ELSEVIER

Journal of Chromatography A, 872 (2000) 247–258

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Utilization of fluorescein sodium salt for the indirect fluorimetric detection in micellar electrokinetic chromatography

C.J. Morin, N.L. Mofaddel, A.M. Desbène, P.L. Desbène*

Université de Rouen, Laboratoire d'Analyse des Systèmes Organiques Complexes, UPRES EA 2659, IRCOF and IFR No. 23, 55 Rue Saint-Germain, 27000 Evreux, France

Received 6 August 1999; received in revised form 6 October 1999; accepted 2 November 1999

Abstract

The potential of fluorescein sodium salt to generate a background signal in indirect fluorimetric detection in micellar electrokinetic chromatography (MEKC) has been studied by using a sample test containing various alcohols (methanol, ethanol, propan-1-ol, propan-2-ol, 2-methylpropan-1-ol, pentan-3-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol), acetaldehyde, acetone and ethyl acetate. In order to optimize sample test resolution and detection sensitivity, we have studied different parameters: sodium dodecyl sulfate concentration, electrolyte ionic strength, applied voltage as well as that of the addition of an organic cosolvent within the running electrolyte. Although the micellar pseudo-phase was not able to be saturated with the fluorophore in order to obtain the maximal detection sensitivity, the detection thresholds were satisfactory. By way of example, the detection threshold reached for 3-methylbutan-1-ol is 0.03% (v/v). The quantitative aspect was approached with ethanol titration in a commercial white wine. Finally the repeatability relating to, on the one hand, the effective electrophoretic mobilities and, on the other hand, the corrected areas has also been studied.

Keywords: Fluorescence detection; Indirect detection; Detection, electrophoresis; Wine; Food analysis; Fluorescein; Alcohols

1. Introduction

The two techniques mainly used in high-performance capillary electrophoresis (HPCE) are capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). For each of these two techniques, the indirect detection offers interesting potential due to its universality. But, while much researches have been performed in CZE, especially for the analysis of inorganic ions, only a few papers have been published in MEKC with UV [1–3] or fluorimetric detection [4–7]. In these studies, the chromophore or the fluorophore, providing the background signal, is systematically different from the surfactant used to form the micelles, except in the work of Erim et al. [2]. Indeed these authors have used the dodecylbenzenesulfonate both as a chromophore and as a surfactant. Amankwa and Kuhr have shown [4], on the basis of the work carried out by Takeuchi and Yeung [8], in reversed-phase liquid

*Corresponding author. Tel.: +33-2-3229-1538; fax: +33-2-3229-1539.

E-mail address: paul-louis.desbene@univ-rouen.fr (P.L. Desbène)

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01152-8

chromatography, that the analyzed solute has to alter the partition of the visualizing agent between the micelles and the aqueous phase to be detected. Consequently the solute has to modify either the formation of ion pairs between the visualizing agent and the micelle, or the solubilization of this visualizing agent in the hydrophobic core of the micelle.

In order to obtain a greater sensitivity, one can use indirect fluorimetric detection. Indeed the fluorophore micellization or the formation of ion pairs between the fluorophore and the micelle results in inducing significant displacements in excitation spectra and emission spectra [4] and enhances the fluorescence intensity [9–11]. Indeed the micelles protect the excited fluorophore towards non radiative deactivation and change the fluorescence quantum yields [9]. Thus the introduction of a solute, that reduces the interactions between the micelles and the fluorophore and causes a net reduction of fluorescence quantum efficiency in the sample zone [4], will induce a decrease of the background fluorescence. The latter results from the difference between the fluorescence emitted by the fluorophore in the aqueous phase and the more intense fluorescence of the micelle–fluorophore complex. On the other hand, if the solute does not induce any change in the fluorophore partition between the aqueous phase and the micelles, one peak can appear if the solute quenches the fluorescence emitted by the fluorophore contained within the aqueous phase.

In our previous studies, which have dealt with the indirect fluorimetric detection of inorganic ions [12,13] or organic ions [14], we have used fluorescein sodium salt as visualizing agent. As shown by Kennedy et al. [5], this fluorophore possesses a fluorescence intensity slightly higher within micelles than in aqueous phase. Therefore, this fluorophore offers some interesting potentialities with regard to the indirect fluorimetric detection in MEKC. Although this difference of fluorescence is slight [5] and therefore can be a limiting factor concerning the detection sensitivity, we have decided to optimize such an electrophoretic system in MEKC. Indeed the technical characteristics of our commercial fluorimetric detector are in perfect adequacy with the use of fluorescein as a fluorophore [12]. To carry out this study, a mixture was made up of methanol, ethanol, propan-1-ol, propan-2-ol, 2-methylpropan-1-ol, pentan-3-ol, 2-methylbutan-1-ol, acetone, acetaldehyde and ethyl acetate. It is interesting to note that the totality of these compounds, except the acetone, is commonly quantified in analyses of alcoholic drinks. As for acetone, ethanol, acetaldehyde and the two isomers of propanol are titrated in blood analyses. These compounds are currently quantified by using many techniques, especially gas phase chromatography although the handling of the headspace injection mode, that is often used, proves to be delicate for the quantification. In the case of complex matrices, other analytical problems, such as the compulsory pre-treatment of the sample or the capillary fouling and blockages, make the use of this technique equally delicate [1]. So the quantitative aspect will be approached in this paper with the ethanol titration in a commercial white wine. The effect of the addition of an organic cosolvent within the running electrolyte will be considered in qualitative or quantitative approach.

