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Determination of C_8–C_{20} saturated anionic and cationic surfactant mixtures by capillary isotachophoresis with conductivity detection

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ABSTRACT

Isotachophoresis combined with conductivity detection was systematically investigated as an alternative method for the determination of various classes of C_8–C_{20} saturated ionic surfactants, including sulphates, sulphonates and quaternary ammonium cations. Its major interest is the ability to perform separations in free electrolyte media (*i.e.*, without any stationary phase) and in capillaries of fairly large inner diameter (typically 0.5 mm), which reduces the possibility of deleterious adsorption, often encountered in other techniques with ionic surfactants. Methanol–water mixtures of appropriate compositions were used to prevent micellization of the surfactant from occuring, thus yielding separations according to their free-form electrophoretic mobilities. Compounds differing in one carbon atom only can be fully resolved. The steady-state zone stability was ascertained by plotting analyte zone lengths *versus* injected amounts and by determinations of separation capacities for standard binary mixtures in various proportions. Conductimetry provides a convenient and universal detection method, with acceptable detection limits (*ca.* 0.1–1 nmol for a 10-μl volume injected), owing to the absence of background electrolyte in analyte zones, inherent in isotachophoretic principles. The determination of the quaternary ammonium ions contained in an industrial surfactant formulation is presented as an example.

INTRODUCTION

During the last decade, surface-active compounds have gained widespread applications that now encompass much more than detergency. They are involved, generally as mixtures, in many industrial separation processes (liquid–liquid extraction, flotation of ore minerals, enhanced oil recovery) and in many formulations of products of agricultural, food, pharmaceutical or health interest such as body creams and vesicle encapsulation of medicines. They even have entered the class of fine reagents for analytical purposes to promote ion-pair or micellar pseudo-phase formation. A wide variety of surface-active agents, both ionic and non-ionic, are becoming commercially available to meet these needs and, consequently, this places great demands on high-performance analytical methods capable of discriminating homologues, isomers or any other small structural differences.

Chromatographic techniques overall have been considered to meet these requirements, especially for the non-ionic class of the most often oligomeric

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or polymeric surfactants, such as the derivatized polyoxyethylenes [1–4].

In contrast, less has been published on the various classes of ionic surfactants with chain lengths ranging from eight to about twenty carbon atoms, whether they are anionic (alkyl sulphates and sulphonates, carboxylates) or cationic (trimethylalkylammonium, alkylimidazolium ions), probably because of increased difficulties owing to their ionic character. Apart from when previous derivatization of the ionic moiety is applied [4], these products cannot be submitted to capillary gas (GC) or supercritical fluid chromatography (SFC) owing to their low volatility. Moreover, their poor solubility in supercritical carbon dioxide makes the use of a polar modifier necessary in SFC and precludes one of the major advantages of this techniques, *viz.*, its compatibility with flame ionization detection. Thus, only the separation of C_8–C_{24} even carbon number free fatty acids on a packed microbore column has been reported so far [5]. In addition to their ionic character, the amphiphilic properties make the choice of suitable phase systems in liquid chromatography (LC) that would prevent strong or even irreversible adsorption on a stationary phase difficult. The most successful techniques reported are reversed-pase [6,7] and ion-pair [8–14] chromatography.

Various modes of detection have been combined with the LC separations of ionic surfactants. The most common, direct UV absorbance, is virtually restricted to alkylbenzene suphates and sulphonates [8], imidazolines [9] or ester derivatives [7]. Indirect UV absorbance was used for qualitative detection of ammonium ion with xylene sulphonate as a UV-absorbing counter-ion [13]. On-line extraction of a postcolumn-formed, UV-absorbing [10] or fluorescent [6] ion pair was also implemented for cationic [10] and anionic [6] surfactants, but required complex equipment. Differential refractive index monitoring would constitute a versatile means of detection [8,14], but suffers from a lack of sensitivity. Light scattering appears to be well adapted to the detection of heavy compounds, but is not consistent with separations under ion-pairing conditions. Hence its use is limited to non-ionic surfactants or a mere class separation of the ionic and non-ionic types under reversed-phase conditions [15]. Finally, ion-pair chromatography associated with conductivity detection has been reported for aliphatic and olefinic anionic surfactants, but a device allowing the suppression of the mobile phase ions is necessary in order to obtain a level of detectability of practical interest [11]. This device considerably reduces the choice of compatible counter ions and gives a certain degree of complexity to the method. None of these LC methods has turned out to be completely satisfactory because of limitations in either the separation itself or the detection modes. Few quantitative determinations have been reported [5,6,14], which is a hint that they remain a problem. Detection limits are commonly in the 1–10 nmol range for *ca.* 10–20-μl injections [7,10,11,13] and more seldom in the 0.1–1 nmol range [5,6].

