ELSEVIER

Journal of Chromatography A, 716 (1995) 279–290

JOURNAL OF CHROMATOGRAPHY A

Utilization of fluorescein sodium salt in laser-induced indirect fluorimetric detection
II. Application to organic anions

A.M. Desbène[a,b,*], C.J. Morin[a,b], N.L. Mofaddel[a,b], R.S. Groult[c]

[a]*Laboratoire d'Analyse des Systèmes Organiques Complexes, Université de Rouen, I.U.T., 43 Rue Saint Germain, 27000 Evreux, France*

[b]*I.F.R.M.P., Université de Rouen, 76134 Mont Saint Aignan Cedex, France*

[c]*Beckman France, 92 Chemin des Bourdons, 93220 Gagny, France*

Abstract

Previously, we have used the fluorescein sodium salt to generate the background signal in the indirect fluorimetric detection of inorganic cations (alkaline, alkaline earth metals and transition metals) separated by capillary zone electrophoresis (CZE). In this work, we report that the use of fluorescein sodium salt can be extended to the detection of organic anions. A study of the detection thresholds and of reproducibility (elution times and effective electrophoretic mobilities) was performed. As samples we used, on the one hand, a mixture of *n*-alkane sulfonates (from C_1 to C_{10}) and, on the other hand, a mixture of carboxylic acids (from C_1 to C_{18}). Under the optimal conditions, detection thresholds of about 700 to 800 ppb were obtained and the reproducibility of effective electrophoretic mobilities was characterized by a relative standard deviation of about 0.5%. Calibration curves were also established (correlation coefficients were found to be between 0.995 and 0.9998). Finally, we compared the evolution of the experimental response coefficients of these two series of samples as a function of alkyl chain length with theoretical response coefficients obtained from findings reported by Ackermans et al. [J. Chromatogr., 549 (1991) 345].

1. Introduction

High-performance capillary electrophoresis (HPCE) is a rapidly expanding separation technique which has been successfully used in a wide range of analytical problems involving both inorganic anions and cations and neutral or potentially ionizable molecules [1,2]. A disadvantage, however, is the lack of a sensitive, generally applicable detector. An interesting way of overcoming this drawback was to use indirect detection. Indirect detection is now widely used in capillary electrophoresis for the determination of both organic and inorganic compounds that do not possess a suitable detection property [3,4].

In ionic capillary electrophoresis, which is used for the determination of both anions [5–15] and cations [16–33], detection is essentially performed via indirect UV detection.

As laser-induced fluorimetric detection is now commercially available, we recently considered the development of a new electrophoretic system with which indirect fluorimetric detection could be used, and without detector modification.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00786-5

Taking into account the characteristics of the laser associated with the commercial fluorimetric detector, we investigated the potential of the sodium salt of fluorescein as a fluorescent reagent in order to detect alkaline, alkaline earth and transition metals separated by capillary zone electrophoresis (CZE) [34]. In view of the very satisfactory results, we have now decided to extend this work to organic anions which have no chromophoric or fluorophoric group (*n*-alkane sulfonates and linear carboxylic acids) to study the sensitivity of indirect fluorimetric detection.

In the first instance, *n*-alkane sulfonates, which are consumed on a large scale in industrial and commercial formulations, were determined without derivatization in CZE. Various strategies had already been developed in indirect UV detection [5,35,36], with and without an electroosmotic flow reverser. Recently, with the aim of optimizing detector sensitivity, Shamsi and Danielson [36] tested various naphthalene sulfonates as chromophoric ions.

Determination of carboxylic acids in a similar way, to which much effort is being devoted since they are commonly used for the preparation of ionic and zwitterionic surfactants, is difficult because: (i) on the one hand, they are amphiphiles as a result of the strong polarity of the carboxylic group and of the weak affinity of their alkyl chain for water ($>C_{12}$), (ii) on the other hand, they have no chromophoric group which would allow detection in the classical UV range.

A variety of techniques has been proposed for such analyses [37–46]; all, however, have drawbacks. For example, in gas-phase chromatography, derivatization is required to obtain satisfactory sensitivity or volatility. And absorption phenomena are a hazard, in particular with the techniques in which a solid stationary phase is used.

In such conditions, capillary electrophoresis has already been investigated as an alternative method. The carboxylic acids have been analyzed using isotachophoresis with hydro-alcoholic media [46,47], MEKC [48] and, in particular, CZE. With respect to this last technique, different detection methods have been reported: direct UV detection at low wavelength [49], conductimetry [17–19] and, in by far the greatest number of studies, indirect UV detection [5,8,36,48,50,51].

Moreover, different systems have been proposed for the determination of mono-, di- and even tricarboxylic acids. In the case of polycarboxylic acids the electroosmotic flow has often been modified by the addition of cationic surfactant [5,8,48].

In view of the dearth of analyses for long-chain carboxylic acids (from C_8 to C_{18}), we undertook the study of their separation and of the potential of fluorimetric detection in this application. We used the electrophoretic medium based on the utilization of the fluorescein sodium salt that we had previously developed to analyze cations [34].

In order to establish its broader applicability, we tested this new electrophoretic medium in analyses to determine the whole range of commercially available *n*-alkane sulfonates, i.e. from C_1 to C_{10}.

2. Experimental

2.1. Reagents

All solutions, electrolytes and standards were prepared using 18 MΩ water generated by an Alpha Q Laboratory water-purification system (Millipore, Bedford, MA, USA). The *n*-alkane sulfonates (purity >98%) and linear carboxylic acids (purity >98%) were obtained, respectively, from Aldrich (Aldrich France, La Verpillière, France) and from Janssen (Janssen Chimica, Noisy le Grand, France). Sodium tetraborate (99.999% purity), used for the preparation of buffers, was purchased from Aldrich France. The cosolvents used, on the one hand, to modify the electroosmotic flow and, on the other hand, to solubilize the long-chain carboxylic acids were all of RS grade for high-performance liquid chromatography (HPLC) and were purchased from Carlo Erba (Rueil Malmaison, France). In order to solubilize the longest carboxylic acids, a polyoxyethylenic surfactant resulting from the

condensation of ethylene oxide on a C_{12} fatty alcohol was used. This hexaethylene glycol mono-*n*-dodecyl ether (purity >99%) was purchased from Nikko (Nikko Chemicals, Tokyo, Japan). Finally, the fluorescein sodium salt providing the fluorescence background was purchased from Aldrich France. All chemicals were used without further purification.