2. Experimental

2.1. Chemicals

All solutions were prepared by using the 18 MΩ water produced by means of an Alpha Q purification system (Millipore, Bedford, MA, USA). The sodium tetraborate and the fluorescein sodium salt were of analytical purity and came from Aldrich (Aldrich France, La Verpillère, France). The sodium dodecyl sulfate (SDS; purity 98%) was provided by Sigma (Sigma France, L'Isle d'Abeau Chesnes, France). Methanol, ethanol, propan-2-ol, tetrahydrofuran (THF) were of RS HPLC purity and were obtained from Carlo Erba (Carlo Erba France, Rueil Malmaison, France). Acetaldehyde, propan-1-ol, acetone, 2-methylpropan-1-ol, pentan-3-ol, 2-methylbutan-1-ol and 3-methylbutan-1-ol were all of analytical purity and were purchased from Aldrich France. It should be noted that all these reagents were used without any purification.

2.2. Equipment

All analyses were carried out on a P/ACE 2100 system (Beckman, Fullerton, CA, USA) fitted with a

fluorimetric detector using an argon laser (excitation=488 nm, emission=520 nm). The acquisition and the processing of data were performed with a PS/2 computer (IBM, Greenwock, UK) by means of P/ACE or a Gold 7.11 softwares (Beckman). The samples were systematically injected in hydrodynamic mode (injection pressure: 0.5 p.s.i. or 3.4 kPa) and their analysis was achieved on a fused-silica capillary of 57 cm (50 cm effective length to detection window)×50 μm I.D. Injections were performed on the anodic end of this capillary and the temperature was systematically fixed at 30°C. The pH of the solutions was measured before utilization, to the temperature of the experiment, with a Model Φ pH meter (Beckman). Finally the electrolytes were systematically degassed by sonication by means of a Brandson device purchased from Touzart and Matignon (Touzart and Matignon, Ivry sur Seine, France).

3. Results and discussion

It is interesting to note that initially the fluorescein concentration introduced into the electrolyte, as fluorophore, was 10^{-5} *M*. This concentration has allowed us, during our previous studies by using an untreated fused-silica capillary [12,14], to obtain the best detection sensitivity.

3.1. Optimization of the SDS concentration

The methanol and the acetaldehyde, being the less hydrophobic compounds of the analyzed sample test, constitute the pair of compounds whose resolution is somewhat difficult. Indeed if their interactions with the SDS micelles are too weak, these compounds will coelute with the electroosmotic flow and as a result they will not be able to be separated. So the studies dealing with the organic solvent analysis by MEKC conclude that SDS concentrations superior than 10^{-1} *M* are necessary in order to increase solute–micelle interactions [1,3,4]. Furthermore the detection of this type of compound is possible only if they possess, as previously reported in this paper, either the capacity to quench the fluorescence emitted within the aqueous phase by the fluorophore, i.e., the fluorescein, or the possibility to modify the partition of the fluorophore between the micellar and the aqueous phase. As a result, in order to solve both separation and detection problems induced by these compounds, we have studied at the same time the effect of the SDS concentration in the electrolyte on the resolution of the methanol–acetaldehyde pair and on the evolution of the signal-to-noise ratio.

Methanol and acetaldehyde appear on the electropherogram, as negative peaks distinct from that of the electroosmotic flow only for SDS concentrations superior or equal to 10^{-1} *M*. The effect of the SDS concentration on the resolution of this pair of compounds as well as on the detection sensitivity has been studied only from this concentration (Fig. 1).

So Fig. 1A clearly shows that the resolution of this pair of compounds increases with the concentration of the surfactant introduced within the electrolyte. Unfortunately the detection sensitivity (see Fig. 1B) is nearly divided by a factor of 3 when the SDS concentration rises from 10^{-1} *M* to $1.6\cdot10^{-1}$ *M*. Therefore it is necessary to compromise between sensitivity and resolution by choosing a medium SDS concentration. A SDS concentration of $1.33\cdot10^{-1}$ *M* appears adequate, the resolution of the methanol–acetaldehyde pair being near 1.5 while the detection sensitivity is again notable.

3.2. Influence of the ionic strength of the electrolyte

The high SDS concentration within the carrier electrolyte, that is necessary to obtain a satisfactory resolution between methanol and acetaldehyde, notably rises the ionic strength of the electrolyte. Consequently we can observe on the one hand, a decrease of the electroosmotic flow and therefore an increase of the analysis time, and on the other hand, an increase of Joule heating. To dissipate this heat that causes sample zone broadening and increases baseline noise, we studied resolution and detection sensitivity as a function of the sodium tetraborate concentration in the running electrolyte.

The resolution of the sample matrice does not seem to be affected by tetraborate concentration. Contrarily, the analysis time is divided by approximately 1.5 and the sensitivity increases, when the $Na_2B_4O_7$ concentration decreases from $3\cdot10^{-2}$ *M* to

