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Analysis of sulphonated polyphenols, synthetic tanning agents in heavily polluted tannery wastewaters

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ABSTRACT

A method is presented for the analysis of sulphonated polyphenols, acting as synthetic tanning agents, in heavily polluted tannery wastewater. It consists of ion-pair solid-phase extraction (RP-18) with tetrabutylammonium bromide followed by reversed-phase ion-pair liquid chromatography on a C_{18} stationary phase with UV detection. The detection limit is 15 μg/l. The influence of the wastewater matrix on extraction and the effects of pH and temperature on the HPLC separation are discussed. The procedure is not affected by the high contents of dissolved organic carbon and inorganic salts of tannery wastewater. The tannery effluents investigated contained around 40 mg/l of sulphonated polyphenols. These compounds appear to be refractory against both anaerobic and aerobic biological wastewater treatment. They might, thus, contribute to the dissolved organic sulphur content of surface waters.

INTRODUCTION

Sulphonated polyphenols (SPPs) are employed as synthetic tanning agents in leather production. Many of them contain naphthalenesulphonate-related structural components (Fig. 1), which are known to be hardly degradable by biological treatment [2]. Moreover, removal of less polar aromatic sulphonates such as linear alkylbenzenesulphonates (LASs) in municipal wastewater treatment plants was shown to be due to adsorption, rather than to biodegradation [3]. Hydroxylated aromatic sulphonates might form a substantial portion of dissolved organic sulphur (DOS) in surface waters, the nature of which is still poorly understood [4,5]. SPPs of tannery effluents, if refractory to a similar extent, would provide an additional source of DOS.

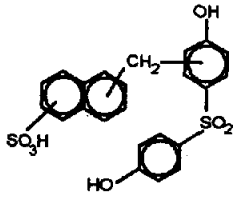

Fig. 1. Structure of a SPP syntane (according to ref. 1).

LASs, which are used as detergent products, are the most widely investigated group of aromatic sulphonates [6]. They were analyzed by anion-exchange chromatography [7], by RP-HPLC from river water [6,8], wastewater [9] and marine water [10]. Reversed-phase ion-pair liquid chromatography (RP-IPC) was invented for the analysis of biodegradation products of LASs [11].

However, other substances contributing to the pool of aromatic sulphonates are less thoroughly investigated. Amino- and hydroxynaphthalenesulphonates, which are employed in dye production and which do appear in effluents of textile industries, might also be analyzed by anion-exchange chromatography [7,12]. Nowadays, RP-IPC is more frequently employed [5,13,14] and was only recently investigated in detail [15].

In analogy to the colorimetric determination

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of anionic surfactants as methylene-blue active substances (MBASs), liquid–liquid extraction with the methylene blue cation was first employed for the isolation of LASs from water [16]. It was superseded from solid-phase extraction [9] and ion-pair solid-phase extraction with tetraalkylammonium cations for the more polar compounds [5]. Enrichment on XAD resins [17] or freeze-drying was occasionally reported [12].

Commonly, the analysis of industrial wastewater constituents has to face a complex mixture of various organic substances, that hamper the extraction and analysis of the compounds under investigation. This is especially true for tannery wastewater, that might be loaded with natural and synthetic dissolved organics, comprising up to 3000 mg/l of dissolved organic carbon (DOC). Furthermore, it exhibits a high content of inorganic salts of up to 20 g/l.

We developed a method for the analysis of SPPs from tannery wastewater, in order to determine their amount, the *in plant* sources and to study their behaviour towards biological wastewater treatment. This publication presents an extension of ion-pair techniques towards other groups of aromatic sulphonates and to heavily contaminated waters.

EXPERIMENTAL

Extraction

Samples were stored frozen at −20°C until analyzed. They were filtered over 0.45-μm cellulose acetate membrane filters (Sartorius, Göttingen, Germany) before extraction and adjusted to pH 6.5 with phosphoric acid or sodium hydroxide. A 1-mmol amount of tetrabutylammonium bromide (TBABr) was added to the sample (10–50 ml), which was then applied to 500 mg Supelclean LC18 extraction cartridges (Supelco, Bellefonte, USA) and eluted from the wet column with 3 ml of methanol and 3 ml of acetonitrile. The extracts were dried in a Speed-Vac A 160 rotary evaporator (Savant, Farmingdale, USA) and dissolved in 1.0–1.5 ml of distilled water. Methanol or acetonitrile solutions obstruct the HPLC separation.

