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# Simultaneous quantitative trace analysis of anionic and nonionic surfactant mixtures by reversed-phase liquid chromatography ☆

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**Abstract**

The aim of this work was to simultaneously analyse mixtures of a polydisperse polyethylene oxide (PEO) nonionic surfactant and an anionic surfactant (sodium dodecylsulphate, SDS) in water containing sodium chloride in order to quantify trace amounts of these mixtures after their adsorption at water–solid interfaces. A fractional factorial design was then used to optimise the separation by ion-pair reversed-phase liquid chromatography as a function of six factors: the chain length of the tetraalkylammonium salt used as ion-pairing reagent which varied from methyl ($C_1$) to *n*-propyl ($C_3$); the concentration of this ion-pairing salt; the acetonitrile percentage in water used as organic modifier; the flow-rate; the temperature of analysis and also the sodium chloride concentration. The factorial design enabled in a limited number of analyses, not only to determine which factors had significant effects on retention times or on resolution between a pair of nonionic oligomers, but also to modelize and then find the interesting and rugged area where this resolution was optimal as well as the conditions where time of analysis was not prohibitive. After optimisation of HPLC analysis, we used a trace enrichment procedure to quantify very low concentrations of SDS and $C_{12}E_9$ polydisperse PEO in water. A $C_{18}$ cartridge and a strong anionic exchange cartridge were coupled and the conditions of elution were optimised in order to obtain concentrated samples which were injected in the same eluent than the HPLC mobile phase. Under such conditions, we were able to quantify, in a single run, mixtures of anionic and nonionic surfactants at concentrations as low as 3.6 μg $l^{-1}$ for SDS and 2.5 μg $l^{-1}$ for each PEO oligomer in water. 

*Keywords:* Factorial design; Extraction methods; Surfactants; Poly(ethylene oxide); Sodium dodecylsulphate

## 1. Introduction

Research on surfactant mixtures is of considerable interest for various industrial applications (such as detergency, wetting, flotation . . . ) because surfactant mixtures enhance the performance of these compounds as compared to the use of single surfactants for practical applications. Almost all commercial and industrial formulations are made of mixtures of

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different type of surfactants such as nonionic and ionic surfactants. Both of these surfactant families are used in large quantities and are rejected in the environment. Therefore the determination of mixtures of surfactants is not only important in order to understand specific properties for industrial applications but also for environmental monitoring. The polyethoxylated alcohol surfactants (polyethylene oxide, PEO) are the most important nonionic surfactants used today. Commercial PEOs are mixtures of oligomers of different ethylene oxide (EO) number but also of various hydrophobic alkyl chains. Very efficient analytical techniques are required to determine not only the degree of oligomerisation but also the alkyl chain length.

Chromatographic separation techniques have been widely used for many years to analyse nonionic surfactants. Gas chromatography (GC) and even special high-temperature (HT) GC is not applicable for polyethylene oxides with high degree of polymerisation [1]. Supercritical fluid chromatography has been investigated for nonionic surfactant analysis, using capillary columns [2] or packed microcolumns [3]. However high-performance liquid chromatography (HPLC) seems to be the most efficient tool in the case of highly condensed chains in order to obtain total polymer characterisation, using normal-phase conditions [4–7], reversed-phase liquid chromatography [8–10], ion-exchange chromatography [11–13] or thin-layer chromatography [14].

The level of PEO in untreated municipal sewage is in the 0.5–5 mg $l^{-1}$ concentration range and can be lower after water treatments [15]. Only the combination of trace enrichment procedures and HPLC could solve the problem of the determination of PEO surfactants in the aqueous phase at total concentrations below 1 mg $l^{-1}$ [16]. Solid-phase extraction (SPE) is a rapid and quantitative method that permits selective extraction and concentration of traces of analytes [17]. When the samples are mixtures of nonionic and ionic species, the choice of different solid phases can also permit a separation of the different analytes prior to HPLC analysis.

In this context, we have developed a strategy for the simultaneous analysis of mixtures of PEOs with an anionic surfactant in a very large concentration range, down to the trace levels in water, with the aim of a subsequent quantitative analysis of these mixtures after their adsorption at water–solid interfaces. Our goal was to quantify precisely the concentration of the nonionic and the ionic species remaining in the aqueous supernatant after their adsorption on a solid surface. Three cases could then occur. If the polydisperse PEO and the anionic surfactant were at concentrations above the limit of quantification of the HPLC method developed, they could be directly and simultaneously analysed and their concentration determined. If one of the two species was at trace level in the supernatant, we should concentrate one or the other on an appropriate SPE cartridge before the HPLC analysis. Finally, if the two species were at trace levels, a simultaneous enrichment method should be developed to quantify them in water.

Indeed the surfactant properties of these complex mixtures of surfactants have to be more thoroughly investigated to understand their behaviour in the environment or in industrial formulations [18].

## 2. Experimental

### 2.1. Apparatus

The analyses were performed using a Gold liquid chromatography system (Beckman, Fullerton, CA, USA) equipped with a 200-μl injection loop, a pulse damper from Touzart et Matignon (Les Ulis, France) and a RID-6A differential refractometric detector (Shimadzu, Kyoto, Japan). Reversed-phase HPLC analyses were performed using an octyl Ultrasphere Beckman column (250×4.6 mm I.D., $d_p$=5 μm). The column was thermostated by a Sup-Rs Stabitherm oven from Prolabo (Fontenay-sous-Bois, France).

The liquid–solid extraction cartridges were Sep-Pak plus $C_{18}$ and Sep-Pak accell plus QMA (Waters France, Saint-Quentin-en-Yvelines, France). SPE cartridges from Touzart et Matignon (Isolute PSA and Isolute $NH_2$ type) were also tested.

### 2.2. Reagents and samples

Water was purified and deionized using an Alpha Q system (Millipore France, Molsheim, France). Acetonitrile of HPLC-grade (SDS, Paris, France) was used without previous purification.

The mobile phases used in HPLC were degassed prior to use in a 2510 Branson Ultrasonic system purchased from Touzart et Matignon.

The polydisperse PEO surfactant studied is described according to one of the usual terminologies, i.e., $C_mE_n$, where $m$ is the number of carbons in the fatty chain ($m=12$) and $n$ is the number of condensed ethylene oxide units. The average degree of ethoxylation is denoted by $\bar{n}$ ($\bar{n}=8.8$). This polydisperse lauryl ether (noticed $C_{12}E_9$) was obtained from Nikko (Tokyo, Japan). We also used *n*-dodecanol, $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_5$ and $C_{12}E_8$ as standards of 98% or better purity from Fluka France (L'Isle d'Abeau, France). The anionic surfactant, i.e., sodium laurylsulphate (SDS, purity>99%), was purchased from Sigma France (L'Isle d'Abeau, France).

NaCl (purity>99%) was obtained from Aldrich France (L'Isle d'Abeau, France).

Three alkylammonium salts were used (purity> 99%): tetramethylammonium bromide, tetraethylammonium bromide and tetra-*n*-propylammonium bromide, from Acros Organics France (Noisy le Grand, France).

### 2.3. Chromatographic conditions

Different chromatographic conditions were tested in order to analyse directly and simultaneously a mixture of ionic and nonionic surfactants that remained in an aqueous phase containing NaCl after a previous step of adsorption on a solid phase.

Different mobile phases were prepared with a definite percentage of acetonitrile in water and definite concentrations of NaCl and ion-pairing reagent. The anionic surfactant (SDS) was not retained on a $C_8$ column with mobile phases containing only acetonitrile and water and was eluted at the column dead volume. The addition of the cationic ion-pairing reagent made the separation possible simultaneously with the nonionic surfactant. Other mobile phases were prepared without salt in order to rinse the column between two different analysis. The column was conditioned before analysis firstly with the mobile phase without NaCl nor the ion-pairing reagent (during approximately 45 min). It was then equilibrated for approximately 1 h with the same eluent but in the presence of the salts (until a stable baseline was reached). The chromatographic system was regularly rinsed with the mobile phase without salt in order to avoid a rapid degradation of the column.

### 2.4. Data handling

Retention times $t_R$, retention factors $k'$, resolution $R_s$ and selectivity $\alpha$, deduced from the chromatograms, were handled using JMP 3.2 software from the SAS Institute (Cary, NC, USA); statistical calculations and modelling were performed.