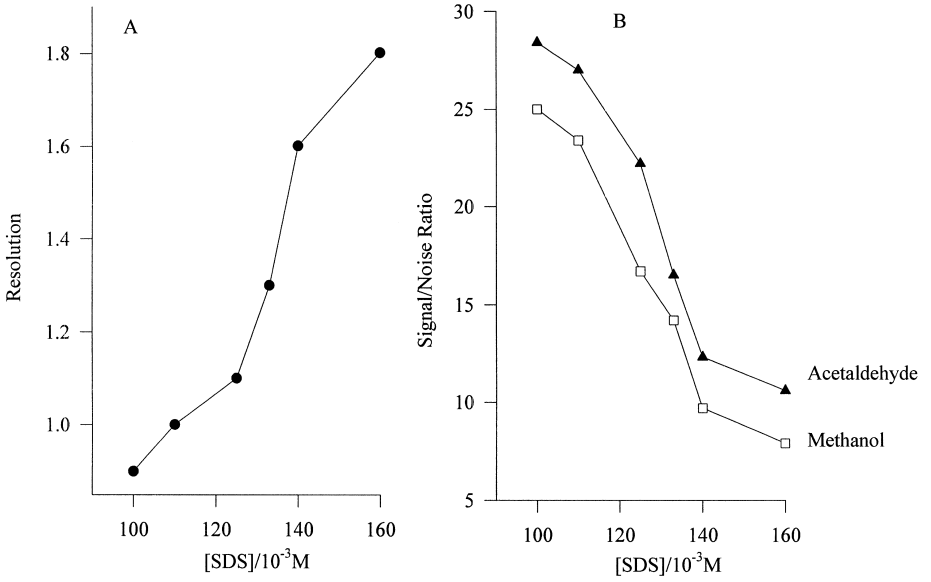

Fig. 1. Optimization of the SDS concentration within the running electrolyte. Operating conditions: capillary: 57 cm length (50 cm effective length to detection window)×50 μm I.D.; temperature: 30°C; applied voltage: 15 kV; electrolyte: $[Na_2B_4O_7]=10^{-2}$ *M*/[fluorescein]=10^{-5} *M*, the SDS concentration varying; hydrodynamic injection 4 s of an aqueous solution containing 1% (v/v) MeOH and 2% (v/v) acetaldehyde. (A) Effect of the SDS concentration on the resolution of the methanol–acetaldehyde pair. (B) Effect of the SDS concentration on the detection sensitivity.

$2.5\cdot10^{-3}$ *M* (Fig. 2). As in our previous works in CZE [12,14], the loss of sensitivity when the sodium tetraborate concentration increases can be explained, not only by the baseline fluctuation which occurs when the conductivity of the running electrolyte increases, but also by the decrease of the electronic delocalization of the fluorescein anion when the sodium concentration increases. This last effect of the sodium cation is similar to pH effect with regard to the fluorescence of the fluorescein [12].

Consequently it appeared suitable to use a sodium tetraborate concentration equal to $2.5\cdot10^{-3}$ *M* so as to obtain a short analysis time and a satisfactory detection sensitivity.

3.3. Influence of the applied voltage

Although the ionic strength of the running electrolyte has been drastically decreased an applied voltage of 30 kV could appear unsuitable if one considers the Joule heating. Indeed, as previously noticed, the latter not only results in a broadening of the sample bands but an increase in background noise. As well, following the evolution of the signal-to-noise ratio as a function of the applied voltage (see Fig. 3), we have been able to evaluate its effect on the sensitivity detection.

So by decreasing the applied voltage from 30 kV to 10 kV we can notably increase the detection sensitivity by a factor 3 to 4 according to the considered solute. With regard to the analysis time we had to find a compromise between this analysis time and the detection sensitivity. So an applied voltage at about 15 kV seemed suitable.

3.4. Optimization of the fluorescein concentration

Amankwa and Kuhr [4] have evidenced that the signal corresponding to any solute is maximal when the concentration of the fluorophore equals that of the micelles. So, the fluorescein has to be introduced within the running electrolyte at a concentration which allows the saturation of all the micelles. This concentration can be easily obtained by calculating the micelles concentration within the solution [5] by means of the following equation:

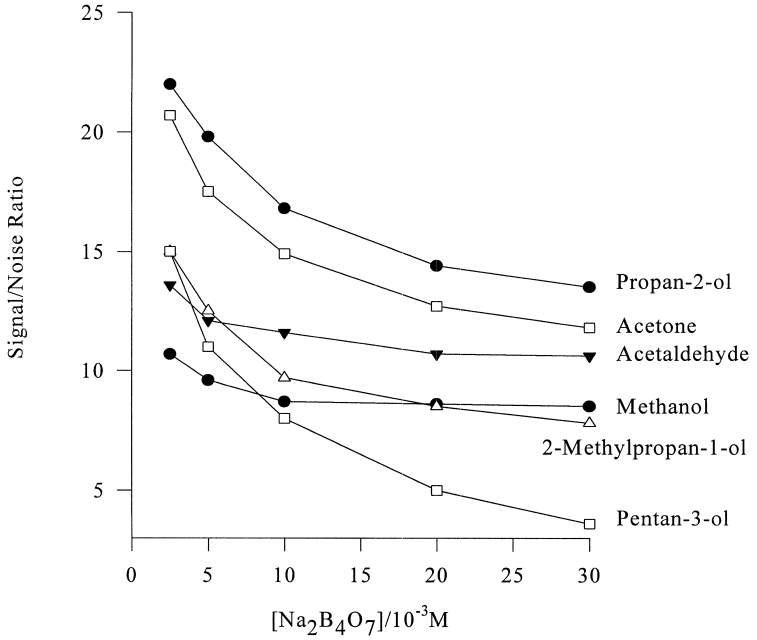

Fig. 2. Effect of the ionic strength of the running electrolyte on the detection sensitivity. The analyzed sample contains, in aqueous solution, all the compounds at the 1% (v/v), except acetaldehyde 2% (v/v). Operating conditions as in Fig. 1, except the electrolyte. Electrolyte: variable concentration of $Na_2B_4O_7$ (pH 9.2 to 9.4), [fluorescein]$=10^{-5}$ *M* and [SDS]$=0.133$ *M*.

$$C_{mic} = \frac{C_{SDS} - CMC_{SDS}}{n_{SDS}}$$

where C_{mic} is the micelle concentration within the electrolyte, C_{SDS} is the SDS concentration within the electrolyte, CMC_{SDS} is the critical micellar concentration of SDS ($8.1 \cdot 10^{-3}$ *M* in pure water at 25°C), and n_{SDS} is the number of SDS molecules constituting a micelle ($n_{SDS}=62$ [5]).

