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Comparison of Sorbents Using Semipolar to Highly Hydrophilic Compounds for a Sequential Solid-Phase Extraction Procedure of Industrial Wastewaters

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Untreated and biologically cleaned industrial wastewaters contain huge amounts of both hydrophilic and hydrophobic compounds. Due to breakthrough effects, only a few percent of the dissolved organic carbon is usually extracted by solid-phase extraction methods. Methods for screening analysis have not faced that problem sufficiently yet. To extract more hydrophilic compounds, solid-phase sorbent characteristics had to be evaluated in this study. Recovery studies were carried out with 21 hydrophilic aromatics including carboxy acids, sulfonates, aldehydes, ketones, thiols, and phenols at a level of 1−2 μmol/L. Both silica gel based sorbents and polymeric materials were tested at neutral to acidic pH values and extraction volumes up to 200 mL. It was found that poly(styrene−divinylbenzene) sorbents are a powerful tool for the enrichment of hydrophilic aromatic substances. Most of the tested compounds could be extracted at recovery rates of >80%. The quantitative extraction of thiols as well as (aminohydroxy)disulfonic acids remains an unsolved problem. Finally, combination of different solid sorbents and pH changes is suggested for sequential extraction of heavily loaded industrial wastewaters. Advantages and limitations are discussed and an example of its usefulness is given.

Industrial wastewaters may contain high amounts of dissolved organic carbon (DOC). In conventional chemical screening analysis such as liquid−liquid extractions (LLEs) followed by GC/MS, the detected compounds normally account for only a few percent of the original sample−DOC.[1−3] It is assumed that the missing DOC does not include compounds of special interest. However, industrial chemicals like sulfonated organics are highly hydrophilic.[4] Others may not be found after biological or chemical transformation in wastewater treatment plants due to their increasing polarity.[5,6]

High preconcentration factors for nonpolar to medium polar analytes have been well-known for SPE methods for ∼20 years. SPE has been applied for nontarget analysis like surface water monitoring,[7,8] toxicity tracking procedures,[9−11] or, most regularly, analysis of compounds of special interest. Its drawbacks, however, have been rarely pointed out clearly.

For example, it has often been impossible to extract polar (hydrophilic) organic solutes with octanol−water partition coefficients of log K_{ow} < 2 quantitatively by means of SPE without addition of ion-pairing reagents, when more than 100 mL was extracted.[12] Compounds more hydrophilic than phenol have mostly not been included in recovery studies or were not extractable.[13−15] However, carboxylic acids as well as hydroxy compounds, amines, or organic sulfonates have been detected quite regularly in analysis of both anaerobic and aerobic treated wastewater streams[16] when LLEs were applied.

Further, losses of semivolatile substances have been reported during drying or incomplete elution from solid sorbents.[17−19] Sampling rates, solvent permeation times, pH values, and effects of salts, solvents, or detergents have been rarely studied systematically.[20,21] Therefore, LLE methods are often favored for GC/MS screening analysis.[22,23]

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Recently some new methods for the extraction of polar organics using non silica gel based sorbents have been reported. Phenol was efficiently extracted using a polymeric material.[24] However, breakthrough occurred at extraction volumes exceeding 100 mL, even when counterions were added. Aromatic sulfonates in textile wastewaters were selectively extracted using graphitized carbon black and ion-pairing,[25] but recoveries of compounds with mixed polar functionalities were generally low. Breakthrough volumes were severely influenced by matrix effects, so extraction volumes were suggested not to exceed 50 mL.

Following these studies, breakthrough volumes and pH dependencies of the extraction of both hydrophilic and semipolar compounds were studied. Substances with different functionalities, fugacities, and reactivities had to be employed to assess the applicability of solid sorbents. The SPE materials were expected to reveal different characteristics in respect to capacity and breakthrough volumes of the analytes. Ion exchange properties were not relevant since inorganic salt contents in industrial wastewaters can be up to 40 g/L.

At least six suppliers offer poly(styrene−divinylbenzene) polymers (PS-DVB) for extraction purposes. Some of these polymers, however, exhibit exactly the same physical properties[26] (PRP1 and PL-RPS), or extraction efficiencies given in the literature were not satisfying (XAD resins). Differences between the others are not pointed out clearly by the suppliers. So, only two of them were chosen for a comparative study.

It was an aim of this study to develop an extractive fractionation procedure for industrial wastewaters followed by toxicity testing. Method development was constrained to suitable solvents and conditions. Mild conditions were required to prevent artifactual toxicity, and complete solvent exchange was avoided due to losses of semivolatile organics. Therefore, recovery tests were carried out with HPLC/UV and not with GC analysis, which would have required water-free conditions. SPE eluates were restricted to methanol, which is known to be suitable for biotests at contents up to 1% (v/v) after back-dilution into water.