2.2. Electrolyte and standard preparation

The electrolytes used were prepared from solutions made up fresh each day containing the sodium tetraborate buffer. After dilution, electrolytes were systematically degassed for 20 min using sonication and their pH was measured before use. All the vessels used for the preparation of the solutions to be analyzed and of the electrolytes were made of polypropylene (Polylabo Block, Strasbourg, France). The disposable sample vials were made of siliconized polypropylene and were also purchased from Polylabo Block. Finally, the dilutions were performed using Gilson electronic automatic pipettes (Gilson France, Villiers le Bel, France) equipped with disposable polypropylene cones.

2.3. Apparatus

All experiments were carried out on a P/ACE 2100 System (Beckman, Fullerton, CA, USA) fitted with an on-column Argon-laser-based fluorescence detector ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 520 nm) and monitored by PS/2 computer (IBM, Greenwock, UK) using the PACE or GOLD 7.11 software (Beckman). Data collection was performed using the same software. Samples were loaded by pressure injection [injection pressure 3447.38 Pa (0.5 psi)] into a fused-silica capillary. Before use the following solutions were flushed through the capillary, in the order given: 0.1 *M* NaOH, water and finally the buffer solution. The untreated fused-silica capillaries were 50 μm I.D. and 57 cm in length. Injections were done at the high-voltage anode and organic anions were eluted to the earthed electrode. Indirect fluorimetric detection was performed at λ = 520 nm through the capillary at a distance of 50 cm from the inlet. Finally, the pH values of the electrolytes were measured using a Beckman Model ϕ pH meter at the analysis temperature.

3. Results and discussion

In a previous study [34], we showed that the sensitivity of the detector response was a function of: (i) on the one hand, the pH of the electrophoretic medium, because of the nature of the fluorescent reagent used to generate the background signal, i.e. fluorescein; (ii) on the other hand, as also found by Yeung and Khur [4], the concentration of the fluorophoric agent (here, fluorescein).

In the present work, therefore, we systematically performed the separations of *n*-alkane sulfonates and of carboxylic acids in basic media (pH = 9), at a 10^{-5} *M* concentration of the sodium salt of fluorescein, i.e. under the best conditions with respect to the sensitivity of the detection system [34].

3.1. Analysis of a mixture of C_1 to C_{10} *n*-alkane sulfonates

In order to optimize the separation of this complex mixture of surfactants we studied the influence of ionic strength on the electrophoretic behaviour of the analytes. The effect of buffer concentration was investigated in the range 2.5–30 m*M*, 2.5 m*M* being the minimum that gives a buffer effect. The evolution of the effective electrophoretic mobilities of the different *n*-alkane sulfonates as a function of the ionic strength of the electrophoretic medium is reported in Fig. 1.

This figure shows clearly that the selectivity of the electrophoretic system, which is measured by the difference between the effective electrophoretic mobilities of the analytes taken two by two [1,2], evolves only to a very small extent, or perhaps even not at all, as a function of ionic strength. However, whatever the *n*-alkane sulfonate, its effective electrophoretic mobility increases when the ionic strength of the electrolyte decreases. Considering that the aim of this study

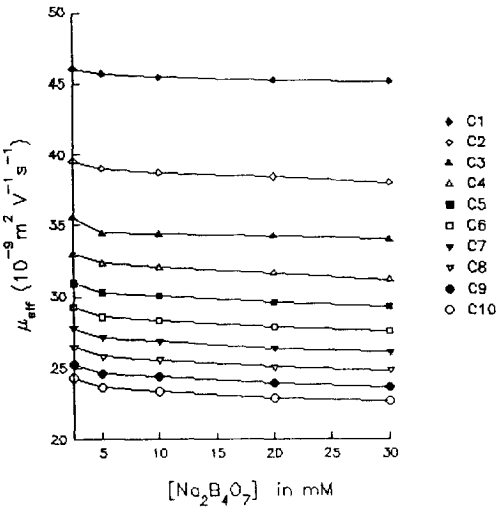

Fig. 1. Evolution of the effective electrophoretic mobilities of alkane sulfonates as a function of the borate concentration in the electrophoretic medium. Experimental conditions: fused-silica capillary: 57 cm × 50 μm I.D.; electrolyte: $Na_2B_4O_7$ buffer (pH = 9.2), [fluorescein] = 10^{-5} *M*; temperature: 30°C; applied voltage: 30 kV.

was not only to separate the *n*-alkane sulfonates, but also to achieve the greatest possible sensitivity, a compromise optimum value of this parameter must exist.

(i) Retention must be sufficient for satisfactory separation of the analyte components to be obtained. Consequently, the electroosmotic flow must be perfectly adapted.

(ii) However, in our recent study [34], in which we reported for the first time the utilization of fluorescein as the fluorophore generating the background signal, we showed that the sensitivity of the detector response was greater at smaller ionic-strength values. To obtain optimum sensitivity, therefore, ionic strength should be minimal, whereas for optimum separation it should be maximal. After optimization, we found that the best compromise is obtained at a sodium borate concentration of 5 m*M*. Sodium tetraborate concentrations lower than this improve system efficiency and give shorter analysis times and maximal detector sensitivity. However, as a result of the almost constant selectivity as a function of the ionic strength, the discrimination of the longest-chain *n*-alkane sulfonates, C_9 and C_{10} (which are the first eluted), is not sufficient. On the other hand, at borate concentrations greater than 5 m*M*, the discrimination between the different peaks corresponding to C_9 and C_{10} is greater, and migration times increase as a result of the decreasing electroosmotic flow; this does not mean, though, that the peak-to-peak resolution is increased, because the system efficiency falls. Moreover, as mentioned above, using such conditions leads to a noticeable loss of sensitivity and to a degradation of the analysis quality.