The purpose of this work was to develop and assess an alternative method for the determination of the various classes of C_8–C_{20} saturated anionic and cationic surfactants, based on electrophoresis in its isotachophoretic mode combined with conductivity detection. The interest in this approach is first, the ability to perform separations in free electrolyte solutions, *i.e.*, without any stationary phase, often responsible for strong adsorption, and second, the acceptable sensitivity of conductivity detection when used in the absence of a background electrolyte medium, as in the case with isotachophoresis (ITP), the displacement mode of electrophoresis. Further no specific sample treatment or derivatization should be required. Nevertheless, the application of ITP to surfactant analysis has remained uncommon and no method allowing the total determination of C_8–C_{20} saturated alkyl sulphates or ammonium ions has been proposed. Previous work in this direction dealt with C_1–C_8 [16] or C_1–C_{18} [17] fatty acids, decyl and dodecyl sulphonates and inorganic ions in shampoo formulations [18] and complex alkyl sulphonate mixtures formed in the peroxodisulphate-initiated polymerization of butadiene [19]. All of them seem very promising. This is why we carried out a systematic investigation of the operational electrolyte systems, including various aqueous–organic solvent mixtures, to assess better the performances of ITP with respect to surfactant mixture analysis. The optimum conditions found for cationic surfactants were applied to trimethylalkylammonium ion mixture of industrial interest synthesized by alkylation of natural copra oil.

EXPERIMENTAL

Apparatus and products

The separations were performed with an LKB 2127 Tachophor apparatus (Pharmacia–LKB, Uppsala, Sweden) fitted with solvent-resistant polymethylpentene (TPX) pieces in direct contact with the solutions. A single column configuration was used with 22 cm × 0.55 mm I.D. polytetrafluoroethylene (PTFE) tubing. The separation unit was equipped with a conductivity detector, comprising linear and differential signal outputs, and a UV absorbance detector operated with a 254-nm wavelength filter. The signals were recorded on a Kipp and Zonen (Delft, Netherlands) BD 100 three-channel chart recorder. The driving current was delivered by the Tachophor power supply unit and a laboratory-made pilot unit capable of delaying the start of the recorder and switching the current intensity to a lower value a short time before the solutes migrate past the detector cell.

The electrolytes were prepared from ultrapure deionized water (18 MΩ cm resistivity) produced by a Millipore Milli-Q system and from high-performance liquid chromatographic-grade solvents (methanol, 1- and 2-propanol, acetonitrile) purchased from Prolabo (Paris, France). Buffer solutions were prepared from the highest available grades of analytical reagents, used as received. The standard surfactants studied were supplied by Fluka (Buchs, Switzerland) or Lancaster Synthesis (Morecambe, UK) and used as received after solubilization in water or methanol. Noramium, an industrial mixture of quaternary ammonium ions manufactured by CECA (Paris La Défense, France), was kindly provided by Orsan (Orsay, France). All solutions were degassed for 10–15 min in an ultrasonic bath.

Electrolyte preparation

The leading solutions were prepared from 0.1 *M* stock buffer solutions of potassium acetate–acetic acid or L-histidine–hydrochloric acid in the appropriate proportions and in various aqueous organic solvent mixtures. A 5-ml volume of these stock solutions were added to 1 ml of pure (99%) tetraethylene glycol (Aldrich) and adjusted to 50 ml with the appropriate aqueous organic solvent mixture to obtain the desired 10 m*M* leading electrolyte solutions. The pH of the final electrolyte solutions was measured using a classical pair of glass and reference electrodes for aqueous media. For methanol, the effect of organic solvent proportion was studied between 0 and 80% (v/v). The terminating electrolyte solutions were prepared simply by dissolving the appropriate weight of reagent in the same solvent mixture as that of the leading electrolyte. The compositions of the electrolytes yielding the best results for the sulphates, sulphonates and ammonium ions are given in Table I.

TABLE I

OPERATING ELECTROLYTE SYSTEMS FOR ANIONIC (SULPHATES AND SULPHONATES) AND CATIONIC (AMMONIUM IONS) ANALYSIS OF SATURATED C_8–C_{20} SURFACTANT MIXTURES

Parameter	Anionic separations	Cationic separations
Leading electrolyte		
Leading ion	Cl^- 10 m*M*	K^+ 10 m*M*
Counter ion	L-Histidine 13.3 m*M*[a]	Acetate 20 m*M*[a]
pH	4.88[b]	5.64[b]
Additive	Tetraethylene glycol 2% (v/v)	Tetraethylene glycol 2% (v/v)
Terminating electrolyte	Picolinic acid 10 m*M*	Creatinine 10 m*M*
Solvent composition[c]	Methanol–water (80:20)	Methanol–water (50:50)

[a] Total concentration of acidic and basic forms.

[b] The unusual pH values of the L-histidine and acetate buffers can be accounted for by the acid–basic p*K* shifts in methanol–water mixtures. The p*K* of acetate is greatly increased whereas that of histidinium remains roughly unchanged. In addition, the purely aqueous feature of the electrode filling solutions is stressed here (see Experimental).

[c] For both the leading and terminating electrolytes.

