1   **QUATERNARY AMMONIUM-FUNCTIONALIZED SILICA SORBENTS FOR THE**
2   **SOLID-PHASE EXTRACTION OF AROMATIC AMINES UNDER NORMAL PHASE**
3   **CONDITIONS**

4
5   Lorena Vidal^{a,b*}, Orlane Robin^a, Jevgeni Parshintsev^a, Jyri-Pekka Mikkola^{c,d} and Marja-Liisa
6   Riekkola^{a*}

7
8   ^a Laboratory of Analytical Chemistry, Department of Chemistry, P.O. Box 55, University of
9   Helsinki, FI-00014 Helsinki, Finland

10   ^b Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de
11   Materiales, Universidad de Alicante, Apdo. 99, 03080 Alicante, Spain

12   ^c Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Åbo
13   Akademi University, Åbo-Turku, FI-20500, Finland

14   ^d Technical Chemistry, Department of Chemistry, Chemical-Biological Centre, Umeå
15   University, SE-901 87, Umeå, Sweden

16   *Corresponding authors: Tel.: +34965903400 (3117); +358 9 191 50268; fax: +358 9 191 50253.
17   E-mail addresses: lorena.vidal@ua.es (L. Vidal); marja-liisa.riekkola@helsinki.fi (M.-L.
18   Riekkola)

19
20   **Abstract**

21   Quaternary ammonium-functionalized silica materials were synthesized and applied for solid-
22   phase extraction (SPE) of aromatic amines, which are classified as priority pollutants by US
23   Environmental Protection Agency. Hexamethylenetetramine used for silica surface modification
24   for the first time was employed as SPE sorbent under normal phase conditions. Hexaminium-
25   functionalized silica demonstrated excellent extraction efficiencies for o-toluidine, 4-ethylaniline
26   and quinoline (recoveries 101-107%), while for N,N-dimethylaniline and N-isopropylaniline
27   recoveries were from low to moderate (14-46%). In addition, the suitability of 1-alkyl-3-(propyl-
28   3-sulfonate) imidazolium-functionalized silica as SPE sorbent was tested under normal phase
29   conditions. The recoveries achieved for the five aromatic amines ranged from 89 to 99%. The
30   stability of the sorbent was evaluated during and after 150 extractions. Coefficients of variation
31   between 4.5 and 10.2% proved a high stability of the synthesized sorbent.

Elution was carried out using acetonitrile in the case of hexaminium-functionalized silica and water for 1-alkyl-3-(propyl-3-sulfonate) imidazolium-functionalized silica sorbent. After the extraction the analytes were separated and detected by liquid chromatography ultraviolet detection (LC-UV). The retention mechanism of the materials was primarily based on polar hydrogen bonding and π - π interactions. Comparison made with activated silica proved the quaternary ammonium-functionalized materials to offer different selectivity and better extraction efficiencies for aromatic amines. Finally, 1-alkyl-3-(propyl-3-sulfonate) imidazolium-functionalized silica sorbent was successfully tested for the extraction of wastewater and soil samples.

Keywords

Solid-phase extraction; hexamethylenetetramine; ionic liquid-functionalized silica; imidazolium; zwitterionic; aromatic amines.

1. Introduction

Organic, aromatic bases such as aniline and aniline derivatives are of environmental concern because of their potential toxicity, carcinogenicity, and the large quantities of annual production [1]. These chemicals are used in the manufacturing of dyes, synthetic polymers, cosmetics, medicines, rubber products, agricultural chemicals and varnishes [1-4]. Moreover, the actual industrial production of aniline is mainly carried out via a two-step procedure starting from benzene, itself a carcinogenic hydrocarbon, although alternative synthesis routes e.g. from phenol and ammonia exist [5]. Anilines are found in wastes from coal gasification and shale oil extraction processes and as transformation products in soils treated with carbamate herbicides [3]. Aromatic amines may be released from the manufacturing processes and power generators and as a result, these residues are considered to be contaminants in environment and effluent waters. Furthermore, these species are also known to enter the soil through various routes including accidental spills, improper waste disposal, and conversion of azo-dyes to the parent aromatic amines through bacterial action [2]. Obviously, they have been classified as priority pollutants [6] by US Environmental Protection Agency (EPA) and due to their extensive and increasing use, monitoring of their levels in industrial effluents is regulated [7]. Therefore, the development of simple, sensitive, rapid and reliable analytical methods is required. Gas chromatography (GC) [7], capillary electrophoresis (CE) [8], and high-performance liquid chromatography (HPLC) [9] have traditionally been used as the analytical techniques of choice for determining aromatic amines in water samples. However, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have been frequently employed in the extraction of amines. SPE is today widely used because it overcomes most of the disadvantages of LLE. The most commonly employed non-polar sorbents for the SPE of amines have been octadecylsilanes and silica from polar phases. In addition, sorbents with cationic-exchange properties have been used [10].