In the present case, a fluorescein concentration of about 10^{-3} *M* appears necessary to saturate all the micelles. As this concentration was unable to be reached because of saturation of the detector, we have studied the evolution of the signal-to-noise ratio (S/N) as a function of the fluorescein concentration introduced within the running electrolyte in a concentration range compatible with our electrophoretic system (see Fig. 4).

Fig. 4 shows that the detection sensitivity is optimal and near constant, within the limit of experimental errors, for fluorescein concentration in the range from $5 \cdot 10^{-6}$ *M* to 10^{-4} *M* ($S/N \pm 0.7$ for the ethanol peak, $S/N \pm 0.3$ for the ethyl acetate peak and $S/N \pm 1$ for the peak of 3-methylbutan-1-ol), the precision being determined from five consecutive injections. It resulted that a fluorescein concentration equal to 10^{-5} *M*, which we had determined in our previous works [12,14], appeared suitable in the present case.

3.5. Influence of the cosolvent addition

As it can be observed from Fig. 5, corresponding to the analysis of the sample test in conditions defined up till now, on the one hand, the ethyl acetate and the 2-methylpropan-1-ol and, on the other hand, 2-methylbutan-1-ol and 3-methylbutan-1-ol are coeluting. The resolution of this last pair is very interesting if one considers that their ratio allows to detect an alteration of alcoholic beverage [15].

Therefore it would be advisable to envisage the addition of an organic cosolvent to the carried electrolyte in order to modify the partition coefficients of these compounds between the micelles and

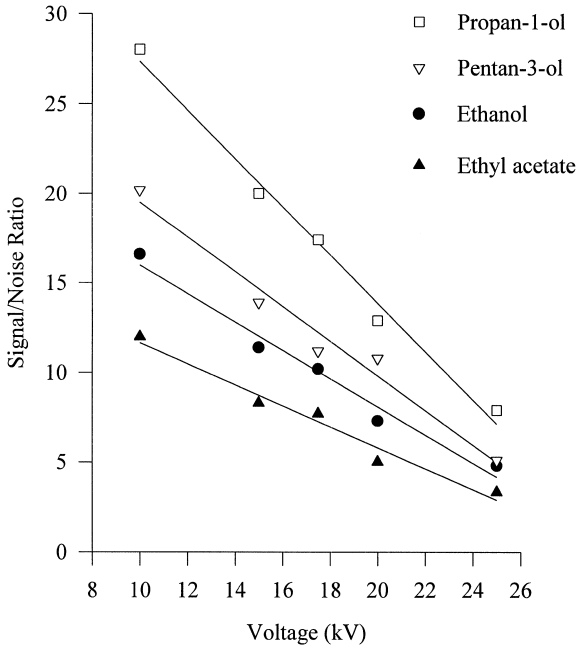

Fig. 3. Effect of the applied voltage on the detection sensitivity. Operating conditions: 57 cm length (50 cm effective length to detection window)×50 μm I.D.; temperature: 30°C; electrolyte: $[Na_2B_4O_7]=2.5\cdot10^{-3}$ *M*/[fluorescein]$=10^{-5}$ *M*, [SDS]=0.133 *M*; variable applied voltage. The concentration of all the analyzed compounds were equal to 1% (v/v).

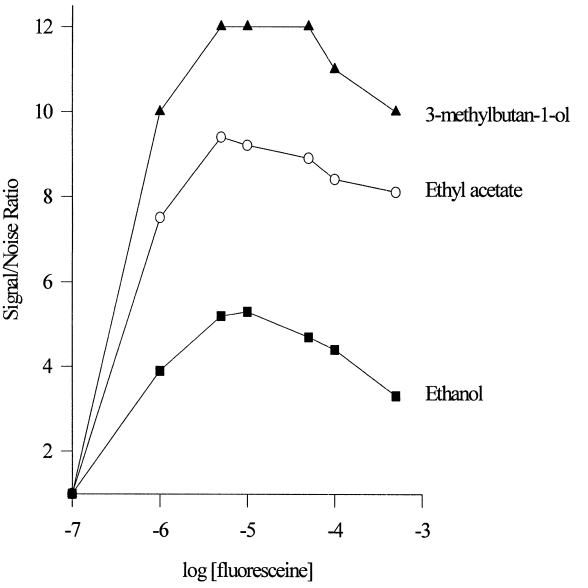

Fig. 4. Optimization of the fluorescein concentration within the running electrolyte. Operating conditions: as in Fig. 3, except the applied voltage: 15 kV and the fluorescein concentration (variable), the studied sample containing: 1% (v/v) ethanol, 1% (v/v) ethyl acetate and 0.5% (v/v) 3-methylbutan-1-ol.

the aqueous phase and to obtain a complete resolution for the sample test.

3.5.1. Addition of acetonitrile

The first organic cosolvent introduced in the running electrolyte was acetonitrile. The best resolution by time unit has been achieved with a content equal to 7.5% (v/v). The electropherogram obtained in these conditions is presented in Fig. 6.

In these operating conditions, the 2-methylpropan-1-ol and the ethyl acetate are satisfactorily resolved although the resolution of the 2 methylbutan-1-ol–3-methylbutan-1-ol pair is still poor.

Moreover this acetonitrile addition is coupled by the occurrence of a system peak that makes impossible the ethanol quantification. We have proved that this positive peak can be attributed to the acetonitrile by matching the migration time so obtained from the injection of this solvent into the running electrolyte free of any organic cosolvent. The occurrence of any organic solvent within the carried electrolyte systematically induces a positive peak on the corresponding electropherogram. As a result the cosolvent introduced into the running electrolyte has to be chosen by taking in account the retention times of the analyzed compounds.