### 2.5. Solid-phase extraction

When PEO and/or SDS were too diluted in the aqueous phase after the adsorption experiments to be directly and simultaneously analysed with HPLC, a trace enrichment procedure was developed.

Traces of PEO could be concentrated using a $C_{18}$-bonded silica cartridge, such as the one described in a previous paper [19], whereas traces of SDS could be enriched using a strong anion-exchange cartridge (QMA cartridge) of the quaternary ammonium type. Weak anion exchangers: aminopropyl phase ($NH_2$ cartridge) and ethylenediamine-*n*-propyl phase (PSA cartridge) were also tested for SDS enrichment. The SDS diluted in a volume $V_{aq}$ of water was percolated (by vacuum) through QMA, $NH_2$ or PSA cartridges at a flow-rate less than 10 ml $min^{-1}$. Under the same experimental conditions, SDS was quantitatively absorbed on the strong anion exchanger but not on the weak anion exchangers. As a consequence we chose the QMA cartridge for quantitative enrichment of SDS.

When anionic and nonionic surfactants were both at trace levels in water, they were simultaneously extracted with on-line cartridges of the $C_{18}$ and the QMA type. Diluted aqueous solutions ($V_{aq}$) were percolated first through the QMA cartridge and then through the $C_{18}$ cartridge. To respect this order was of great importance because a non negligible fraction of SDS remained on the $C_{18}$ cartridge if it was in the first position. Vice versa, when QMA was in the first position, PEO was not significantly retained on this ionic cartridge and only a negligible amount of SDS passed through the QMA solid phase to be retained on the second cartridge, i.e., $C_{18}$ cartridge. As the

quantification limit of SDS is largely lower than that of PEO, only a known fraction of the total aqueous volume was percolated on QMA cartridge. After this first step of enrichment, the cartridges could be separated and the rest of the aqueous volume could be percolated on the $C_{18}$ cartridge only. Finally, anionic and nonionic surfactants were desorbed, respectively, from QMA and $C_{18}$ cartridges with different enrichment factors. The two surfactants were then analysed with the same mobile phase.

Desorption from a solid phase of the PEO surfactant, diluted in an aqueous volume ($V_{aq}$), was optimised in a previous paper [19]: PEO was quantitatively desorbed from the $C_{18}$ cartridge with 4 ml of pure acetonitrile. A fraction of this concentrated organic solution was diluted with water containing the ion-pairing reagent in order to inject the solutes in a solvent mixture of the same composition as the mobile phase used in the following chromatographic analysis. Consequently the concentration factor $F$ to consider in the quantitative studies was:

$$F = \frac{V_{aq}}{4} \cdot \left( \frac{\%\ CH_3CN \text{ in mobile phase}}{100} \right)$$

Similarly, we optimised the desorption of SDS from the QMA cartridge, with acetonitrile containing the ion-pairing reagent as desorption solvent ($V_{solv}$). This concentrated organic solution was next diluted with water in order to obtain the composition of the mobile phase. So, the concentration factor $F'$ to consider was:

$$F' = \frac{V_{aq}}{V_{solv}} \cdot \left( \frac{\%\ CH_3CN \text{ in mobile phase}}{100} \right)$$

## 3. Results and discussion

SDS and various anionic surfactants are generally analysed and separated by ion-exchange chromatography [20], but reversed-phase liquid chromatography may also be used whenever a high ionic strength mobile phase is employed [21–23].

Ion-pair reversed-phase chromatography is based upon the use of an apolar stationary phase, such as $C_8$ or $C_{18}$ bonded silicas, that is dynamically modified by an ion-interaction reagent added to the aqueous–organic mobile phase. Anionic species that are not retained in reversed-phase systems can thus be retained because of the formation of an electroneutral complex between the anionic sample and the ion-pairing reagent of opposite charge. The most commonly used ion-pairing reagents present a large hydrophobic moiety and a small ionised group whose charge is opposite to that of the samples. The effect on the retention of ionic samples is interpreted by the adsorption of the ion-interaction reagent on the hydrophobic surface leading consecutively to the formation of charged sites. Ionic samples are then retained by electrostatic forces in the layer adjacent to the surface of the particles of the stationary phase [24,25] but all reversed-phase sites are not modified. Ion-interaction and conventional reversed-phase mechanisms coexist, permitting simultaneous separation of ionic and neutral hydrophobic species.

In our case, a method using $C_8$-bonded silica and acetonitrile–water mobile phase was developed [19] in order to obtain the separation of nonionic surfactant mixtures which differ in their alkyl chain length and their degree of ethoxylation. Consequently, we chose to adapt these experimental conditions to ion-interaction chromatography, adding a suitable ion-interaction reagent of alkylammonium type in order to separate, in a single run, the SDS and the $C_{12}E_9$ polydisperse nonionic surfactant. It was particularly important to individually separate the SDS and the single oligomers of the PEO in order to investigate the effects of the individual compounds on their respective adsorption at water–solid interfaces as it is the adsorption of global mixtures of surfactants which are generally studied.

Many variables are involved in ion-pair reversed-phase chromatography such as the nature and the concentration of alkylammonium salts, the organic cosolvent percentage, flow-rate, temperature of analysis. The optimisation of the experimental conditions can be very time-consuming if we make use of the traditional approach consisting on the systematic and univariate study of one factor. A chemometric approach is based on the use of a matrix of experiments which allows studying the impact of the simultaneous variation of all the factors considered to influence the chromatographic separation.

### *3.1. Optimisation of analysis by means of factorial design*

Many papers deal with the development and the

optimisation of HPLC methods using experimental designs, that permit to fully characterise the effects and the interdependence of all studied parameters [26–29]. A mathematical model is then built which relates the observed chromatographic responses to the various factors and to their combinations [30–32].

The factors chosen for the investigation were: the chain length of the alkylammonium salt ($C_n$), which varied from methyl ($C_1$) to *n*-propyl ($C_3$), the concentration of the ion-pairing reagent ([salt]), the acetonitrile percentage (% $CH_3CN$), the flow-rate (Flow) and the temperature of analysis ($T$) [33,34]. Because NaCl was used to keep a fixed ionic strength in adsorption experiments of surfactant mixtures at water–solid interfaces, it could be present in water with traces of SDS and nonionic surfactant before the chromatographic analysis. In such conditions, we added to the study of operating parameters reported above, the influence of NaCl concentration on the chromatographic separation.

In the factorial design method each factor is investigated at two fixed levels, denoted by $+1$ and $-1$. In our case, with six factors investigated and supposedly to be influent, a full factorial design would have required $2^6=64$ trials. Such a full factorial design would have given all combinations of six variables, each at two levels, but it would have induced a too high number of HPLC analyses. To perform a lower number of experiments, a fractional factorial design can be used. It is possible to include one, two or more factors in the design by associating them to higher order interaction columns of the matrix. This operation complicates the interpretation of the results and leads to a partial loss of information because it is then impossible to discriminate between the original effects of a factor and the interactions of the effects between different factors. However we decided first to run a $2^{6-3}$ factorial design, associating three factors to two second-order interaction columns and to the third-order interaction column of the matrix. Then, eight experiments had to be performed to calculate the magnitude of the effects of the six factors and of their interactions. With our experimental results, we could build a model of the type: $Y_i = h_0 + \Sigma_i \ h_i X_i + \Sigma_{ij} h_{ij} X_i X_j$ where $Y_i$ is the experimental response, $X_i$ the factors studied, $X_i X_j$ the second-order interaction factors and $h$ the coefficients of each term calculated by multiple regression analysis (higher order interaction factors, like $X_i X_j X_k$, were neglected).

Preliminary experiments allowed the identification of the experimental space. Table 1 gives the selected values for $-1$, 0 and $+1$ levels for the six different factors. Seven experiments were performed at level 0 to estimate the effect $h_0$. The $-\alpha$ and $+\alpha$ levels correspond to a star design that is necessary to determine eventual second-order effects (a squared term is then added to the equation reported above: $\Sigma_i h_{ii} X_i^2$) if the response is not linear on the defined experimental space. In the case of a $2^{6-3}$ design, the value of the $\alpha$ level is equal to 1.684. The star design contains only the first-order factors and squared factors, but no interaction terms. The juxtaposition of a two-level factorial design with a star design, when the centers of the two separated experimental designs coincide, is said to be a central composite design.