As demonstrated in our recent review [11], conventional SPE materials (i.e., silica and polymers) have been modified in several studies to increase their specific selectivity, affinity and capacity. One of the recent modification trends has been to immobilize or graft ionic liquid (IL) moieties onto silica surface [11-13]. The power of these materials arises from their dual nature as evidenced by many studies [11]. Although the interactions of IL-functionalized silica sorbents in SPE have been proved to be mainly of anion exchange nature; other interactions, such as hydrogen bonding, hydrophobic and π type also play an important role [14]. Our previous work,

that was focused on the SPE of several relevant compounds for atmospheric particles, included also two aliphatic amines and one aromatic amine [14]. From the three IL-functionalized SPE silica sorbents employed, the best recoveries were obtained at pH 12 with 1-alkyl-3-(propyl-3-sulfonate) imidazolium zwitterionic IL-functionalized silica (SiImPS). However, the low recoveries (6-32%) achieved demonstrated that the sorbent or the extraction conditions were not optimal for the extraction of amines. Furthermore, at pH 12, the sorbent was not anymore stable, and almost half of the imidazolium groups were detached [14]. Working at lower pH than the pK_a value of these basic compounds could not be considered due to positive charge in the imidazolium ring of IL-functionalized silica that would have caused repulsion toward the protonated amines. For this reason, one of the aims of this work was to study the behaviour of IL-functionalized silica material under normal phase extraction conditions in order to test if higher recoveries could be achieved. Normal phase SPE materials vary from mid- polar to polar ones, being suitable for the extraction of analytes with a wide polar range [15]. In this study, a new silica-based, IL-modified sorbent was synthesized and its applicability to the extraction of amines was studied. In the new sorbent a hexamethylenetetramine (hexamine) was covalently bonded onto the silica surface [16], including a quaternary ammonium group, like in IL-functionalized silica materials. Opposite to imidazolium IL-functionalized silica, hexaminium-functionalized silica is not aromatic. Moreover, it contains more nitrogen atoms and hexamine has a symmetrical cage-like structure that can be expected to give more selectivity to the sorbent. However, the weakness of this compound is that it can lose its symmetry by the protonation of one nitrogen atom, resulting in lower stability.

The goal of the present work was to study the applicability of hexaminium-functionalized silica as SPE sorbent and the suitability of SiImPS and hexaminium-functionalized silica materials under normal phase conditions for the extraction of amines. For the comparison, silica gel was used throughout the study. Finally SiImPS sorbent was applied to the extraction of aromatic amines in complex wastewater and soil samples.

2. Experimental part

2.1. Materials and reagents

The reagents for preparation of imidazolium-based zwitterionic IL-functionalized silica material were silica gel 60 (0.063-0.2 mm, 500 m² g⁻¹) from Fluka (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), nitric acid (65%) from Riedel-de Haën (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany), and imidazole (puriss. p.a.), (3-chloropropyl)trimethoxysilane (97%), 1,3-propane sultone and triethylamine (99%) which were purchased from Sigma-Aldrich (Steinheim, Germany).

The reagents for preparation of hexaminium-functionalized silica material were silica gel 60, nitric acid, (3-chloropropyl)trimethoxysilane, and hexamethylenetetramine (hexamine) (puriss. p.a.) from Sigma-Aldrich.

HPLC grade toluene was purchased from LAB-SCAN Analytical Sciences (Gliwice, Poland), acetone (>99%), hexane, chloroform and acetonitrile (HPLC grade) were from VWR International bvba. (Leuven, Belgium). The solvents methanol (HPLC grade), ethanol (AA grade, 99.5%) and dichloromethane were supplied by Aldrich (Steinheim, Germany), Altia Plc (Helsinki, Finland) and Sigma-Aldrich, respectively.

