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Short communication

Immobilized humic substances and immobilized aggregates of humic substances as sorbent for solid phase extraction

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ABSTRACT

In this work, humic substances (HS) immobilized, as a thin layer or as aggregates, on silica gel were tested as material for solid phase extraction. Some triazines (simazine, atrazine, therbutylazine, atrazine-desethyl-desisopropyl-2-hydroxy, ametryn and terbutryn), have been selected as test analytes due to their environmental importance and to span a large range of solubility and octanol/water partition coefficient (logP). The sorbent was obtained immobilizing a thin layer of HS via physisorption on a pre-coated silica gel with a cationic polymer (polybrene). While the sorbent could be used as it is, it was demonstrated that additional HS could be immobilized, via weak interactions, to form stable humic aggregates. However, while a higher quantity of HS could be immobilized, no significant differences were observed in the sorption parameters. This sorbent have been tested for solid phase extraction to concentrate triazines from aqueous matrixes. The sorbent demonstrated performances equivalent to commercial alternatives as a concentration factor between 50 and 200, depending on the type of triazines, was obtained. Moreover the low cost and the high flow rate of sample through the column allowed using high quantity of sorbent. The analytical procedure was tested with different matrixes including tap water, river water and estuarine water.

1. Introduction

Pollution of the water resources is one of the problems created by moderns farming practices as well as by industries [1,2]. However, analysis of water is costly in time and resources; not only the matrixes are complexes but target compounds have to be monitored at very low concentrations [3,4]. In solid phase extraction (SPE), probably the most common technique for the treatment of aqueous sample [5], compounds of interest are extracted from the matrix by interactions with an immobilized sorbent. While various commercial sorbents are available, there is still need for further research to obtain products with a different selectivity and/or a higher sample loading [6–8].

Humic substances (HS) are the stable degradation products of the natural organic matter and are composed of a mixture of not well-defined structures [9] of organic molecules and macromolecules; most of them extractable from the soil using alkaline solution or isolated from water using hydrophobic resins. Of their many properties, they are the main sorbent in soil for organic and inorganic chemicals. The first attempt to use humic substances as a sorbent in analytical techniques was in the early 2000s [10]. Since then, humic based sorbents have been used in HPLC [11–16], capillary electrochromatography [17,18] and solid phase extraction [19]. In this latter work, humic substances were immobilized covalently to 3-aminopropyltriethoxysilane (APTS)-silica gel and used as SPE sorbent for the analysis of benzo[*a*]pyrene from edible oil. While most often humic substances are immobilized covalently [20–24], physisorption and/or chmisorption, because of its easiness, are also interesting approaches [11,17]. We previously used such strategy to immobilized humic acid in the inner wall of a silica capillary to study sorption with key chemicals using open tubular capillary electrochromatography [17]. More importantly, we also demonstrated that this stable, immobilized layer of HS can be used to self-assembled humic aggregates as it is believed to naturally occur [25–28]. Higher retention was observed with aggregates of humic substances in comparison to a thin layer.

The aim of this work is to test immobilized humic substances, both as thin-layer and aggregates, as sorbent in solid phase extraction to isolate and concentrate pesticides in aqueous matrixes. For this work, triazines were chosen as test compounds and were separated using micellar electrokinetic chromatography (MEKC) [29].

2. Materials and methods

2.1. Apparatus

The separation and quantification of the triazines were done using a capillary electrophoresis instrument, P/ACE MDQ

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instrument (Beckman Coulter Fullerton, CA, USA) equipped with a UV–vis photo-detector. Polyimide coated fused-silica capillaries with a total length of 80 cm (70 cm to detector) and 75 μm of internal diameter were used. Electropherogram were recorded at 214 nm. All separations were done at 20 kV and the temperature was maintained at 25 °C. Concentration of humic substances was measured using a T90+ (PG instrument Ltd.) UV–vis spectrometer at 250 nm.

2.2. Chemicals and reagents

All chemicals were analytical reagent grade: sodium hydroxide was from Merck (Lisboa, Portugal), hexadimethrine bromide (polybrene), humic acid (HA) and sodium tetraborate from Sigma Chemical Co (St Louis, MO, USA), atrazine (atz), simazine (sim), atrazine-desethyl-desisopropyl-2-hydroxy (add), ametrym (ame), terbuthylazine (tba) and terbutryn (tbt) were from Riedel de Haen (Seelze, Germany), sodium dodecyl sulfate (SDS) from Sigma–Aldrich. Triazines stock solutions were prepared in acetonitrile with a final concentration of 500 mg L^{-1} and were stored in dark, at 4 °C, for no more than 1 month. Ultra pure water was obtained using a Milli-Q system (Milli-Q plus 185) from Millipore (Bedford, MA, USA).

