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Microporous membrane liquid–liquid extraction coupled on-line with normal-phase liquid chromatography for the determination of cationic surfactants in river and waste water

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Abstract

Membrane-based continuous liquid–liquid extraction combined on-line with normal-phase liquid chromatography is proposed for the determination of cationic surfactants in complex aqueous samples. The technique has the potential for complete automation. Selective enrichment of cationic surfactants from spiked river water and waste-water samples with simultaneous removal of matrix constituents, followed by a quantitative transfer of the extract onto a liquid chromatographic column and separation of the surfactant homologues yielding low detection limits, has been realised. The homologues of alkyldimethylbenzylammonium chloride (Dodigen 226) were chosen as model compounds in the method development. Dodigen homologues were ion-paired with heptanoic acid and extracted into chlorobutane by means of microporous membrane liquid–liquid extraction. It was thereby possible to attain an enrichment of over 250 times for one of the homologues, viz. the concentration in the organic liquid is 250 times higher than in the original sample. Detection limits for the three best-detected homologues of the mixture were in the range 0.7–5 μg/l in spiked river water samples. Ion-pair normal-phase liquid chromatography, again with heptanoic acid as counter-ion, gave the necessary separation of the surfactant homologues.

Keywords: Microporous membrane liquid–liquid extraction; Extraction methods; Water analysis; Environmental analysis; Surfactants

1. Introduction

Cationic surfactants are widely used as, for example, fabric softeners, anti-static agents and antiseptic components. Hence, they may be present in waste-water in large amounts. Although more than 90% of all surfactants are removed in waste-water treatment plants, trace amounts may be released into the environment [1]. Cationic surfactants have rather low toxicity for mammals, except in high doses, but they can irritate skin and eyes as well as the neural system, especially with continuous exposure. At the same time the toxicity for microorganisms is high and this property makes these surfactants useful as biocides and antiseptics [2]. The quaternary ammonium compounds are toxic in the μg/ml range and even lower to many aquatic organisms like algae, fish, shrimp and starfish, [3]. However, there is no evidence for accumulation in higher aquatic life-forms [4].

To this end, it is of importance to be able to determine the amount of cationic surfactants in natural waters. As the concentrations are often low and other compounds might interfere with the analy-

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sis, a selective extraction step is usually needed. However, due to the high adsorption enthalpies, extracting cationic surfactant is obliging to supplementary caution. For the determination of cationic surfactants various methods have been proposed. A non-specific method is the colorimetric disulfine method by Waters and Kupfer [5] and later modified by Osburn [6]. In the early 1980s, Wee and Kennedy [7] described a liquid chromatography method for the determination of cationic surfactants. The detection was made with conductometry in an organic eluent. Some other LC methods were later to be presented [8–11]. The main problems associated with the analysis of cationic surfactants include detection of non-aromatic surfactants and separation of homologues at low concentrations. There are several applications that utilise on-line post column ion-pair formation with a counter-ion to form a coloured complex, followed by extraction into an organic phase and UV or fluorescence detection [12,13].

Continuous flow liquid–liquid extraction (LLE) can be achieved using a hydrophobic microporous membrane. The membrane is well wetted by an organic solvent, filling the pores of the membrane. When an aqueous solution, immiscible with the organic solvent, is in contact with the membrane, an interfacial contact area is established at the membrane surface. The use of microporous membrane liquid–liquid extraction (MMLLE) generates a great potential for automation of typical LLE applications. It has been found that the membrane exhibits little resistance to mass transfer from the aqueous solution into the organic extractant since the solutes have higher solubility in the membrane solvent [14,15]. Shen et al. used MMLLE combined with capillary GC for the determination of some local anaesthetics [16]. More recently, the potential of using MMLLE combined with HPLC has been evaluated in a series of articles [17].

We suggest an MMLLE method for ion-pair extraction of cationic surfactants, which easily can be connected on-line to normal-phase liquid chromatography. The method proposed comprises of certain actions to minimise analyte losses, encompassing, as well, collection and preservation of the sample, as an entire analysis scheme.

2. Experimental

2.1. Chemicals

The cationic surfactant used as model compound were the commercial product Dodigen 226, obtained from Hoechst (Frankfurt, Germany). According to the producer, Dodigen 226 is a dimethyl alkyl benzyl ammonium chloride and has five homologues with chain-lengths C_{10} (2%), C_{12} (57%), C_{14} (23%), C_{16} (11%), C_{18} (7%), see Fig. 1. The content of the commercial product is 30–100% of surfactant. For quantitative measurements in this work, a range of concentrations is given, corresponding to this interval.