Acetonitrile, Milli-Q water (DirectQ-UV, Millipore Corp., Billerica, MA, USA) and acetic acid (99%) from Fluka were used for the HPLC analysis.

Anhydrous sodium sulphate from Riedel-de Haën was used for the drying of organic solvents. Hydrochloric acid (37%, HCl) and sodium hydroxide from VWR were used to adjust the pH of extraction cartridge regeneration solution and mobile phase.

Dipotassium hydrogen phosphate from J.T. Baker Chemicals B.V. (Deventer, Holland), potassium dihydrogen phosphate from Fisher Scientific (Fair Lawn, NJ, USA) and orthophosphoric acid (85%) from Merck KGaA (Darmstadt, Germany) were purchased for the buffer preparation.

Five aromatic amines were selected for the evaluation of the sorbents: Quinoline (98%) from Sigma (Steinheim, Germany); o-toluidine ($\geq 99.5\%$), 4-ethylaniline ($\geq 98\%$), N-isopropylaniline ($\geq 97\%$) and N,N-dimethylaniline ($\geq 99.5\%$) from Fluka. The structures, pK_a and logP values of the analytes are listed in Table 1. Standard stock solutions (3000 mg L⁻¹) of each analyte were prepared in hexane. Working solutions were prepared in hexane before the extraction. All the solutions were stored at 4°C.

2.2. Environmental samples

The applicability of SiImPS material under normal phase condition was evaluated for the SPE of amines in complex wastewater and soil samples after the collection and pretreatment of the samples. Effluent and influent wastewater samples were collected from a wastewater treatment plant (HSY, Viikinmäki, Helsinki, Finland), stored at 4°C and filtered before use with Millipore system (0.45 µm filter). Humus and mineral soil samples were collected at Station for Measuring Forest Ecosystem Atmosphere Relations (SMEAR II) in Hyytiälä (61°51'N, 24°17'E, 180 m above sea level), which is 230 km north of Helsinki. The forest consists of Scots pines (*Pinus sylvestris* L.). The trees were 30-40 years old and homogeneously spread through 200 m in all directions from the measurement site. Soil samples were stored at 4°C until needed.

Wastewater samples were treated before SPE as follows: sample was filtered and pH value measured (7.75 and 7.47 for influent and effluent, respectively). pH adjustment was not necessary since these values were two units higher than pK_a values of the aromatic amines (Table 1). Then, 10 mL wastewater sample was liquid-liquid extracted with 2 mL of hexane using vortex for 30 seconds. The procedure was repeated two times and the portions were collected and passed through the SPE cartridge according to the conditions described in Section 2.6. Finally, 20 µL of the aqueous extract was injected in the LC-UV system.

Soil samples (humus and mineral soil) were extracted with 20 mL NaOH solution (pH 11) due to the acidity of the soil and 10 mL hexane, and sonicated for 90 min in an ultrasonic bath Eurosonic 44 (J. Dienes Anwendungstechnik, Offenbach, Germany). After the solid-liquid extraction, the hexane portion was filtered with 0.45 µm filter and passed through the SPE cartridge according to the conditions described in Section 2.6. 20 µL of the extract was injected in the LC-UV system. The extracts obtained from wastewater and soil samples were then analyzed, and because no aromatic amines were detected, the water samples were spiked with 5 µg of the amines before filtering, and in case of soil samples, organic solvent was evaporated for 60 minutes.

2.3. Preparation of quaternary ammonium-functionalized silica

According to our previous work [14], the silica gel used as support material was activated to enhance the number of silanol groups on the surface, and to eliminate metal and nitrogenous impurities. Silica gel (18 g) was stirred with 200 mL nitric acid-water (50:50 v/v) at room

temperature for 2 h and refluxed for 8 h. The activated silica was filtered and washed thoroughly with deionized water until the effluent pH was neutral followed by the flushing with acetone, and drying overnight at 60°C [17].