2.3. Immobilization of the humic acids and self-assembling of the humic aggregate

The procedure involved a pre-coating of the silica gel with a cationic polymer (solution of 0.2% (w/v) of polybrene, 1 g of gel for 25 mL of solution), thus reversing the polarity of silica gel. The silica gel was then incubated with an alkaline solution of humic substances (25 mg L^{-1} of HA in 25 mM Na_2HPO_4 (pH 9.5), 1 g of gel for 25 mL of solution) overnight to form a stable, thin, layer of humic substances [17]. The coated silica gel was extensively washed (25 mM Na_2HPO_4) with an alkaline solution and then water to only keep the stable layer. This sorbent will be now be referred as SG:HA. Supramolecular structures were self assembled using successive incubations. Incubation solutions were made dissolving HA in Na_2HPO_4 (25 mg L^{-1}) and adjusting the pH to the required value with H_3PO_4, in this case pH 4. Incubation (25 mL of solution per gram of SG:HA) was done for no less than 24 h, the slurry being gently shaken during all time. This step was repeated 10 time has it was observed that a higher quantity of substances could be immobilized with each new incubation. The amount of immobilized substances by supramolecular interactions was quantified by removing the aggregate using an alkaline solution. A final concentration of 37 mg of HA by gram of silica gel was obtained. The gel obtained after immobilization of HA by aggregation will be now referred as SG:HA_{agr}. SG:HA and SG:HA_{agr} were washed with water and acetonitrile, dried at 25 °C and stored in a desiccators until further use.

2.4. MEKC analysis

New capillaries were first conditioned with 1 M NaOH for 30 min followed by Milli-Q water for 15 min. The capillary was washed with NaOH 0.1 M for 10 min followed by Milli-Q water for 10 min at the beginning of each working day and with Milli-Q water for 5 min at the end of the day. Between runs the capillary was flushed with BGE for 3 min. All the capillary conditioning steps were performed at a pressure of 138 kPa (20 psi). The running BGE was composed of 30 mM SDS, 6.25 mM sodium tetraborate and 5% ACN. Before use, the BGE was sonicated and filtered through a 0.22 μm filter (Millex-127GV from Millipore). Samples were prepared or re-dissolved in 10 mM SDS and injected for 10 s at a pressure of 0.5 psi.

2.5. SPE procedure

The cartridges used in this work were prepared with 1 g of sorbent (SG:HA or SG:HA_{agr}) enclosed in a 6 mL polypropylene SPE-tube. The sorbent was retained using a polyethylene frit. The cartridges were conditioned with 5 mL of acetonitrile, 5 mL of 1% of H_3PO_4 and 25 mL of Milli-Q water. 50 mL of sample was flown and then eluted with 5 mL acetonitrile. The eluates were evaporated under nitrogen and dissolved in 100 μL of 10 mM SDS.

3. Results and discussion

3.1. Test triazines and separation by MEKC

The triazines were separated and quantified using a method modified from our previous works [29] (see experimental). While the separation is relatively long, excellent analytical performances have been obtained. In particular the relative standard deviation (*RSD*) in the determination of the peak area (excluding add) is between 0.8 and 2.4. The limits of detections, calculated as three times the background noise, were between 60 and 250 μg L^{-1}. This compares well with similar method (75 μg L^{-1} in [30], higher than 150 μg L^{-1} in [31], around 100 μg L^{-1} in [32]). $[HA_{dis}]_{eq}$.

3.2. Stability and sorption isotherms

While SG:HA shows good stability this is not the case of SG:HA_{agr} where, depending on the conditions, important leaching can be observed. The stability of the aggregates in various solvents were assessed using UV–vis spectroscopy. While the aggregates remain whole if using pure water, acetonitrile or low concentrated solution of phosphoric acid, when using solutions with high ionic strength or in alkaline conditions, important quantities of sorbent are lost. As acetonitrile is often a solvent of choice to elute pesticides from various matrixes [33], it was selected as our elution solvent.

Conditioning of the sorbent was optimized using different washing procedures. The conditioning was always followed by a wash step with 25 mL of Milli-Q water and then 50 mL of the sample containing 50 μg L^{-1} of the six triazines. The cartridge was eluted using 5 mL of acetonitrile and the eluate recovered in a polypropylene conical tube to be air dried under a gentle nitrogen stream. After complete dryness, the samples were re-dissolved using 0.1 mL of the sample buffer and injected for analysis. In Fig. 1 shows the results obtained with different conditioning solvents (A: water, B: 5 mM $CaCl_2$, C: 5 mM acetonitrile, D: 1% of H_3PO_4, E: 5 mM acetonitrile followed by 1% of H_3PO_4) using SG:HA_{agr}. For each of the five triazines (because of it lowest intensity, add was excluded of this part), the percentage of triazines that has been sorbed was calculated as

$$\%_{sorbed} = \frac{c_{obs}}{c_{exp}} \times 100 \quad (1)$$

where c_{exp} is the expected concentration (that is the concentration that will have been obtained if all analytes that were initial present were recovered) and c_{obs} is the observed concentration. Each experiment (including SPE step) was repeated four times, and for each experiment, the CE analysis was run in duplicate. While the percentages of sorbed compounds are similar within each condition, important variation in the repeatability is observed. The last condition was selected for SG:HA_{agr} and SG:HA (5 mL of acetonitrile followed by 5 mL of 1% of H_3PO_4) as it provide the lowest RSD (average over the five triazines of 15% in condition A, 15% in condition B, 18% in condition C, 8% in condition D and 6% in condition E).

Affinity of the two sorbents (SG:HA and SG:HA_{agg}) with the triazines was assessed using batch experiments to measure the