As the chromatographic eluent a mixture of chloroform, ammonia, both analytical-reagent grade (Merck, Darmstadt, Germany), heptanoic acid (ICN Biomedicals, Aurora, OH, USA) and ethanol (ETAX 99.7%, v/v, Primalco, Rajamäki, Finland), were used. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Other solvents used were: *n*-undecane (analytical-reagent grade, Merck), heptane (LabScan, Dublin, Ireland) and 1-chlorobutane (LabScan).

2.2. Treatment of glassware

To avoid adsorption of surfactants to glass sur-

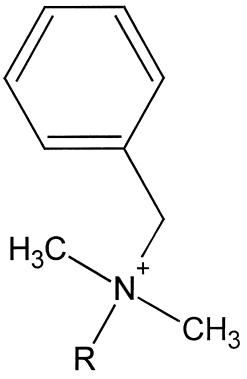

Fig. 1. The commercial product Dodigen 226 represents an alkyldimethylbenzylammonium compound with an alkyl chain (R) length distribution of: $C_{10}=2\%$, $C_{12}=57\%$, $C_{14}=23\%$, $C_{16}=11\%$ and $C_{18}=7\%$.

faces, all glassware was pre-treated with a 5 μg/ml surfactant (Dodigen) solution for 24 h, followed by thorough rinsing with warm tap water and distilled water. Finally, the glassware was rinsed with methanol and left to dry. This is in line with the advice given in the literature [18]. Osburn et al. [6] showed that the adsorption of surfactant to the walls of the sample container can be minimised by a modest addition of formaline and an etoxylated alcohol to the sample, but this was not found necessary in this work.

2.3. Apparatus

A schematic system configuration is shown in Fig. 2. The LC system consisted of a model 2150 HPLC-pump (LKB, Bromma, Sweden), a two-position ten-port high-pressure valve (Valco, Houston, TX, USA) and a UV detector (Spectroflow 757, Kratos, Ramsey, NJ, USA). The monitoring wavelength was 264 nm. Chromatographic software (JCL6000 Chromatography Data System, Jones Chromatography, Hengoed, UK) was used for collection of data and control of the high-pressure valve connected to the MMLLE system. A column oven (Kontron Instruments, Milan, Italy) kept the column at 50°C.

The membrane unit consisted of two identically machined grooved blocks with dimensions 0.25×1.5×150 mm, forming a channel on each side of the membrane with a nominal volume of 56 μl. Clamped between the blocks was a thin (25.4 μm) porous membrane (Celgard 2400, Hoechst Celanese, Charlotte, NC, USA) with pore dimension 0.04×0.12 μm and a porosity of 41%.

Syringe pumps were used for sucking the aqueous samples (donor phase) (P-500 Pharmacia, Uppsala, Sweden) and for transferring the organic acceptor phase (CMA/100, CMA Microdialysis, Stockholm, Sweden).

The liquid chromatographic analysis was performed as normal-phase chromatography with a mixture of chloroform (70%), ethanol (28%), ammonia (1%) and heptanoic acid (1%) as the eluent and a 250×2.1 mm I.D. cyanopropyl column (LiChrosorb, Merck). The flow-rate used in all experiments was 0.2 ml/min.