2.3.1. 1-Alkyl-3-(propyl-3-sulfonate) imidazolium-functionalized silica (SiImPS)

Synthesis route of the zwitterionic IL-functionalized silica followed that described in our recent work [14]. Briefly, activated silica (10 g) was suspended in 100 mL of dry toluene, and 10 mL of (3-chloropropyl)trimethoxysilane was added, followed by 1 mL of triethylamine (added as a catalyst). The suspension was mechanically stirred and refluxed at 115°C for 24 h. The reaction was then stopped and the functionalized silica was cooled to room temperature, filtered and washed with toluene, ethanol-water mixture (50:50, v/v), deionized water and methanol. The chloropropyl silica (SilprCl) was dried overnight at 60°C. Next, SilprCl silica (5 g) was mixed with 5 g of imidazole in 60 mL of dry toluene. The mixture was refluxed with stirring for 24 h. The reaction was stopped and the IL-functionalized silica (SilprIm) was cooled to room temperature, filtered and washed with methanol, deionized water and again with methanol, and dried overnight at 60°C. Finally, 3 g of SilprIm was allowed to react with 3.3 mL of 1,3-propane sultone in 50 mL of dry toluene [18]. The mixture was refluxed with stirring for 24 h. After refluxing, the reaction was stopped and the IL-functionalized silica (SiImPS) was cooled to room temperature, filtered, washed with toluene, ethanol and acetone, and finally dried overnight at 60°C.

2.3.2. Hexaminium-functionalized silica

(3-chloropropyl)trimethoxysilane (2 g) was mixed with hexamine (1.2 g) in 10 mL of dry chloroform. The mixture was refluxed (70°C) with stirring for 24 h. The reaction was stopped and the 1-(3-trimethoxysilyl)propyl-1,3,5,7-tetraazaadamantan-1-ium chloride (hexaminium propyltrimethoxysilane chloride) (Fig. 1) was cooled to room temperature, filtered, washed with dry chloroform, and dried overnight at 70°C. It is important to notify that the yield of the reaction is generally quite poor.

Hexaminium propyltrimethoxysilane chloride (400 mg) was dissolved in 50 mL of dry chloroform and mixed with activated silica (150 mg). The mixture was refluxed (70°C) with stirring for 24 h. The reaction was quenched and the hexaminium-functionalized silica (Fig. 1) was cooled to room temperature, filtered and washed with dry acetone and dry chloroform, followed by a drying in air overnight at 60°C.

2.4. Characterization of materials

To confirm the immobilization reaction, the relative amounts of nitrogen, carbon and hydrogen in the activated silica, SiImPS and hexaminium-functionalized silica materials were determined by elemental analysis performed on a Vario MICRO analyser from Elementar Analysen Systeme GmbH (Hanau, Germany). In addition, FT-IR spectra between 650 and 4000 cm⁻¹ were measured with a Spectrum one FT-IR spectrometer from Perkin Elmer (Waltham, MA, USA).

2.5. Stability study of quaternary ammonium-functionalized silica

The extraction of the aromatic amines was carried out under normal phase conditions, avoiding the stability problems of the sorbent at basic pH. However, after the extraction, the pH value of the solution used for the conditioning was fixed at 0.4 (see next Section), and therefore control of SiImPS was essential. For this reason, repeatability was evaluated during and after 150 extractions with the same cartridge.

Because hexaminium salts are unstable in protic media and decompose to give various products depending on the pH value of the solution according to Blazevic et al. [16], hexaminium-functionalized silica (50 mg) was suspended in 6 mL of protic media (i.e. water, methanol, acetic acid and buffer solutions at pH 2 and 12) for 24 hours. Mixtures were mechanically stirred during the whole process. After the stirring, sorbent was filtered and dried overnight at 100°C. Elemental analysis was carried out to determine the hexaminium groups still attached to the silica surface.

2.6. Solid-phase extraction

Empty polypropylene cartridges (1 mL) equipped with two polyethylene discs were filled with 40 mg of SiImPS, hexaminium-functionalized silica and activated silica individually, and equilibrated by flushing with 6 mL of acetonitrile. Cartridges were connected to an SPE manifold (*IST* VacMaster-20, Biotage AB, Uppsala, Sweden), which was connected to a vacuum pump. The samples prepared in hexane (500 μ L, 6 mL or 10 mL) were uploaded onto the cartridges and washed with 1 mL of hexane. For the comparison purposes, elution was done with 500 μ L of acetonitrile and cleaning with 2 mL acetonitrile followed by 2 mL of hexane. After the selection of the optimum phase for the extraction (SiImPS), the elution was performed with 500 μ L of water. Cleaning of the cartridge was carried out with 1 mL water, regeneration with 4 mL HCl (pH 0.4) and conditioning with 1 mL acetonitrile and 1 mL hexane. Extracts were directly injected to the LC-UV system.