Consequently, our experimental space was defined with the calculated values corresponding to the $-\alpha$ and $+\alpha$ levels as limits. For example, if we choose to study the effect of flow-rate between the $-\alpha$ level ($x_{-\alpha}=0.8$ ml min$^{-1}$) and the $+\alpha$ level ($x_{+\alpha}=1.2$ ml min$^{-1}$), the value corresponding to a level less than 0 ($-0.6$ for example) is given by $x_{-0.6}=x_0-0.6/\alpha(x_0-x_{-\alpha})=0.93$ ml min$^{-1}$. Moreover, it is to

Table 1
Experimental space for the six factors studied as a function of five levels of investigation

| Level | A: $C_n$ | B: [Salt] (mol l$^{-1}$) | C: [NaCl] (mol l$^{-1}$) | D: Flow (ml min$^{-1}$) | E: $T$ (°C) | F: % $CH_3CN$ (%) |
|---|---|---|---|---|---|---|
| $-\alpha$ | – | $5\cdot10^{-4}$ | $10^{-3}$ | 0.80 | 21 | 50 |
| $-1$ | $(CH_3)_4NBr$ | $1.3\cdot10^{-3}$ | $2.5\cdot10^{-3}$ | 0.88 | 23 | 52 |
| 0 | $(C_2H_5)_4NBr$ | $5\cdot10^{-3}$ | $10^{-2}$ | 1.00 | 26 | 55 |
| $+1$ | $(C_3H_7)_4NBr$ | $2\cdot10^{-2}$ | $4\cdot10^{-2}$ | 1.12 | 29 | 58 |
| $+\alpha$ | – | $5\cdot10^{-2}$ | $10^{-1}$ | 1.20 | 31 | 60 |

be noticed that we considered the decadic logarithm of the concentrations in order to calculate the concentrations corresponding to each level reported in Table 1.

Two chromatograms are shown in Fig. 1 to illustrate two experiments of the initial $2^{6-3}$ design (chromatograms performed, respectively at level +1 and 0). All the analyses were carried out in random to avoid block effects. We could see that the retention of $C_{12}E_n$ nonionic oligomers decreased regu-

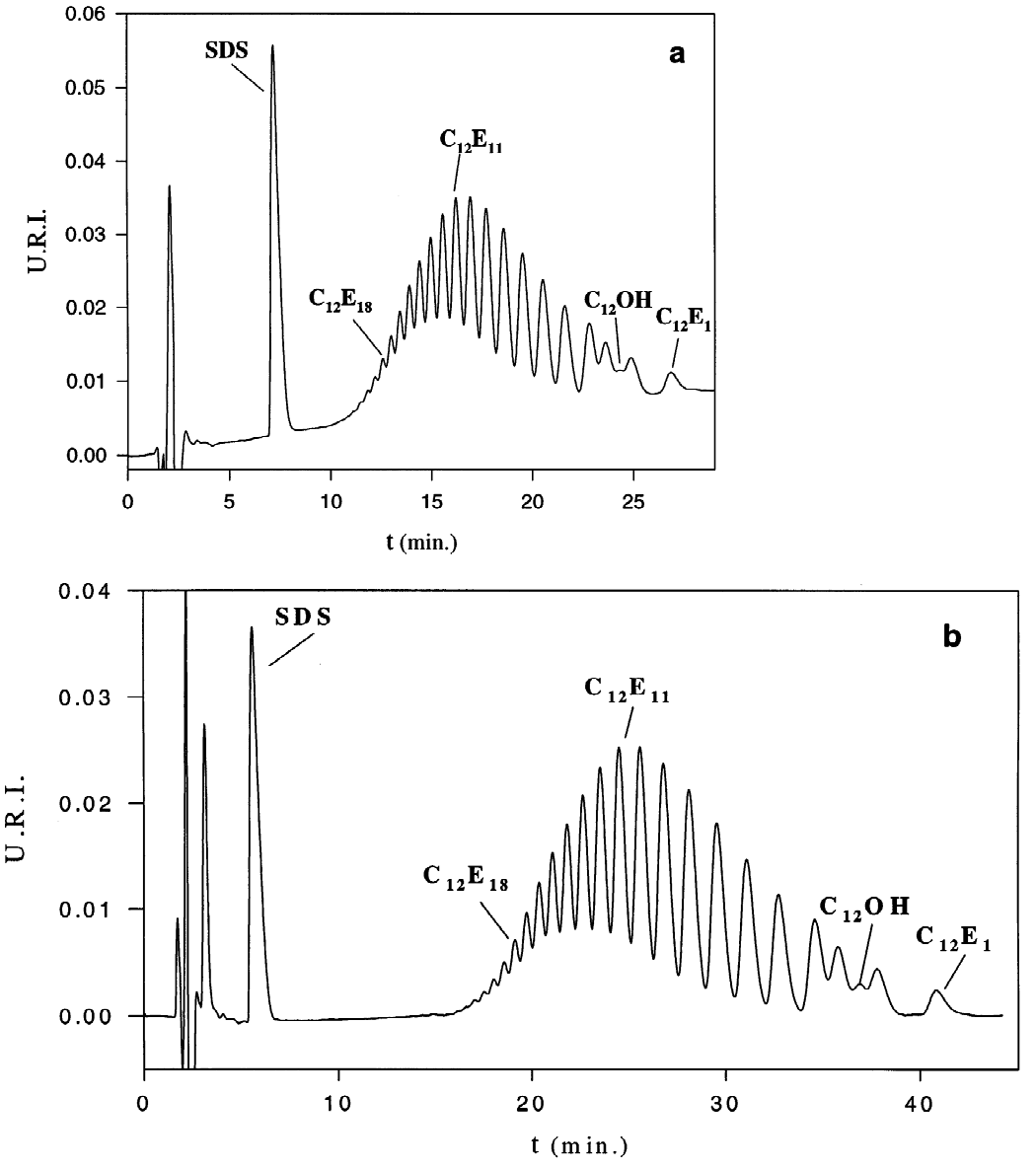


Fig. 1. Analysis of a mixture of SDS (0.2 g $l^{-1}$) and $C_{12}E_9$ (2 g $l^{-1}$) on $C_8$ Ultraspher column (250×4.6 mm, $d_p$=5 μm) under the following operating conditions: (a) mobile phase $CH_3CN$–water (58:42, v/v)+$(C_3H_7)_4NBr$ $2\cdot10^{-2}$ mol $l^{-1}$+NaCl $4\cdot10^{-2}$ mol $l^{-1}$, flow-rate 1.12 ml $min^{-1}$ and temperature 29°C; (b) mobile phase $CH_3CN$–water (55:45, v/v)+$(C_2H_5)_4NBr$ $5\cdot10^{-3}$ mol $l^{-1}$+NaCl $10^{-2}$ mol $l^{-1}$, flow-rate 1 ml $min^{-1}$ and temperature 26°C.

larly as the number of ethylene oxide units increased, in agreement with earlier experiments on $C_8$ or $C_{18}$ columns [19,35].

We chose different chromatographic responses $Y_i$ to evaluate the separation quality. First, the resolution factor, which reflects the degree of separation of a pair of peaks, was selected as the quality criteria of the nonionic surfactant separation. Chromatograms reported in Fig. 1 show a zone where the peaks resolution, approximately between $C_{12}E_{10}$ and $C_{12}E_{20}$ oligomers is less than unity. So we chose to study the resolution of the $C_{12}E_{11}$–$C_{12}E_{12}$ pair that gives the smaller value for the resolution.

Secondly, we decided to include an information concerning the time of analysis. This criteria was given by the retention time of $C_{12}E_1$, $C_{12}E_1$ being the last peak to elute. Other retention times, like $t_R$ of $C_{12}E_{18}$, could also be studied to apprehend the behaviour of compounds, homologous of $C_{12}E_1$, but very different from this one.

Finally, it was obviously important to measure the effects of the different operating factors on the retention time of the anionic SDS species, since the retention mechanisms of this compound were supposed to be quite different from those of nonionic oligomers.