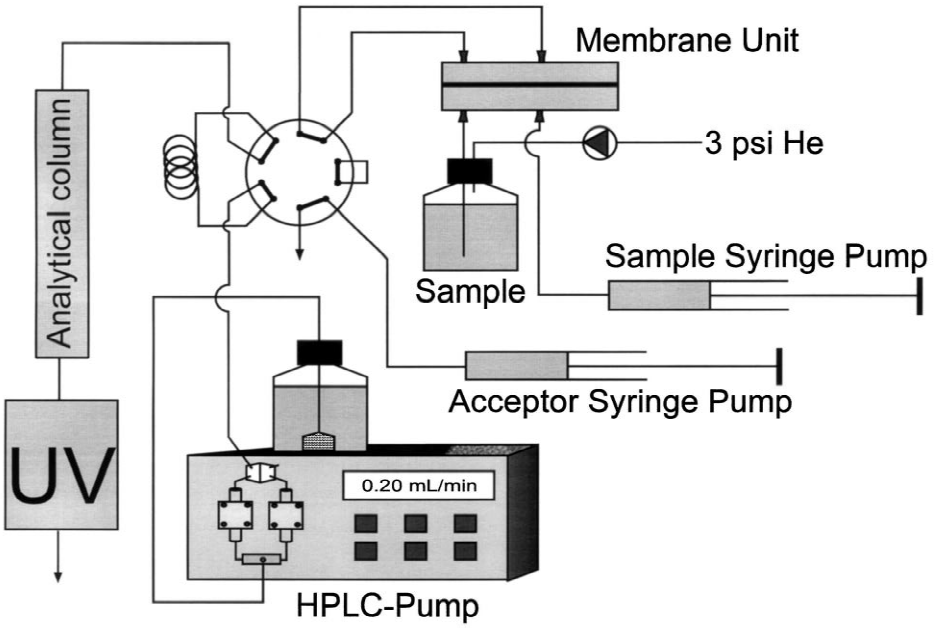

Fig. 2. Experimental set-up with the high pressure valve in position for MMLLE extraction.

2.4. Analytical procedure

Please refer to Fig. 2 when reading this section.

Milli-Q or river water samples, spiked with the surfactants under study, were most often sucked at a flow-rate of 1–8 ml/min. This was feasible only when pressurising the sample container with He at 3 p.s.i., otherwise the membrane was sucked into the flow-path, disrupting the flow (1 p.s.i.=6894.76 Pa). The reason for sucking the sample, rather than pumping it, is to avoid contact between the sample and the pump syringes/pistons, where adsorption of surfactants otherwise might occur. The organic acceptor phase was undecane, 1-chlorobutane or chloroform with up to 10% heptanoic acid added as ion-pairing agent. The acceptor was kept stagnant during the extraction. After completed extraction, the injection valve was switched and 200 μl of solvent passed through the acceptor channel and the connecting tubing (V_{tubing}=100 μl), transferring the sample to a 130 μl injection loop. Upon switching the valve, the content of the loop is transferred by the eluent stream onto the column, where the analytes are initially focused (see Section 3.2) and subsequently separated. Meanwhile, the donor side of the membrane unit is washed with a 1% solution of ammonia. After 4 min of separation, the valve is again switched allowing for rinsing of the acceptor compartment and attached tubing. Finally the valve is switched back and a new extraction cycle can commence. The total time for one analysis is approximately 25 min.

3. Result and discussion

3.1. Extraction

3.1.1. Organic solvent and ion-pairing agent

Quaternary ammonium ions can be extracted from an aqueous solution to an organic solution as ion pairs with anions as counter-ions. The counter ion may be solvated in the organic solvent:

$$NR^{+}_{4(aq)} + X^{-}_{(org)} \leftrightarrow NR^{+}_{4}\ X^{-}_{(org)}$$

or in the aqueous sample

$$NR^{+}_{4(aq)} + X^{-}_{(aq)} \leftrightarrow NR^{+}_{4}\ X^{-}_{(org)}$$

The organic solvents chloroform, 1-chlorobutane, undecane and heptane were tested for this extraction scheme. As ion-pairing agents, heptanoic acid, sodium phenol sulfonate and *N*-2-hydroxyethyl piperazinepropanolsulfonic acid were tried. Although the highest distribution coefficients were obtained with chloroform, this solvent was troublesome with leakage into the aqueous phase. These problems were negligible using the other solvents and 1-chlorobutane gave the highest distribution coefficient. It was chosen to include the counter-ion in the organic liquid. This is a straightforward option, as it does not necessitate any premixing or T-connector in the system. Furthermore, it minimises the risk for foam or emulsion formation which otherwise might lead to analyte losses. Of the complexing agents tested, only heptanoic acid exhibited no problems with solubility in the organic phase. Hence, the combination of 1-chlorobutane and heptanoic acid was chosen for the further experiments.

3.1.2. Microporous membrane liquid–liquid extraction

In MMLLE an organic solvent fills the pores of a hydrophobic membrane and an acceptor compartment, into which extraction occurs. Hence, the membrane serves as an interfacial support between the aqueous sample solution and the organic solvent of the acceptor phase. With the sample solution in continuous motion and the acceptor stagnant, high enrichment factors are readily and rapidly achieved. This is obvious considering the large volume ratio ($V_{sample}/V_{organic}$) attainable in this set-up, which together with large K_p values promises high preconcentration factors. Moreover, this approach sets aside a number of problems usually encountered in liquid–liquid extraction. It prevents the formation of foam and emulsions, enables a minimal consumption of organic liquid and, which is even more important, facilitates an on-line connection to analytical instrumentation.