We report in Fig. 2 the analysis of the ten *n*-alkane sulfonates under these optimal ionic-strength conditions. As can be seen from this figure, the resolution is satisfactory, with the exception of the C_9–C_{10} pair. In order to obtain good resolution of all the components of this complex mixture we investigated the consequences of adding an organic cosolvent.

We studied the influence of the cosolvent on the detector sensitivity and on the electrophoretic behaviour of the *n*-alkane sulfonates, as it is known that such a strategy makes it possible to modify the electroosmotic flow [1,2]. We used

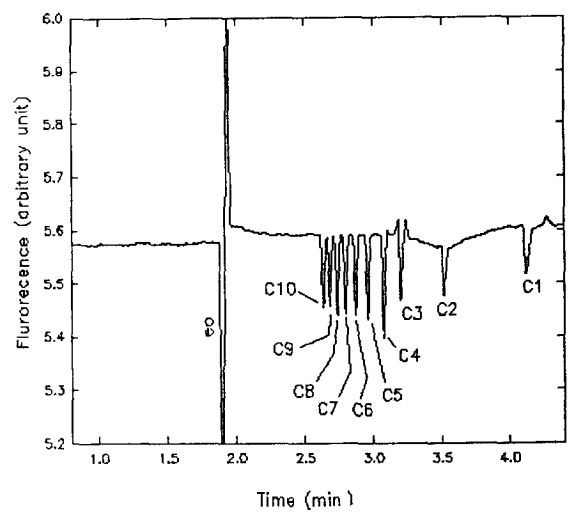

Fig. 2. Electropherogram corresponding to the analysis of the 10 *n*-alkane sulfonates in the optimal ionic-strength conditions (operating conditions identical to those reported in Fig. 1, except that the $Na_2B_4O_7$ concentration is fixed at 5 m*M*).

essentially 2-propanol and acetonitrile, which are widely used in MEKC and are perfectly adapted to capillary electrophoresis.

To begin with, we verified that the addition of organic cosolvent (2-propanol, ethanol or acetonitrile) does not affect the sensitivity of the detector response. Whatever the solvent used, we did not observe any decrease in the background signal for a cosolvent percentage lower than 40%. Beyond this limit, baseline perturbations appear and the noise increases appreciably.

We then examined the influence of the nature and the percentage of organic cosolvent on the electrophoretic behaviour of the *n*-alkane sulfonates. We report in Fig. 3 the evolution of the peak-to-peak resolution of seven of the ten *n*-alkane sulfonates studied, as a function of the quantity of organic cosolvent, for two solvents possessing very different chemical characteristics: 2-propanol (Fig. 3a) and acetonitrile (Fig. 3b).

It can be seen clearly that, whichever of the cosolvents (2-propanol or acetonitrile) was used and whatever the *n*-alkane sulfonate pair analyzed, the resolution increased when the percentage of organic cosolvent increased. However, this improvement is not identical for all the *n*-alkane sulfonate pairs. Moreover, it is greater in the case of 2-propanol. This applies most markedly in the case of the *n*-nonyl sulfonate–*n*-decyl sulfonate pair. In fact, these two compounds are not completely resolved in aqueous solutions and present the limiting resolution in the mixture analyzed. Therefore, it seems, at first sight, that 2-propanol should be used as the cosolvent. However, this conclusion is not totally correct because the change in the electroosmotic flow as a function of the cosolvent percentage differs according to the nature of the cosolvent, as shown in Fig. 4.

It can be seen clearly that the addition of acetonitrile in the electrophoretic buffer results in a smaller modification of the electroosmotic flow than the addition of 2-propanol or ethanol (which have similar behaviour). Consequently, it is possible to use higher amounts of organic cosolvent in the case of acetonitrile while maintaining satisfactory analysis times and detection sensitivity. Comparison of Figs. 3a and 3b shows that an equivalent resolution of the limiting pair (*n*-nonyl sulfonate–*n*-decyl sulfonate) is obtained with 10% of 2-propanol or 20% of acetonitrile. It would seem, then, that acetonitrile is the cosolvent required in order for the best res-

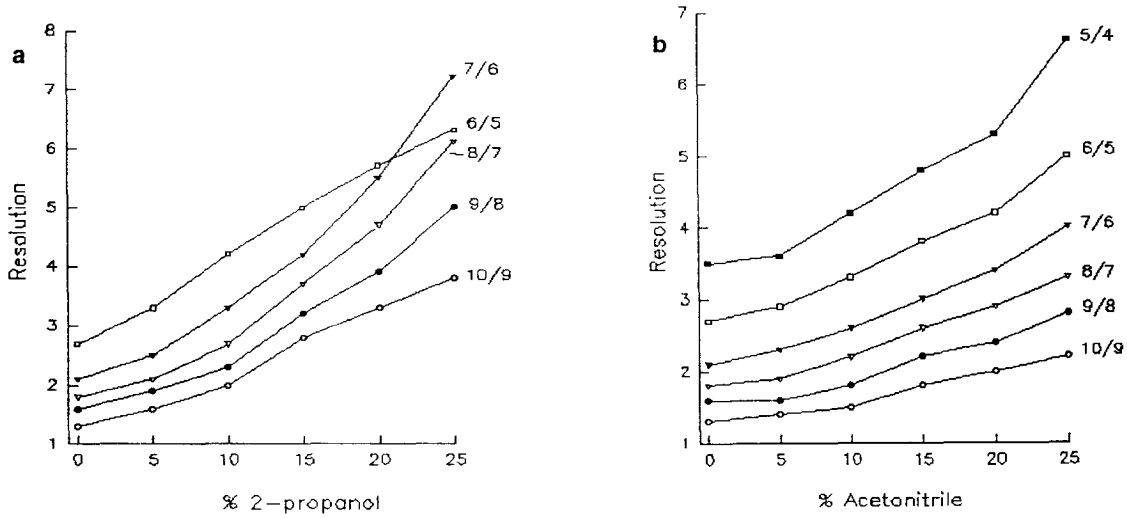

Fig. 3. Evolution of the peak-to-peak resolution as a function of the nature and percentage of the organic cosolvent in the electrolyte. (a) 2-Propanol, (b) acetonitrile. Operating conditions: fused-silica capillary: 57 cm × 50 μm I.D.; electrolyte: 5 m*M* $Na_2B_4O_7$ at pH = 9.2, [fluorescein] = 10^{-5} *M*; temperature: 30°C; applied voltage: 30 kV.