In the initial $2^{6-3}$ fractional design, values of $h_i$ gave in fact the effects of the principal factors $X_i$ combined with second-order interaction factors $X_iX_j$. It was impossible to determine which of the principal factors or the interaction factors were predominant. So we decided to add to the $2^{6-3}$ design three series of eight experiments, executing a $2^{6-1}$ design (see Table 2). With these complementary designs all ambiguities about two-factor interactions were removed.

The results of the experiments enabled to estimate the effects of the principal factors, the squared factors and their second-order interactions for the retention time of $C_{12}E_1$ and $C_{12}E_{18}$ oligomers. As shown in Table 3, the main effects on nonionic oligomers retention times were due to the flow-rate and the organic solvent percentage, as is usual for a reversed-phase mechanism. Retention of nonionic species did not practically depend on ionic strength (NaCl and ion-pairing salt concentrations) and very little on the temperature of the analysis. However, it can be noticed that only retention times of homologous compounds with high degrees of ethoxylation ($C_{12}E_{18}$ as an example) slightly depend on temperature of analysis, contrary to the retention time of short ethoxylated homologues (like $C_{12}E_1$) which do not depend at all on temperature.

As far as the sign of the temperature effect is concerned, it is possible to distinguish a different behaviour of $C_{12}OH$ native alcohol with respect to the ethoxylated homologues. A positive value indicates that the retention time increases when temperature increases, that is the case for highly ethoxylated surfactants, whereas a negative value indicates the opposite effect as it is the case for *n*-dodecanol. $C_{12}OH$ peak identification was carried out by spiking the polydisperse nonionic surfactant with a pure $C_{12}OH$ standard. We noticed that *n*-dodecanol coeluted with the $C_{12}E_2$ peak at a lower temperature (i.e., 21°C, corresponding to level $-\alpha$). When the temperature was increasing, the tendency of $C_{12}OH$ was to coelute with the less lipophilic $C_{12}E_3$, the coelution taking place at 31°C (level $+\alpha$). At the opposite, highly ethoxylated surfactants showed a slightly increasing retention with temperature which was not the case for shorter ethoxylated oligomers. This behaviour could be interpreted by assuming the existence of two conformers of PEO in a water–acetonitrile medium [36]. This phenomenon would lead to a narrower distribution when temperature increased and would have consequences on the resolution of the nonionic surfactant peaks, that will be described later.

Statistical analysis revealed also an interaction effect between the flow-rate, the acetonitrile percentage and a squared effect (see Table 3). These were nevertheless negligible in comparison to the flow-rate and acetonitrile percentage factors. Finally, the retention time responses of the nonionic species were quasi-linear with flow-rate and organic modifier percentage (see Fig. 2).

The case of the retention of the ionic surfactant was obviously more complex. As expected, the effects of the concentration of ion-pairing reagent [salt] and of the chain length ($C_n$) on the retention of SDS were the major effects, as can be seen in Table 4. These two principal factors had positive effects, that means the higher the concentration and chain length of ion-pairing reagent, the higher the retention of SDS. Flow-rate factor and organic cosolvent

Table 2
Matrix of the fractional factorial design $2^{6-1}$ and chromatographic responses

| No. exp. | $C_n$ A | [Salt] B | [NaCl] C | AB | Flow AC=D | % $CH_3CN$ BC=F | T ABC=E | I | $t_r$ SDS (min) | $t_r$ $C_{12}E_1$ (min) | $t_r$ $C_{12}E_{18}$ (min) | $R_{s\ 11\text{-}12}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | − | − | − | + | + | + | − | + | 3.20 | 29.09 | 12.92 | 0.76 |
| 2 | + | − | − | − | − | + | + | + | 5.25 | 35.06 | 16.89 | 0.71 |
| 3 | − | + | − | − | + | − | + | + | 6.10 | 48.18 | 25.07 | 0.67 |
| 4 | + | + | − | + | − | − | − | + | 17.61 | 61.15 | 28.18 | 0.83 |
| 5 | − | − | + | + | − | − | + | + | 6.95 | 55.46 | 27.96 | 0.78 |
| 6 | + | − | + | − | + | − | − | + | 6.24 | 48.27 | 21.54 | 0.86 |
| 7 | − | + | + | − | − | + | − | + | 5.81 | 35.23 | 15.27 | 0.90 |
| 8 | + | + | + | + | + | + | + | + | 7.16 | 26.84 | 12.58 | 0.71 |
| 9 | − | − | − | + | − | − | − | + | 4.90 | 60.77 | 27.69 | 0.83 |
| 10 | + | − | − | − | + | − | + | + | 5.68 | 45.61 | 22.79 | 0.71 |
| 11 | − | + | − | − | − | + | + | + | 5.88 | 35.60 | 17.52 | 0.69 |
| 12 | + | + | − | + | + | + | − | + | 8.69 | 28.80 | 12.91 | 0.80 |
| 13 | − | − | + | + | + | + | + | + | 4.26 | 27.18 | 13.16 | 0.66 |
| 14 | + | − | + | − | − | + | − | + | 5.66 | 33.86 | 14.51 | 0.81 |
| 15 | − | + | + | − | + | − | − | + | 6.16 | 46.98 | 21.08 | 0.90 |
| 16 | + | + | + | + | − | − | + | + | 14.49 | 57.25 | 28.99 | 0.73 |
| 17 | − | − | − | + | + | − | + | + | 3.96 | 46.54 | 24.05 | 0.71 |
| 18 | + | − | − | − | − | − | − | + | 7.24 | 62.01 | 27.97 | 0.88 |
| 19 | − | + | − | − | + | + | − | + | 4.64 | 29.01 | 12.71 | 0.76 |
| 20 | + | + | − | + | − | + | + | + | 10.84 | 35.56 | 17.68 | 0.68 |
| 21 | − | − | + | + | − | + | − | + | 5.43 | 36.06 | 15.57 | 0.77 |
| 22 | + | − | + | − | + | + | + | + | 4.71 | 27.84 | 13.34 | 0.60 |
| 23 | − | + | + | − | − | − | + | + | 8.10 | 59.67 | 30.72 | 0.78 |
| 24 | + | + | + | + | + | − | − | + | 11.86 | 47.57 | 21.34 | 0.89 |
| 25 | − | − | − | + | − | + | − | + | 3.98 | 35.91 | 15.89 | 0.77 |
| 26 | + | − | − | − | + | + | + | + | 4.31 | 28.21 | 13.73 | 0.63 |
| 27 | − | + | − | − | − | − | + | + | 8.23 | 62.03 | 32.91 | 0.69 |
| 28 | + | + | − | + | + | − | − | + | 14.08 | 48.35 | 22.34 | 0.68 |
| 29 | − | − | + | + | + | − | + | + | 5.72 | 47.64 | 24.30 | 0.60 |
| 30 | + | − | + | − | − | − | − | + | 7.94 | 60.04 | 26.85 | 0.82 |
| 31 | − | + | + | − | + | + | − | + | 4.65 | 27.94 | 12.20 | 0.69 |
| 32 | + | + | + | + | − | + | + | + | 9.32 | 34.36 | 16.36 | 0.59 |

percentage factor also had significant effects on the SDS retention but temperature had not. The ionic strength was represented by the interaction factor [salt][NaCl]. Finally, three other second-order interactions appeared to influence significantly the retention time of SDS. Fig. 3 shows three models correlating the retention time of SDS to the different influent experimental factors. In these examples, NaCl concentration and flow-rate were fixed, respectively, at level +0.5 and at level 0. We can see on these models that modifying the ion-pairing reagent concentration, at a fixed percentage of acetonitrile, has a larger effect on the SDS retention when one uses tetra-*n*-propylammonium bromide (level +1) than tetramethylammonium bromide (level −1).