In Fig. 3 the enrichment factors (E_e) versus the extracted volume for three of the homologues of Dodigen 226 are plotted. Eighty to three hundred milliliters spiked reagent grade water was extracted at a flow-rate of 8 ml/min. The partition coefficient seems to increase with increasing chain-length of the homologues. As can be seen in the figure con-

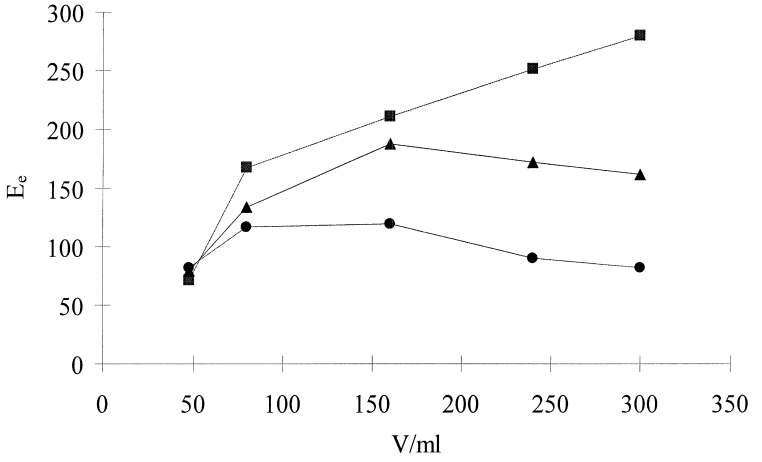

Fig. 3. Enrichment factor, E_e, versus the extracted volume, V, for three of the homologues of Dodigen 226 (C_{16} ■, C_{14} ▲, C_{12} ●) in reagent grade water.

centration factors of 100–250 times are readily possible.

3.2. Chromatographic separation and interfacing with MMLLE

A normal-phase liquid chromatographic separation system was developed, capable of separating the homologues of the model compound. The chromatography is based on ion-pair formation between the surfactant and the dissociated heptanoic acid.

The concentration of ammonia in the mobile phase is important. It acts as a modifier interacting with residual silanol functions on the cyanopropyl stationary phase, hence reducing unwanted adsorption. With a mobile phase composition of 28% (v/v) ethanol and 1% (v/v) heptanoic acid in chloroform, the retention times decreased with the ammonia concentration up to 1%. At 2% ammonia, the separation between homologues impaired and the peak shape deteriorated. Optimal conditions were obtained at 1% ammonia. The concentration of ion-pairing agent also strongly influences the retention, as expected. A heptanoic acid concentration of 1% was selected.

In this work an MMLLE unit with an acceptor volume of 56 μl was chosen to be appropriate for the application. This called for an injection loop of 130 μl to ensure complete transfer of the extract. With the sample now contained in a non-eluting (eluotropic) solvent, it is focused upon entering the beginning of the column. Large volume injections are favoured by large differences in eluent strength between the loop content and the eluent itself. The assessments of injection volume limits have been described by Mills et al. [19].

Since it was found that a mid-bore chromatographic column (2.1 mm I.D.) could provide the necessary focusing, resulting in better mass sensitivity for the same injected mass compared to a conventional 4.6 mm I.D. column, the narrower column was employed. Furthermore, the consumption of solvent is decreased which is especially valuable in normal-phase chromatography as such systems generally consume more hazardous solvents than do reversed-phase systems. The situation with an eluotropic acceptor solvent is by no means unique for the present application. On the contrary the opposite holds true in the majority of possible MMLLE applications, giving the opportunity of large volume injection with practically any extraction protocol.

3.3. Detection

For Dodigen, which contains a UV-absorbing benzene ring, detection is conveniently made using a UV detector at 264 nm. However, most cationic surfactants do not absorb UV radiation and, therefore, the detection is not straightforward [8]. In a non-aqueous eluent in normal-phase chromatography, conductometric detection can be used for

Table 1
Limits of detection for Dodigen homologues in spiked river water. The results are given in ranges, due to the uncertainty in the concentration of the reference material (see Section 2.1)

Homologue	LOD (μg/l)
C_{12}	1–4
C_{14}	0.7–2
C_{16}	1–5

ionic compounds as described in the literature [7]. Some experiments with this detection have been made in our laboratory. However, the detection limits were about 50 times higher than with UV detection, which is not practical. Even if post-column detection set-ups have been described [12,13], LC–MS will be a more realistic alternative for future work.