EXPERIMENTAL SECTION

Chemicals. Solid-phase extraction tubes from the following companies were investigated: 500 mg cartridges Supelclean LC-8, LC-Ph, LC-NH_2, 250 and 500 mg Supelclean EnviChrom from Supelco (Bellefonte, PA); 500 mg cartridges Chromabond NO_2, PA, and DMA from Macherey-Nagel (Düren, FRG); 500 mg LiChrolut RP-18, 500 mg LiChrolut RP-18e, and 250 mg LiChrolut EN from Merck (Darmstadt, FRG). All solvents were delivered by Merck in gradient-grade quality. Double-distilled water was employed for standard solutions. Test compounds as given in Table 1 were purchased from following companies: compounds **1**, **2**, **6**, **10**, **17**, **18**, and **20** from SAF (Deisenhausen, FRG) and **3**−**5**, **7**−**9**, **11**−**16**, **19**, and **21**, and **22** from Merck. Cellulose acetate filters were obtained from Sartorius (Göttingen, FRG).

Samples. Stock solutions of equal amounts of **1**−**6**, and **8** (mix I) and **9**−**22** (mix II) in methanol were prepared (Table 1). NaCl (4 g/L) was added to pure water and pH values were adjusted with 1 mmol/L Na_2HPO_4, NaH_2PO_4, and H_3PO_4 for pH 7 and 4.5 and 2.5, respectively. The water was spiked with aliquots of the stock solutions to reach concentrations given in Table 1. Aliquots (25, 50, 100, and 200 mL) of these solutions were extracted in single tests. A combination of mixes I and II containing **2**−**6**, **8**, and **11**−**20** (mix III) was prepared for final recovery tests with sequential extractions of spiked waters.

All cartridges were conditioned with 7 mL of methanol and 10 mL of water prior to use. Extraction rates were 1−2 mL/min. After extraction, the tubes were washed with 10 mL of water of the pH value and ionic strength of the sample and eluted with 7 mL of methanol. They were vacuum-dried for only ∼30 s to prevent losses of volatile organics. Methanolic eluates were concentrated in a speed vacuum concentrator at a chamber temperature of 40 °C to reach a volume of ∼0.7 mL. Water content in the resulting mixtures was estimated to reach up to 300 μL following this procedure, once determined by addition of 500 μL of water-immiscible dichloromethane.

A treated tannery wastewater (250 mL) was extracted and analyzed following the proposed method (see below) after filtration over 0.45 μm cellulose acetate filters. The tannery wastewater treatment pilot plant is described in detail elsewhere.[27]

Analytical Apparatus and Procedure. Dissolved organic carbon content of the wastewater samples was determined with an AstroLiquiTOC 2001-MB analyzer (Foss-Heraeus, Hanau, FRG) and UV absorbance on a Lambda-2 spectrophotometer (Perkin-Elmer, Überlingen, FRG).

HPLC analysis were carried out on a 30 cm × 4.6 mm i.d. LC-ABZ, column 5 μm (Supelco) (mix I); a 30 cm × 4.6 mm i.d. Eurosphere 80 C_4 column, 7 μm (mix II); and a 25 cm × 4 mm i.d. Eurosphere 100 C_8 column, 5 μm (mix III and wastewater eluates); the latter two columns were supplied by Knauer, Berlin. The HPLC system was equipped with an L-6200A gradient pump, an AS-2000 A autosampler, and a T-6300 column thermostat (all from Merck-Hitachi, Darmstadt, FRG). UV detection was performed with a Shimadzu SPD-10AV UV−VIS detector (Kyoto, Japan) and a Waters 990 diode array detector (Milford, MA). Data storage and quantification was carried out using ChromStar software provided by Bruker (Bremen, FRG). Samples were concentrated in a speed vacuum concentrator from by Savant (Farmingdale, NY).

LC separations were performed with acetonitrile−water gradients. Pure water containing 1 mmol/L NaH_2PO_4 was adjusted to exactly pH 2.40 with H_3PO_4 and served as solvent A. Solvent B was an acetonitrile−water mixture (95:5). Elutions for mixes I and II were at constant flow rates of 1 mL/min and were performed at constant column temperatures of 40 °C. Elution for mix I started with 85% solvent A and was linearly shifted to 30% A at 12.5 min and 40% A at 20 min. Elution for mix II started with 88% solvent A and was linearly shifted to 58% A at 10 min and 21% A at 22 min. Mix III was eluted starting with 90% A, followed by a linear gradient to 74% A at 15 min, 65% A at 20 min, and a 10 min isocratic run. Chromatographic separations of wastewater eluates started with 80% solvent A which was linearly diminished to a content of 70% A at 15 min and 30% A at 20 min, followed by a 20 min isocratic run. A 10 min equilibration time back to start conditions followed for all elution procedures.