Running parameters

Volumes of 5 μl of sample mixtures were normally injected into the separation unit at the interface between the leading and the terminating electrolytes. A high voltage was then applied. The separations were performed under a driving current intensity of 120–130 μA for a typical time of 10 min. Then the current was switched to 20–30 μA just prior to zone recording. The total analysis time varied between about 20 and 30 min, according to the sample.

RESULTS AND DISCUSSION

Constraints arising from micellization phenomena

Industrially, ionic surfactants are mainly employed in neat aqueous media. It is therefore natural to seek purely aqueous separation conditions for their analysis by ITP, as first proposed earlier [18,19]. This approach is well documented by the values of absolute electrophoretic mobilities in water, m^0, available in the literature, which vary from $27.1 \cdot 10^{-5}$ to $20.6 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} on going from octyl- to octadecyltrimethylammonium ions and from $30.1 \cdot 10^{-5}$ to $24.9 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (at 25°C) on going from octyl to dodecyl sulphate [20]. From these mobility values, the possibility of separating surfactants differing in one carbon atom only can be contemplated.

For the case of cationic surfactants, a leading electrolyte consisting of an aqueous potassium acetate–acetic acid buffer was first tested. The interest in this buffer lies in the fact that, as soon as the voltage is applied, the analyte, most often in the

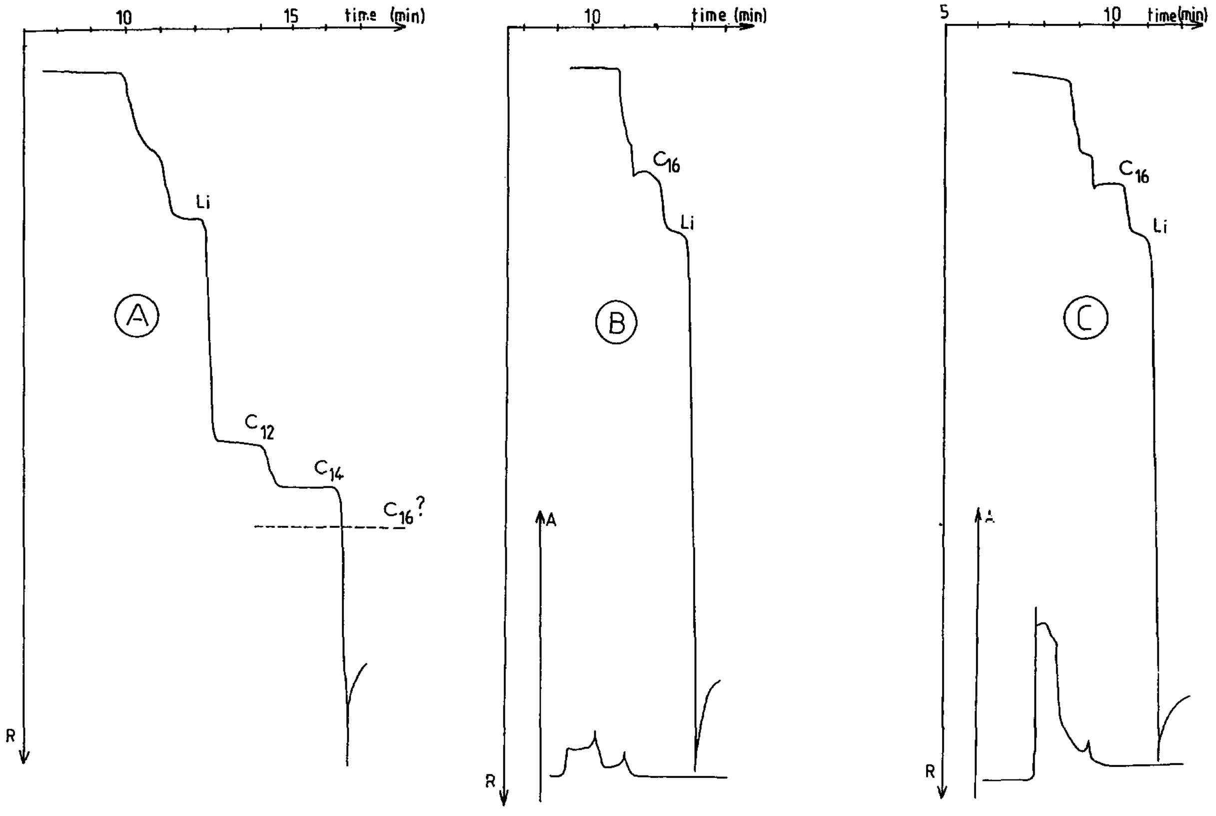

Fig. 1. Isotachophoretic migration of alkyltrimethylammonium ions with a purely aqueous electrolyte system. (A) C_{12}–C_{14} mixture; (B) C_{16}-ammonium ion; (C) C_{16} ion in the presence of cerol orange. Li$^+$ ion was added to all analyte solutions as a reference mobility marker. Leading electrolyte, 10 m*M* K$^+$, acetate buffer (pH 4.7), terminating electrolyte, 10 m*M* acetic acid; detection current, 20 μA; samples, 20 nmol each of C_{12}-, C_{14}- and C_{16}-ammonium surfactants, 5 nmol Li$^+$, 2 nmol cerol orange (injected volume 5 μl). Dashed line: expected step height of the C_{16}-ammonium ion.

