Fresenius J Anal Chem (1993) 345:767–772

Fresenius' Journal of Analytical Chemistry

HPLC separation and quantification of anionic surfactants using an automated on-line ion pair extraction system*

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Received August 25, 1992; revised November 19, 1992

Summary. Aliphatic anionic surfactants, like alkylsulfonates, alkyl sulfates, α-sulfo alkanoic acids and their esters, were separated with respect to their alkyl chain length by high performance liquid chromatography (HPLC) using reversed phase (RP) columns. For sensitive and specific detection of these classes of compounds the HPLC separation was combined with a post-column ion-pair extraction system. The limit of detection is in the range of 3–30 ng. Previously reported detection systems of this kind were modified by changing the inner set-up of the phase separator and the extraction capillary and integrating a purge function, which led to a higher system stability and made automatization possible. In order to optimize the experimental set-up, the influence of the reagent concentration as well as different sizes and materials of the extraction capillary were evaluated. The calibration curve of surfactant concentrations versus fluorescence emission is discussed for sodium decylsulfonate. Since both the electrolyte concentration and the percentage of organic solvents in the mobile phase may adversely affect the detection system, different eluent compositions were tested.

Introduction

Anionic surfactants which are used in detergent formulations for washing purposes are largely discharged into the aquatic environment via the waste water system, even after proper use [1]. Due to the better biodegradability of aliphatic compounds, there is a trend to increase the use of aliphatic anionic surfactants in detergent formulations, which furthermore may be produced from renewable resources. Therefore, compounds like alkyl sulfates, alkylethoxylated sulfates or methyl α-sulfo alkanoates are potential substitutes for linear alkylbenzenesulfonates [2].

In order to quantify individual anionic surfactants specifically, complex mixtures of homologues and potentially isomers have to be separated chromatographically. Reversed phase (RP) HPLC [3–5] as well as ion pair chromatography (IPC) [6–8] are usually employed.

After chromatographic separation of anionic surfactants, sensitive detection methods are only available for light absorbing or fluorescent surfactant classes, like linear alkylbenzenesulfonates. For aliphatic compounds, like alkylsulfonates or alkyl sulfates, either a refractive index detector or a conductivity detector must be applied. Only for macroscopic amounts (in process control, for example) the evaporative light scattering detector may also be used [9]. None of these possibilities can be used for amounts less than 1 μg; thus large concentration factors have to be achieved in environmental analyses by use of suitable isolation and separation procedures. Refractive index and conductivity detectors furthermore require isocratic elution which is ineffective for most environmental matrices. Applying indirect photometric or indirect conductivity detection, the limits of detection achievable for anionic surfactants under isocratic elution conditions are as low as 5–20 ng [3, 6, 10, 11]. However, using a mobile phase gradient limits of detection rise to larger than 1 μg.

In order to obtain sufficiently small limits of detection, an extremely sensitive detection system which can also be operated using gradient elution must be used. This can be achieved by an on-line coupling of HPLC separation and ion pair formation with fluorescent counter ions. Extraction of the ion pair formed by the addition of the cationic dye to the HPLC column effluent is performed on-line by further addition of an immiscible apolar organic solvent and the application of a continuously operating phase separator. Thus, aliphatic anionic surfactants may be determined selectively and sensitively [4, 12].

In continuation of our efforts to determine aliphatic cationic surfactants [13, 14] we present an analytical procedure and equipment for the determination of aliphatic anionic surfactants which can be realized incurring low costs and upgraded to work automatically. As a fluorescent cationic dye we use 1-cyano-[2-(2-trimethylammonio)ethyl]-benz(f)isoindole (CTBI) which has a high fluorescence quantum efficiency and thus enables highly sensitive detection [15, 16].

