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Anion Exchange Solid Phase Extraction of Humic Substances for the Determination of Complexed Heavy Metals in Natural Waters with High Dissolved Organic Carbon Contents

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Summary. A solid phase extraction material based on a copolymer of styrene and divinylbenzene was developed for the extraction of heavy metals complexed by humic and fulvic acids in water. The sorbent was synthesized by a modified multistep activated swelling polymerization procedure; anion exchange functionalities were introduced by chemical modification or by coating of the particle surface. These materials allowed the extraction of humic substances from water samples at their natural *pH*. The extract or the sorbent containing the humic substances was digested by dry ashing with sulfuric acid, and the metals Cu, Zn, Pb, and Cd were determined by differential pulse anodic stripping voltammetry. With this method, the four metals could be determined with recoveries between 87 and 99% at concentrations down to 50 ng/l.

Keywords. Humic substances; Heavy metals; Solid phase extraction; Anodic stripping voltammetry.

Anionenaustausch-Festphasenextraktion von Huminstoffen zur Bestimmung der komplex gebundenen Schwermetalle in natürlichen Gewässern mit hohem Gehalt an gelöstem organischem Kohlenstoff

Zusammenfassung. Für die Festphasenextraktion von Humin- oder Fulvinsäure-Schwermetallkomplexen aus Wasser wurde ein Sorbens auf Basis eines Copolymeren von Styrol und Divinylbenzol entwickelt. Das Material wurde durch eine modifizierte mehrstufige Schwellmethode synthetisiert; die Anionenaustauschfunktionalitäten wurden durch chemische Modifizierung oder durch Belegung der Partikeloberfläche eingeführt. Diese Sorbentien ermöglichten die Extraktion von Huminstoffen bei ihren natürlichen *pH*-Werten; das Eluat oder die Festphase gemeinsam mit den Huminstoffen wurde mittels Schwefelsäure verascht (Trockenveraschung). Die Bestimmung der Metalle Cu, Zn, Pb und Cd erfolgte nach dem Verfahren der inversen Differentialpulsvoltammetrie. Mit dieser Methode konnten die vier Metalle mit Wiederfindungsraten zwischen 87 und 99% bei Konzentrationen bis hinunter zu 50 ng/l bestimmt werden.

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Introduction

Due to the high complexing capacity of humic substances, heavy metals in natural waters exist to a considerable extent in the state of complexes with humic or fulvic acids. As the behaviour of free and complexed metals differs greatly with regard to solubility and reactivity, these complexes are of major analytical interest. To a high degree humic substances are responsible for the transport of heavy metals in natural waters and exert a great influence on their bioavailability. Therefore, a lot of research work has been spent on the isolation of these complexes and on the determination of their metal content.

One the most common methods for the isolation of humic substances from natural waters is solid phase extraction (SPE) on macroreticular Amberlite XAD resins [1–8] after acidifying the solution to a *pH* of 2–3. These resins are nonionic, macroporous polystyrene/divinylbenzene copolymers or polymethacrylate materials which possess large surface areas [1]. In comparison with techniques like ultrafiltration [9, 10] or gel permeation chromatography (GPC) [11], the apparative requirements are minimal. The extraction of large sample volumes is possible within a reasonable time; therefore, low detection limits can be achieved for the determination of complexed metals. The SPE procedure can also be used for on-site preconcentration during the sampling process; this technique allows easy transportation and storage on the small SPE columns.

Despite being the most widely used material for SPE isolation of humics from water, XAD resins suffer from several disadvantages, especially when used for the determination of complexed metals [12–15]. These are the size exclusion effects for macromolecules exhibited by most XAD resins, the fact that irreversible aggregation may occur in the pores leading to physical trapping of the adsorbed material, the retention of free metals on polar impurities in the resins [14], and the partial loss of the complexed metals upon acidification.

Ways to overcome these problems include grinding of the particles to enlarge the accessible surface [5–7], the use of sodium dodecyl sulfate to improve elution [4], treatment of the resins with a solution of indium to occupy cation retaining sites [5], and the use of weak anion exchanging sorbents like diethylaminoethyl cellulose [15] or diethylaminoethyl-Sephadex A-25 [7, 13] for extraction at higher *pH* values.

The present paper describes the synthesis of a solid phase extraction material for the extraction of humic and fulvic acids in water samples at their natural *pH* (*i.e.* without acidification of the sample). This material was intended to be used for the determination of heavy metals complexed by these organic kryptands. The final goal was to produce a material with a high capacity for humic substances but no adsorption sites for free metals. Differential pulse anodic stripping voltammetry (DPASV) was selected for the final determination of Cu, Pb, Cd, and Zn after a digestion step by dry ashing.