Analytical apparatus

The HPLC system consists of a Merck-Hitachi L 6200 gradient pump with low-pressure mixing, a Merck-Hitachi AS 2000-A autosampler and a Merck-Hitachi T 6300 column oven (all from Merck, Darmstadt, Germany). A 250 × 4 mm LiChrospher 100 RP-18 (5 μm) column (Merck) with a 4 × 4 mm precolumn of identical material was employed for the separation. Substances were detected at 260 nm by a Linear UVIS 204 (Linear, Reno, NV, USA) or a Shimadzu SPD-10A (Shimadzu, Kyoto, Japan) detector. UV spectra were obtained by a Waters 990 diode array detection (DAD) system (Millipore, Milford, MA, USA). Chromatographic data were stored and calculated with the Chromstar software (Bruker-Franzen, Bremen, Germany). A 20-μl volume of aqueous sample was injected.

Separation

Final chromatographic conditions were the following: solvent A: 40 μl H_3PO_4 (85%), 24 mmol NaH_2PO_4, 2 mmol TBABr, 1000 ml water; solvent B: 80 μl H_3PO_4 (85%), 12 mmol NaH_2PO_4, 2 mmol TBABr, 200 ml water, 800 ml MeOH; linear gradient from 45% B (0 min) to 69% B (40 min) at a flow of 1 ml/min, followed by 7 min equilibration. The column temperature was held at 40°C. t_0 was determined by the baseline signal obtained from the aqueous solutions.

Peak assignment and quantitation

The various components of the syntane mixtures are not separately available. Quantitation is, therefore, related to the peak areas of standard solutions of the technical mixtures with known total SPP content. Peak assignment in the wastewater extracts is based on retention times and UV spectra, gained by DAD analysis.

Chemicals

Chemicals for extraction were of analytical grade, and solvents for chromatography were of HPLC grade from various companies. TBABr of synthetical grade (Merck) was found to be less UV absorbing than analytical-grade TBABr of other companies (Fluka, Buchs, Switzerland;

Aldrich, Gillingham, UK). Further clean-up of TBABr, if necessary, can be easily performed by recrystallization from hot acetic acid ethyl ester. Technical SPP mixtures were manufactured by Ciba-Geigy (Basel, Switzerland) and Bayer (Leverkusen, Germany), while tannic acid was obtained from Fluka.

RESULTS AND DISCUSSION

Extraction

Although the amounts of SPPs in tannery effluent later detected (see below) would have allowed the direct HPLC analysis without prior enrichment, an extraction step appeared to be necessary, in order to free the SPPs from the complex organic mixture present in the tannery effluent and the high load of inorganic salts.

Freeze-drying and resolution of the compounds under investigation with methanol [12] did not yield reproducible results or sufficient recovery. Apparently, the complex sample matrix of tannery effluent hampered the dissolution of SPPs from the precipitate.

We expected ion-pair extraction of SPPs with TBABr on C_{18}-covered solid phases to be more satisfactory. This method has been employed for the extraction of naphthalenesulphonates and related compounds from water with a comparatively low DOC content [5]. The influence of pH, DOC and inorganic salts upon extraction was studied.

Variations of the pH of the samples between 4 and 6.5 did not substantially influence the overall recovery of two technical SPP mixtures (1–5 mg/l) from distilled water and wastewater (Table I). However, the relative standard deviation for individual components was lower at pH 6.5. Furthermore, extraction of SPPs at pH 6.5 from distilled water was less affected by the addition of salts (30 g/l), than at pH 4.

The recovery of SPPs from wastewater might be affected by the nature and the amount of the accompanying DOC. However, only little information is available on the nature of dissolved organics in tannery wastewater and, thus, studying its influence on SPP recovery by adding a synthetic DOC mixture to distilled water would have been rather speculative. Therefore, we determined the amount of DOC to be loaded on the extraction cartridges before SPP breakthrough. Untreated wastewater was applied to an extraction cartridge and 10-ml portions of the filtrate were subsequently reextracted for SPP analysis. Although 80 ml of wastewater (1200 mg/l DOC) were extracted, no SPPs became detectable in the filtrate. Accordingly, more than 100 mg DOC might be applied onto a 500-mg extraction cartridge, without breakthrough. A 10-ml volume of the sample would have been sufficient for SPP analysis. Solid-phase extraction with TBABr, hence, provides a reliable means for the extraction of SPPs, even from wastewater with a high organic and inorganic load.