Experimental

Materials

Naphthalene-2,3-dicarboxy aldehyde and chloroform "Chrom AR" were purchased from Promochem GmbH,

* This article is part of the planned dissertation of Markus Schoester at the Mathematical Natural Science Faculty of the University of Düsseldorf

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Wesel, FRG. (2-Aminoethyl)trimethyl ammonium chloride hydrochloride and sodium tetradecylsulfonate were obtained from Aldrich Chem. Co., Milwaukee, USA. Sodium decylsulfonate was purchased from Fluka AG, Buchs, CH. Sodium dodecylsulfonate, sodium dodecylsulfate, sodium cyanide, disodium tetraborate decahydrate, acetonitrile "LiChrosolv gradient grade", water "LiChrosolv" for HPLC, trisodium citrate dihydrate and 5 mol l^{-1} hydrochloric acid were obtained from Merck, Darmstadt, FRG. n-Butanol, glass distilled grade, was supplied by Burdick & Jackson Laboratories Inc., Muskegon, MI, USA. Methanol was purchased from Riedel de Haën AG, Seelze, FRG. Sodium tetradecylsulfate and sodium hexadecylsulfate were obtained from Lancaster, Morecambe, GB. Disodium α-sulfo hexadecanoate, disodium α-sulfo octadecanoate, sodium α-sulfo hexadecanoic acid methylester and sodium α-sulfo octadecanoic acid methylester were supplied by Henkel, Düsseldorf, FRG. All chemicals were of p.a. quality unless otherwise stated.

A solution of the fluorescent dye CTBI was synthesized according to [17]. A 100% reaction yield corresponds to a CTBI concentration of 279 μmol l^{-1}. Stored at 4°C in the dark the solution was stable for at least 2 months.

Mobile phases and reagent solution

Mobile phase component A was prepared by adding 1 ml 5 mol l^{-1} hydrochloric acid to 0.01 mol l^{-1} trisodium citrate dihydrate and filling up with "LiChrosolv" water to 1000 ml. Component B was prepared in the same way, but 500 ml acetonitrile were added before finally filling up with water.

The reagent solution was prepared freshly every day by filling up 10 ml of the CTBI stock solution to 200 ml with 0.011 mol l^{-1} trisodium citrate dihydrate solution to reach a final CTBI concentration of approximately 14 μmol l^{-1}.

Apparatus

Trouble-free operation as well as reproducible results are depending on a lot of technical requirements which therefore have to be presented in detail.

For high performance liquid chromatography an HPLC pump 2150, an HPLC controller 2152 and a degasser 2156 from Pharmacia Biosystems, Freiburg, FRG were used. For injection of the samples a six-port valve from Valco, Houston, TX, USA equipped with a 50 μl injection loop of stainless steel was applied. Chromatographic separation was performed with a 40 × 4.0 mm stainless steel column filled with Spherisorb S5-C1 from Phase Sep, GB. The detector was a fluorescence spectrophotometer F1000 from Merck-Hitachi, Darmstadt, FRG, with a 12 μl cell set to an excitation wavelength of 285 nm and an emission wavelength of 485 nm. The chromatograms were recorded on a Merck-Hitachi D-2500 chromato integrator. For delivering the reagent, purge fluid and extraction fluid a 3-channel pump 655A-13 from Merck-Hitachi was used combined with three unlatched 3/2-way electromagnetic valves Type 364 from Asco GmbH, Ratingen, FRG.

The mixing capillary was a 500 mm × 0.5 mm i.d. polytetrafluorethene (PTFE) capillary coiled to a helix with a diameter of 15 mm. As extraction capillary a 1000 mm × 0.5 mm Tefzel[1] capillary coiled to a helix with a diameter