3.5.2. Addition of THF

By injecting the THF into the electrolyte free of this cosolvent, it appeared that this solvent was potentially interesting in this type of analysis, because it seems not to have the same retention time that of the considered compounds. Its occurrence within the running electrolyte induces the solubilization of the polyimide covering the external wall of the separation capillary, so it has been necessary to remove the latter by combustion from each end of the capillary so as to prevent any contamination of the electrolyte.

The electropherogram obtained, respectively, with 3% and 7% (v/v) of THF in the running electrolyte are represented in Fig. 7a and b.

From Fig. 7a one can note that the positive peak, due to THF, elutes immediately after propan-1-ol.

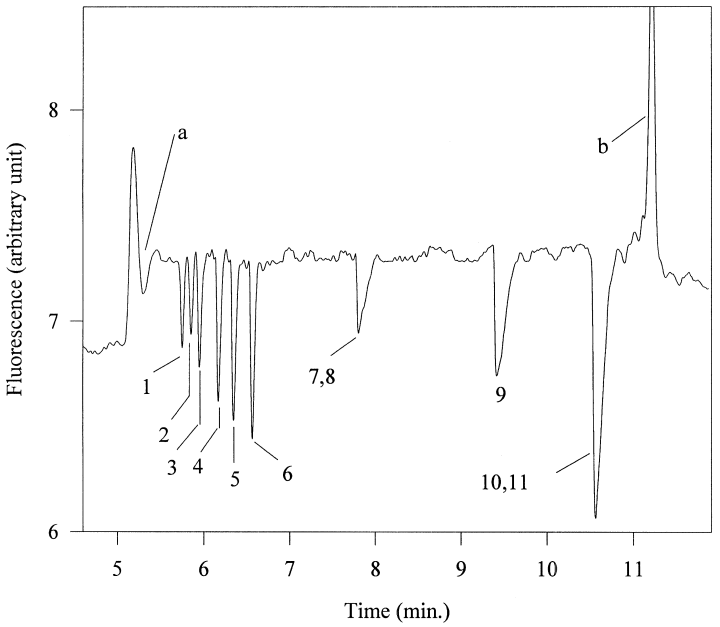

Fig. 5. Separation of the sample test by using an electrolyte without any cosolvent. Operating conditions: 57 cm length (50 cm effective length to detection window)×50 μm I.D.; temperature: 30°C; electrolyte: $[Na_2B_4O_7]=2.5\cdot10^{-3}$ *M*/[fluorescein]$=10^{-5}$ *M*, [SDS]=0.133 *M*; applied voltage: 15 kV. Peaks: (1) methanol 1%; (2) acetaldehyde 2%; (3) ethanol 1%; (4) acetone 1%; (5) propan-2-ol 1%; (6) propan-1-ol 1%; (7) ethyl acetate 0.5%; (8) 2-methylpropan-1-ol 0.5%; (9) pentan-3-ol 1%; (10) 2-methylbutan-1-ol 0.25%; (11) 3-methylbutan-1-ol 0.25%. (a) Water peak (electroosmotic flow); (b) system peak corresponding to the fluorescein.

Thus the quantification of this solute became impossible [16]. In these experimental conditions, i.e., 3% of THF within the running electrolyte, the resolution of the ethyl acetate–2-methylpropan-1-ol pair is very good while that of the 2-methylbutan-1-ol–3-methylbutan-1-ol pair is again poor.

In order to completely resolve this last pair of compounds, we envisaged to increase the THF content within the running electrolyte. The best results were obtained by adding 7% (v/v) of THF. The electropherogram obtained in these conditions is reported in Fig. 7b. While the resolution of the ethyl acetate–2-methylpropan-1-ol pair remains very good, that of the 2-methylbutan-1-ol–3-methylbutan-1-ol pair appears noticeably improved, although it is not entire. Besides the positive peak, due to THF as an organic cosolvent, coelutes now with the propan-1-ol, the quantification of this compound remains impossible.

Finally, it has been able to evidence that the detection sensitivity decreases with the increasing of the THF content as we can observe it from date gathered in Table 1, that relates to detection thresholds obtained with and free THF within the running electrolyte.

Indeed while the detection sensitivity is satisfactory when an running electrolyte free of THF is used, it becomes low as soon as 7% of THF are added to the electrolyte. In these conditions the injection volume has to be reduced because of notable perturbations that rapidly appear on the baseline. It has to be noted that for a same injection volume the detection sensitivity is divided by a factor from 4 to 6 when 7% of THF are added to the electrolyte. Besides a 20% loss of the background fluorescence has been evidenced when 7% of organic solvent is added to the free THF electrolyte.

To explain this loss of sensitivity it is interesting to consider the following equation which define the concentration limit of detection, C_{LOD} [4]:

$$C_{LOD} = \frac{C_{FLU}}{DR \cdot TR}$$

where DR is the dynamic reserve, TR the transfer

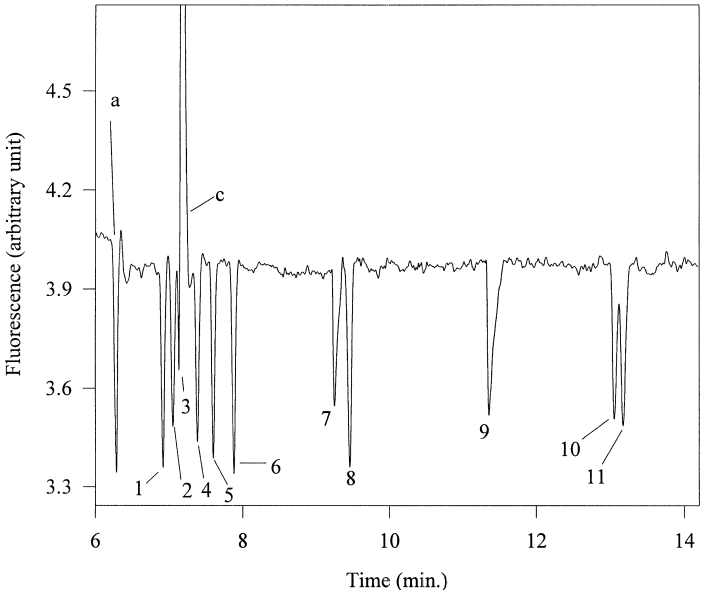

Fig. 6. Separation of the sample test by using a running electrolyte containing acetonitrile (7.5%, v/v). Operating conditions: as in Fig. 5 except the content of 7.5% (v/v) of acetonitrile within the running electrolyte. Peaks: (1) methanol 1%; (2) acetaldehyde 2%; (3) ethanol 1%; (4) acetone 1%; (5) propan-2-ol 1%; (6) propan-1-ol 1%; (7) ethyl acetate 1%; (8) 2-methylpropan-1-ol 1%; (9) pentan-3-ol 1%; (10) 2-methylbutan-1-ol 0.5%; (11) 3-methylbutan-1-ol 0.5%. (a) Water peak; (c) system peak due to the acetonitrile.

number and C_{FLU} the concentration of the fluorophore.

Indeed, in the operating conditions of Fig. 5, i.e., without any organic solvent in the running electrolyte, the dynamic reserve (DR) is equal to 304. This DR decreases when the THF percentage increases in the running electrolyte. It becomes equal to 256 and 177 with 3% and 7% of THF in the carrier electrolyte, respectively. This decrease of DR would explain the loss of sensitivity observed in Table 1. We can note that, for identical percentage in the running electrolyte, acetonitrile leads approximately the same decrease of DR than THF but it does not enable the resolution of the 2-methylbutan-1-ol–3-methylbutan-1-ol pair.

The addition of organic cosolvent appears therefore uninteresting with regard to the analysis of this sample test: (i) on the one hand, in view of the quantification, because of the occurrence of a positive peak that could coelute with one of the analyzed compounds, (ii) and on the other hand, in view of the detection sensitivity, that proves noticeably reduced.

Moreover the use of an electrolyte containing 3 to 7% of THF involves the complete renewal of this medium after each analysis. Indeed, after about 30 min, under a 15 kV applied voltage, this electrolyte bleaches at the cathodic end of the capillary and the fluorescence intensity becomes nearly zero. It appeared impossible to reproduce the analysis without renewing the running electrolyte. Actually, the exact cause for this phenomenon have not been found, but it might result from an electrochemical reaction taking place between the THF and the fluorescein.

3.6. Repeatability and quantification

The repeatability of retention times and of the effective electrophoretic mobilities has been determined by using washing process of the capillary described hereafter: 1 min with water; 20 min with 1 *M* sodium hydroxide; 1 min with water and finally 5 min with running electrolyte, namely $[Na_2B_4O_7] = 2.5 \cdot 10^{-3}$ *M*/[SDS] $= 1.33 \cdot 10^{-1}$ *M*/[fluorescein] $= 10^{-5}$ *M*, containing or not containing THF.

The repeatabilities determined for retention times for a free THF electrolyte vary from 0.6% for methanol to 1.2% for 3-methylbutan-1-ol while those obtained in the case of effective electrophoretic