form of an iodide or bromide salt in the samples, exchanges its counter ion for acetate, which greatly improves its solubility. As the analytes remain fully ionized over in whole operational pH range, pH is not a key parameter from the viewpoint of separability. Therefore the pH was adjusted to 4.7, which enables one simply to use acetic acid as the terminating electrolyte, with H^+ as the terminating ion. Likewise, promising results were easily obtained with dodecyl- and tetradecyltrimethylammonium ions, as shown in Fig. 1A. However, for alkyl chain lengths of 16 or more carbon atoms, apparently inconsistent isotachopherograms were obtained, insofar as the corresponding surfactant zones were detected before that of lithium ion used as a reference mobility marker ($m^0 = 40.1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} at 25°C [20]), and with a lower step height (Fig. 1B). This phenomenon can be attributed to the formation of a more mobile species in equilibrium with the free surfactant, *i.e.*, to the micellization of the long-chained surfactants. Using an ITP steady-state simulation program [21] and ignoring micellization phenomena, the equilibrium concentration of the hexadecyltrimethylammonium ion was evaluated to be 4.93 m*M*, while the critical micellar concentration (CMC) of the C_{16}-ammonium ion with acetate as counter ion was previously determined to be 1.6 m*M*. Under these conditions, the apparent mobility of the C_{16}-ammonium ion is much higher than those of both the C_{14} and C_{12} ions, and the prediction of the C_{16} step height derived from the measurements of the corresponding C_{12} and C_{14} step heights no longer holds true (Fig. 1A and B). Further, it has been shown that the electrophoretic mobility of an ionic micelle is *ca.* 2–3 times higher than that of the free ionic surfactant [22]. The presence of the aggregates was confirmed by injecting a mixture of hexadecyltrimethylammonium ion and cerol orange, a very slightly water-soluble neutral dye that can be solubilized by micelles (Fig. 1C). The UV detector trace revealed the presence of a UV-absorbing species in the zone of the C_{16} surfactant, which indicates that the neutral dye had migrated inside the hydrophobic micelle cavity (it should be noted that a constant delay was observed between the records of the UV absorbance and conductivity signals, owing to the distance between the location of the sensing cells, along the capillary). Further investigations on the isotachophoretic behaviour of ionic micelles, reported elsewhere [23], have shown that ITP turns out to be a technique well adapted to the characterization of such micelles and the study of micellization phenomena.

As the purpose of this work was the determination of free surfactants, conditions preventing the formation of aggregates had to be designed. In this respect, Abe *et al.* [18] chose simply to lower the concentration of their leading ion to 5 m*M*, so that the equilibrium concentration of dodecyl sulphate in its zone would not reached the CMC of this species of *ca.* 8 m*M*. However, this approach is limited insofar as the concentration of the leading ion cannot be easily lowered to a sufficient extent to match the CMC values of longer alkyl-chain surfactants. This prompted us to explore the possibilities of using binary aqueous organic mixtures as solvents for the leading electrolyte. Methanol, 1- and 2-propanol and acetonitrile were successively tested in various proportions. For quaternary ammonium ions, mixtures containing up to 70% of methanol were required to prevent micellization below 10 m*M* with alkyl chain lengths up to C_{20}. The free surfactants can then be separated in the order of their electrophoretic mobilities.

Selection of the operating conditions

The optimization of the operating conditions is aimed at minimizing the zone disturbances mainly caused by electroosmotic flow or analyte adsorption on the capillary surface. Electroosmotic flow is usually suppressed in ITP by adding 0.1–0.3% of a neutral polymeric surfactant to the leading electrolyte. This solution is not applicable with ionic surfactant analytes, which could interact with these additives. However, electroosmosis is almost entirely suppressed, without the need of a neutral surfactant, when using electrolytes containing large proportions of methanol. Working under low pH conditions may further improve electroosmosis suppression, but the final choice of pH was mainly dictated by the solubilizing properties of the counter ion and by elimination of deleterious adsorption effects.

Hence, for quaternary ammonium ions, use of an acetate buffer was maintained with aqueous–organic electrolytes for the solubility reason already discussed. For alkyl sulphates, analyte adsorption on the capillary surface and subsequent mixing with the terminating zone occurs when using a low pH leading electrolyte (*e.g.*, a β-alanine buffer with an aqueous pH of 3.7). Such a phenomenon was avoided with a pH 5.5 aqueous histidine buffer. This improvement can be explained by the better protective role played by the increased number of fixed negative charges on the capillary surface at higher pH, as is apparent from the observation of the sharp resulting separation boundary between the last surfactant zone and the terminating zone. This boundary can be conveniently checked by systematically using a UV-absorbing species for the terminating ion such as creatinine for cationic separations and picolinic acid for anionic separations.