[1] Trade mark

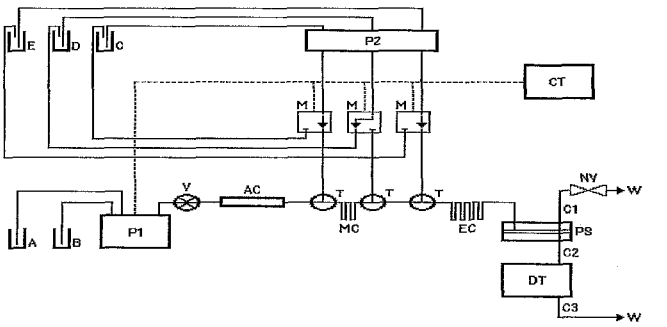

Fig. 1. Scheme of the assembly of the analytical equipment. *A* = mobile phase component A; *AC* = analytical column; *B* = mobile phase component B; *C* = CTBI reagent; *C1* = 2800 × 0.50 mm i.d. PTFE capillary; *C2* = 250 × 0.30 mm i.d. PTFE capillary; *C3* = 2800 × 0.30 mm i.d. PTFE capillary; *CT* = HPLC controller; *D* = methanol; *DT* = detector; *E* = chloroform; *EC* = extraction capillary; *M* = electromagnetic valve; *MC* = mixing capillary; *NV* = needle valve; *PS* = phase separator; *P1* = HPLC pump; *P2* = 3-channel pump; *T* = T-piece; *V* = injection valve; *W* = waste

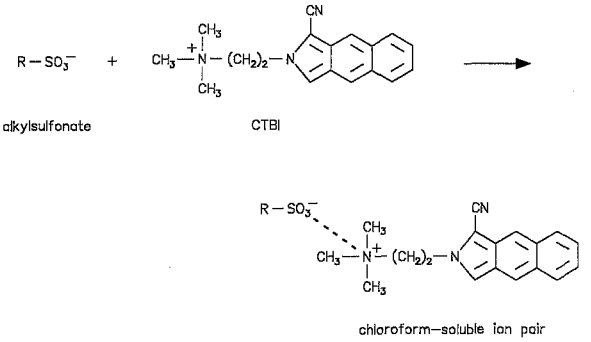

Fig. 2. Formation of extractable fluorescent ion pairs of anionic surfactants (for example: alkylsulfonate) and CTBI

of 40 mm was used unless otherwise stated. All other capillaries downstream of the analytical column consisted of PTFE; all capillaries before the column were of stainless steel. Supplier for the capillary material was Chromatographie Service GmbH, Langerwehe, FRG.

The phase separator was constructed in our workshop according to [18]. As a modification we used a groove with a circular cross-section of 2 mm diameter and a length of 12 mm. Influent and effluent capillaries were installed at a distance of 1 mm from both ends of the groove, respectively [14]. A G6Y needle valve from Hoke GmbH, Frankfurt, FRG was installed as restriction.

Procedures

Figure 1 shows a scheme of the assembly of the analytical equipment. During analysis the fluorescent dye CTBI (C) is continuously added just behind the analytical column (AC). In a short mixing capillary (MC) it is mixed with the eluent stream in order to form extractable fluorescent ion pairs (according to the reaction scheme in Fig. 2) during elution of anionic surfactants. After addition of chloroform as extraction solvent (E) the two-phase mixture is transported through an extraction capillary (EC). The major part of the organic phase is separated in the continuously operating sandwich-type phase separator (PS) and then transported (via C2) to the fluorescence detector (DT). Separation of the

Table 1. Program for system operation. Standard mobile phase gradient and the consecutive purge and equilibration steps (actuated by the HPLC controller). Flow of reagent, methanol and chloroform: 0.8 ml min^{-1}

Time/min	Mobile phase		CTBI-reagent	Methanol	Chloroform
	Flow ml min^{-1}	Composition % B			
0	0.80	20	on-line	off-line	on-line
20	0.80	100	on-line	off-line	on-line
22	0.80	100	on-line	off-line	on-line
23	0.80	20	on-line	off-line	on-line
25	off	–	off-line	on-line	off-line
27	off	–	off-line	off-line	on-line
29	off	–	on-line	off-line	on-line
30	0.80	20	on-line	off-line	on-line
32	0.80	20	on-line	off-line	on-line

two phases is effected by exploitation of the different wetting characteristics of the aqueous and chloroform phases towards different materials (stainless steel and PTFE) of the phase separator faces. The portion of the chloroform phase separated is set to 50–60% (of the total chloroform) by adjustment of the needle valve (NV). After the end of a chromtographic run methanol (D) is pumped through all parts of the system that had been in contact with the two-phase eluent stream, so that they will be thoroughly cleaned. After a priming sequence and a short equilibration time the equipment is ready for the next chromatographic separation. All control operations are actuated by the HPLC controller (CT), thus, automatic operation is achieved (Table 1).