Results and Discussion

Synthesis of PS/DVB particles for solid phase extraction

The particles used for the extraction procedure had to fulfill several requirements. The size had to be sufficiently high (>15 μm) to allow flow rates of at least 2 ml/

min even during the extraction of real samples which tended to clog the sorbent bed. To achieve optimum bed characteristics, a spherical shape and a narrow particle size distribution was desirable without fine particles which would lead to a high backpressure. To get a large accessible surface for the humic macromolecules, a high proportion of macropores was necessary. A high capacity for the humic substances necessitated a high anion exchange capacity. Last not least, the matrix had to be free of polar impurities to prevent the adsorption of uncomplexed metals.

These objectives could be achieved by modifying the multistep activated swelling polymerization method commonly used to produce uniformly sized particles with a diameter of 3–5 μm for use in high performance liquid chromatography [16, 17]. The original method includes emulsion polymerization of styrene droplets resulting in uniformly sized latex particles with a diameter of about 1 μm, the activation of these latex particles by 1-chlorododecane followed by growing with a mixture of styrene, divinylbenzene, and toluene, and finally polymerization and crosslinking. A drawback of this method was the fact that it was not possible to achieve uniformly sized particles with a size larger than 5 μm. In the present work, an SPE material with a diameter of about 19.5 μm could be developed by combining the original procedure with a second activating and growing sequence. Even after this second sequence the particle size distribution was quite narrow (±1 μm). The non-crosslinked polystyrene particles of the first growing procedure served not only as seeds for a second growing step but also as polymeric porogen, resulting in a sufficient number of macropores with a diameter larger than 100 nm. The pressure stability necessary for the use in SPE was ensured by crosslinking the particles with divinylbenzene in the second growing and polymerization sequence.

The introduction of anion exchange functional groups was achieved either by chemical modification *via* nitration in acetic acid, nitric acid, and sulfuric acid, reduction with tin in HCl, and quaternization with iodomethane [18] or by coating the particles with hydrophobic amines or ammonium salts. As discussed below, both methods yielded satisfying anion exchange capacities, but the chemical modification led to some destruction of the particles due to the mechanical stress during the chemical reactions.

Solid phase extraction

To prevent the loss of complexed metals, no adjustment of the *pH* of the sample solution was done before the extraction step. The *pH* of the standard solution was 6.6, and that of the real sample 5.2, but no difference in the retention behaviour was observed. The capacity for the retention of humic acid exhibited by the PS/DVB sorbents synthesized in this work as well as that exhibited by silica particles

Table 1. Anion exchange capacities of different styrene/divinylbenzene particles

Sorbent modification	Capacity
Chemically modified with quaternary ammonium groups	0.45 mequ/g
Coated with decylamine	1.24 mequ/g
Coated with cetyltrimethylammonium bromide	0.35 mequ/g

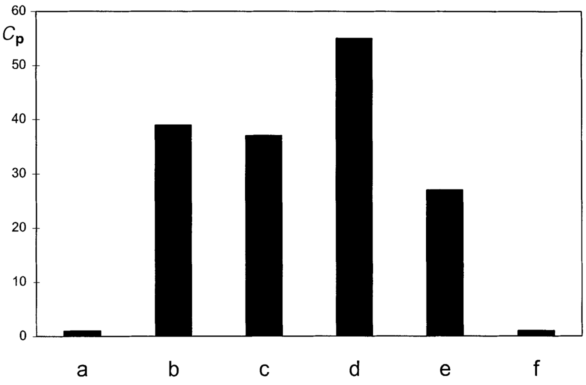

Fig. 1. Capacities (c_p; mg humic acid/g anion exchange polymer) for the retention of humic acid of different SPE materials (a: PS/DVB unmodified, b: PS/DVB–NR_3, c: PS/DVB–CTAB, d: PS/DVB–decylamine, e: silica–NR_3, f: silica–propylamine); breakthrough curves were measured by UV absorbance at 400 nm

modified with aminopropyl groups or quaternary amino groups was measured by recording the breakthrough curves *via* monitoring the UV absorbance at 400 nm. The anion exchange capacities are given in Table 1, the capacities for the retention of humic acid in Fig. 1. Optimum capacities could be achieved only for flow rates not higher than 3 ml/min. Whereas the PS/DVB particles modified with decylamine gave the highest capacities of all tested materials, the aminopropyl modified silica gave no retention at all. This was most probably caused by the differing sorbent matrix. The PS/DVB sorbents containing quaternary ammonium groups yielded a high capacity for humic acid only if existing in the iodide or bromide form, but no capacity at all if existing in the hydroxide form. The reason for this behaviour may be the change of the *pH* value of the solution during the extraction caused by the release of hydroxide ions.