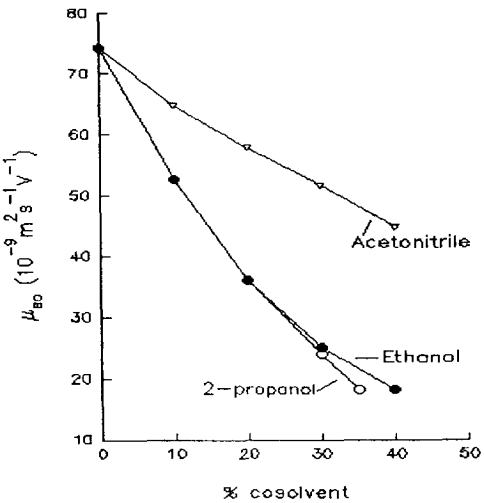

Fig. 4. Evolution of the electroosmotic flow as a function of the nature and the percentage of organic cosolvent added to the electrophoretic buffer. Operating conditions similar to those reported in Fig. 3.

olution/time unit ratio to be obtained. The comparison of the two electropherograms reported in Fig. 5, which were obtained using

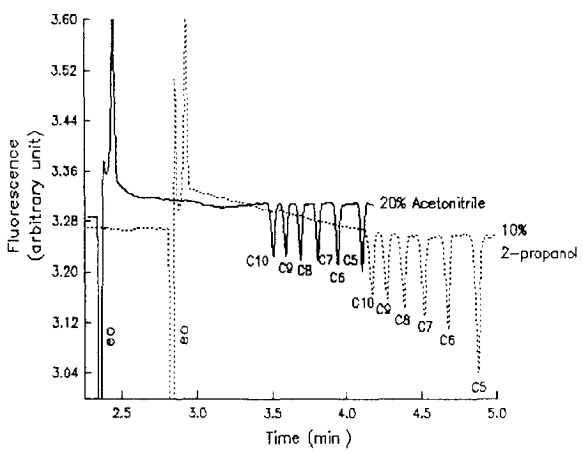

Fig. 5. Analysis of a mixture of alkane sulfonates containing all the homologous compounds from C_5 to C_{10}. Operating conditions: fused-silica capillary: 57 cm × 50 μm I.D.; electrolyte: 5 m*M* $Na_2B_4O_7$ at pH = 9.2, [fluorescein] = 10^{-5} *M*; temperature: 30°C; applied voltage: 30 kV; detection: λ_{exc} = 488 nm, λ_{emis} = 520 nm; hydrodynamic injection: 1 s. Dashed line: 20% 2-propanol added in the electrophoretic buffer; solid line: 10% acetonitrile added in the electrophoretic buffer.

electrophoretic buffers containing respectively 10% 2-propanol and 20% acetonitrile, confirms the validity of this strategy.

Since the optimum conditions for the analysis of *n*-alkane sulfonates were being determined in order to obtain also the maximal sensitivity of the detector response, we studied the quantification of this complex mixture and we estimated the reproducibility, response coefficients and detection thresholds for some of the *n*-alkane sulfonates concerned.

The reproducibility of effective mobilities of the *n*-alkane sulfonates on which the analysis was performed was more than satisfactory: we obtained a relative standard deviation (R.S.D.) better than 0.5% from 19 independent analyses. Similarly, the reproducibility of retention times was quite acceptable: the R.S.D.s determined from 19 independent analyses were at most 1% and varied somewhat as a function of the *n*-alkane sulfonate being studied. Finally, the reproducibility of peak areas was greatly dependent on the dilution in the concentration range explored, i.e. from $2.5 \cdot 10^{-4}$ to $2 \cdot 10^{-3}$ *M*. Generally speaking, at an individual concentration of 10^{-3} *M* for each *n*-alkane sulfonate, the R.S.D. obtained in peak areas was about 3%, and it reached 6% for $2.5 \cdot 10^{-4}$ *M* concentration.

We established the response factors using the calibration curves obtained from the corrected peak areas. The number of independent analyses was from 6 to 10 for each dilution, depending on the *n*-alkane sulfonates being analyzed. We obtained satisfactory calibration curves between corrected areas of peaks and the corresponding concentrations. The analytical expressions of these calibration curves are reported in Table 1.

Except in the case of methane sulfonate (correlation coefficient of the linear regression = 0.995) the correlation coefficients are systematically equal to 0.9995. Moreover, an evolution of the response coefficients of *n*-alkane sulfonates as a function of their side-chain lengths is in evidence. The response coefficient increases with alkyl chain length, i.e. when the absolute electrophoretic mobility of the *n*-alkane sulfonates decreases. This behaviour is in perfect

Table 1
Calibration curves, response coefficients in indirect fluorimetric detection and effective electrophoretic mobilities for a number of the *n*-alkane sulfonates analyzed

n-Alkane sulfonates	Effective electrophoretic mobilities ($m^2\ s^{-1}\ V^{-1}$)	Response coefficients	Intercept	Correlation
C_{10}	23.1	3392	−0.05	0.9993
C_9	24.1	3266	−0.002	0.9991
C_8	25.3	3233	0.05	0.9998
C_7	26.6	3081	0.04	0.9995
C_6	28.0	3097	−0.02	0.9997
C_5	29.7	3031	0.02	0.9997
C_1	44.69	2484	0.01	0.995

agreement with the theoretical treatment of indirect detection developed by Ackermans et al. [51], that we have previously transposed to the indirect fluorimetric detection of cations, using the fluorescein sodium salt [34].