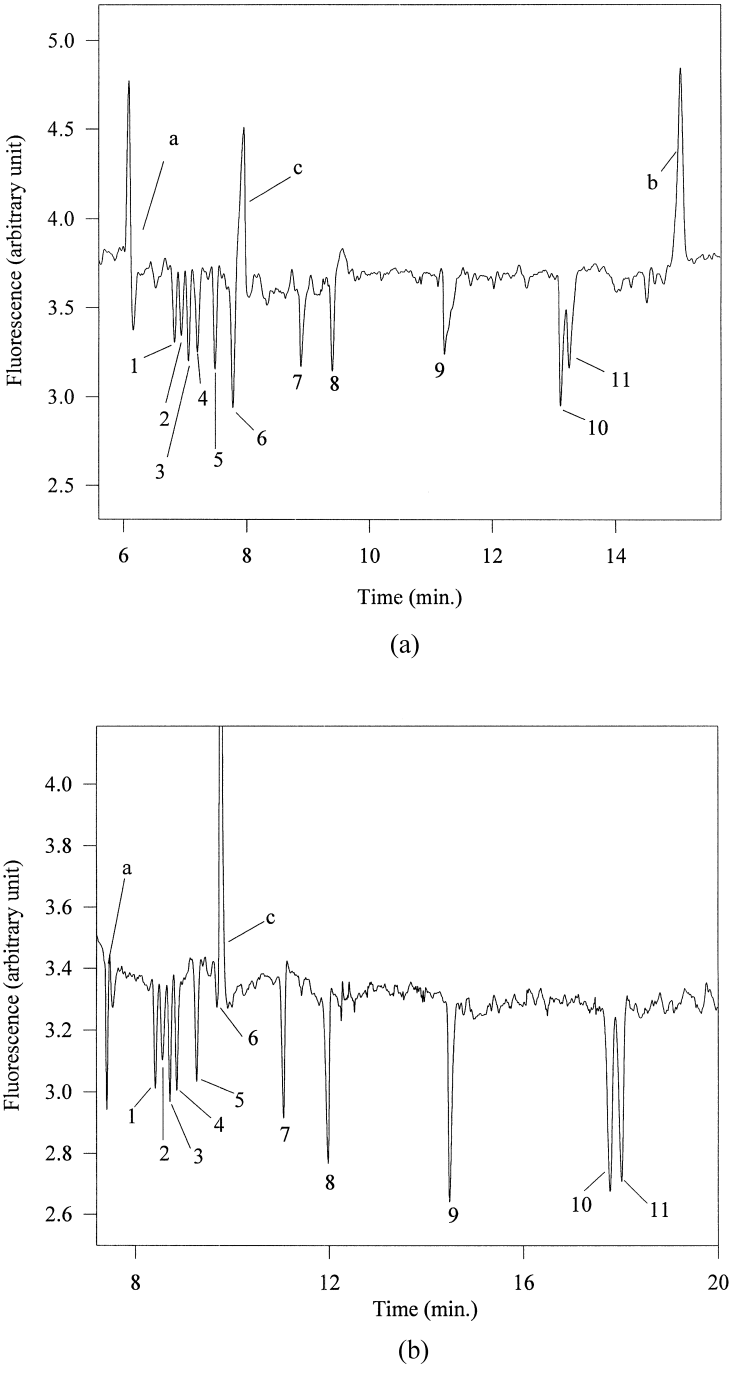

Fig. 7. Separation of the sample test by using a running electrolyte containing tetrahydrofuran. Operating conditions: as in Fig. 5, except the content of THF in the running electrolyte (a: 3%, v/v, b: 7%, v/v). Peaks: (1) methanol 1%; (2) acetaldehyde 2%; (3) ethanol 1%; (4) acetone 1%; (5) propan-2-ol 1%; (6) propan-1-ol 1%; (7) ethyl acetate 1%; (8) 2-methylpropan-1-ol 1%; (9) pentan-3-ol 1%; (10) 2-methylbutan-1-ol 0.5%; (11) 3-methylbutan-1-ol 0.5%. (a) Water peak; (b) system peak corresponding to the fluorescein; (c) system peak due to the THF.

Table 1
Comparison of the detection thresholds obtained for some organic compounds by using a running electrolyte containing or not containing THF (operating conditions: as in Fig. 5)

Compound	Detection limit (volumic percentage)[a]	
	Without THF[b]	With 7% THF[c]
Methanol	0·10	1.00
Acetaldehyde	0.20	1.50
Ethanol	0·10	0.50
Acetone	0.08	0.50
Propan-2-ol	0.08	0.50
Propan-1-ol	0.08	Undetermined
Ethyl acetate	0.08	0.50
2-Methylpropan-1-ol	0.08	0.33
Pentan-3-ol	0.50	0.20
2-Methylbutan-1-ol	0.03	0.13
3-Methylbutan-1-ol	0.03	0.13

[a] Detection threshold established for a signal-to-noise ratio equal to 3.
[b] Hydrodynamic injection, time varying from 20 to 30 s.
[c] Hydrodynamic injection, time varying from 8 to 20 s.

mobilities vary from 0.2% for 3-methylbutan-1-ol to 1% for the methanol.

With regard to the performed analysis with an electrolyte containing 7% THF, the repeatabilities on effective electrophoretic mobilities are comparable with those observed in the case of a free THF electrolyte while the retention time repeatabilities are approximately five-times less good.

The very satisfactory repeatabilities obtained in this study allow us to envisage the titration of ethanol within a white wine without any cosolvent in the electrolyte to prevent inherent loss of detection sensitivity.

So the calibration curve has been determined for ethanol percentages varying from 0.5 to 5% (v/v). A very good linearity has been obtained as indicated by the correlation coefficient that is equal to 0.9997. The studied white wine was diluted three times with ultra pure water then it was injected in hydrodynamic mode during 5 s. The electropherogram obtained is presented Fig. 8.

Because of the absence of interference due to the various components of the sample matrix, the ethanol content which was calculated from five independent analyses is equal to 11.6% (v/v) and so it appears to be identical with the content indicated by the wholesaler.

At last in the case of ethanol the repeatability with regard to the corrected areas is also satisfactory since

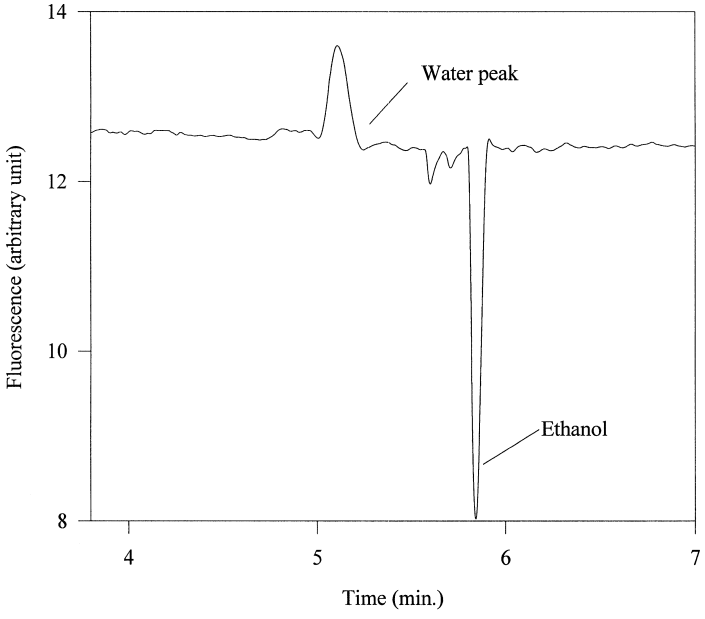

Fig. 8. Analysis of a commercial white wine without any organic solvent in the running electrolyte. Same operating conditions as in Fig. 5 except the injection: hydrodynamic injection of a commercial white wine diluted three times with ultra pure water. Injection time: 5 s.

it varies from 3% to 8%, according to the concentration of the sample test.