The elution of the humic acid was much more difficult. Elution was possible from the sorbents chemically modified with quaternary ammonium groups by alternately using eluent 1 (methanol/25% aqueous sodium hydroxide = 1:2 and eluent 2 (5% nitric acid) in portions of about 1 ml. 5 ml were enough to elute all adsorbed humic acid. Complete elution was not possible from the styrene/divinylbenzene particles coated with decylamine or cetyltrimethylammonium bromide; the sorbent retained a brown color even after applying more than 20 ml of the eluents. The addition of SDS to eluent 1 resulted in a somewhat faster extraction from the sorbents chemically modified with quaternary ammonium groups, but could not improve the results for the coated particles. Thus, when using the coated materials the sorbents had to be digested together with the humic acid. This was possible as the styrene/divinylbenzene matrix could be digested without any problems under the same conditions as used for the humic acid in eluates. Ashing the SPE material together with the sample had the additional advantage of saving the time necessary for elution and evaporation of the eluent without having to change the ashing procedure.

On the other hand, the PS/DVB particles chemically modified with quaternary ammonium groups contained a considerable amount of the metals Cu, Pb, and Zn which were introduced during the surface modification. Even after washing the sorbent several times with hydrochloric acid, the washing solution still contained the metals. This sorbent was therefore not suited for the determination of these metals.

As several authors mention the possibility of the retention of free metals on XAD resins, this was checked for the styrene/divinylbenzene sorbents. No measurable adsorption of free metals could be observed under the conditions used for the SPE of the humic acid. The sorbents based on a silica matrix, however, still had a considerable retention capacity for metals and therefore had to be ruled out. This left the coated PS/DVB particles as the only materials that could be used for the determination of complexed metals in humic substances. As the decylamine coated particles gave considerably higher capacities, they were used for all further experiments.

Digestion and polarography

Several methods were tested for digestion of the humic acid including pressure ashing, wet ashing with mixtures of sulfuric acid and hydrogen peroxide or nitric acid and perchloric acid, and dry ashing with or without ashing aids. More sophisticated methods like high pressure ashing or microwave assisted ashing might be preferable but were not available for the investigations reported in this paper. It should be emphasized that the aim of this work was the synthesis of appropriate solid phase extraction materials and not the optimization of ashing procedures or methods for metal determination. Thus, polarography was chosen for the final determination of Cu, Pb, Cd, and Zn only for reasons of convenience. Atomic absorption or emission techniques should be likewise applicable. Among the digestion methods investigated, satisfactory results could be achieved by dry ashing using sulfuric acid as an ashing aid. A polarogram recorded by differential pulse anodic stripping voltammetry (DPASV) after using the optimized digestion procedure is shown in Fig. 2. Quantitative recovery of the metals could be achieved without any discernible interference from incompletely dissolved organic material. The method was found to be suitable for digestion of the humic acid standard material, of the eluates, and of the styrene/divinylbenzene sorbents containing the humic acid. Some disadvantage originated from the long sample preparation time.

The metal content of humic acid in water was determined for a solution of the humic acid standard material with a known content of the four metals and for a real sample. The metal content of the standard material had been determined before by DPASV and by AAS. The decylamine coated PS/DVB particles were digested together with the humic acid as complete elution of the humic acid was neither possible nor practical. This could be done without a change in the ashing procedure. The recoveries for solutions of the standard material are given in Table 2, the metal content of the humic substances from the real sample in Table 3. Whereas the standard material consisted solely of humic acid, the real sample contained a mixture of humic and fulvic acids as could be shown by precipitation of the humic acid at $pH = 2$. A large part of the humic substances stayed in solution and so had to be, per definition, fulvic acid. During the SPE the fulvic acid was retained to the same extent as the humic acid. Accordingly, a differentiation