Under the optimized operating conditions given in Table I, the ionic surfactants are separated into well defined, classical isotachophoretic zones, displaying increasing electrical resistance with increasing alkyl chain length. These conditions provide satisfactory qualitative resolution of any class of saturated C_8–C_{20} alkyl-chain ionic surfactants, as shown in Fig. 2. The resolving power of the technique enables one to distinguish surfactants differing in one carbon atom only, as is the case for the

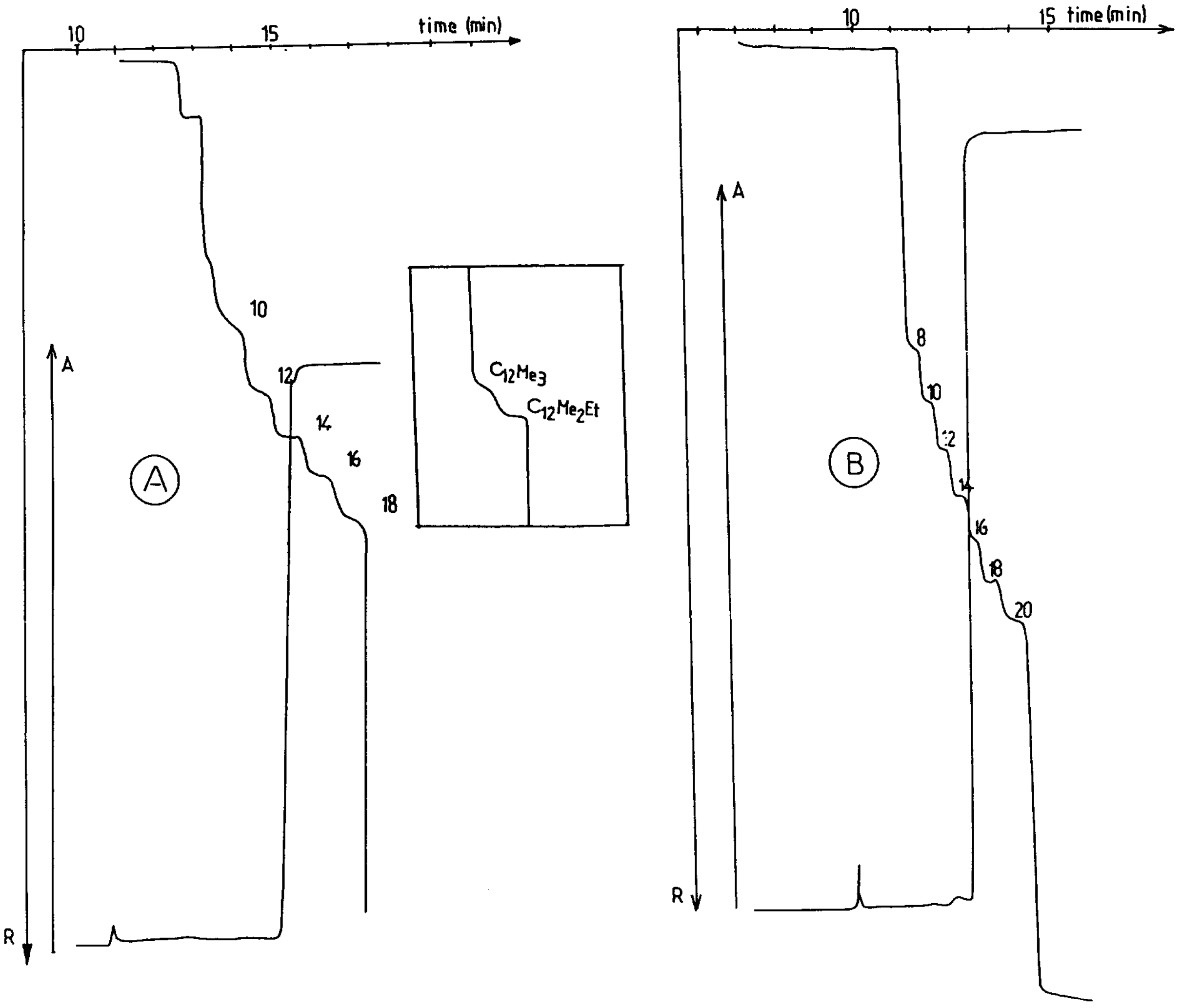

Fig. 2. ITP separation of C_8–C_{20} alkyltrimethylammonium ions (A) and sulphonates (B). Electrolyte systems as in Table I. Detection current, 30 μA; sample, standard mixtures containing 5 nmol of each surfactant (5 μl injected). Inset: separation of dodecyltrimethylammonium and dodecylethyldimethylammonium ions under conditions identical with those for the above ammonium ions.