3.4. Linearity and limit of detection

The linearity and limit of detection were investigated extracting 80 ml of spiked river water (Höje River, Sweden) for 20 min. Starting at the highest (300 μg/l Dodigen) and ending with the lowest (10 μg/l Dodigen) concentration, the most abundant homologue (C_{12}) had an $r^2>0.999$ and an intercept of 58 ± 61 ($P=0.05$, $n=5$). The fact that the intercept does not deviate significantly from origo, and that it is quite feasible to extract starting with the sample of highest concentration, indicates low or no adsorption or carry-over effects. This holds true both for the extraction procedure and the chromatographic system.

Limits of detection (LODs) in river water, approximated with three times the baseline noise, for three of the homologues are presented in Table 1. The corresponding spiking concentration was 2–7 μg/l per homologue.

3.5. Application

Processed real samples included spiked river water collected from Höje River, Sweden and spiked waste-water from a sewage plant in Portugal. As can be seen from the chromatograms in Figs. 4 and 5, the selectivity is extremely high. No interfering compounds are visible even at such low concentrations as μg/l and no analyte peaks were found in the corresponding blank chromatograms. However, the quantitation is still uncertain in the difficult matrix of wastewater, so those results have to be considered as semi-quantitative.

Fig. 4 shows the enrichment of 80 ml river water spiked with 10 μg/l of Dodigen, i.e. 2–5 μg/l of the C_{12} homologue and 0.7–2 μg/l of the C_{14} homologue. Because of the rather polar eluent, potentially interfering compounds are eluted at the beginning of the chromatogram, thus not interfering with the analytes of interest.

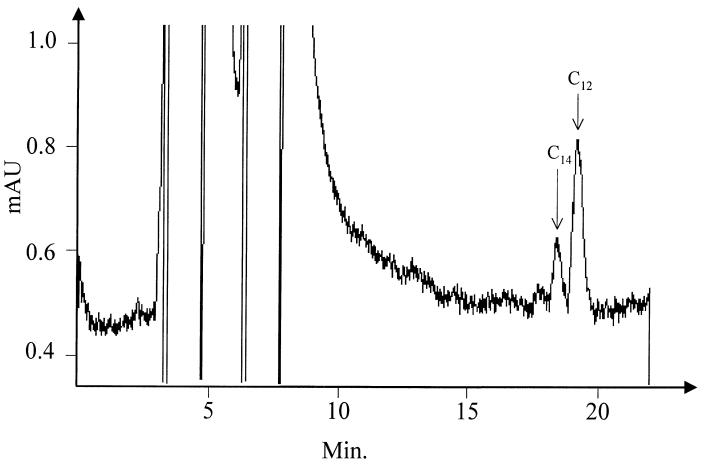

Fig. 4. Enrichment of 10 μg/l Dodigen (2–5 μg/l of the C_{12} homologue and 0.7–2 μg/l of the C_{14} homologue) in natural water followed by NP-HPLC with UV detection. No peaks were found in the corresponding blank.

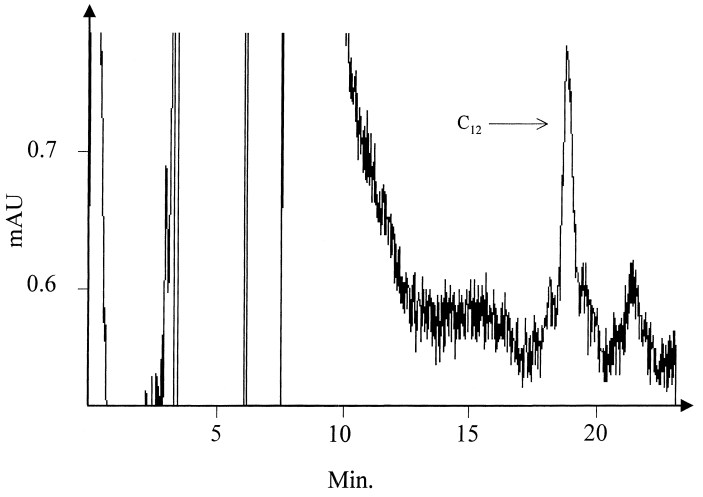

Fig. 5. Enrichment of 12.5 μg/l Dodigen (2–7 μg/l of the C_{12} homologue) in waste-water followed by NP-HPLC with UV detection. No peaks were found in the corresponding blank.

Fig. 5 shows the enrichment of 12.5 μg/l Dodigen (2–7 μg/l of the C_{12} homologue) in waste-water (Porto, Portugal).

4. Conclusions

A system for sensitively and selectively enriching and separating cationic surfactants even at homologue level from complex matrices, with detection limits in the low μg/l region has been demonstrated. The cumbersome adsorption of cationic surfactants has been circumvented both in the extraction as well as in the chromatographic procedure.

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