UV detection was performed in the dual-wavelength mode at 206 and 238 nm. Peak identification was based on the retention times of the corresponding standards combined with the area

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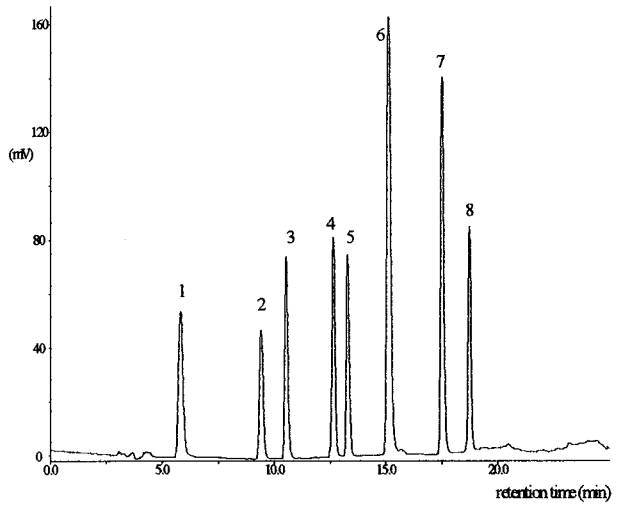

Figure 1. Standard HPLC chromatogram (206 nm) of mix I, separated on an LC-ABZ column with an acetonitrile−water gradient: 1, hydroquinone; 2, 4-hydroxyphenylacetic acid; 3, 4-hydroxybenzoic acid; 5, phenylacetic acid; 6, 2,4-dihydroxybenzoic acid; 7, toluene (int std); 8, indene-2-carboxylic acid.

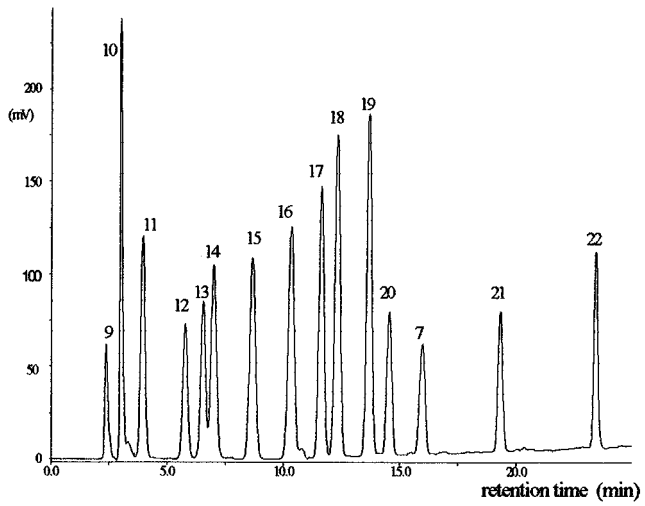

Figure 2. Standard HPLC chromatogram of mix II (206 nm), separated on a C_4 column with an acetonitrile−water gradient: 9, 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid; 10, benzylamine; 11, 3′-aminoacetophenone; 12, 4-amino-2-hydroxybenzoic acid; 13, phenol; 14, 2-naphthylamine; 15, 2-naphthylsulfonic acid; 16, 2-hydroxy-5-methoxybenzoic acid; 17, *m*-cresol; 18, acetophenone; 19, 3-methylbenzoic acid; 20, cinnamic aldehyde; 7, toluene, 21, 2-aminothiophenol; 22, 2-naphthylthiol.

ratios of the peaks detected at 206 and 238 nm. Quantification was carried out using external standards. To all recovery test samples was added 10 μL of a stock solution of toluene in methanol prior to injection for sample volume correction using internal standards. Aliquots (20 μL) were injected into the HPLC system.