dodecyltrimethylammonium and dodecylethyldimethylammonium ions (see inset in Fig. 2). However, in practice the mass of the species seems to act as the major discriminating parameter, as surfactant ions of equal mass but different organic structures, such as the cetylpyridinium and benzyldimethyldodecylammonium ions (common MW 304.5), are not resolved from one another. This constitutes a limitation to the method performance compared with that of GC, which is capable of separating branched-chain isomers. In an attempt to define the discriminating principle better, the migration behaviour of perfluorooctyl sulphonate (MW 499) was investigated and compared with that of octyl sulphonate (MW 193). No resolution was observed using the conditions for anionic separations given in Table I. This result shows that the sizes of the CH_2 and CF_2 moieties are about the same, which in turn can only be explained by strong solvation of the carbonaceous chains. In conclusion, the actual basic discriminating parameter is not the mass, but rather the size of the surfactants, including solvation effects, even if size is uniquely related to mass or number of carbon atoms for the most commonly encoutered hydrocarbonaceous chains. In addition, an improvement in the resolution of branched-chain isomers might be expected from these results by using solvents or electrolyte additives capable of inducing interactions directly related to chain length. However, this approach was not successful as neither investigations on organic solvents (such as 1- and 2-propanol and acetonitrile) nor the replacement of acetate counter ion by longer chain carboxylates (such as propanoate, butyrate, valerate and hexanoate) provided a noticeable improvement in the separation performances.

Quantitative aspects

The quantitative aspects of the method were first studied by injecting various amounts (0–50 nmol) of pure compounds. The linear relationship between amount injected and zone length was verified for octyl, dodecyl and eicosyl sulphate and also for decyl- and dodecyltrimethylammonium ions (Fig. 3A and B). It is noteworthy that the slopes of the straight standardization lines obtained are all of the same order of magnitude and differ only slightly for close homologous surfactants. This behaviour is in agreement with the approximate theoretical equation between zone length, l_i, and injected amount, n_i, of a solute i [21]:

$$l_i = [F\,U\,/\,I]\,[1 + m_c^0\,/\,m_i^0]n_i \qquad (1)$$

where F is the Faraday constant, U the chart speed of the recorder, I the current intensity at the time of detection and m_c^0 and m_i^0 the absolute mobilities of the counter ion c and solute i, respectively. Eqn. 1

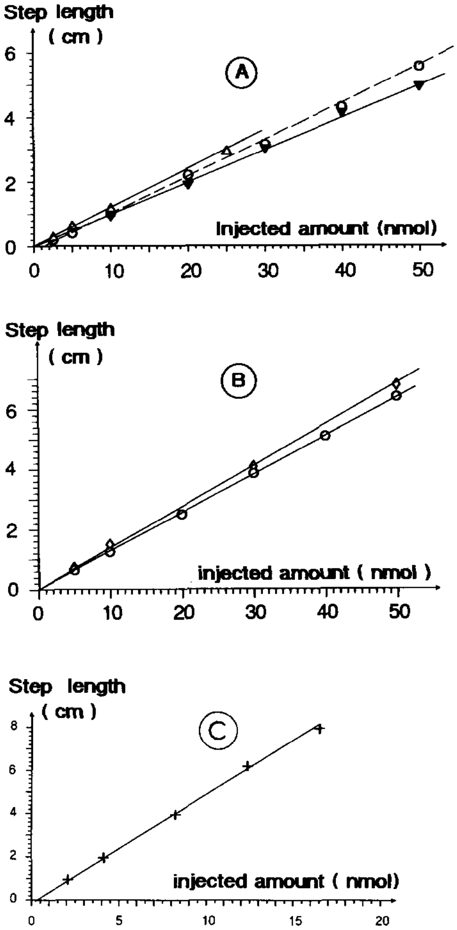

Fig. 3. Experimental plot of recorded step length *versus* amount of single solute injected for (A) alkyl sulphate and (B) ammonium surfactants. Electrolyte conditions as in Table I. Detection current, 30 μA; recorder chart speed, 10 mm/min. (C) Extended precision plot relative to dodecyltrimethylammonium ion for a reduced range of injected amount. Operating conditions as for (A) and (B), except that the purely aqueous electrolyte system was used with 20 μA as detection current and 20 mm/min as recorder chart speed. (A) ▼ = Octyl sulphate; ○ = dodecyl sulphate; △ = eicosyl sulphate; (B) ◇ = $C_{12}H_{25}N(CH_3)_3$ Br; ○ = $C_{10}H_{21}N(CH_3)_3$ Br.

further indicates a slight increase in zone length with decreasing solute mobility, *i.e.*, with increasing chain length. This point was also supported by the experimental results (Fig. 3A and B). Hence conductivity detection provides the advantage over direct or indirect absorbance, often used in LC, that a single calibration point, realized with any surfactant standard of roughly intermediate chain length, suffices for a first estimate of the proportions of an unknown mixture. Moreover, to obtain an accurate analysis of a previously identified mixture, the availability of a reference mixture containing all the components of the mixture to be determined in known proportions is not required, as effective mobility is the only solute parameter influencing the zone length. For this purpose, only the plot between zone length and step height needs to be determined, and this can be done using a few commercially available surfactants belonging to the same class.