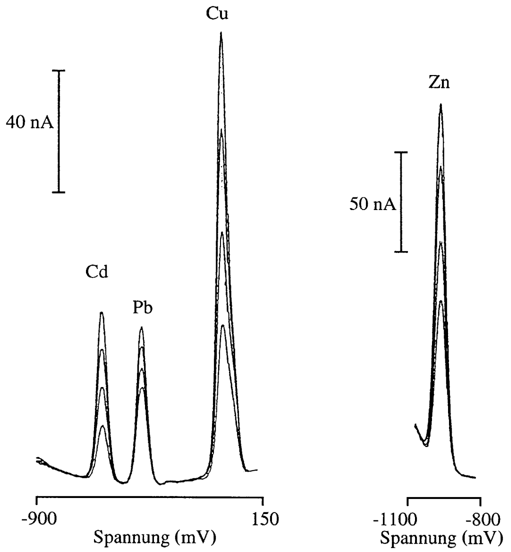

Fig. 2. Inverse voltammetric determination of Cu, Pb, Cd, and Zn complexed by humic substances in a water sample enriched from 150 ml by SPE; concentrations in the original sample: Cd 0.47 µg/1, Pb 1.47 µg/1, Cu 1.17 µg/1, Zn 5.08 µg/1 (quantification by threefold standard addition)

Table 2. Recoveries for the heavy metals complexed with the humic acid standard material after SPE with styrene/divinylbenzene particles coated with decylamine; digestion by dry ashing, determination by DPASV, *R.S.D.* from 3 measurements

Metal	Recovery (%)	*R.S.D.*
Cd	96	4.4
Pb	99	3.9
Cu	87	5.8
Zn	93	4.4

Table 3. Metals complexed by humic substances in water from the *Tanner Moor*, Upper Austria; SPE with styrene/divinylbenzene particles coated with decylamine, digestion by dry ashing, determination by DPASV, *R.S.D.* from 3 measurements

Metal	Metal content (µg/1)	*R.S.D.*
Cd	<0.05	
Pb	5	6.3
Cu	2.5	5.4
Zn	21.3	6

between these two components of the humic substances is not possible by this method. The same behaviour can be expected for other organic complexants of low polarity which would likewise be adsorbed at the resin. The method is therefore not suited to differentiate between large and small complexants which would have to be done by techniques like ultrafiltration or GPC. As in many cases the interest lies on the sum of the complexing organic substances, such a differentiation was believed to be unnecessary in the present work.

Conclusions

The well known advantages of SPE, as for example the short sample preparation time, high preconcentration factors, and the simplicity of the method, make it a highly attractive approach for the extraction of humic substances to determine the amount of metals complexed by humic or fulvic acids in water samples. Spherical shape of the particles and narrow size distribution at about 19.5 μm allow the preparation of SPE columns with optimal bed properties of the solid phase that can readily be used for routine sampling purposes, thereby combining the sampling and the preconcentration step. The problems mentioned for the extraction with XAD resins (losses of complexed metals due to the low *pH* necessary to achieve adsorption, incomplete elution because of irreversible adsorption of the humic acid, and positive errors resulting from uncomplexed metals retained on the sorbent) could be solved or avoided by using the new PS/DVB based anion exchange particles described in this paper for the extraction of the complexed metals. The recoveries between 87 and 99% with standard deviations between 4 and 6% show the suitability of the method for the determination of heavy metals complexed by humic substances in water. The number of the metals to be determined can easily be expanded by adapting the DPASV parameters or by employing atomic spectroscopic techniques. Although the suitability of dry ashing for the trace analysis of heavy metals complexed with humic or fulvic acid could be ascertained, the long sample preparation time resulting from this procedure is the main disadvantage of this method. A more sophisticated digestion procedure like for example high pressure ashing could serve to shorten the sample preparation time considerably.

Experimental

Apparatus

Breakthrough characteristics of the sorbents were measured by connecting the extraction columns to a Perkin Elmer LC-75 spectrophotometric detector. UV spectra were recorded with a Hewlett Packard 8452A diode array detector. For digestion, an Ika combimag RCH heating plate and a Heraeus MC170 furnace were used. Polarographic measurements were done on a Metrohm 646 VA processor with a 647 VA stand. Size distribution measurements were performed with a Reichert Biover microscope and by scanning electron microscopy.

Reagents

The humic acid standard material was from Roth, Germany; real samples were taken from the *Tanner Moor* in Upper Austria. The humic acid standard solution was prepared by dissolving the standard material in water to achieve a concentration of 100 mg/1. The solution was filtered through 0.45 μm HVLP filters from Millipore before use. All acids were of suprapure quality from Merck; decylamine prakt. and cetyltrimethylammoniumbromide purum were obtained from Fluka.

Deionized and doubly distilled water was used throughout this work; all other solvents were of analytical grade quality. The metal standard solutions were prepared by dissolving the nitrates or sulfates (p.a. quality, Merck) in 1% nitric acid to achieve concentrations of 1 g/1. The diluted solutions used for standard addition were prepared freshly before use.