Results and discussion

Influence of CTBI concentration

The detector signal was recorded using varying concentrations of CTBI at three different amounts (20, 100 and 1000 ng) of sodium decylsulfonate as model substances. As can be seen from Fig. 3, the amount of CTBI available for ion pair formation seems to control signal heights at low ratios of CTBI to anionic surfactant; at higher excess of CTBI over anionic surfactant the ion pair formation leading to observable detector signal seems to be controlled primarily by the amount of anionic surfactant, as required for a reliable quantification procedure. The plateau of the curves delineates the region where CTBI excess is sufficient to effect complete ion pair formation.

As baseline noise (due to extraction of non-ion-paired CTBI) exceeds the signal height at higher CTBI concentrations and a low amount of analyte, no measurement is possible in this region (broken lines in Fig. 3). For this reason, a compromise has to be found leading to a maximum dynamic range along with a minimum baseline noise: for sodium decylsulfonate above its detection limits (see Table 2) a CTBI concentration of 14 µmol l^{-1} was found to be optimal.

Variation of extraction capillary material

Our previous experiments using the post-column ion pair extraction system for the determination of cationic surfactants [14] demonstrated that some capillary materials require longer equilibration times and that memory effects were

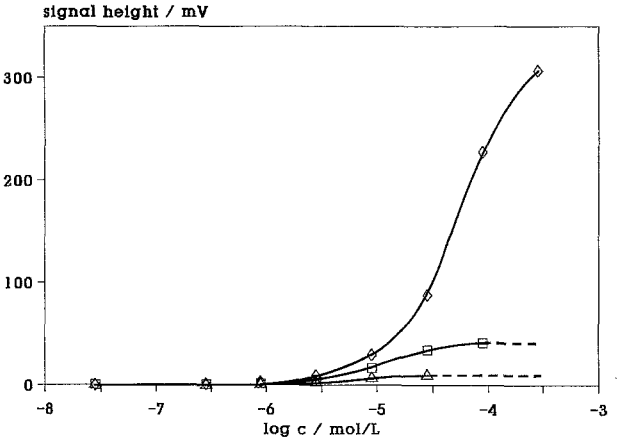

Fig. 3. Signal height versus concentration of CTBI reagent at three different amounts of sodium decylsulfonate injected. △ = 20 ng; □ = 100 ng; ◇ = 1000 ng. Mobile phase: isocratic 45% component B. Flowrate: 0.80 ml min^{-1}

obvious for other materials. Thus, our present system was evaluated for these effects.

Up to now, four different materials (stainless steel, PTFE, PEEK and Tefzel) were tested using consecutive injections of 100 ng sodium decylsulfonate each every two minutes under isocratic conditions (retention time about 2 min) and recording subsequent detector responses.

Favourably, no changes in detector response for subsequent injections were observed for any of the materials investigated. In the direct comparison, however, differences in signal heights (and signal height variation) were obvious. Stainless steel capillaries resulted in particular low signal heights, they therefore were exluded from further investigations. PTFE capillaries exhibited the largest signal heights, but a noisy baseline and large signal height variations were distinct disadvantages. Using PEEK or Tefzel capillaries, acceptable and comparable signal heights were observed with only small variations. Since it is easier to bend Tefzel to form a coil, it was used for practical reasons.