Table 3
Sum of squares, due to the influent principal factors and second-order interactions, for the retention time of two nonionic oligomers and for *n*-dodecanol

| | $C_{12}OH$ | $C_{12}E_1$ | $C_{12}E_{18}$ |
|---|---|---|---|
| Flow | 697 | 934 | 200 |
| % $CH_3CN$ | 3478 | 4612 | 1202 |
| *T* | 28 | – | 35 |
| Flow·% $CH_3CN$ | 46 | 42 | 16 |
| % $CH_3CN$·% $CH_3CN$ | 55 | 75 | 32 |

At first glance, if we choose to optimise the separation with an experimental constraint on $t_R$ (SDS), i.e., a retention time higher than 5 min, in order to avoid baseline disturbances that occur just

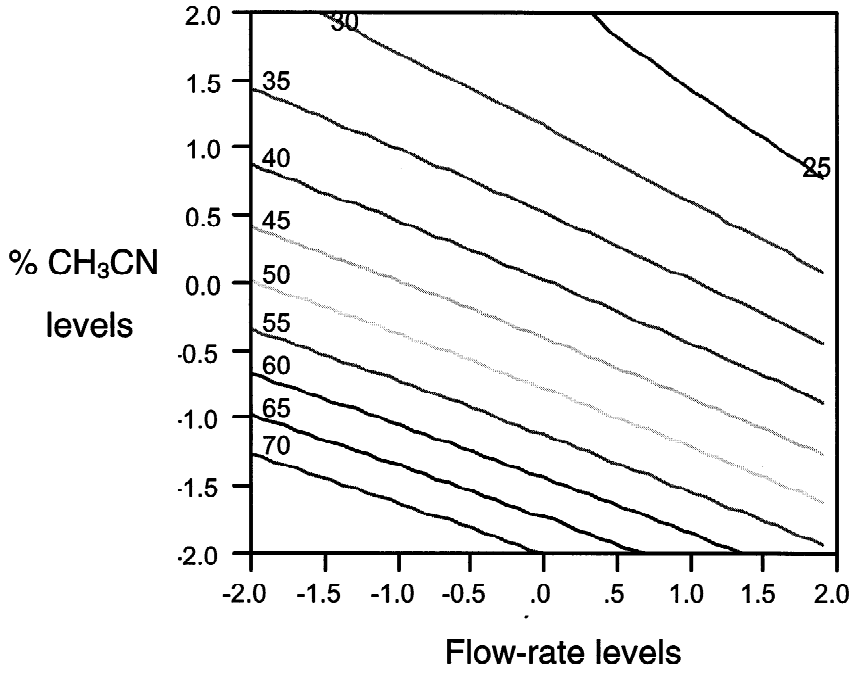


Fig. 2. Response surface correlating retention time of $C_{12}E_1$ oligomer (in minutes) to the most influent factors. Fitted model: $t_R$ ($C_{12}E_1$) = 40.41 − 4.98 (flow) − 11.07 (% $CH_3CN$) + 1.14 (flow·% $CH_3CN$) + 1.91 (% $CH_3CN)^2$.

after the dead volume, and lower than 8 min, in order to obtain the narrow peak which is necessary to quantify very low concentrations, tetra-*n*-propylammonium bromide seems to be the best choice as an ion-pairing reagent. In fact, with this ion-pairing reagent, we can obtain a maximum effect on $t_R$ (SDS) with a lower salt concentration.

Being now able to draw satisfactorily a model from the raw data of SDS and of $C_{12}E_n$ retention times, we had to study, in a second step, the evolution of the resolution between $C_{12}E_{11}$ and $C_{12}E_{12}$ oligomers ($R_{s\ 11\text{-}12}$) in the whole experiment space. It appeared that only one of the principal factors played a significant part on the resolution, i.e., the analysis temperature. An other important effect is that of the ionic strength, i.e., the interaction factor [salt][NaCl]. Finally, two smaller squared terms represented by [NaCl][NaCl] and % $CH_3CN$· % $CH_3CN$ appeared after the statistical treatment.

In order to determine the best conditions of separation, we decided to select only the conditions for which the resolution was larger than 0.85 and whenever possible close to 0.9. The effect of this constraint was dramatic for the range of high temperatures of analysis (levels up to −1, that is to say for temperatures greater than 23°C), where the resolution was always lower than 0.85. It could be explained by the fact suggested earlier: increasing temperature causes a narrowing of the distribution of the nonionic oligomers which is not compensated by an increasing efficiency. Consequently, the most suitable temperature of analysis was the lowest one.

As pointed out previously, $R_s$ was also dependent upon other factors: Fig. 4 presents some models with

Table 4
Sum of squares, due to the most influent principal factors and second-order interactions, for the SDS retention time

| $C_n$ | [Salt] | Flow | % $CH_3CN$ | $C_n$[salt] | [Salt][NaCl] | % $CH_3CNC_n$ | % $CH_3CN$[salt] |
|---|---|---|---|---|---|---|---|
| 92 | 109 | 24 | 56 | 40 | 9 | 9 | 10 |

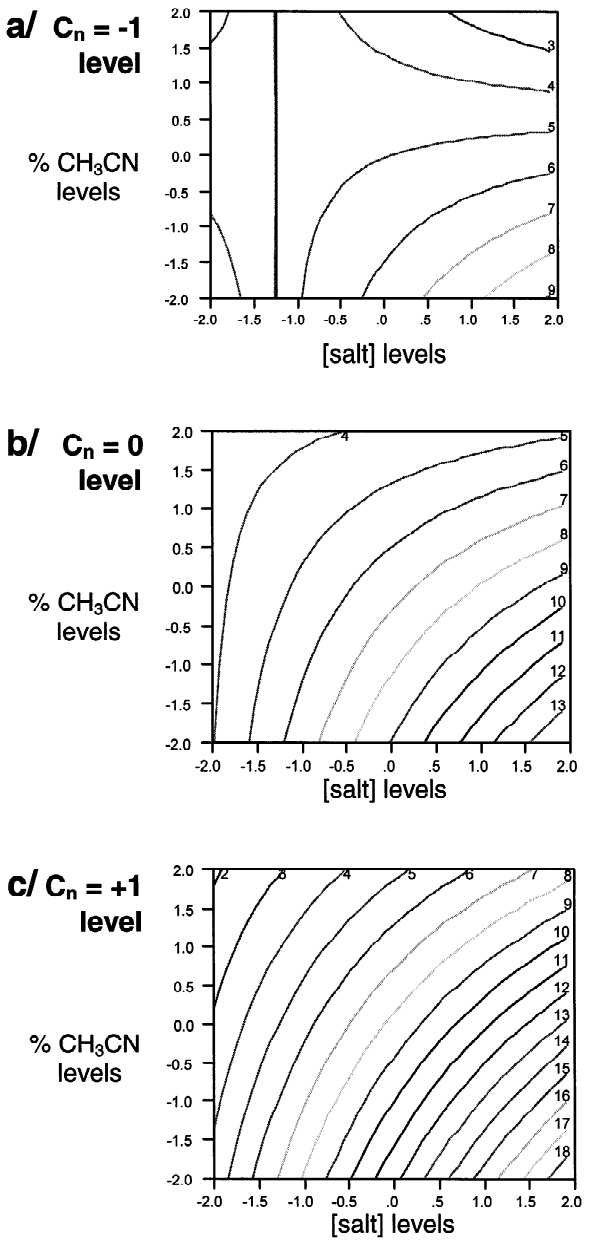


Fig. 3. Response surface correlating SDS retention time (in minutes) to the most influent factors, with a fixed NaCl concentration ($2\cdot10^{-2}$ mol $l^{-1}$) and a fixed flow-rate (1 ml $min^{-1}$). (a) $(CH_3)_4NBr$, (b) $(C_2H_5)_4NBr$, (c) $(C_3H_7)_4NBr$, as ion-pairing reagents. Fitted model: $t_R$ (SDS) = 6.62 + 1.64 ($C_n$) + 1.70 ([salt]) − 0.79 (flow) − 1.22 (% $CH_3CN$) + 1.12 ($C_n$[salt]) − 0.53 ([salt][NaCl]) − 0.53 ($C_n\cdot$% $CH_3CN$) − 0.56 ([salt]·% $CH_3CN$).

a fixed temperature of 21°C. One can see an interesting area appearing with a resolution equal to 0.9. This area was large at low salt concentrations (ion-pairing reagent and NaCl) and decreased with increasing salt concentration (see Fig. 4a and b). The change of the $C_{12}E_{11}$ and $C_{12}E_{12}$ retention factors was modelled and showed essentially a quasi-linear decrease upon increasing both the acetonitrile percentage and the NaCl concentration. At first glance we could conclude that high concentrations of salts (NaCl and tetraalkylammonium bromide) are not recommended to perform analysis with acceptable resolutions for nonionic oligomers. However Fig. 4c and d show evidences that increasing the ion-pairing reagent and NaCl concentrations even further (when concentrations of ion-pairing reagent and NaCl are, respectively greater than $10^{-2}$ mol $l^{-1}$ and $5\cdot10^{-3}$ mol $l^{-1}$) leads to an increase of the area where the resolution is maximal. The correlation between the statistical model and the experimental data being satisfactory (the predictive ability of the model was very well verified with chromatograms obtained in these salts concentration ranges), we studied the salts effects on efficiency and selectivity.