RESULTS AND DISCUSSION

Analysis. RP-HPLC separations on C_{18} silica gels of mixtures of highly hydrophilic compounds normally result in low retention times and bad separations. Normal-phase separations are not preferable due to bad peak shapes for basic compounds and lack of compatibility to aqueous injections or elution conditions. The carboxylic acids and hydroxy compounds of mix 1 could not be fully separated with C_{18}-, C_8-, C_4-, and C_1-modified silica gels, even using gradient elutions and different pH values. Using a newly developed deactivated column, LC-ABZ, a reliable separation could be achieved (Figure 1). Sulfonic acids, however, were not able

Table 1. Concentrations and Peak Numbers of Model Compounds and Comparison of Their Recoveries (%)[a] from 200 mL of Spiked Pure Water at Different pH Values with Four SPE Phases

conc (μmol/L)	compd name and peak no.		C_{18}			C_8			EN			EnviChrom		
			pH 7	pH 4.6	pH 2.5	pH 7	pH 4.6	pH 2.5	pH 7	pH 4.6	pH 2.5	pH 7	pH 4.6	pH 2.5
	mix I													
0.91	hydroquinone	**1**	5	1	−	−	−	−	5	70	80	1	3	4
0.66	4-hydroxyphenylacetic acid	**2**	−	−	−	−	−	7	10	97	96	−	45	94
0.73	4-hydroxybenzoic acid	**3**	−	−	−	−	−	7	29	97	102	−	58	97
0.61	4-hydroxycinnamic acid	**4**	1	−	6	−	25	99	52	97	98	11	90	100
0.74	phenylacetic acid	**5**	2	−	8	−	10	29	48	96	94	6	80	90
0.65	2,4-dihydroxybenzoic acid	**6**	1	−	−	−	3	15	6	90	96	−	12	97
int std	toluene	**7**												
0.62	indene-2-carboxylic acid	**8**	4	1	23	−	19	98	59	94	94	92	72	99
	mix II													
0.37	4-amino-5-hydroxynaphthalene-2,7-disulfonic acid	**9**	−	−	−	−	−	−	−	−	−	−	−	−
2.29	benzylamine	**10**	7	−	−	9	1	−	−	5	6	14	9	2
1.47	3′-aminoacetophenone	**11**	51	32	2	24	24	1	57	52	22	88	88	91
0.88	4-amino-2-hydroxybenzoic acid	**12**	13	1	4	1	2	7	4	98	49	−	50	51
2.35	phenol	**13**	5	4	6	4	4	8	42	83	101	85	87	91
1.02	2-naphthylamine	**14**	80	80	12	96	63	24	52	27	12	88	42	51
0.60	2-naphthylsulfonic acid	**15**	15	20	46	1	10	1	46	95	96	97	98	105
1.04	2-hydroxy-5-methoxybenzoic acid	**16**	27	29	31	23	23	20	44	85	98	85	90	89
2.69	*m*-cresol	**17**	10	23	101	9	20	90	−	96	106	33	98	101
1.92	acetophenone	**18**	56	71	73	68	68	52	34	78	91	58	76	56
1.33	3-methylbenzoic acid	**19**	2	65	95	3	50	100	53	86	102	43	87	96
1.78	cinnamic aldehyde	**20**	75	75	96	83	81	86	29	60	80	61	60	68
1.72	2-aminothiophenol	**21**	−	−	−	−	−	−	−	−	−	−	−	−
0.93	2-naphthylthiol	**22**	−	−	−	−	−	−	−	−	−	−	−	−

[a] −, below 1%.

to pass this column, so another one had to be found for mix II, which contained amines, thiols, sulfonic acids, phenols, carboxylic acids, aldehydes, and ketones, both with one functional group and in combination with another polar functionality. Even more, some of the selected compounds, such as acetophenone or phenol, were semivolatile whereas others were easy to oxidize (thiols) or susceptible to nucleophilic attack (cinnamic aldehyde). Separation of this complex mixture was successful using a C_4 column (Figure 2). For final extraction tests, 16 of these compounds could be reliably separated on a new C_8 column in one run (mix III, not shown).

Detection was performed at 206 nm to get low detection limits, which were between 1 and 2 ng per analyte injected onto the column. Calibration curves were linear from 10 to 1000 ng per analyte injected. Assuming 200 mL aliquots of samples are extracted and concentrated to 700 μL of solvent prior to injection of a 20 μL aliquot, this results in a detection limit of ~1.5 μg/L per analyte or 1% recovery of the spiked model mixtures.

Extraction of Standard Solutions. Volume reduction of SPE eluates is commonly performed using a rotary evaporator followed by heating at 40 °C under a gentle stream of N_2. In preliminary tests (not shown) it was found that compared to a speed vacuum concentrator this method is somewhat less effective for recoveries of semivolatile organics such as phenol or acetophenone. Minimum volumes should not be lower than 750 μL due to the water content of the SPE eluates; otherwise semivolatile compounds are at least partially lost. Following these precautions, phenol can be almost quantitatively preconcentrated. However, thiophenol, originally intended to be included in recovery studies, could not be preconcentrated by either method. Some of the cartridges showed huge peaks in HPLC/UV analysis after blank extractions.