Influence of the size of the extraction capillary

For constant amounts of sodium decylsulfonate and CTBI the detector signal was recorded in relation to a varying

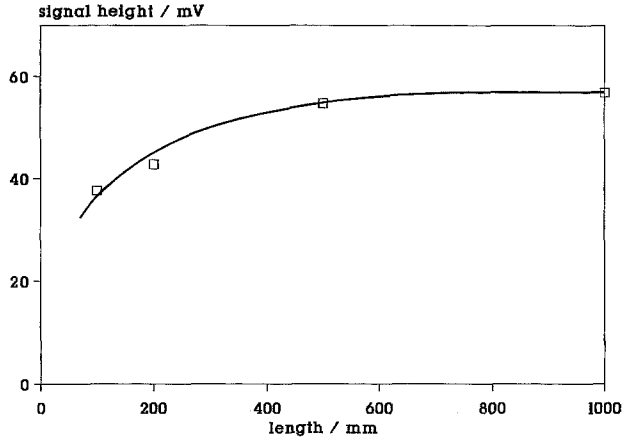

Fig. 4. Signal height versus extraction capillary length. Capillary: Tefzel, 0.50 mm internal diameter. Amount injected: 100 ng sodium decylsulfonate. Mobile phase: isocratic 45% component B. Flowrate: 0.80 ml min^{-1}

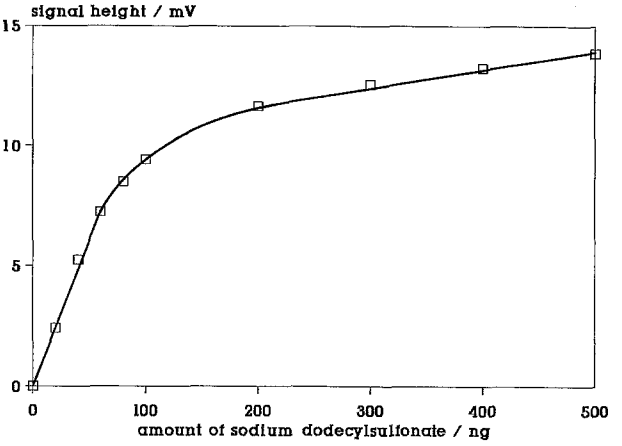

Fig. 6. Calibration graph of fluorescence signal peak height versus sodium dodecylsulfonate amount injected. Mobile phase: isocratic 60% component B. Flowrate: 0.80 ml min^{-1}

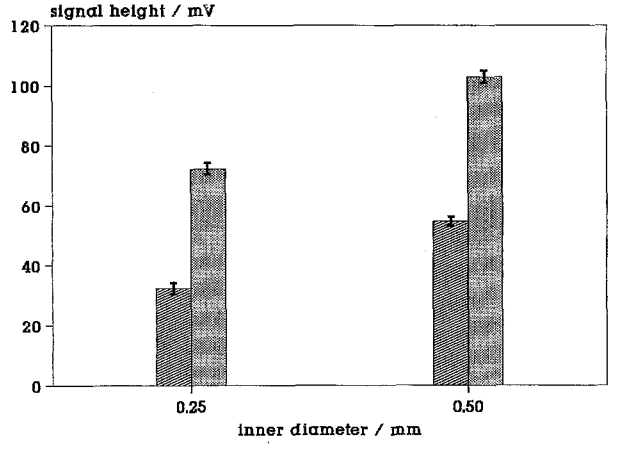

Fig. 5. Signal heights at two different internal diameters of the extraction capillary and two different amounts of sodium decylsulfonate. Amount injected: ▨ = 100 ng; ▩ = 1000 ng. Capillary: Tefzel, 500 mm length. Mobile phase: isocratic 45% component B. Flowrate: 0.80 ml min^{-1}

Table 2. Limits of detection and limits of quantification of some aliphatic anionic surfactants (as sodium salts, numbers in ng). C_x = alkyl chain with x carbon atoms; $-SO_3^-$ = sulfonate group (in position 1); $-SO_4^-$ = sulfate group (in poition 1); $C_x$aa = alkanoic acid with x carbon atoms; α−s- = sulfo group (in position α); −me = methylester group. Mobile phase gradient: 0 to 20 min: 20% to 100% component B. Flowrate: 0.80 ml min^{-1}

Analyte	Limit of quantitation	Limit of detection
$C_{10}-SO_3$	14	5
$C_{12}-SO_3$	12	4
$C_{14}-SO_3$	17	6
$C_{12}-SO_4$	14	5
$C_{14}-SO_4$	27	10
$C_{16}-SO_4$	50	17
α−s−C_{16}aa	29	11
α−s−C_{18}aa	66	27
α−s−C_{16}aa−me	20	5
α−s−C_{18}aa−me	24	9

length of a 0.5 mm i.d. Tefzel extraction capillary. As can be seen from Fig. 4, a length of 500 mm is sufficient for maximum signal height.

