactivity (dpm/filter) of the filters as a function of the filter number *i* and ○ shows the radioactivity (dpm) of aliquots of 0.5 ml of the effluent. The radioactivity of the eluant was 7560 dpm/ aliquot

Table 1 Molecular and solution characteristics of the complexed humic acid separated by surface-area-exclusion chromatography. In experiment d aluminium ions were in slight excess after mixing. The degree of dissociation α (see Eq. 1) was 0.10

Experiment	[HA-COOH] × 10^4 (mol/l)	[Al $^{n+}$] × 10^5 (mol/l)	[H $^+$] × 10^5 (mol/l)	n^+	α_{comp}
a	2	10	2.74	2.3	0.20
b	1	3	1.40	3.0	0.15
c	1	5	2.39	2.79	0.28
d	1	6	2.59	2.64	0.28

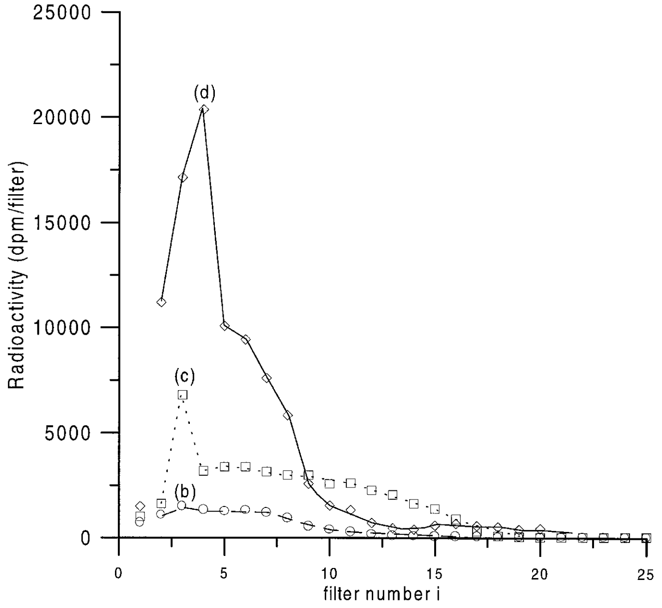

Fig. 2 Chromatograms of the elution of complexed humic acids at a concentration of 10^{-4} mol/l in aqueous solutions of 10^{-3} mol/l KCl at pH 5.0 and different $AlCl_3$ concentrations of 3×10^{-5} mol/l (○) (experiment b), 5×10^{-5} mol/l (□) (experiment c) and 6×10^{-5} mol/l (◇) (experiment d) on a column constituted of stacked glass fibre filters modified by adsorption of aluminium ions

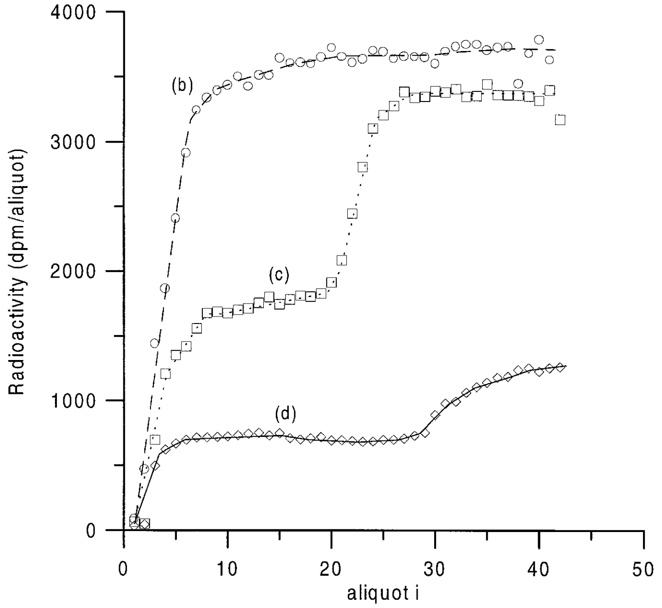

Fig. 3 The radioactivity of the effluent (dpm/aliquot of 0.5 ml) corresponding to the elution of complexed humic acids at a concentration of 10^{-4} mol/l in aqueous solutions of 10^{-3} mol/l KCl at pH 5.0 and different $AlCl_3$ concentrations of 3×10^{-5} mol/l (○) (experiment b), 5×10^{-5} mol/l (□) (experiment c) and 6×10^{-5} mol/l (◇) (experiment d) on a column constituted of stacked glass fibre filters modified by adsorption of aluminium ions. The radioactivity of the eluant was 3780 dpm/aliquot

humic acid is 0.15 and the amount of humic acid adsorbed on the stationary phase only represents 8% of the amount which was injected. In the presence of 5×10^{-5} mol/l $AlCl_3$ (experiment c) and 6×10^{-5} mol/l $AlCl_3$ (experiment d), the mean degree of complexation of humic acid is a maximum and the amount of humic acid adsorbed on the stationary phase represents 30 and 65%, respectively, of the amounts which were injected. It should be noted that in experiment d, aluminium ions were in slight excess in the eluant, so it cannot be excluded that residual ions finally modify the system characteristics in an unknown way.