TABLE I

RECOVERY (%) AND R.S.D. OF SPP EXTRACTION FROM WASTEWATER

Syntane	pH 4		pH 6.5	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
1	93	13.7	109	5.3
2	107	16.9	104	4.5

It was frequently observed that the extraction efficiencies of nominal identical solid-phase extraction cartridges of different manufacturers strongly differed. This is also true for the procedure presented here. For example, extractions with Adsorbex RP-18 (Merck) yielded only those components which elute comparatively late in RP-IPC analysis. The more polar, early-eluting components of the syntane mixtures, were not at all or not sufficiently extracted.

Chromatography

RP-IPC with cations such as cetyltrimethylammonium or tetrabutylammonium has formerly been employed for the separation of degradation products of LASs [11] and for other aromatic sulphonates [5,15]. Corresponding to the ion-pair extraction described above, we used TBABr for the separation of SPP mixtures, combined

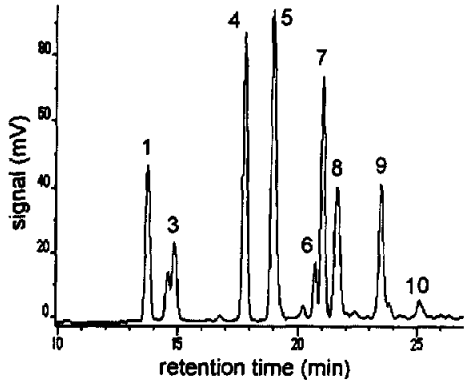

Fig. 2. Chromatogram of a SPP mixture (for chromatographic conditions see text). Peaks were not identified; peak numbers are given only to ease the peak assignment in the corresponding wastewater extract (Fig. 5).

with a phosphate buffer and a water–methanol gradient (Fig. 2).

The relation between the pK values of the acids under investigation and the appropriate pH value of the eluents for IPC is still a matter of debate [13,14]. We studied the influence of pH variation between 3 and 7 on the capacity factors of SPP components (Fig. 3). They all tend to increase above pH 4. These results are consistent with the data obtained for LASs [11], while, astonishingly, the adverse effect was reported for naphthalenesulphonates [13]. In order to obtain the most reproducible chromatographic conditions we employed a buffer system of pH 3.6 in the aqueous solvent.

Under these conditions, not necessarily all sulphonate groups within the molecules need to

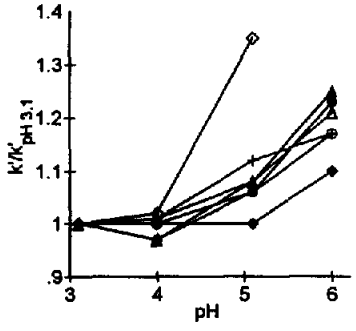

Fig. 3. Variation of capacity factors (k') in RP-IPC with the pH of eluents. Symbols refer to the peaks in Fig. 2: △ = peak 1; ● = peak 2; ◆ = peak 3; ◇ = peak 4; ▲ = peak 5; ○ = peak 6; + = peak 7.

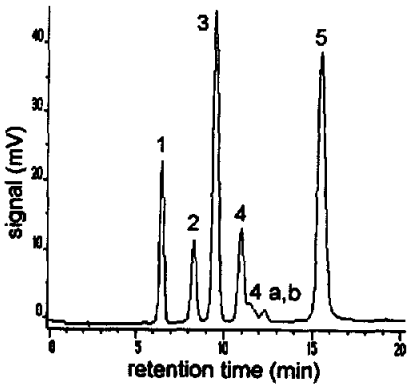

Fig. 4. Chromatogram of naphthalenesulphonates. Peaks: 1 = napththalene-1,5-disulphonate; 2 = 1-naphthol-4-sulphonate; 3 = 2-naphthol-3,6-disulphonate; 4 = naphthalene-1,3,6-trisulphonate; 4a, b = 1,3,5- and 1,3,7-isomers; 5 = naphthalene-2-sulphonate.

be ion-paired. This is illustrated by the elution order of naphthalenesulphonates under identical conditions (Fig. 4). If completely ion-paired, they should elute according to the degree of sulphonation. However, naphthalenedisulphonate elutes first, followed by naphthalenetrisulphonate and -monosulphonate.