2.7. Liquid chromatography

A LC system from Hewlett-Packard (Palo Alto, CA, USA) equipped with a HP series 1050 pump, autosampler and a HP 1100 diode array detector set at 280 nm was employed. Chromatographic separation was carried out in a Ascentis C18 column from Supelco (150 mm $\times$ 4.6 mm, I.D. 5 μ m, Bellefonte, PA, USA) with the following gradient: 0-2 min 70% of A (1% acetic acid in water adjusted to pH 4 with NaOH), 2-5 min 60% A, 5-7.5 min 50% A, 7.5-15 min 100% B (acetonitrile), 15-20 min 70% A. Flow rate was 1 mL min⁻¹ and injection volume was 20 μ L. The actual analysis was performed at room temperature.

3. Results and discussion

Before the applicability of hexaminium-functionalized silica material synthesized could be tested as an extraction sorbent, its detailed characterization was needed for the confirmation of its structure. In addition, to clarify the suitability of a new material at different pH values, the stability tests were made. Furthermore, before the new hexaminium-functionalized silica material for the SPE of amines in complex environmental samples could be used, the recoveries obtained for amines were compared to those achieved with other materials. Hexaminium-functionalized silica was compared to SiImPS material for the extraction of five target amines under normal

phase conditions, and finally the better sorbent was subjected to further optimization and to the extraction of environmental samples.

3.1. Characterization of materials

3.1.1. FT-IR analysis

FT-IR spectra of the hexamine, hexaminium propyltrimetoxysilane chloride and hexaminium-functionalized silica were recorded between 650 and 4000 cm^{-1} . Fig. 2 shows the spectra recorded from 1000 and 1500 cm^{-1} for hexamine and hexaminium propyltrimetoxysilane chloride (first reaction Fig. 1), and the difference between both spectra is clearly seen. Among the different bands of hexaminium propyltrimetoxysilane chloride, the band around 1220-1170 cm^{-1} (2nd dotted circle) can be related to a $\text{Si-CH}_2(\text{CH}_2)_x\text{CH}_3$ group and the band around 1100-1080 cm^{-1} (1st dotted circle) can be related to a Si-OCH_3 group [19] meaning that the reaction was successfully carried out and hexaminium product was obtained.

Fig. 3 shows a comparison of the spectrum of activated silica and hexaminium-functionalized silica recorded from 600 and 1700 cm^{-1} . According to Ref. [20], trimethyl ammonium quaternary groups usually give rise to several bands in the 980-900 cm^{-1} and near to 1410, 1485 and 3020 cm^{-1} . In Fig. 3 two clearly distinguished bands (dotted circles), one around 980 cm^{-1} and the second one around 1475 cm^{-1} , can be related to the trimethyl ammonium quaternary groups. These results together with those obtained by the elemental analysis (results are shown in next Section) confirm that the activated silica was successfully modified by hexaminium propyltrimetoxysilane chloride. For SiImPS, as demonstrated in our previous work [14], the FT-IR analysis results confirmed the covalent bonding of the imidazole to the activated silica and the bonding of the 1,3-propane sultone to the imidazolium ring.

3.1.2. Elemental analysis

Nitrogen, carbon and hydrogen data from elemental analyses and the surface coverage of nitrogen and carbon on functionalized silica gel are summarized in Table 2. Carbon and nitrogen coverage was calculated as in our previous work according to Ref. [18]. From the percentage of carbon (%C), the concentration of organic groups attached to the silica surface was calculated to

be as 3.0 $\mu\text{mol}/\text{m}^2$ for SiImPS and 1.1 $\mu\text{mol}/\text{m}^2$ for the hexaminium-functionalized silica. From the percentages of nitrogen (%N), the concentration was 3.0 $\mu\text{mol}/\text{m}^2$ for SiImPS and 1.3 $\mu\text{mol}/\text{m}^2$ for hexaminium-silica. For SiImPS and hexaminium-functionalized silica, the presence of nitrogen enabled successful immobilization of the imidazole ring and hexamine ring to the silica surface, respectively. However, the coverage was lower for hexaminium-functionalized silica than for SiImPS attributing to the probable steric hindrance of the hexamine ring.