From this theoretical treatment of indirect detection it appears that the response coefficient must evolve as a function of the absolute or effective electrophoretic mobility of the anion being detected as:

$$C \cdot \frac{m_B^0 + m_X^0}{m_X^0}$$

where m_B^0 and m_X^0 are the respective absolute electrophoretic mobilities of the sodium ion and the anion being detected (here an alkane sulfonate), and C is a constant given by the ratio of the absolute electrophoretic mobility of the fluorescein anion to the sum of the absolute mobilities of the sodium cation and of the fluorescein anion.

It is therefore interesting to study the variation of the experimental response coefficients of the different *n*-alkane sulfonates being determined as a function of their effective electrophoretic mobility (see Table 1), and then to compare this variation to the computed theoretical variation for the same *n*-alkane sulfonates. This comparison is reported in Fig. 6. There is good agreement between the theoretical and the experimental estimations.

Finally, we completed this quantitative study by determining the detection thresholds of these organic anions using our electrophoretic system. These detection thresholds are about 700 ppb.

These results show that the electrophoretic system based on the utilization of fluorescein in a basic medium is suitable for the analysis of organic anions lacking chromophoric or fluorophoric groups, which at present can be detected routinely only by using indirect detection in capillary electrophoresis.

The analysis of only one mixture is not sufficient to assess objectively the potential of this new electrophoretic medium for the analysis of

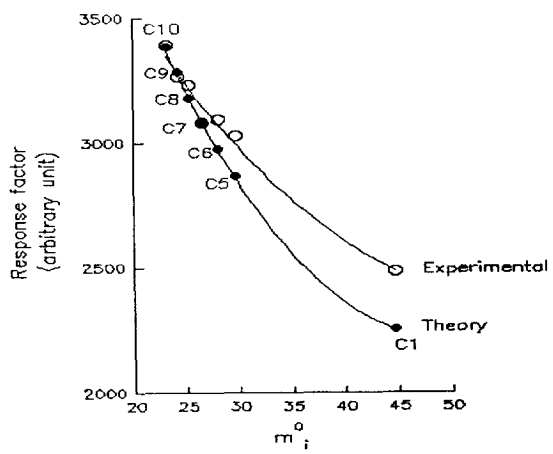

Fig. 6. Comparative study of the evolution of the theoretical and experimental response factors of *n*-alkane sulfonates as a function of their effective electrophoretic mobility.

such ions, and we used it to perform both qualitative and quantitative analyses of another mixture. We studied the analysis of carboxylic acids, with a view to concentrating on the mixtures containing both the carboxylic acids previously analyzed (from C_1 to C_8) [47,48] and carboxylic acids possessing longer chains, particularly the C_{16} and C_{18} carboxylic acids.

3.2. Analysis of a mixture of carboxylic acids containing all the homologues between C_1 and C_{12} and the C_{16} and C_{18} carboxylic acids

This mixture is quite similar to the *n*-alkane sulfonates mixture, as it is totally ionized in basic medium, at a pH allowing the maximum sensitivity of the detector. Under these conditions, the first phase of the optimization procedure developed previously in this paper for *n*-alkane sulfonates can be transposed directly. Consequently, we started the optimization to resolve this complex mixture from the pH and ionic strength selected previously for the analysis of *n*-alkane sulfonates. The electropherogram obtained in these conditions is reported in Fig. 7.

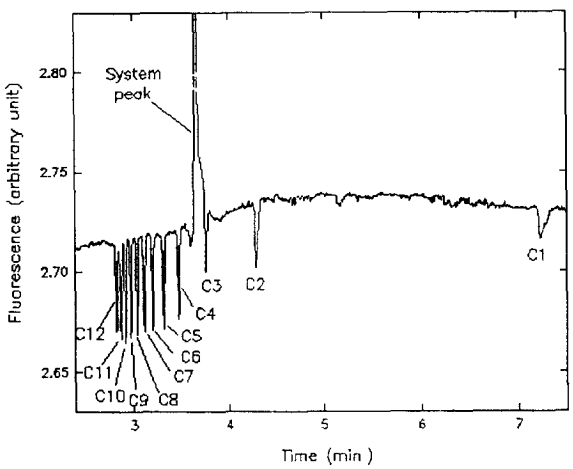

Fig. 7. Analysis of a mixture of carboxylic acids by capillary electrophoresis with laser-induced indirect fluorimetric detection. Experimental conditions: fused-silica capillary: 57 cm × 50 μm I.D.; electrolyte: 5 m*M* $Na_2B_4O_7$ at pH = 9.2, [fluorescein] = 10^{-5} *M*, temperature: 30°C; applied voltage: 30 kV; detection: λ_{exc} = 488 nm, λ_{emis} = 520 nm; hydrodynamic injection: 1 s.

Thus, it is possible to separate in about 7 min all the homologues of carboxylic acids from C_1 to C_{12}, with a practically satisfactory resolution. Unfortunately, the analysis of carboxylic acids possessing chains longer than C_{12} is impossible using this electrophoretic buffer, because they are not soluble in this medium. As natural oils contain C_{16} and C_{18} carboxylic acids, both saturated and unsaturated, it was compulsory to develop operating conditions which would permit analysis for these acids to be performed.

To begin with, we attempted to solubilize these compounds using a non-ionic surfactant; if successful, this strategy could result in similar or even shorter analysis times. We used hexaethylene glycol mono-*n*-dodecyl ether ($C_{12}E_6$), this having the longest hydrocarbon chain of the commercially available non-ionic surfactants of acceptable purity.

We then proceeded to study the evolution of resolution as a function of the concentration of this surfactant in the electrophoretic medium. The results are reported in Fig. 8.