The results on resolution could not be completely explained by considering the salt effects on efficiency because efficiency was not really affected by the ion-pairing reagent concentration whereas NaCl concentration was a major factor. It turned out that increasing NaCl concentration led to a maximum in efficiency in the approximate range from $5\cdot10^{-3}$ to $2\cdot10^{-2}$ mol $l^{-1}$, but it was followed by a decrease at higher NaCl concentrations. Furthermore it seemed that this decrease in efficiency was compensated by a slight increase in selectivity at high ion-pairing salt concentrations. The role of the ion-pairing reagent in increasing selectivity of nonionic species in reversed-phase systems could be based on the modification of the polarity of the mobile phase and of the properties of the stationary phase. This phenomenon has been explained by the fact that the adsorption of the ion-pairing cation in the stationary phase is not linear but follows a Langmuir isotherm [25]. Thus, at low ion-pairing salt concentration, the tetraalkylammonium cation concentration in the stationary phase is proportional to its concentration in the mobile phase in the steep portion of the Langmuir isotherm. In these concentration ranges, the polarity of the

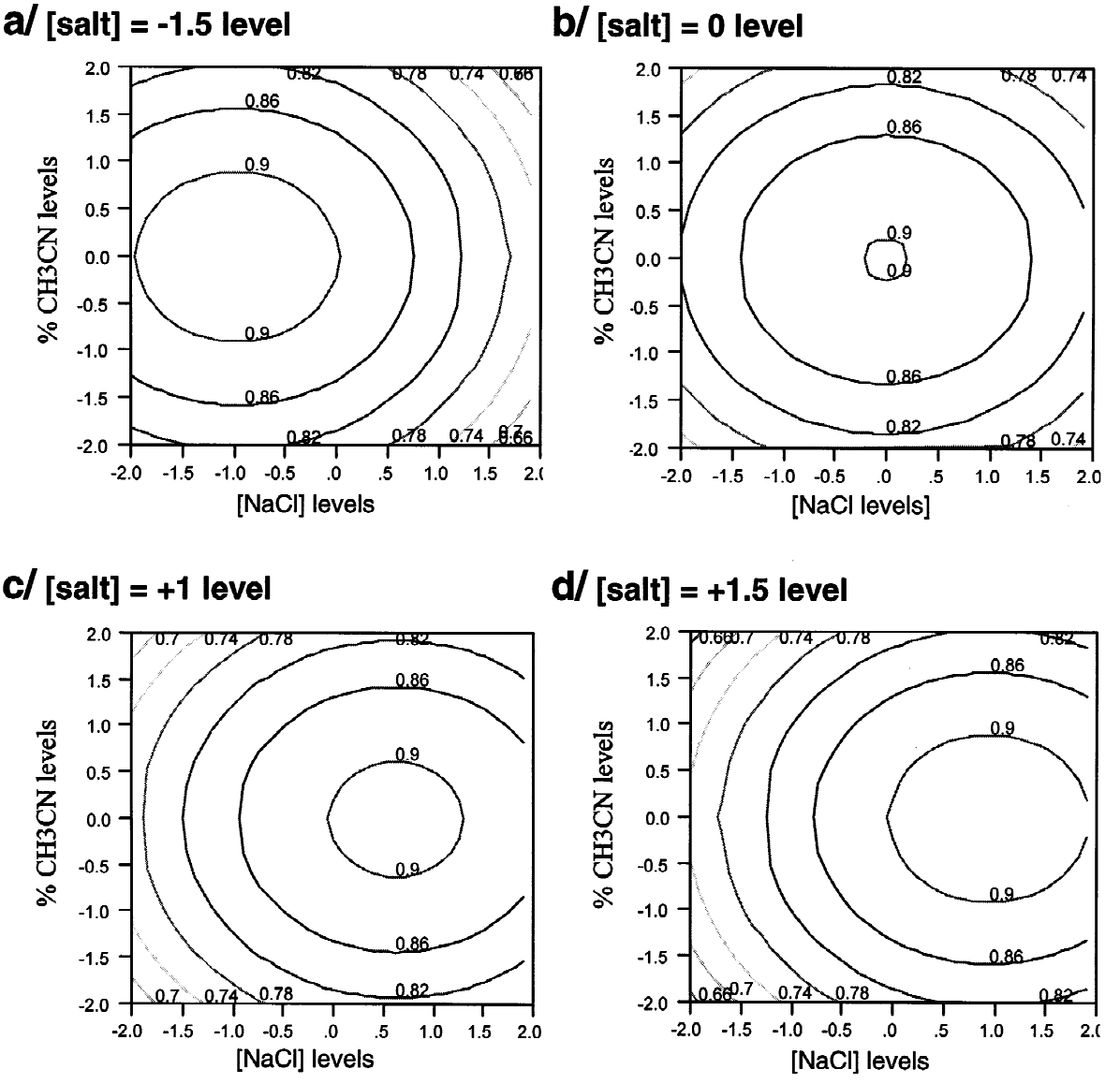


Fig. 4. Response surfaces correlating the resolution of the $C_{12}E_{11}$–$C_{12}E_{12}$ pair to the most influent factors, at a fixed temperature (21°C). (a) [Salt]$=6.4\cdot10^{-4}$ mol $l^{-1}$, (b) [salt]$=5\cdot10^{-3}$ mol $l^{-1}$, (c) [salt]$=2\cdot10^{-2}$ mol $l^{-1}$, (d) [salt]$=3.9\cdot10^{-2}$ mol $l^{-1}$. Fitted model: $R_{s\ 11\text{-}12}=0.801-0.067\ (T)+0.025\ ([\text{salt}][\text{NaCl}])-0.020\ ([\text{NaCl}])^2-0.024\ (\%\ CH_3CN)^2$.

mobile phase in relation to that of the surface of the column packing material does not really vary. However, when the shallow part of the Langmuir isotherm is reached, at high concentrations of salt, the adsorbed amount in the stationary phase is lower than in the mobile phase, resulting in an increase of the polarity of the mobile phase. This slight effect seems sufficient to slightly increase the selectivity of the separation of the nonionic compounds and to enhance the resolution when the ion-pairing salt concentration is larger. Finally, the concomitant effect of NaCl on efficiency and of tetraalkylam-

monium cations on selectivity leads to a maximal resolution when the concentrations of the two salts are both relatively large (see Fig. 4d).

As a final point, Fig. 4 shows very well that the best choice for the optimisation of SDS and polydisperse $C_{12}E_9$ separation is the level zero for acetonitrile (55%, v/v, in water) because it corresponds to favourable conditions considering the resolution but also the ruggedness. Moreover it appears in Fig. 4d that, in terms of concentration of salts, the most suitable levels seem to be at +1.5 for the ion-pairing reagent and +1 for NaCl, i.e., $4 \cdot 10^{-2}$ mol $l^{-1}$ for each salt. These selected concentrations had obviously a consequence on the choice of the ion-pairing cation. As mentioned earlier, constraints on SDS retention time (between approximately 5 and 8 min) resulted in the choice of the salt with shorter alkyl chains, tetramethylammonium bromide or tetraethylammonium bromide. In fact, $t_R$ SDS was always up to 8 min with tetrapropylammonium bromide whatever the flow-rate. Deciding between methyl or ethyl chains was easily performed after a new experimental constraint arising from the liquid–solid extraction conditions.

### 3.2. Trace enrichment

As recalled earlier, our goal was to analyse and to quantify with good precision SDS and polydisperse $C_{12}E_9$ surfactants in a large range of concentrations in dilute aqueous solutions containing NaCl, with a view to study their behaviour and eventual synergisms at liquid–solid interfaces. These compounds do not possess chromophore moieties and we wanted to avoid any supplementary derivatisation step which could have resulted in unsatisfactory quantitative determinations at trace levels. Therefore we chose differential refractometry as detector to analyse mixtures of SDS and polydisperse $C_{12}E_9$ surfactants.