3.2. Stability of quaternary ammonium-functionalized silica

According to the stability study of the SiImPS carried out at different pH values (2, 8, 10 and 12) in our previous work, it was confirmed that at pH 2 the imidazole ring was still attached to the silica surface after the treatment, showing that the sorbent was stable at low pH values [14]. In the present work precision value was used as an indicator for the stability of the sorbet at pH 0.4. Repeatability values determined as variation coefficient and estimated after 150 extractions with the same cartridge, ranged between 4.5 and 10.2% (the complete results are shown in Section 3.5.) demonstrating the high stability of SiImPS even at extremely pH.

The stability of the hexaminium-functionalized silica was investigated with water, methanol, acetic acid as well as buffer solutions at pH 2 and 12 (Table 3). The nitrogen percentage of close to zero obtained for all the media proved that hexamine groups were unfortunately released after the treatments. Accordingly, hexaminium-functionalized silica was not stable in protic solvents demonstrated by Blazevic et al. [16]. As a consequence, hexaminium-functionalized silica was not used in the comparison study of the sorbents when protic solvents were used (see Section 3.3.).

3.3. Comparison of the sorbents

The comparison between the three sorbents was executed with aprotic solvents. As described in Section 2.6., the elution was done with acetonitrile. As can be seen from Table 4, the highest recoveries were obtained with activated silica followed by hexaminium-functionalized silica. The recoveries ranged from 7 to 68% for SiImPS and from 73 and 117% for activated silica, and those for hexaminium-functionalized silica were 103, 107 and 101% for o-toluidine, 4-

ethylaniline and quinoline, respectively. However, for N,N-dimethylaniline and N-isopropylaniline, the recoveries obtained were as low as 14% and 46%, respectively.

To clarify whether the low recovery values achieved with silica sorbents were caused by adsorption or elution, the sample effluents (passed through the cartridges) were also analyzed. Quinoline was found in the case of activated silica, which means that it was not completely retained (Table 4). The reason for the low recoveries obtained for N,N-dimethylaniline and N-isopropylaniline with hexaminium-functionalized silica was that the compounds almost totally penetrated through the cartridge without any retention. Because none of the aromatic amines were detected in sample effluents with SiImPS sorbent, aromatic amines were completely retained.

Retention of analytes under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface via hydrogen bonding, π - π interaction, dipole-dipole interactions, and dipole-induced dipole interactions, among others [15]. The low recoveries of hexaminium-functionalized silica for some of the amines can be related to the electronic properties and steric hindrance of the substituent nitrogen groups. The interaction possibilities of N,N-dimethylaniline and N-isopropylaniline including alkyl groups that caused steric hindrance, most probably decreased, opposite to the other three amines whose interaction capability was enhanced. The stronger retention seen with SiImPS could be caused by the π - π interaction in addition to hydrogen bonding with the sulfonic group.

As explained above, the low recovery values obtained with SiImPS were related with the elution strength of the solvent that disrupts the binding mechanism. The hydrogen bonding between the sulfonic group and the amines most presumably cause the strongest interaction. Therefore, the elution with de-ionized water that deprotonates the sulfonic group ($pK_a \sim 1$) and disrupts the interaction was studied. In this study, activated silica was also tested for the comparison, and the results obtained are shown in Table 5. As can be seen, the recoveries obtained with SiImPS sorbent ranged between 89 and 99%. The recoveries achieved with activated silica material were lower and ranged from 51 to 92%. The extraction recovery for quinoline obtained with SiImPS sorbent when water was used as an eluent, was higher than that obtained with extremely hydrophilic activated silica with acetonitrile eluent (Table 4). As a whole, the recoveries obtained with SiImPS showed that the aromatic amines dissolved in hexane were completely adsorbed and then eluted with water, a fact that rendered the procedure more environmental

friendly. For all the previously discussed reasons, SiImPS was selected as the stationary phase for the further extraction of the five aromatic amines.

3.4. Conditions of the solid-phase extraction

SiImPS was selected for the further extraction of aromatic amines that were released from the sorbent with water to deprotonate the sulfonic group and break the bonding. Since the sulfonic group must be protonated before the next extraction, the regeneration was performed with an acidic solution at pH that should be equal or below the pK_a value (~ 1) of sulfonic group. Accordingly, 4 mL of two acidic solutions of HCl, one of pH 1 and the second of 0.4, were tested. After the regeneration and conditioning with acetonitrile and hexane, the sample solution with the aromatic amines was passed through the cartridge, and the effluent from sample was collected and analyzed by LC-UV. At pH 1 small peaks were still seen in the effluent from sample, but at pH 0.4 no peaks were detected. Therefore, the regeneration was made at lower pH in further studies. To assure that the amines were completely eluted from the sorbent the elution was done with as high as 500 μ L of water. Finally, 20 μ L of the extract was directly injected into LC-UV. 1 mL of water was used for the cleaning of the sorbent to avoid possible memory effects. Then, 4 mL of hydrochloric acid at pH 0.4 was used for the regeneration of the sorbent, and 1 mL of acetonitrile as well as 1 mL of hexane were used to precondition the cartridge for the next extraction.