Separation capacity

A well known practical limitation of any ITP system is the finite amount of sample mixture that the system can separate. This limitation corresponds to the theoretical concept of separation capacity. If the injected amounts of two closely migrating solutes are beyond the respective capacities for these solutes, an ITP steady state is not reached at the time of detection and only a partial separation occurs, leaving an unresolved mixed zone. Thus, an intermediate conductivity step is detected between those of the pure species. In practice, pure and mixed zones can be differentiated by injecting two widely differing amounts of the sample mixture: when a mixed zone is still present, its length varies with the amount injected whereas the lengths of the resulting pure zones remain constant in length. The separation capacity, n_s, was experimentally determined for the model binary mixture of C_{12}- and C_{14}-sulphates and -trimethylammonium ions; n_s is defined as the maximum amount of the more mobile C_{12} surfactant that can be separated from the less mobile C_{14} surfactant under given isotachophoretic conditions (capillary dimensions, leading electrolyte composition and binary mixture proportions). Fig. 4 shows that linear plots were obtained for the inverse of the separation capacity, $1/n_s$, *versus* the ratio of molar C_{14} of C_{12} amounts injected, δ. This result is in agreement with theoretical expectations [21] and is a proof that both anionic and cationic surfactants exhibit normal ITP behaviour. Fig. 4 also shows that the percentage of methanol in the electrolyte has only a very slight influence on the separation capacity, which remains of the order of 10 nmol for equimolar binary mixtures. In contrast, it was ascertained that 1- or 2-propanol in place of methanol is very unfavourable with respect to separation capacity and hence separability. This phenomenon can be attributed to the fact that solvation of surfactant alkyl chains is stronger with 1- or 2-propanol than with methanol, resulting in more hindered, heavier structures. Consequently, the difference in electrophoretic mobilities of two close homologues such as the C_{12} and C_{14} surfactants tends to level off. These results again illustrate the significant role played by solvation effects in discriminating carbonaceous chains.

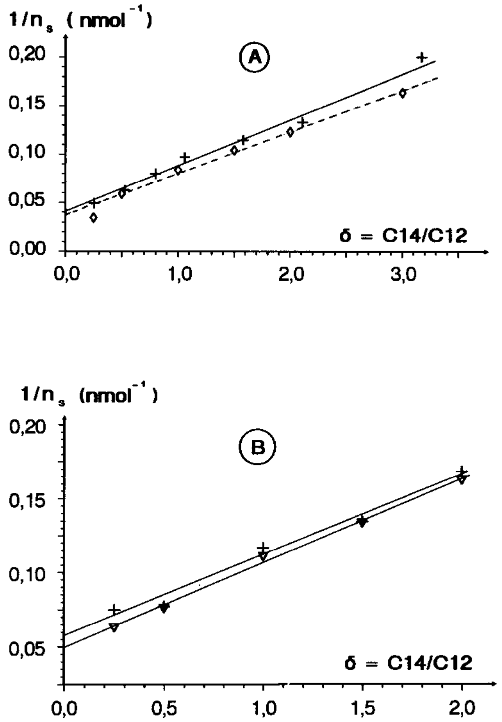

Fig. 4. Plot of the inverse of separation capacity, n_s, *versus* the molar ratio of C_{14} to C_{12}-alkyl surfactant amounts injected, δ. (A) Alkyltrimethylammonium ions; (B) alkyl sulphates. Electrolyte systems as in Table I, except for the solvent compositions: (A) + = water; ◇ = water–methanol (50:50); (B) ▽ = water–methanol (20:80); + = water–methanol (40:60).

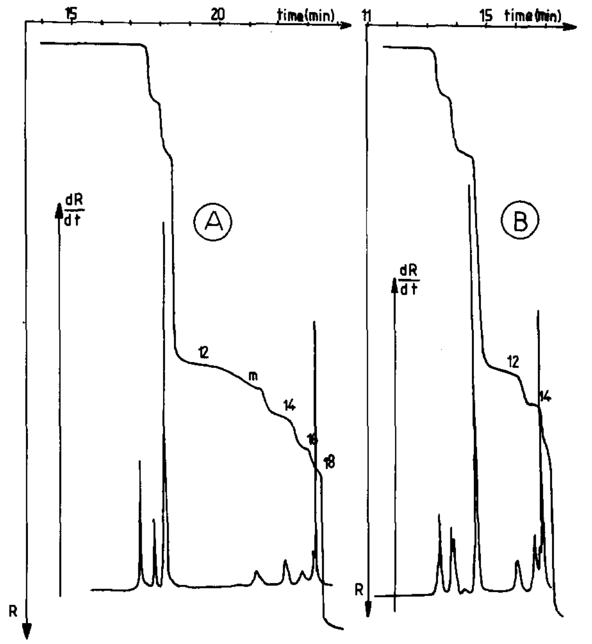

Fig. 5. Determination of the minor (A) and major (B) components of Noramium, a commercial quarternary ammonium surface-active agent. Electrolyte systems: see Table I but diluted to $6 \cdot 10^{-2}$ *M*. Sample: (A) 5 μl of a 2.915 mg/ml Noramium solution in water; (B) 5 μl of a 1.615 mg/ml solution. The symbol m denotes a mixed zone between the C_{12} and C_{14} surfactants.