3.7. Response mechanism

As mentioned in the introduction, the mechanism of indirect fluorimetric detection in MEKC is thought to be based on the alteration of the partition of the fluorophore in the micelles and/or on the fluorescence quenching [4–7]. In the Figs. 5, 6 and 7, the response factors of the solutes are more or less constant or increase with retention factors. As a consequence, the response mechanism seems to be based essentially on the alteration of the partition of the fluorophore in the micelles. Indeed, as reported by Amankwa and Kuhr [4] fluorescence quenching by an analyte leads to increased sensitivity. These authors have observed that, unlike aliphatic alcohols, phenol is able to quench the fluorescence of quinine sulfate. According to this study [4], the increase of response factor is due to the fact that the singlet state of phenol is close to the one of the fluorophore. Moreover, two other experimental results would suggest that detection is essentially based on the alteration of the fluorophore partition in the micelles: (1) on the one hand, we observed a very good response linearity versus ethanol content when quenching leads to non-linear response [8], (2) on the other hand, in Table 2, we compared the relative fluorescence intensities of fluorescein solutions, with or without micelles and propan-1-ol, to those obtained, by Amankwa and Kuhr in the case of the quenching of the quinine sulfate fluorescence by phenol [4].

In this table, it can be seen that the fluorescence of fluorescein is only slightly increased in micellar solution compared to the one observed by Amankwa and Kuhr in the case of quinine sulfate. Nevertheless, this result is consistent with those reported by Kennedy et al. [5]. The fluorescence decrease due to the introduction of the solute within micellar solutions containing the fluorophore is much pronounced in the case of phenol addition than in our study i.e., with propan-1-ol. Thus propan-1-ol, unlike phenol, is shown to have the same poor quenching efficiency than the unsaturated alcohols analyzed by Amankwa and Kuhr [4]. All these data tend to suggest that the response mechanism is mainly based on the alteration of the fluorophore partition in the micelles.

4. Conclusion

The intensity of fluorescence emitted by the fluorescein sodium salt being enhanced within the micelles, this fluorophore has been introduced in micellar phase in order to achieve the indirect fluorimetric detection in micellar electrokinetic chromatography. Although the operating conditions required for the resolution of the sample test, analyzed in this paper, are not favorable to such a detection: (i) high ionic strength, inducing certain difficulties to dissipate the Joule heating, (ii) no saturation of the micelles by the fluorescein, (iii) introduction of an organic cosolvent within the electrolyte generating the occurrence of positive system peaks that able to interfere with the analyzed compounds, the results obtained during this study appear satisfactory in regard to the sensitivity detection and the quantitative approach. They allow us to envisage the resolution of complex analytical problems by means of the indirect fluorimetric detection in MEKC without technical modification of the currently available commercial fluorimetric detector.

Table 2
Relative fluorescence intensities (RFIs) of various solutions of 10^{-5} *M* fluorescein (F) compared to those obtained by Amankwa and Kuhr with $5 \cdot 10^{-4}$ *M* quinine sulfate (Q) [4]

Solutions[a]	RFI (%)	Solutions	RFI (%)
F + 0.133 *M* SDS	100	Q + 0.1 *M* SDS	100
F	94	Q	87
F + 0.133 *M* SDS + 20% (v/v) propan-1-ol	88	Q + 0.1 *M* SDS + 0.112 *M* phenol	25

[a] These solutions contained $2.5 \cdot 10^{-3}$ *M* $Na_2B_4O_7$.

References

[1] K.D. Altria, J.S. Howells, J. Chromatogr. A 696 (1995) 341.
[2] F.B. Erim, X. Xu, J.C. Kraak, J. Chromatogr. A 694 (1995) 471.
[3] R. Szucs, J. Vindevogel, P. Sandra, J. High Resolut. Chromatogr. 14 (1991) 692.
[4] L.N. Amankwa, W.G. Kuhr, Anal. Chem. 63 (1991) 1733.
[5] S. Kennedy, B. Caddy, J.M.F. Douse, J. Chromatogr. A 726 (1996) 211.
[6] T. Kaneta, T. Imasaka, Anal. Chem. 67 (1995) 829.
[7] D.F. Swaile, C.L. Copper, M.J. Sepaniak, D.E. Burton, L.L. Powell, Talanta 41 (1994) 1499.
[8] T. Takeuchi, E.S. Yeung, J. Chromatogr. 366 (1986) 145.
[9] W.L. Hinze, H.N. Singh, Y. Baba, N.G. Harvey, Trends Anal. Chem. 3 (1984) 193.
[10] D.W. Armstrong, W.L. Hinze, K.H. Bui, H.N. Singh, Anal. Lett. 14 (1981) 1659.
[11] W. Beayens, B. Lin, V. Corbisier, Analyst 115 (1990) 359.
[12] P.L. Desbène, C.J. Morin, A.M. Desbène-Monvernay, R.S. Groult, J. Chromatogr. A 689 (1995) 135.
[13] P.L. Desbène, C.J. Morin, A.M. Desbène, Analusis 26 (1998) 107.
[14] A.M. Desbène, C.J. Morin, N.L. Mofaddel, R.S. Groult, J. Chromatogr. A 716 (1995) 279.
[15] J. De Zeeuw, N. Vonk, P. Saint-Martin, presented at SEP 97 Congress, Paris, 1997, poster.
[16] J.L. Beckers, J. Chromatogr. A 679 (1994) 153.