SPE materials

Prepacked aminopropyl modified silica was obtained from Applied Separations, PA, USA, silica modified with quaternary ammonium groups, also in prepacked columns, from J. T. Baker, NJ, USA. Each of the extraction columns contained 500 mg of the sorbent. Particles synthesized according to a procedure given below were packed into 6 ml disposable columns (60×10 mm I.D.) equipped with polyethylene frits (Applied Separations). All columns were washed with methanol, water and hydrochloric acid and activated with methanol before use.

Synthesis of polystyrene/divinylbenzene (PS/DVB) particles

The procedure for the synthesis of PS/DVB particles has been described in detail recently [19]. It includes the preparation of primary latex particles with a size of 2 μm by emulsion polymerization of styrene from a solution containing the monomer, NaCl to adjust the ionic strength, and the water soluble initiator potassium peroxodisulfate. The solution was stirred and heated to 78°C to start the polymerization. After 16 h the mixture was filtered to remove large agglomerates and stored in an erlenmeyer flask with slow stirring to prevent agglomeration of the particles.

The particles were activated by swelling with 1-chlorododecan and grown with styrene and the water insoluble initiater dibenzoylperoxide. After removal of the monomer not taken up by the particles, they were polymerized at 72°C for 60 h. The resulting macrolatex, consisting of non-linked polystyrene chains, had a very narrow size distribution with a mean diameter of 7 μm. These particles served as seed particles for a second activation and growing procedure and as polymeric porogens to achieve the desired macroporosity. After the second growing and polymerization sequence which differed from the first one by the addition of divinylbenzene and toluene together with styrene before growing, the particles, now with a mean diameter of 19.5 μm, were *Soxhlet* extracted with toluene to remove the polymeric porogen. The anion exchange functional groups were introduced either by a sequence of nitration, reduction and quaternization as described in Ref. [18], or by adsorbing hydrophobic amines or ammonium salts on the apolar surface. The latter was performed by packing 500 mg of the sorbent into a column and passing 500 ml of a solution containing either 500 mg/1 of *CTAB* in water or 500 μl/1 decylamine in water : methanol = 95 : 5 (v/v) through the column. The anion exchange capacity of the sorbents was determined by acid/base titration in the case of the chemically modified particles, by potentiometric titration of the remaining bromide in the coating solution after the coating process in the case of cetyltrimethylammonium bromide, and by capillary electrophoretic determination of decylamine remaining in the coating solution.

SPE procedure

The samples were filtered through a 0.45 μm filter to prevent clogging of the column and passed through the column at flow rates of 2–3 ml/min by applying an adequate pressure. The capacity of the sorbent was checked by connecting the extraction column to a UV detector and measuring the absorbance of the solution at 400 nm. Breakthrough was defined as the point where the absorbance reached 5% of that of the initial solution.

Methanol: 25% aqueous sodium hydroxide = 1:2 (eluent 1) and 5% nitric acid (eluent 2) were used for the elution. The addition of 0.1 mol/1 sodium dodecyl sulfate (*SDS*) to eluent 1 was investigated to enhance the elution efficiency.

Ashing and DPASV

The eluate or the sorbent containing the humic acid was transferred into a 10 ml quartz vessel and brought to dryness on a heating plate at a temperature of about 300°C after adding 500 µl of concentrated sulfuric acid as an ashing aid. After the white fumes ceased to evolve, the vessel was transferred into the furnace, heated slowly to 550°C, and kept at this temperature for 16 h. The residue was dissolved in 200 µl of nitric acid and 5 ml water, brought to dryness to complete the digestion and to convert the sulfates into nitrates, and redissolved in 100 µl of nitric acid and 5 ml of water.

The solution obtained after the ashing procedure was transferred into a Teflon polarographic vessel and mixed with 15 ml water. For determination of zinc, the *pH* was adjusted to 2 by addition of ammonium hydroxide. The following parameters were used for the measurements: drop size 0.55 mm^2; stirring speed 2445 rpm; puls amplitude 50 mV.

Cu, Pb, and Cd: preconcentration potential -800 mV, preconcentration time 30 to 120 sec, stripping from -800 to 200 mV at 10 mV/sec; Zn: preconcentration potential -1100 mV, preconcentration time 30 to 120 sec, stripping from -1100 to -800 mV at 10 mV/sec.

Quantitation was done by the standard addition method. A teflon vessel had to be used, and the vessel and the electrodes had to be washed with hydrochloric acid before and after each measurement to avoid blanks.

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