Nitrophenyl-modified silica gels (Chromabond NO_2) were intended to extract aprotic polar aromatics. However, they eluted with a yellow color. Repeating the conditioning step twice did not result in clear HPLC chromatograms, so these cartridges were not investigated any further. As polyamide phases (Chromabond PA) eluted with strongly absorbing compounds at 220 nm after blank extractions, they were not included in further studies, too. Blank extractions using phenyl-modified cartridges (LC-Ph) eluted with a single peak in HPLC chromatograms with a peak height of more than 1.0 aufs. Peak identity was not confirmed but assumedly was due to weakly bonded phenyl residues.

Recoveries were below 10% for most of the analytes using aminopropyl-modified silica gels (LC-NH_2), despite of varying pH conditions and extraction volumes. Since these sorbents usually serve as normal-phase material in clean up steps, irreversible adsorption might have taken place using them as RP extraction cartridges. The only exception was *m*-cresol with 26% recovery at an extraction volume of 100 mL. Similar results were obtained using (dimethylamino)propyl-modified sorbents (DMA). Therefore, recoveries reached with these sorbents (NO_2, PA, Ph, NH_2, and DMA) are not given in detail.

Recoveries of 21 analytes at three pH values comparing the remaining four sorbent materials (C_{18}, C_8, EnviChrom, and EN) are given in Table 1 for the extraction volume of 200 mL. All tests were carried out in single runs. As they were only done for the evaluation of sorbent characteristics and not to establish routine analysis, determinations of standard deviations were not necessary. However, they can be estimated using data at different extraction volumes for analytes that appeared not to exhibit breakthrough effects. In these cases, standard deviations were below 8% for nonvolatile compounds and below 10% for acetophenone, phenol, and cinnamic aldehyde.

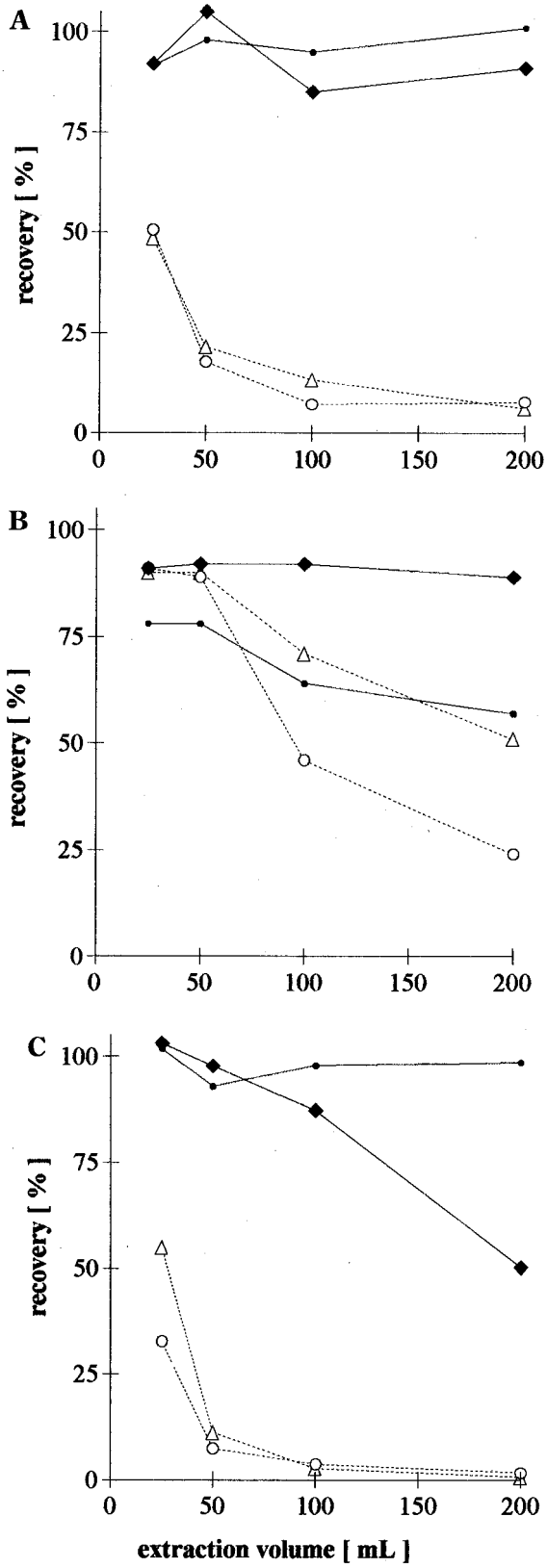

Figure 3. Breakthrough volumes of selected compounds at suitable pH values using different SPE sorbents (concentration 1−2 μmol/L (see Table 1): (A) phenol, pH 2.5; (B) 3′-aminoacetophenone, pH 7; (C) 4-amino-2-hydroxybenzoic acid, pH 4.5. △, C_{18}; ○, C_8; ◆, EnviChrom; ●, EN.