In Fig. 5, signal heights determined at two different inner diameters of the extraction capillary (0.25 mm and 0.5 mm) are shown for two different amounts of sodium decylsulfonate (100 or 1000 ng). As expected, the narrower capillary results in slightly lower signal heights for identical amounts of sodium decylsulfonate. Increasing the amount of sodium decylsulfonate by a factor of ten, however, leads to signals of only double the height for both inner diameters. Thus, we conclude that the time available for ion pair formation is not the limiting factor for signal generation during the determination of the 1000 ng injection.

Calibration graphs and detection limits

After optimizing all experimental parameters a calibration graph of fluorescence signal peak area versus sodium dodecylsulfonate amount was generated. As can be seen from Fig. 6, a linear calibration graph was obtained for 1 to 60 ng sodium dodecylsulfonate. Between 60 and 200 ng a non-linear region was apparent which was followed by a second linear section of significantly lower slope above 200 ng. We suspect that for amounts larger than 60 ng an increasing depletion of CTBI may be the reason for non-linearity. Calibration beyond the steep linear range, however, is tedious and time-consuming, even though correct results may be obtained for amounts as large as 500 ng when using a suitable internal standard (data not shown). We therefore prefer to work in the first linear range of the calibration graph where linear regression can be performed using few standard injections.

In Table 2, the limits of detection (signal/noise-ratio = 3) and limits of quantification (signal/noise-ratio = 10) are shown for some selected aliphatic anionic surfactants. These data were obtained by using standard mixtures at increasing levels of dilution using optimum gradient elution programs.

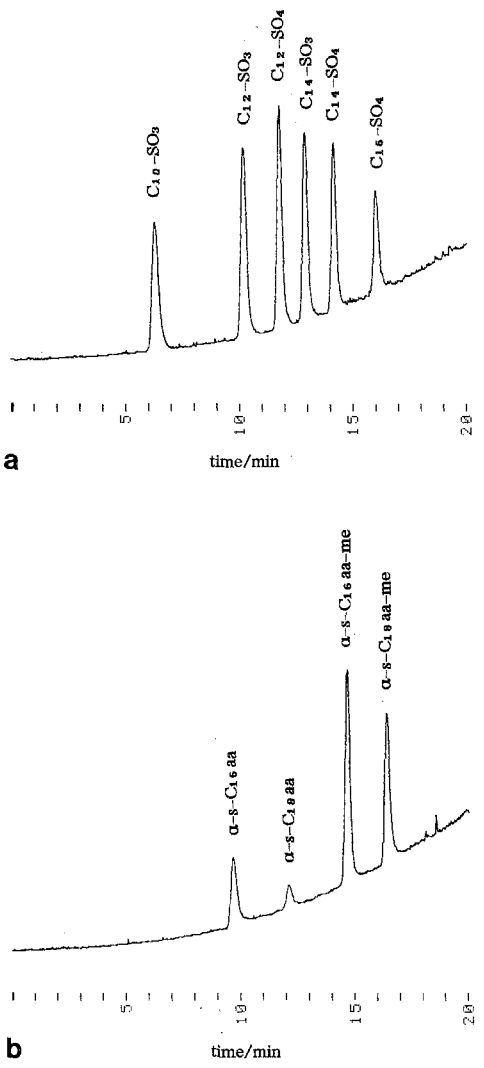

Fig. 7. Chromatograms of standard mixtures: (**a**) alkylsulfonates and alkylsulfates; (**b**) α-sulfo alkanoic acids and methyl α-sulfo-alkanoates. C_x = alkyl chain with x carbon atoms: $-SO_3^-$ = sulfonate group (in position 1); $-SO_4^-$ = sulfate group (in position 1); $C_x$aa = alkanoic acid with x carbon atoms; α−s- = sulfo group (in position α); −me = methylester group. Amount injected: 100 ng (as sodium salt) of each component. Mobile phase gradient: 0 to 20 min: 20% to 100% component B. Flowrate: 0.80 ml min^{-1}