3.5. Performance of the extraction

Linearity, recoveries and repeatability were studied with the SiImPS sorbent by using the same cartridge. The linearity was evaluated from 0.5 to 50 μ g of each aromatic amine and the results are shown in Table 6. Correlation coefficient ranged from 0.995 for N-isopropylaniline to 0.9996 for 4-ethylaniline, demonstrating a good linearity in the range studied. The recovery values were between 88% for o-toluidine and 97% for N-isopropylaniline, confirming a good extraction efficiency (Table 6).

The repeatability was studied intraday with five replicates; in three consecutive days (interday); and in five different days, with one of them after 150 extractions (interday); and finally intraday

with three different cartridges. The results are listed in Table 7. Coefficients of variation were lower than 12%, and almost a half of them below 5%. These results demonstrate that the method developed for the extraction of aromatic amines by SiImPS under normal phase conditions provided a good precision within the same day, for different days, with different cartridges and even after 150 extractions. In addition, 150 successive extractions after the conditioning with HCl at pH 0.4 proved the stability of the sorbent.

3.6. *Extraction of amines in environmental samples*

Finally, the suitability of the SiImPS sorbent for the extraction of the five aromatic amines in complex environmental matrices such as wastewater (influent and effluent) and soil (mineral and humus) samples was studied. As demonstrated in Section 2.2., samples did not contain aromatic amines at concentrations above their detection limits. Although, it is well known that spiking does not necessarily provide the complete information of the matrix effects in environmental samples, the technique was used, and the samples were spiked with 5 µg of each aromatic amine to give at least some idea about possible matrix interactions.

Wastewater samples were filtered after spiking, and LLE and SPE procedures were carried out as described in Section 2.2. The results obtained for influent and effluent wastewater samples are presented in Table 8. As assumed, the recoveries achieved in effluent were higher than in influent due to the differences in the matrix complexity. Recoveries ranged from 23 to 96% for influent and from 43 to 97% for effluent. Low recoveries were obtained for o-toluidine and N,N-dimethylaniline, in both wastewater samples and for N-isopropylaniline in influent wastewater. Before evaluating these results it is important to point out that in the procedure used the LLE carried out as well might affect the recoveries. For this reason, recoveries of the LLE procedure with amines dissolved in de-ionized water were determined, being 59% for o-toluidine, 70% for N,N-dimethylaniline, and between 88 and 101% for the rest of the aromatic amines. Table 8 demonstrates the recoveries after the loss caused by LLE. As can be seen from the corrected recoveries, influent samples caused high matrix effects for N,N-dimethylaniline and N-isopropylaniline. For the other three compounds no matrix effects were observed for influent samples, and only N,N-dimethylaniline was slightly affected by effluent matrix.

Soil samples were also spiked with 5 μg and extracted according the procedure described in Section 2.2. Low recoveries below 38% obtained for humus and mineral soil could be caused by a poor extraction from the soils or by matrix effects (Table 9). However, according to previous studies [2,21] the extraction of amines from soil is usually quite poor under acidic conditions. Active acidic sites on the sand surface are e.g. responsible for the lower recoveries of amines, and the matrix interactions have been found to be even stronger when humic soil is extracted [21]. Nevertheless, our results demonstrate the applicability of SiImPS for the SP extraction of amines from complex matrices.

4. Conclusions

A novel, successfully synthesized SPE sorbent material, namely hexaminium-functionalized silica, provided much higher extraction efficiencies for o-toluidine, 4-ethylaniline and quinoline than SiImPS. Although the extraction of N,N-dimethylaniline was not successful due to the elution through the extraction cartridge without almost any interactions with the sorbent, hexaminium-functionalized silica sorbent opens up opportunities for the development of new SPE materials with different selectivities.