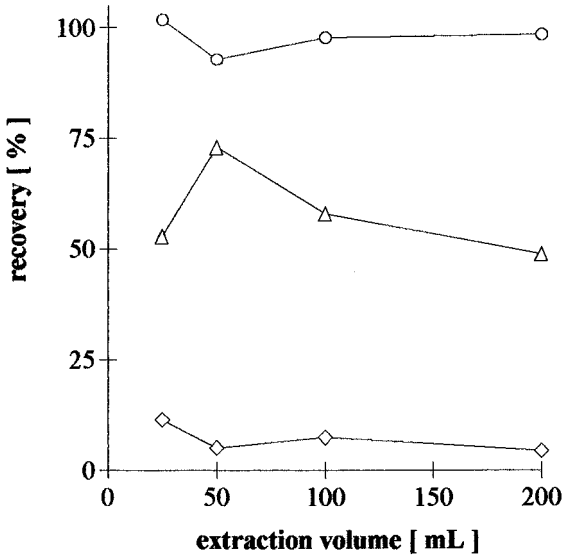

Figure 4. Breakthrough volumes of 4-amino-2-hydroxybenzoic acid (0.88 μmol/L) at different pH values using EN sorbents: △, pH 2.5; ○, pH 4.5; ◇, pH 7.

Three of the analytes could not be recovered at all. First, the thiol compounds **21** and **22** were replaced with some new peaks eluting even later. These peaks could belong to the corresponding disulfides after oxidation though confirmation was not carried out. There was no correlation between loss of thiols and lack of recovery of aldehydes or ketones. Since oxidizing agents were not included in mix II, oxidation reactions must have taken place during the extraction process or the preconcentration step. Reasons for these oxidations remain unclear, so further studies with even milder conditions for the extraction of thiol compounds have to be carried out. Second, the most polar analyte of all (**9**, a dye precursor) was not recoverable with any of the cartridges at any pH value. Sometimes a single peak eluted at the retention time corresponding to this disulfonic acid, but the ratio of areas detected at 238 and 206 nm did not fit. Therefore, ion-pairing agents still have to be used for quantitative extraction of disulfonic acids.

Some remarkable differences in between the SPE tubes have to be pointed out. Extraction of phenols was performed best at acidic conditions (Figure 3A). Highly polar phenols (**1**−**3**, **6**, **12**, **13**) were only recoverable using EN or EnviChrom phases with pH values of at least pH 4.5. Astonishingly, hydroquinone (**1**) could only be extracted sufficiently using EN cartridges. Slight changes in polarity (**4**, **17**) made phenols also accessible to RP silica gels in a strongly acidic milieu.

Amines (**10**, **11**, **14**) were best recovered out of neutral waters. This was especially the case for semipolar compounds such as naphthylamine. As shown for the example of 3′-aminoacetophenone, generally lower breakthrough effects were observed using PS-DVB phases instead of RP silica gels (Figure 3B). EnviChrom phases were superior compared to EN cartridges. Benzylamine, however, was poorly recovered in all cases. Assumedly it was not effectively retained, since extractions of amines and diamines usually require basic conditions. As RP silica gels do not withstand such conditions, the comparison of the SPE tubes was not extended to basic pH values.

Compounds containing both acidic and basic functionalities should give satisfying results at moderate pH values, if they are extractable at all. It is demonstrated that RP silica gels could not recover 4-amino-2-hydroxybenzoic acid satisfactorily whereas it was completely extracted using EN cartridges (Figure 3C). Compared to EN, clearly less retention was observed on EnviChrom phases. Recovery was greatly influenced by pH changes (Figure 4).

Acidic compounds cannot be recovered efficiently with commonly used RP silica gel sorbents, especially at high extraction volumes and at moderate pH values. Although C_8 phases have been expected to reveal better results than C_{18} sorbents, this had been true only partially (**4**, **8**), even in strongly acidic milieus. Compounds were more easily extractable using C_{18} cartridges (**15**, **16**) if they included nonpolar parts in their molecular structure. Mostly, there were only slight differences between C_{18} and C_8 phases. Carboxylic and sulfonic acids were best recovered using polymeric phases. Recoveries were less dependent on pH-values compared to RP silica gels. In fact, 2-naphthylsulfonic acid could be quantitatively extracted using EnviChrom cartridges without breakthrough even at pH 7, where it is clearly anionic. Mean recoveries of this analyte for all pH values were 99 ± 4%. However, extraction of acidic compounds at pH 4.5 with EN phases mostly gave better results as compared to EnviChrom phases (**1**−**8**, **12**).