Coupling of HPLC separation and on-line ion pair extraction (complete analytical sequence)

When coupling reversed phase HPLC with an on-line ion pair extraction system the main problem is the content of organic components in the eluents (especially with gradient elution) which strongly affects the function of the ion pair extraction system. As these organic components are normally miscible with the extraction solvent chloroform, increased solubility of the CTBI in the organic phase is the consequence. Thus, detector baseline is increased and the dynamic range for quantification is reduced concomitantly. Furthermore, baseline noise will also be increased, adversely affecting limits of detection. In RP-HPLC a certain amount of organic components is unavoidable for efficient separation of individual compounds. To avoid problems within the ion pair extraction system, water has to be added to the eluent immediately after the separation step. Since CTBI is easily soluble in water, this step is combined with the addition of CTBI reagent to the eluent. As RP columns with short alkyl chains (RP-1) were sufficiently effective to separate individual homologues of anionic surfactants, those elution solvents were employed that only require minimum addition of organic components for gradient elution.

Apart from organic components in the eluent, electrolytes may also cause problems because some anions, like perchlorate, will form ion pairs with CTBI that can be extracted into chloroform to a significant extent in the absence of anionic surfactants, which results in the same problems concerning baseline or limit of detection, as discussed above for organic components. As the addition of electrolytes, however, is indispensable for efficient RP-HPLC separation of organic ionic compounds, different electrolytes, eluents and RP columns were tested for their mutual compatibility. Using an eluent combination of water/methanol/sodium perchlorate efficient separation was possible with a methanol gradient; limits of detection and quantification, however, were not yet convincing. Most probably this deficiency was caused by the formation of extractable ion pairs (CTBI perchlorate). Combinations of phosphate buffer/acetone (according to [12]) with a phosphate gradient also did not lead to satisfying results. The same was true for phosphate buffer/methanol combinations, both with methanol or a phosphate gradient. We finally changed to the very hydrophilic anion citrate as electrolyte. Using a citrate buffer/acetonitrile combination, successful separations were obtained with sufficiently low limits of detection and quantification.

A typical chromatogram demonstrating the separation of a standard mixture of alkylsulfonates and alkyl sulfates (Fig. 7a) as well as that of a standard mixture of α-sulfo alkanoic acids and methyl α-sulfo alkanoates (Fig. 7b) is shown in Fig. 7.

Linear alkylbenzenesulfonates can also be separated and detected using our combined system. Since linear alkylbenzenesulfonates may be efficiently detected using their inherent fluorescence properties, limits of detection using our system are improved by only a factor of 3 – 5. For aliphatic anionic surfactants, detection limits are improved by 2 – 3 orders of magnitude in comparison with a conductivity or refractive index detector.

Conclusion

Using an on-line ion pair extraction system coupled to HPLC in conjunction with a fluorescence detector, individual aliphatic anionic surfactants can be determined selectively and sensitively. In contrast to conventional HPLC detectors (like conductivity or refractive index detector) this system performs well under gradient elution conditions and is operated automatically; moreover, it can be assembled from moderately priced components. From its operating principle, further applications for the HPLC determination of amphiphilic compounds are envisaged. At present we are in the process of developing an efficient isolation and concentration procedure for the three different surfactant classes (anionic, cationic and non-ionic) from environmental matrices. In conjunction with an efficient HPLC separation and determination method, such as the one presented here, the effort for isolation and concentration can be somewhat reduced without concomitantly increasing detection limits.

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