These curves show that peak-to-peak resolution varies noticeably as a function of the $C_{12}E_6$ concentration in the electrolyte. Moreover, this evolution is not monotonic; it presents either a

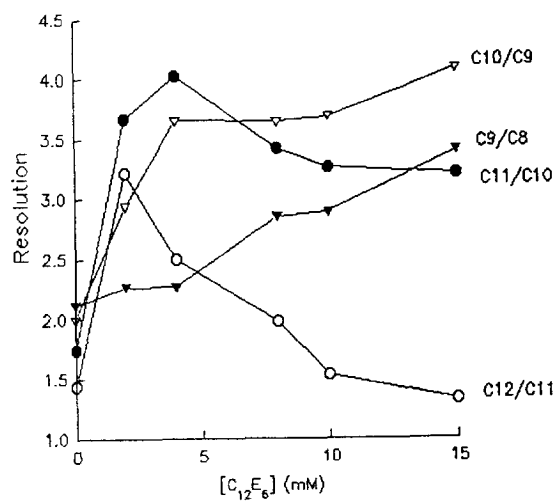

Fig. 8. Evolution of the resolution as a function of the non-ionic surfactant ($C_{12}E_6$) concentration in the electrolyte in the case of some of the carboxylic acids from Fig. 7. Operating conditions similar to those reported in Fig. 7.

step or a maximum, depending on the pair of carboxylic acids being studied. The expectation was that there would be an optimal value of the $C_{12}E_6$ concentration in the electrophoretic buffer which would permit maximal resolution per time unit. It can be seen from Fig. 8 that this optimal concentration of $C_{12}E_6$ is low, about 1 m*M* or even lower. The evolution of the resolution in the case of the C_{11}–C_{12} acids pair demonstrates this more than clearly (Fig. 8). In fact, in this case the optimal value is 0.2 m*M*. Below this value, the heaviest carboxylic acids (palmitic and oleic acids) are no longer solubilized, and the analysis becomes impossible. This analysis is also impossible at higher $C_{12}E_6$ concentrations, for the following reasons: (i) the addition of non-ionic surfactant results in a sharp decline in the efficiency of the electrophoretic system; (ii) migration times decrease sharply with the addition of surfactant to the electrolyte.

The analysis of a carboxylic acids mixture containing particularly palmitic and oleic acids in the presence of non-ionic surfactant ($C_{12}E_6$ at a 0.2 m*M* concentration) is reported in Fig. 9. This electropherogram shows that it is possible to analyze in about 3 min a mixture of carboxylic acids containing all homologous compounds from C_7 to C_{12}, and also palmitic and oleic acids. However, the resolution of the two latter compounds is only partial. This half-check is in fact encouraging: to our knowledge, this is the first analysis in CE of such heavy carboxylic acids. In an attempt to separate totally the pair palmitic–oleic acids, we considered dissolving them in the presence of organic solvents and analyzing them in CZE using an organic cosolvent. The less attractive feature of this strategy is a decrease in the electroosmotic flow, which is of greater or lesser importance depending on the solvent used (Fig. 4) and which lengthens the analysis time.

Among the solvents considered during the optimization of the separation of *n*-alkane sulfonates, only the two alcohols give efficient solubilization of the heaviest carboxylic acids. As we showed previously that the electroosmotic flow is modified to a greater extent by the addition of 2-propanol than by the addition of ethanol, we selected this latter cosolvent. As can be seen from Fig. 10, the addition of ethanol results in a noticeable improvement in the resolution. This increase is more marked in the case of the shortest-chain acids.

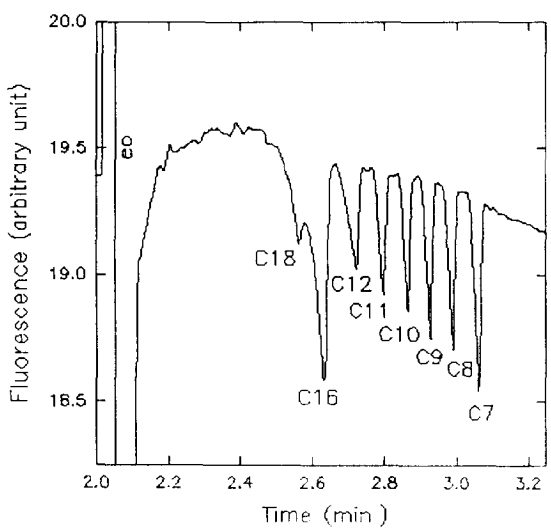

Fig. 9. Analysis of a mixture of fatty acids, containing in particular oleic and palmitic acids, in the presence of $C_{12}E_6$. Operating conditions similar to those reported in Fig. 7, except for the presence of $C_{12}E_6$ (0.2 m*M*) and that the injection time was fixed at 3 s.

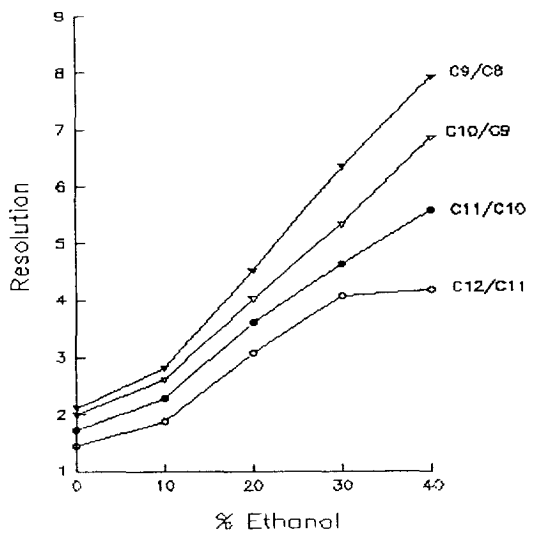

Fig. 10. Resolution in the analysis of some carboxylic acids as a function of the ethanol concentration in the electrolyte. Operating conditions similar to those reported in Fig. 7.

The limiting pair being the oleic and palmitic acids, this last result suggests that very high percentages of ethanol should be used. However, in order to keep the detector sensitivity at an acceptable level, ethanol addition could not exceed 40%. The electropherogram obtained with a mixture of C_7 to C_{18} carboxylic acids under these operating conditions is reported in Fig. 11.