Polar aprotic compounds (**18**, **20**) were best extracted from acidified waters. Mean recoveries for extraction volumes from 25 to 200 mL for cinnamic aldehyde using C_{18} cartridges were 70 ± 5% at both pH 7 and pH 4.5 but 95 ± 2% at pH 2.5. This effect is not completely understood but was not studied any further. EnviChrom material was somewhat more effective compared to EN cartridges, whereas RP silica gels gave even better results. In general, recovery results are satisfactory since both compounds are known as semivolatile and reactive.

Summarizing these results with model compounds, a sequential extraction scheme for nontarget water analysis is proposed. First, a C_{18} phase selects all neutral hydrophobic compounds at pH 7. The use of *end-capped* phases (C_{18}e) ensures that polar compounds completely pass this cartridge. Second, at pH 7 the C_{18}e filtrates pass through an EnviChrom cartridge, where slightly polar compounds are retained. Sorbent mass was doubled to reach 500 mg to avoid sorbent overloading for medium polar compounds, especially for extractions of heavily loaded wastewaters. For the third step, the sample is acidified to pH 4.5 for extraction of compounds with mixed functionalities. Using EN phases in this step, the majority of acidic compounds is expected to be also recovered. After lowering the pH to 2.5 the sample should be passed again over an EN cartridge to ensure completeness of the extraction of acidic compounds.

These predictions were verified by extracting 250 mL of pure water spiked with 16 of the tested compounds using the procedure suggested as above. This test was run in duplicate and mean recoveries are given in Table 2. All analytes with the exception of cinnamic aldehyde could be obtained at total recovery rates exceeding 80% despite their differences in fugacity, reactivity, and polarity. Most of them eluted mainly in one fraction, so a fair polarity separation could be achieved. In fact, the fourth extraction step (EN, pH 2.5) seems not to be needed in regard of recoveries of these model compounds. Extractions of wastewater samples, however, clearly show that this step cannot be left out (see below).

Due to the enlarged sorbent mass, 4-hydroxycinnamic acid (**4**) was eluted to a higher extent into the EnviChrom fraction as could be expected according to the results given in Table 1. In fact,

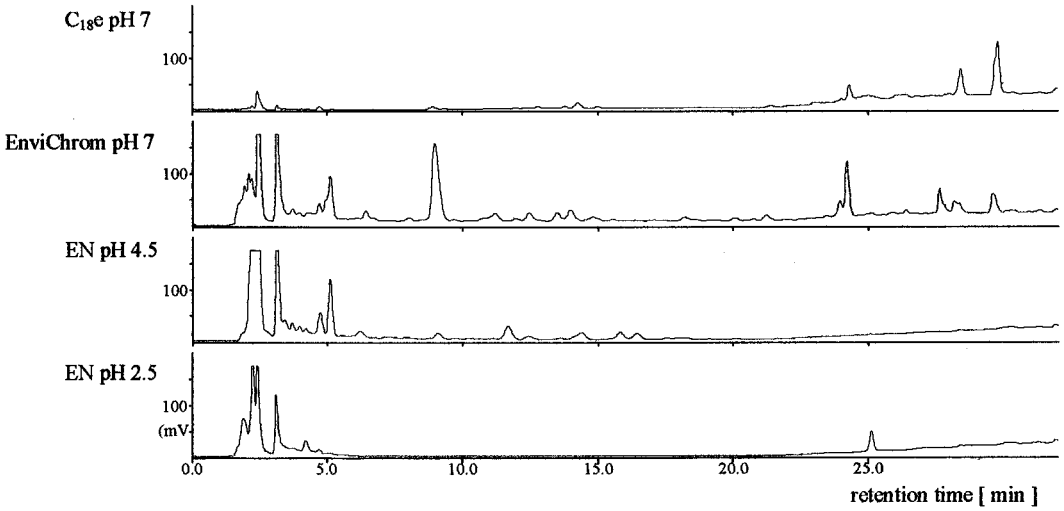

Figure 5. HPLC UV chromatograms (206 nm) of eluates of 250 mL of aerobically treated tannery wastewater following the proposed sequential extraction method.