Differential refractometric detection implied, to keep chromatographic separation quality, that the analytes were dissolved in a solvent strictly identical to the mobile phase used [37]. The problem came from the step of trace enrichment using the QMA cartridge to retain SDS. Experimental parameters were studied such as the percolated volume of aqueous solution containing the SDS, the solvent rinsing the cartridge and its necessary volume to desorb quantitatively the SDS retained on QMA cartridge. We studied the recovery yields after desorption with a solvent less polar than pure water and containing a high concentration of salt to increase the ionic strength. So the QMA cartridge was rinsed with acetonitrile containing the ion-pairing reagent, at a concentration depending on the optimal level chosen for the HPLC analysis. However we noticed that tetramethylammonium bromide was not soluble in acetonitrile whereas tetraethylammonium was completely soluble. This experimental constraint led us to select the latter salt to perform not only the desorption of SDS from QMA cartridge but also the chromatographic analysis. It may also be noted that tetraethylammonium bromide at the level +1.5 ($3.9 \cdot 10^{-2}$ mol $l^{-1}$), which was the optimal and more rugged level for the homologous compounds resolution constituting the PEO surfactant, was too concentrated to obtain a SDS retention time below 8 min in HPLC analysis. Consequently we chose the level +1 for ion-pairing reagent concentration ($2 \cdot 10^{-2}$ mol $l^{-1}$) with a level +0.6 for NaCl concentration ($2.3 \cdot 10^{-2}$ mol $l^{-1}$). These conditions corresponded also to an optimal resolution area ($R_s = 0.9$) although being not the most rugged (see Fig. 4c). Only flow-rates equal or up to level 0 were acceptable to obtain a retention time for SDS below 8 min when the percentage of acetonitrile was optimal (i.e., level 0). As displayed on Fig. 2, the complete analysis was achieved before approximately 42 min with a flow-rate of 1 ml $min^{-1}$ (level 0) and 55% acetonitrile in water.

Going back to the discussion on liquid–solid extraction, it is important to recall that SDS and PEO surfactants were extracted from an aqueous solution containing NaCl, after adsorption at a solid–water interface. As mentioned earlier, the injection should be performed using a water–acetonitrile (45:55, v/v) solvent and at the end of the enrichment procedure, concentrations of ion-pairing reagent and NaCl should be, respectively, $2 \cdot 10^{-2}$ mol $l^{-1}$ and $2.3 \cdot 10^{-2}$ mol $l^{-1}$ in the concentrated solution. Consequently SDS was desorbed from QMA cartridge with acetonitrile containing tetraethylammonium bromide at concentration equal to $2 \cdot 10^{-2} \cdot 100/55 = 3.6 \cdot 10^{-2}$ mol $l^{-1}$. Thus, $V$ ml of this concentrated organic solution was diluted by adding $V \cdot 45/55$ ml of pure water, in order to respect the composition of the

mobile phase used. For the same reasons, the initial concentration of NaCl in water chosen for adsorption experiments at liquid–solid interfaces was, before the enrichment step, $2.3 \cdot 10^{-2} \cdot 100/55 = 4.2 \cdot 10^{-2}$ mol $l^{-1}$, in order to obtain a final appropriate NaCl concentration after solid-phase extraction with acetonitrile and dilution with pure water.

As reported in Table 5, the quantitative recovery of SDS required a minimum of 3 ml of organic eluent, smaller volumes resulting in poorer yields than 100%. Five independent experiments were performed, concentrating SDS (4 mg $l^{-1}$) 46 times. We found a relative standard deviation equal to 7% on concentrations determined by HPLC, with 3% of accuracy.

Finally, we studied the maximal capacity of the QMA cartridge, which contained 360 mg of packing material. It turned out that a maximum of 2 mg of SDS could be concentrated on QMA cartridge without decreasing the recovery yields. So the volume of aqueous phase percolating through the QMA cartridge had to be previously adapted not to exceed this maximal capacity. With a maximum of 1.5 l of percolated aqueous phase (beyond this value, operating times were prohibitive with maximum flow-rates of 10 ml $min^{-1}$), the enrichment factor $F'$ (see the Experimental section) was 275. The detection limit was determined as threefold the background noise ($3.10^{-5}$ refractive index units, U.R.I.) and the quantification limit as 10-times above this value. So quantification limit was 1 mg $l^{-1}$ for SDS before concentration on QMA cartridge and was as low as 3.6 μg $l^{-1}$ after trace enrichment.

The quantification limit was also obtained for the polydisperse $C_{12}E_9$ surfactant. It was approximately 100 mg $l^{-1}$ for the whole mixture and 0.5 mg $l^{-1}$ for the less abundant oligomer before concentration on $C_{18}$ cartridge. With a maximal enrichment factor $F = (1500/4) \cdot 0.55 = 206$, this quantification limit was shifted towards lower concentrations, i.e., 0.5 mg $l^{-1}$ for the whole nonionic surfactant and 2.5 μg $l^{-1}$ for an oligomer. It was reported in a previous work that the enrichment procedure did not cause any distortion in the distribution of the nonionic surfactant as a function of the ethoxylated chain length [19]. Five independent experiments on polydisperse $C_{12}E_9$ (5 mg $l^{-1}$) reconcentrated 206 times gave us results with a relative standard deviation equal to 6% for the quantification of each oligomer (it could only increase to 10% for the less abundant oligomers of the distribution), with an accuracy of 4%.

Table 5
Evolution of the recovery yields for SDS using a QMA cartridge as a function of the acetonitrile volume used for desorption

| Acetonitrile + ion-pairing reagent volume (ml) | Recovery (%) |
|---|---|
| 2.0 | 86 |
| 2.5 | 91 |
| 3.0 | 100 |
| 4.0 | 100 |

Linear correlations were obtained for calibration graphs in the concentration range 0.1–5.5 g $l^{-1}$ for the whole nonionic surfactant (area $= 0.083 \cdot 10^{-3}$ $[PEO]_{g\ l^{-1}} + 1.77 \cdot 10^{-3}$, with a correlation coefficient equal to 0.9991) and 0.001–4 g $l^{-1}$ concentration range for SDS (area $= 0.102 \cdot 10^{-3}$ $[SDS]_{g\ l^{-1}} + 0.44 \cdot 10^{-3}$, with a correlation coefficient equal to 0.998).

In the case of the nonionic surfactant we also studied the evolution of the refractometric response factors as a function of the degree of ethoxylation, using pure commercially available standards and standards with higher degree of ethoxylation obtained by preparative liquid chromatography. The evolution of the relative refractometric response factors was not linear and has been explained in a previous work although under different HPLC analysis conditions [19]. As shown in Fig. 5, under these conditions, we were able to analyse in a very large concentration range, with good accuracy and repeatability, mixtures of anionic and nonionic surfactants diluted in water containing NaCl after their partial adsorption on a silica surface. Aqueous supernatant, after adsorption on a solid surface and centrifugation, was previously injected before any step of SPE to evaluate which of the two surfactants had to be enriched for quantification. For reasons mentioned earlier, 1.64 ml of the aqueous supernatant was diluted with 2 ml of acetonitrile containing tetraethylammonium bromide at a concentration equal to $3.6 \cdot 10^{-2}$ mol $l^{-1}$, giving a direct and simultaneous analysis of SDS and PEO. After this first HPLC analysis, we could choose to concentrate only SDS on QMA cartridge or polydisperse $C_{12}E_9$