Application to the analysis of an industrial cationic surfactant formulation

The above ITP method was applied to the analysis of Noramium, a complex commercial formulation of quaternary ammonium ions of industrial interest. This surface-active agent is available as a viscous concentrated solution containing 49–51% quarternary ammonium ions, 13–15% water and 35–37% 2-propanol (mass percent), according to the technical sheet provided by the manufacturer. It is derived from copra oil and its composition may vary according to season and the origin of the oil. Hence the proportion of some components may vary within 10% and only a mean distribution in the quaternary ammonium ions is available from the manufacturer. To analyse this sample accurately, we carried out four calibrations with pure standards of C_{12}–C_{18} surfactants. The analysis was carried out in two steps. First, a sample size corresponding to an overall dilution of 2.915 mg/ml in water was injected, in order to determine the proportions in minor components, mainly the C_{16}- and C_{18}-alkyltrimethylammonium ions, with good accuracy. It can be seen from Fig. 5A that neither the C_8 nor the C_{10} component was detected under such conditions, so the concentration of each of them can be evaluated to be less than 1% in the mixture, from the minimum detectable amount of the method. For such a sample size, an intermediate zone between those of dodecyl- and tetradecyltrimethylammonium ions was detected and further identified as a mixed zone of these components. Second, a sample of 1.652 mg/ml was injected in the same manner (Fig. 5B), which enabled one to determine the major C_{12} and C_{14} components in the absence of their mixed zone. Table II shows that the results afforded by the isotachophoretic method are in

TABLE II

COMPARISON BETWEEN THE MOLAR PERCENTAGE COMPOSITION OF NORAMIUM IN ALKYLTRIMETHYLAMMONIUM IONS DETERMINED BY ITP (THIS WORK) AND THE AVERAGE CORRESPONDING COMPOSITION GIVEN BY THE MANUFACTURER

The determination "without calibration" is simply the ratio of the step length of the corresponding compound to the sum of the lengths of all the surfactant steps.

Components (alkyltrimethylammonium ions)	ITP determination without calibration (mol%)	ITP determination with complete calibration (mol%)	Average composition according to the manufacturer (mol%)
C_8 and C_{10}	<1	<1	3.5 and 6
C_{12}	59.6	59.8	60
C_{14}	23.2	23.1	16–20
C_{16}	10.8	10.0	6–10
C_{18}	6.1	6.2	5

good agreement with the average composition given by the manufacturer. Further, the zone length calibration enabled us to determine the number of moles and then the mass of each component contained in the 1.652 mg/ml Noramium sample. The total mass of quaternary ammonium ions in this solution, and then the total mass percentage, can be derived. The result obtained, 49.7–50.7% of surfactant (in the chloride form) in the crude Noramium, suffers only from a 1% uncertainty, according to the precision of the method, and are in excellent agreement with the manufacturer's specification. Moreover, we ascertained the absence of free fatty amines by comparing an analysis in acidic medium (Table I) with the same one realized with a β-alanine–KOH buffer in 50% methanol (pH 10.6), which gave an identical result.

CONCLUSION

The absence of any stationary phase, a fairly large capillary inner diameter (*ca.* 0.5 mm) and a proper choice of electrolyte pH and additives has resulted in tail-free, stable steady-state ITP zones with any of the ionic surfactant classes. Although the separation capacity might be further improved by the finding of a still more suitable solvent composition or electrolyte additive, the above isotachophoretic method, associated with conductivity detection, affords analytical performances comparable to those of the classical chromatographic methods. Surfactants differing in only one carbon atom can be separated within an analysis time of *ca.* 20–30 min. The mass limit of detection is of the order of 0.5 nmol for about 10 μl injected, which gives a concentration limit of detection of *ca.* $5 \cdot 10^{-5}$ *M*. However, promising experiments have shown that, by using electrokinetic sample introduction and applying ITP principles, volumes of a few hundred microlitres of dilute samples could be preconcentrated at the inlet of a capillary having such dimensions. This should allow the concentration limit of detection to be lowered by at least a factor of 10.

For the purpose of these analyses, our efforts were aimed at avoiding micelle formation, so that only free surfactants can be present. In addition, the preliminary results on the isotachophoretic behaviour of ionic micelles presented here have suggested a new application area for ITP as a method for the determination of ionic micelle characteristics, such as CMC, charge ratio or neutral solute solubility. Likewise, ITP can be expected to become a method very well adapted to the characterization of small ionic polymers. Work is in progress in both directions.

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