Table 2. Recoveries (%) of 16 Hydrophilic Compounds (Mix III) following the Proposed Sequential Extraction Method from 250 mL of Spiked Pure Water[b]

compd no.	C_{18}e pH 7	Envi pH 7	EN pH 4.5	EN pH 2.5	sum
2	–	–	96	1	97
3	–	–	97	–	97
4	–	38	42	–	80
5	–	–	87	–	87
6	–	–	93	1	94
8	2	84	15	–	101
11	13	76	4	–	93
12	–	–	92	4	96
13	1	76	7	6	90
14	69	22	–	–	91
15	9	87	–	–	96
16	10	53	25	–	88
17	5	39	44	–	88
18	14	54	25	–	93
19	1	39	56	–	96
20	15	44	–	–	59

[a] –, below 1%. [b] Concentration, 1−2 μmol/L (see Table 1).

Table 3. SAC_{280} in Filtrates during Sequential Extraction of 250 mL of Aerobically Treated Tannery Wastewater and Recovery of SAC_{280} in Back-Diluted Eluates (% of Sample SAC)

sample	filtrate	eluate
	100	
C_{18}e pH 7	92	6
Envi pH 7	61	25
EN pH 4.5	38	14
EN pH 2.5	24	7

only those acids seem to pass this cartridge completely that do not include an additional nonpolar part in their molecular structure. For improving polarity separation during the extraction process, use of only 250 mg EnviChrom sorbent could be favorable.

This fractionation scheme has to face two severe restrictions. The behavior of aliphatic compounds cannot be predicted since only aromatic model compounds were tested individually. Assumedly, nonpolar aliphatics such as cyclohexane will be retained by the C_{18}e phase, whereas highly hydrophilic short-chain aliphatics such as oxalic acid probably will not be recovered at all by any phase. Secondly, the extraction and preconcentration procedure for organic thiols has to be somewhat milder. These compounds cannot be recovered unmodified. Extremely basic or extremely acidic compounds (i.e., diamines or disulfonic acids) require more drastic conditions (basic pH for amines, addition of counterions for sulfonates). When basic toxicants are expected, the method should be extended to pH 9 and 12.

Extraction of Tannery Wastewater. The applicability of the method is demonstrated by extracting aerobically degraded tannery wastewater. Table 3 shows the decrease of the UV absorbance at 280 nm (SAC_{280}) during the four steps of the sequential extraction scheme. Only 24% of the SAC_{280} originally included in the sample was not extracted by either step. The extracted compounds could be eluted from the cartridges with good results. However, SAC_{280} recovery from the EN sorbents was somewhat less efficient compared to the C_{18}e and EnviChrom cartridges. Predictions of the behavior of organic solutes were justified. A small part of the SAC_{280} is extracted by using the hydrophobic C_{18}e phase. The main part of the UV-adsorbing compounds was retained by the EnviChrom cartridge at pH 7 and the EN phase at pH 4.5. The following EN cartridge at pH 2.5 could extract additional SAC_{280}. Overall reductions of SAC_{280} values comparing six tannery wastewater samples, both treated and nontreated, and their filtrates were ~70% (54−88%). DOC reduction behaved similarly, but was somewhat less efficient with reduction values in between 40 and 65%. Details of this fractionation scheme concerning SAC, DOC, and toxicity reduction efficiencies will be given elsewhere.

Aliquots (20 μL) of the methanolic eluates of steps 1−4 were analyzed by HPLC as given above (Figure 5). The chromatograms clearly show that the extracted UV-absorbing compounds follow the predicted way of partitioning. With regard to the retention times of the eluted major peaks, C_{18}e material adsorbed low quantities of nonpolar UV-adsorbing compounds. Within the following three PS-DVB cartridges polarity of the eluted substances was increasing. The major part of the UV-absorbing fraction appeared to be extracted with the EnviChrom phase at neutral pH. Most compounds found in the acidic fractions could not be successfully separated on the C_8 column. Peaks of different

fractions but the same retention times in HPLC chromatograms were compared using a diode array detector. Most of them belonged to different compounds. Only a few substances were extracted into two (or even more) fractions. This might not be true for other matrices and different wastewaters but demonstrates the possibilities of the selected procedure.

CONCLUSIONS

A scheme for sequential extractions based on different polarities of individual aromatic compounds has been proposed for the fractionation of the organic content of industrial wastewaters. Sorbents based on poly(styrene−divinylbenzene) copolymers revealed much better extraction efficiencies compared to formerly used RP silica gels or XAD resins. Most of the analytes could be recovered with sufficient results despite their different polarities, fugacities, or reactivities. For basic or extremely acidic compounds, the extraction conditions have to be modified. Reliable recovery of thiols remains an unsolved problem. For GC/MS analysis, however, conditions should be modified to careful drying of the cartridges and elution with suitable solvents such as ethyl acetate.

In summary, screening analysis of industrial wastewaters using SPE cartridges seems to be possible by combining pH changes and polymeric sorbents.

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