Specific adsorption on the modified glass fibres

A distribution in the specific radioactivity of each of the multiple components of humic acid cannot be excluded at present. Nevertheless, to estimate the specific adsorption we took into account the mean specific radioactivity of the humic acid. From the value of the surface area available for adsorption per filter unit, we determined the following relationships between the value of disintegrations per minute per filter and the specific adsorption of humic acid per square meter, and between the value of the disintegrations per minute per aliquot (of volume 0.5 ml) and the humic acid concentration in the eluant, which apply to Figs. 1, 2 and 3:

$$1000\,\mathrm{dpm/filter} \equiv 0.42\,\mathrm{mg/m^2} \qquad (2)$$

$$1000\,\mathrm{dpm/aliquot} \equiv 8.5\,\mu\mathrm{g/ml} \qquad (3)$$

Figure 1 shows an initial very strong adsorption of around 5 mg/m^2, then two plateaux of 2.1 and 1.26 mg/m^2. Figure 2 (curve b) shows no peak and a relatively low adsorption of 0.72 mg/m^2. All these adsorption values are compatible with previous results [13, 18, 25, 26]. It is difficult to compare these two adsorption values owing to the different initial concentrations of aluminium ions and humic acid in the eluant. Comparison is more effective for the two systems in experiments c and d constituted of fully complexed humic acids. Chromatograms c and d show adsorption peaks of 2.9 and 8.4 mg/m^2 and typical plateaux or humps of 1.2 and 3 mg/m^2, respectively. Additionally, curves c and d of Fig. 3 show several typical plateaux which seem to indicate the presence of typical classes of humic acid at variable concentrations which did not adsorb.

The result of the adsorption on aluminium oxide showed the prevailing adsorption of humic acid of high hydrophobic/hydrophilic balance [18, 27]. We believe that this effect did not play a role in the adsorption of complexed humic acid on modified glass fibres. Indeed, no great adsorption or fractionation appears when the degree of complexation is 0.15 at a concentration of 10^{-4} mol/l. Therefore, fractionation should result from the nonuniform distribution of the aluminium complexed segments amongst the different constituents of the humic acid. A first portion does not adsorb certainly because the corresponding macromolecules are characterised by a mean degree of complexation smaller than 0.10 (experiment c). A second portion adsorbs at a relatively low extent, because the corresponding molecules are characterised by the mean maximum degree of complexation of 0.28. A third portion adsorbs at a high degree, and the corresponding molecules may have a mean degree of complexation greater that 0.28. These data confirm the heterogeneous nature of humic substances whose least certain components apparently adsorb differently even under slightly different conditions. Obviously, usual adsorption isotherms cannot deliver straightforward information concerning natural substances composed of macromolecules displaying various complexation and adsorption abilities. A similar conclusion was reached by Tombacz et al. [28], who investigated the effects of pH and ionic strength on the interaction of humic acid with aluminium oxide. Accordingly, these results confirm the chemical anisotropy of humic acids, which was previously determined by means of acid–base titration [29, 30] and illustrate the corresponding influence on adsorption phenomena.

Conclusion

SAEC which is based on variations in the adsorption behaviour of the constituents of humic acid at the stationary/eluant interface constitutes a new method to evidence chemical diversity in the humic acid composition. The stationary phase was the Whatman glass microfibre filter modified by adsorption of aluminium ions, which thus acquired the surface charge density of modified kaolinite clay [19]. Taking into account the experimental parameters, one notes that SAEC shows the distribution in the degree of humic acid complexation to be responsible for the differences observed in the specific adsorption on peaks, plateaux or humps. As we indicated, the method may serve as an additional procedure to determine ageing effects on adsorption when aged solutions are injected in the column. Finally, it may be possible to isolate, characterise and determine differences in the interfacial behaviour when humic acids of various origins have to be characterised [31, 32, 33, 34].

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