In addition, SiImPS with a high flexibility and stability showed great promises as SPE sorbent under normal phase conditions, and therefore, was selected for the sorbent in further studies. The results demonstrated that SiImPS is an excellent alternative for the extraction of aromatic amines. The advantage of SiImPS is its versatility as the sorbent, it is useful under normal phase conditions for the extraction of aromatic amines and under reverse phase conditions for the extraction of organic acids, as shown in our previous work. The possibility to elute aromatic amines with pure water underlines the value of the developed method in terms of environmental and eco-toxicological aspects.

Acknowledgements

M.-L.R. and J.-P.M. would like to thank the Academy of Finland Centre of Excellence program (project. no.1118615) for financial support, and L.V. thanks "Generalitat Valenciana" (Spain) for her post-doctoral grant. In addition, financial support was provided by Kempe Foundations as well as Knut and Alice Wallenberg Foundations (J.-P.M.).

References

- [1] L.S. Lee, C.T. Jafvert, H. Li, J.R. Fábrega-Duque, Modeling Soil-Water Distribution of Aromatic Amines in Saturated Soil Systems. EPA/600/S-99/005. April 2000.
- [2] C.M. Pace, J.R. Donnelly, J.L. Jeter, W.C. Brumley, G.W. Sovocool, J. AOAC int. 79 (1996) 777.
- [3] H. Li, L.S. Lee, J.R. Fabrega, C.T. Jafvert, Chemosphere 44 (2001) 627.
- [4] R. Li, Y. Zhang, C.C. Lee, R. Lu, Y. Huang, J. Chromatogr. A 1217 (2010) 1799.
- [5] T. Kahl, K.-W. Schröder, F.R. Lawrence, W.J. Marshall, H. Höke, R. Jäckh, Aniline in Ullmann's Encyclopedia of Industrial Chemistry, John Wiley & Sons, New York, 2007.
- [6] M. Kulmala, T. Petäjä, Science 333 (2011) 1586.
- [7] EPA Method 1625, Fed. Reg. US Government Print Office, Washington, DC, September 1994.
- [8] Y. Sun, L. Liang, X. Zhao, L. Yu, J. Zhang, G. Shi, T. Zhou, Water Res. 43 (2009) 41.
- [9] M. Aznar, E. Canellas, C. Nerín, J. Chromatogr. A 1216 (2009) 5176.
- [10] C. Molins-Legua, P. Campins-Falcó, Anal. Chim. Acta 546 (2005) 206.
- [11] L. Vidal, M.-L. Riekkola, A. Canals, Anal. Chim. Acta 715 (2012) 19.
- [12] J.L. Anderson, D.W. Armstrong, Anal. Chem. 77 (2005) 6453.
- [13] F. Zhao, Y. Meng, J.L. Anderson, J. Chromatogr. A 1208 (2008) 1.
- [14] L. Vidal, J. Parshintsev, K. Hartonen, A. Canals, M.-L. Riekkola, J. Chromatogr. A 1226 (2012) 2.
- [15] Guide to Solid Phase Extraction, Bulletin 910, Sigma-Aldrich 1998
<http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf>
- [16] N. Blazevic, D. Kolbah, B. Belin, V. Sunjic, F. Kajfez, Synthesis 3 (1979) 161.
- [17] M. Tian, H. Yan, K.H. Row, J. Chromatogr. B 877 (2009) 738.
- [18] H. Qiu, Q. Jiang, Z. Wei, X. Wang, X. Liu, S. Jiang, J. Chromatogr. A 1163 (2007) 63.
- [19] P.J. Luner, Silicone Compounds Register and Review, Edited by B. Arkles et al Petrarch Systems, 1987. New York. Infrared analysis of organosilicon compounds: spectra-structure correlations.
- [20] N.B. Colthup, L.H. Daly, S.E. Wiberley, Introduction to Infrared and Raman Spectroscopy, 3rd ed., Academic Press, United Kingdom, 1990.
- [21] V. Janda, J. Kríz, J. Vejrosta, K.D. Bartle, J. Chromatogr. A 669 (1994) 241.

Figure Captions

474

475

476 Fig. 1. Synthesis route of the hexaminium-functionalized silica.

477 Fig. 2. FT-IR spectrum of hexamine and hexaminium propyltrimetoxysilane chloride.

478 Fig. 3. FT-IR spectrum of activated silica and hexaminium-functionalized silica.