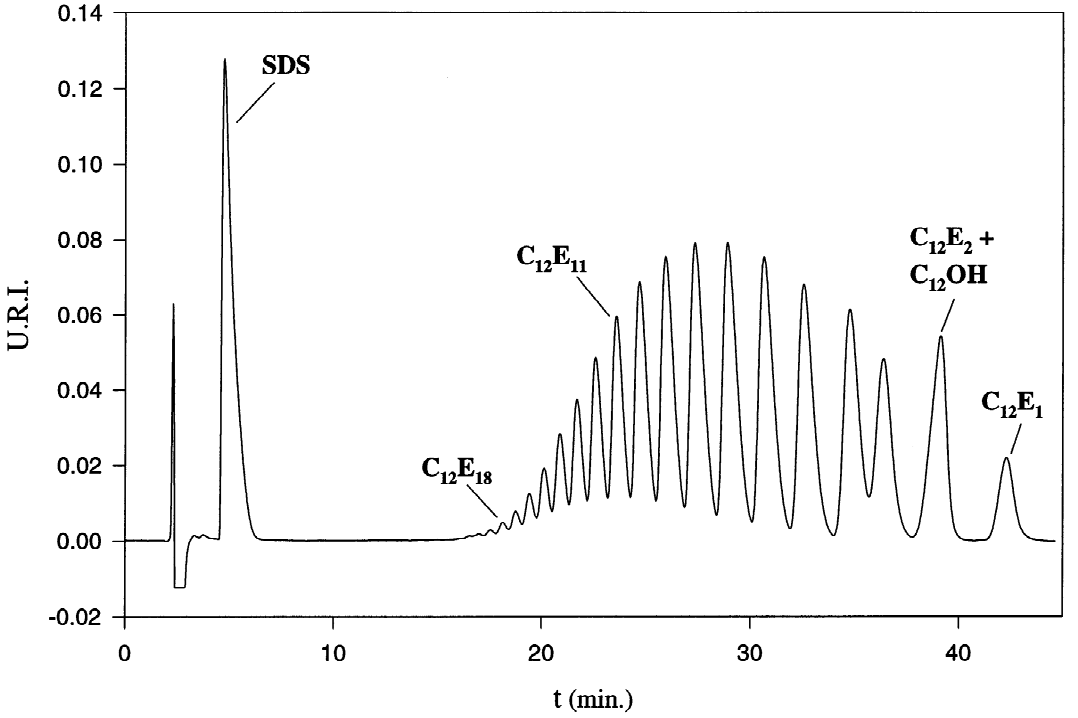


Fig. 5. Analysis of the aqueous supernatant of a polydisperse $C_{12}E_9$–SDS (70:30) mixture (initial concentrations being, respectively 2.1 g $l^{-1}$ and 0.9 g $l^{-1}$) after adsorption at water–silica interface. Operating conditions: stationary phase: $C_8$ bonded silica (250×4.6 mm, $d_p$=5 μm); mobile phase: $CH_3CN$–water (55:45, v/v)+$(C_2H_5)_4NBr$ $2\cdot10^{-2}$ mol $l^{-1}$+NaCl $2.3\cdot10^{-2}$ mol $l^{-1}$; flow-rate 1 ml $min^{-1}$; temperature 21°C. Enrichment on $C_{18}$ cartridge: $F$=10.5.

on $C_{18}$ cartridge, or the two surfactants together with coupled cartridges.

## 4. Conclusion

A method using ion-pair reversed-phase liquid chromatography has been optimised using a fractional factorial design. This factorial design permitted to systematically define on a large experimental space the effects of six factors which could influence the chromatographic separation for nonionic and anionic surfactants mixtures ($C_{12}E_9$ polydisperse polyethylene oxide and SDS). An interesting area was found where the resolution of nonionic oligomers was optimal, the retention time of SDS was not too long and finally the total analysis time was not prohibitive. Eluents containing 55% (v/v) acetonitrile in water with tetraethylammonium as the ion-pairing reagent and NaCl at, respectively, $2\cdot10^{-2}$ mol $l^{-1}$ and $2.3\cdot10^{-2}$ mol $l^{-1}$ concentrations allowed us to obtain accurate and reproducible separations of SDS and polydisperse $C_{12}E_9$ surfactant mixtures on a $C_8$ column at a flow-rate equal to 1 ml $min^{-1}$ and an analysis temperature equal to 21°C.

Moreover, it was possible to analyse simultaneously aqueous solutions containing SDS and PEO even at trace levels owing to a quantitative enrichment on liquid–solid extraction cartridges of cationic and $C_{18}$ type.

Finally, it turned out that HPLC analysis with enrichment procedure was perfectly adapted to quantitative analysis of the aqueous supernatant, containing NaCl and traces of polydisperse $C_{12}E_9$ and SDS, after adsorption of these mixtures of nonionic and anionic surfactants at the water–solid interface.

## References


[1] M. Wisniewski, J. Szymanowski, B. Atamanczuk, J. Chromatogr. 462 (1989) 39.
[2] R.R. Geissler, J. Am. Oil Chem. Soc. 66 (1989) 685.
[3] F. Guerrero, J.L. Rocca, Chromatographia 40 (1995) 563.
[4] K. Rissler, J. Chromatogr. A 742 (1996) 1.
[5] P.L. Desbène, B. Desmazières, J. Chromatogr. A 661 (1994) 207.
[6] N. Martin, J. Liq. Chromatogr. 18 (1995) 1173.
[7] C. Sun, M. Baird, H.A. Anderson, D.L. Brydon, J. Chromatogr. A 771 (1997) 145.
[8] P.L. Desbène, B. Desmazières, J.J. Basselier, A. Desbène-Monvernay, J. Chromatogr. 461 (1989) 305.
[9] R.E.A. Escott, N. Mortimer, J. Chromatogr. 553 (1991) 423.
[10] M. Plaza, R. Pons, Colloids Surfaces A: Physicochem. Eng. Aspects 137 (1998) 287.
[11] B. Desmazières, F. Portet, P.L. Desbène, Chromatographia 36 (1993) 307.
[12] T. Okada, Anal. Chem. 62 (1990) 327.
[13] T. Okada, Anal. Chem. 63 (1990) 1043.
[14] G.A. Cumme, E. Blume, R. Bublitz, H. Hoppe, A. Horn, J. Chromatogr. A 791 (1997) 245.
[15] A. Marcomini, M. Zanette, J. Chromatogr. A 733 (1996) 193.
[16] A.T. Kiewiet, P. Devoogt, J. Chromatogr. A 733 (1996) 185.
[17] M. Castillo, D. Puig, D. Barcelo, J. Chromatogr. A 778 (1997) 301.
[18] J. Bolze, K.D. Hörner, M. Ballauff, Colloid Polym. Sci. 274 (1996) 1099.
[19] P.L. Desbène, F.I. Portet, G.J. Goussot, J. Chromatogr. A 730 (1996) 209.
[20] C.E. Hoeft, R.L. Zollars, J. Liq. Chromatogr. 17 (1994) 2691.
[21] L.M. Nair, R. Saari-Nordhaus, J. Chromatogr. A 804 (1998) 233.
[22] D. Zhou, D.J. Pietrzyk, Anal. Chem. 64 (1992) 1003.
[23] J.A. Boiani, Anal. Chem. 59 (1987) 2583.
[24] J.G. Chen, S.G. Weber, L.L. Glavina, F.F. Cantwell, J. Chromatogr. A 656 (1993) 549.
[25] P. Jandera, J. Urbanek, J. Chromatogr. A 689 (1995) 255.
[26] L.M. Osborne, T.W. Miyakawa, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 501.
[27] Y. Guillaume, C. Guinchard, J. Liq. Chromatogr. 16 (1993) 3471.
[28] C.F. Harrington, D.J. Roberts, G. Nickless, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1773.
[29] H.A. Hansen, C. Emborg, J. Chromatogr. 626 (1992) 171.
[30] S.N. Deming, S.L. Morgan, Experimental Design: A Chemometric Approach, Data Handling in Science Technology, Vol. 3, Elsevier, Amsterdam, 1987.
[31] J. Goupy, La Méthode des Plans d'Expérience, Dunod, Paris, 1988.
[32] G. Sado, M.-C. Sado, Les Plans d'Expérience, AFNOR Technique, Paris, 1991.
[33] E. Marengo, M.C. Gennaro, C. Abrigo, Anal. Chim. Acta 321 (1996) 225.
[34] E. Marengo, M.C. Gennaro, S. Angelino, J. Chromatogr. A 799 (1998) 47.
[35] P. Jandera, J. Chromatogr. 449 (1988) 361.
[36] T. Okada, Anal. Chim. Acta 281 (1993) 95.
[37] Z. Wang, M. Fingas, J. Chromatogr. A 673 (1993) 145.