The influence of column temperature is as strong as the effect of pH. Standard enthalpy changes of solute transfer from the mobile to the stationary phase (ΔH^0) might be calculated from plotting ln k' against $1/T$ (Van 't Hoff plot) [15]. We obtained fairly linear relationships in the investigated temperature range of 22 to 50°C ($r = 0.88$ to 0.98, $n = 4$) and ΔH^0 values of −0.4 to −0.98 kcal/mol (1 cal = 4.14 J) for the individual components. They are in the range of those reported for aminonaphthalenesulphonate separation on a Nucleosil C_{18} column (−2.5 to −5.5 kcal/mol) [15]. For stable chromatographic conditions we selected a column temperature of 40°C.

Fluorescence detection is frequently used for the determination of LASs and naphthalenesulphonates [6]. Unfortunately, SPPs do not exhibit fluorescence activity and must, therefore, be detected by UV measurement.

Evaluation of the whole procedure

The reproducibility of the whole procedure was evaluated by performing four analyses in

TABLE II

RELATIVE STANDARD DEVIATIONS OF SPP COMPONENTS AND TOTAL SPP CONTENTS ($n = 3$)

Total SPP content 5.1–29.6 mg/l.

	Sample No.			
	1	2	3	4
Single components (R.S.D., %)	1–12	3–6	2–8	1–8
Total (R.S.D., %)	4.8	4.2	3.1	3.5

TABLE III

TOTAL SPP CONTENT IN TANNERY WASTEWATER BEFORE AND AFTER BIOLOGICAL TREATMENT

Observation period three months, $n = 9$.

	Syntane (mg/l)	Syntane/DOC (mg/mg)
Untreated	41	0.03
After anaerobic treatment	45	0.11
After anaerobic and aerobic treatment	34	0.13

triplicate (spread over four weeks). Relative standard deviations (R.S.D.s) of individual SPP components vary between 0.5 and 12%, with an average of 3.5% ($n = 26$) (Table II). The R.S.D. of the total SPP content of the four samples was in the range 3.1–4.8%.

The detection limit ($S/N \geq 3$) is 40 ng of total SPP mixture injected onto the column. For quantitation ($S/N \geq 10$) around 130 ng have to be injected. If 500 ml are extracted and redissolved in 100 μl this results in a detection limit around 1.5 μg/l (for 20-μl injections). However, in the case of wastewater loaded with 2000 mg/l DOC, only 50 ml might be extracted in order not to saturate the extraction cartridge. The corresponding detection limit is around 15 μg/l.

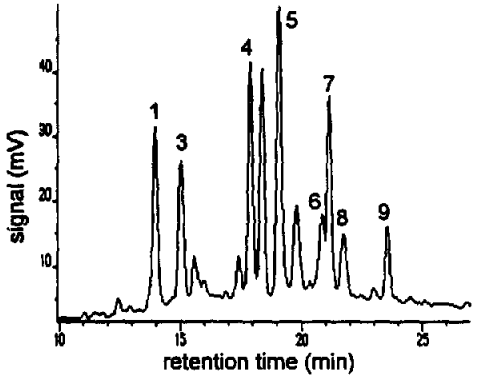

Fig. 5. Chromatogram of an extract from biologically treated tannery wastewater. Peaks as in Fig. 2.

Application

This analytical procedure was subsequently applied to the analysis of SPPs in tannery wastewater as well as in the effluents of a pilot plant, combining anaerobic and aerobic biological treatment [18] over a three-month period (Fig. 5). In fact, SPPs revealed to be highly refractory against both anaerobic and aerobic biodegradation (Table III). Neither their content, nor their composition was altered by the biological treatment. Slight diminution during aerobic treatment might be due to adsorption onto biomass. Consequently, SPPs become substantially enriched during the biological treatment and contribute to about 5% of the final DOC content. Hence, these compounds would pass a biological treatment plant and, finally, become part of the DOS pool of surface waters.

CONCLUSIONS

We developed a method for the analysis of synthetic tanning agents of the sulphonated polyphenol type from tannery wastewater. The method includes ion-pair extraction and RP-IPC with the TBABr. The high salt and DOC contents of the wastewater up to 20 mg/l and 2000 mg/l, respectively, do not affect the analysis. The detection limit is dependent on the DOC content of the wastewater and is in the order of 15 μg/l for 2000 mg/l DOC.

Application of this method to a combined anaerobic/aerobic biological treatment of tan-

nery wastewater revealed that SPPs are highly refractory and have a low adsorption tendency. These compounds will, therefore, contribute to the DOS content of surface waters.

The method described is suggested to be of value for the determination of (polyaromatic) sulphonates and carboxylates from various industrial wastewaters.

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