This electropherogram shows clearly that we were able to resolve correctly the whole of this mixture. However, this satisfactory resolution was obtained at the cost of a longer analysis time (20 min), due to the considerable retardation of the electroosmotic flow under these conditions. Obviously, this buffer also allows the separation of C_1 to C_6 carboxylic acids. Unfortunately, though, analysis of the whole distribution of carboxylic acids from C_1 to C_{18} takes too long. It is therefore advisable to perform two separate analyses, using two different buffers: (i) a 5 m*M* $Na_2B_4O_7$ buffer at pH = 9.2, with a fluorescein concentration of 10^{-5} *M*, allows the separation of all the homologues from C_1 to C_{11}; (ii) a 5 m*M* $Na_2B_4O_7$ buffer at pH = 9.2–EtOH (60:40, v/v), with a fluorescein concentration of 10^{-5} *M*,

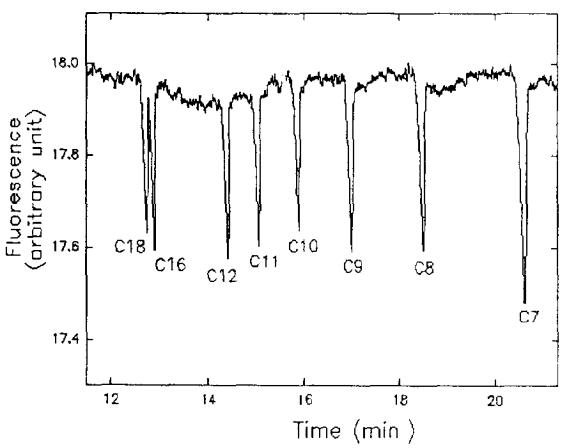

Fig. 11. Analysis by CZE of a mixture of fatty acids containing oleic and palmitic acids. Operating conditions: fused-silica capillary: 57 cm × 50 μm I.D.; electrolyte: 5 m*M* $Na_2B_4O_7$ at pH = 9.2–ethanol (60:40, v/v), [fluorescein] = 10^{-5} *M*; temperature: 40°C; applied voltage: 30 kV; detection: λ_{exc} = 488 nm, λ_{emis} = 520 nm; hydrodynamic injection: 2 s.

allows the separation of the C_{10} to C_{18} homologous acids.

Then, having studied the quantitative aspect of this analysis, we evaluated the reproducibility of the method, the response factors and the detection thresholds.

Reproducibilities of migration times and of effective electrophoretic mobilities are quite satisfactory when analyses are performed using an electrolyte containing only the borate buffer and fluorescein. From 10 independent experiments we obtained an R.S.D. from 0.4 to 0.7% in the case of effective electrophoretic mobilities and from 0.9 to 1.1% for the migration times. On the other hand, in the presence of 40% ethanol, this reproducibility is somewhat worsened. Effectively, the R.S.D.s computed from 16 independent experiments are now respectively 2.2% for effective electrophoretic mobilities and 2.6% for migration times. As previously reported in the case of the *n*-alkane sulfonates, the reproducibility of peak areas is a function of the dilution of the solution analyzed. Its values vary from 4%, at a 2 m*M* concentration, to 10%, at a 0.1 m*M* concentration.

As shown in Table 2, in the concentration range explored, we obtained calibration curves with linear regression coefficients varying from 0.990 to 0.9991. The regression coefficients of calibration curves remained excellent when the electrolyte contained 40% ethanol. Once again, perfect agreement is observed between the theoretical and the experimental response coefficients (Table 2). In fact, the response factors increase with the length of the hydrocarbon chains, i.e. if the absolute or effective electrophoretic mobilities decrease.

To conclude this quantitative study, we determined the detection thresholds in the presence and in the absence of ethanol, and these were about 1 ppm.

4. Conclusions

The indirect fluorimetric detection system based on the utilization of the sodium salt of fluorescein to provide the background signal

Table 2
Calibration curves, response coefficients in indirect fluorimetric detection and effective electrophoretic mobilities for a number of the carboxylic acids analyzed

Carboxylic acids	Effective electrophoretic mobilities ($m^2 s^{-1} V^{-1}$)	Response coefficients	Intercept	Correlation
C_{12}	20.5	7544	0.09	0.9991
C_{11}	21.3	7503	0.09	0.990
C_{10}	22.2	7200	0.08	0.996
C_9	23.2	7335	0.21	0.991
C_8	24.3	7069	0.30	0.994
C_6	26.9	6882	0.16	0.996
C_5	28.5	6870	0.08	0.997
C_4	30.5	6551	0.17	0.994
C_2	38.6	6515	−0.28	0.992
C_{18}	9.8	6094	0.09	0.998

(derived from a system developed previously to analyze inorganic cations) is perfectly adapted to the qualitative and quantitative analysis of organic anions. Analyses with quite acceptable reproducibilities, both of retentions and of quantitative measurements, can be performed. Moreover, this electrophoretic system is highly sensitive, with detection thresholds of a few hundred ppb.

However, if an organic cosolvent is added to the electrophoretic buffer, either to dissolve the analyte or to optimize the electroosmotic flow so that satisfactory resolution can be attained, this sensitivity is slightly decreased at higher cosolvent levels.

Finally, using a buffer rich in ethanol, this electrophoretic system allowed, for the first time, the analysis of heavy carboxylic acids such as oleic and palmitic acids. However, because of a relatively weak electroosmotic flow, the time taken for a single complete analysis of carboxylic acids from C_1 to C_{18} was prohibitive. Under these conditions, it seems reasonable to analyze such complex mixtures by performing two distinct analyses, in different electrophoretic conditions: with a simple borate buffer to analyze for C_1 to C_{11} acids, and with a borate buffer in the presence of organic cosolvent to determine the remaining compounds from C_{12} to C_{18}. In this way, analysis times remain quite acceptable.

Following these very encouraging results, we intend soon to apply this electrophoretic system to the analysis and detection of inorganic anions in capillary zone electrophoresis.

References

[1] S.F.Y. Li, Capillary Electrophoresis–Principles, Practice and Applications, Elsevier, Amsterdam, 1992.
[2] R. Kuhn and S. Hoffstatter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer Verlag, Berlin, 1994.
[3] E.S. Yeung, Acc. Chem. Res., 22 (1989) 125.
[4] E.S. Yeung and W.G. Kuhr, Anal. Chem., 63 (1991) 275A.
[5] J. Romano, P. Jandik, W. Jones and P. Jackson, J. Chromatogr., 546 (1991) 411.
[6] W. Jones and P. Jandik, J. Chromatogr., 546 (1991) 445.
[7] B. Wildman, P. Jackson, W. Jones and P. Alden, J. Chromatogr., 546 (1991) 459.
[8] B.F. Kenney, J. Chromatogr., 546 (1991) 423.
[9] S. Grocott, I. Jefferies, T. Bowser, J. Carnavale and P. Jackson, J. Chromatogr., 602 (1992) 257.
[10] K. Hargadon and B. McCord, J. Chromatogr., 602 (1992) 241.
[11] W. Buchberger and P. Haddad, J. Chromatogr., 608 (1992) 59.
[12] G. Bondoux, P. Jandik and W. Jones, J. Chromatogr., 602 (1992) 79.
[13] D. Salomon and J. Romano, J. Chromatogr., 602 (1992) 219.
[14] J. Nair and C. Izzo, J. Chromatogr., 640 (1993) 445.

[15] M. Harrold, M. Wojtusik, J. Riviello and P. Henson, J. Chromatogr., 640 (1993) 463.
[16] X. Huang, T. Pang, M.J. Gordon and R.N. Zare, Anal. Chem., 59 (1987) 2747.
[17] X. Huang, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61 (1989) 766.
[18] X. Huang, M.J. Gordon and R.N. Zare, J. Chromatogr., 480 (1989) 285.
[19] X. Huang, R.N. Zare, S. Sloss and A.G. Ewing, Anal. Chem., 63 (1991) 189.
[20] M. Aguilar, X. Huang and R.N. Zare, J. Chromatogr., 480 (1989) 427.
[21] M. Aguilar, A. Farran and M. Martinez, J. Chromatogr., 635 (1993) 127.
[22] F. Foret, S. Fanali, A. Nardi and P. Bocek, Electrophoresis, 11 (1990) 780.
[23] L. Gross and E.S. Yeung, Anal. Chem., 62 (1990) 427.
[24] M. Chen and R. Cassidy, J. Chromatogr., 602 (1992) 227.
[25] A. Weston, P. Brown, P. Jandik, W. Jones and A. Heckenberg, J. Chromatogr., 593 (1992) 289.
[26] P. Jandik, W. Jones, A. Weston and P. Brown, LC·GC, 9 (1991) 634.
[27] A. Weston, P. Brown, A. Heckenberg, P. Jandik and W. Jones, J. Chromatogr., 602 (1992) 249.
[28] M. Koberda, M. Kondowski, P. Younberg, W. Jones and A. Weston, J. Chromatogr., 602 (1992) 235.
[29] A. Weston, P. Brown, P. Jandik, A. Heckenberg and W. Jones, J. Chromatogr., 608 (1992) 395.
[30] W. Beck and H. Engelhardt, Chromatographia, 33 (1992) 313.
[31] K. Bächmann, J. Boden and I. Haumann, J. Chromatogr., 626 (1992) 259.
[32] D. Swaile and M. Sepaniak, Anal. Chem., 63 (1991) 179.
[33] P. Morin, C. François and M. Dreux, Analusis, 22 (1994) 178.
[34] P.L. Desbène, C. Morin, A.M. Desbène-Monvernay and R.S. Groult, J. Chromatogr. A, 689 (1995) 135.
[35] S. Chen and D.J. Pietrzyk, Anal. Chem., 65 (1993) 2770.
[36] S.A. Shamsi and N.D. Danielson, Anal. Chem., 66 (1994) 3757.
[37] J.L. Iverson and A.J. Sheppard, J. Chromatogr. Sci., 13 (1975) 505.
[38] J. Krupcik, J. Hrivnak and J. Janak, J. Chromatogr. Sci., 14 (1976) 4.
[39] J.N. Shantha and G.E. Napolitano, J. Chromatogr., 624 (1992) 37.
[40] H.D. Drust, M. Milano, E.J. Kikta, S.A. Connelly and E. Grushka, Anal. Chem., 47 (1975) 1797.
[41] H. Miwa, C. Hiyama and M. Yamamoto, J. Chromatogr., 321 (1985) 165.
[42] Y. Yasaka, M. Tanaka, T. Shono, T. Tetsumi and J. Katakawa, J. Chromatogr., 508 (1990) 133.
[43] Y. Liu, F. Yang and C. Pohl, J. Microcol. Sep., 2 (1990) 245.
[44] C. de Jong and H.T. Badings, J. High Resolut. Chromatogr., 13 (1990) 94.
[45] Y. Tsuyama, T. Uchida and T. Goto, J. Chromatogr., 596 (1992) 181.
[46] M. Koval, D. Kaniansky, M. Hutta and R. Lacko, J. Chromatogr., 325 (1985) 151.
[47] T. Barth, Anal. Chem., 59 (1987) 2232.
[48] R. Szücs, J. Vindevogel and P. Sandra, J. High Resolut. Chromatogr., 14 (1991) 692.
[49] M. Shirao, R. Furuta, S. Suzuki, H. Nakazawa, S. Fujita and T. Maruyama, J. Chromatogr. A, 680 (1994) 247.
[50] S. Hjerten, K. Elenbring, F. Kilar, A.J. Chen, C.J. Siebert and M. Zhu, J. Chromatogr., 403 (1987) 47.
[51] M.T. Ackermans, F.M. Everaerts and J.L. Beckers, J. Chromatogr., 549 (1991) 345.