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Utilization of fluorescein sodium salt in laser-induced indirect fluorimetric detection of ions separated by capillary zone electrophoresis[☆]

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Abstract

Indirect detection, the principles of which were demonstrated some years ago, has attracted renewed interest in both UV-visible and fluorimetric detection. This paper reports the implementation of laser-induced indirect fluorimetric detection using a commercially available detector and its association with high-performance capillary electrophoresis for the determination of inorganic ions. In order to optimize the response sensitivity, the fluorescent agent being fluorescein sodium salt, the influence of the fluorescein sodium salt concentration, the ionic strength, the electrolyte pH and the gain on the signal-to-noise ratio were studied successively, the injection mode being either electrokinetic or hydrodynamic. In the case of a 75 μm I.D. fused-silica capillary, detection limits were in the ppb range or about 10 ppb for alkali metal cations using injection in the electrokinetic or in the hydrodynamic mode, respectively. As the detection system used had its maximum sensitivity at basic pH, the use of a complexing agent was required in order to perform the determination of alkaline earth metal cations, because they precipitate or are adsorbed at basic pH. Therefore, using ethylenediaminetetraacetic acid sodium salt (EDTA), it is possible to analyse at pH 7.5, in less than 5 min, a mixture containing three alkaline earth metal cations (Ba^{2+} , Ca^{2+} and Mg^{2+}) and three transition metal cations (Fe^{3+} , Zn^{2+} and Cu^{2+}) in addition to the previously mentioned alkali metal cations (Li^+ and K^+).

1. Introduction

High-performance capillary electrophoresis is a recent analytical technique which appears to be one of the most powerful techniques for the separation of a broad range of complex matrices

including both inorganic anions and cations and neutral or potentially ionizable molecules [1]. However, it presents some limitations. In particular, it still suffers from the lack of a sensitive and universal detector. A neat way of overcoming the lack of universality of detection systems was to use indirect detection, already widely used in high-performance liquid chromatography [2–25].

Ionic capillary electrophoresis has been used to determine both anions and cations. However,

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there is at present a greater diversity of the mixtures analysed in the case of anions than in the case of cations. Inorganic anion separations were performed in the presence of an electrophoretic buffer containing a chromophore, to obtain the background signal, and an electroosmotic flow reverser. Effectively, in the case of anions and in contrast to cations, the electroosmotic flow must be at least decreased, or even reversed, in order to shorten the analysis time. From literature data, it appears that potassium chromate is the most widely used absorbing agent [26–34]. Other compounds absorbing in the UV region were also used to obtain a background signal, for instance pyromellitic acid [35], potassium phthalate [27,36] and potassium benzoate [27]. The most frequently used modifiers or reversers of electroosmotic flow are quaternary ammonium surfactants such as tetradecyltrimethylammonium bromide [31] or CIA Pak OFM Anion BT (Millipore–Waters) [26–29, 32–34,36]. However, this modifier or reverser of electroosmotic flow can also be diethylenetriamine [30] or hexamethonium hydroxide [35]. Such electrophoretic systems were also used to separate organic anions that do not possess chromophoric groups, e.g., alkylsulfonates and organic acids [27,31].

In the case of cation determination, the analytical approach is akin to that mentioned above for the determination of anions. Effectively, as a great number of cations are transparent in the UV range, it is often necessary to use indirect spectrometric detection (UV or fluorimetric) and less frequently conductimetric detection [37].

Initially, Aguilar et al. [38] separated by capillary zone electrophoresis (CZE) the cations of transition metals, Fe^{2+} , Cu^{2+} and Zn^{2+} , as cyano complexes, spectrophotometric detection being performed at 214 nm. More recently, the same group separated the Au^+ and Ag^+ cations, again complexed, and with UV detection (214 nm) [39]. However, the visualization of most separations of cations is performed by means of indirect photometric detection techniques. Foret et al. [40] separated a mixture of fourteen lanthanides using an electrophoretic buffer containing α -hydroxyisobutyric acid (HIBA), as

complexing reagent, and creatine, as a chromophoric agent, to generate the background signal. Effectively, because of the similitude of the electrophoretic mobilities of some cations, it is necessary to introduce a supplementary mechanism of separation. According to the literature, complexation is frequently used with this aim. Chen and Cassidy [41] analyzed a mixture of lanthanides by CZE using as complexing agent HIBA, the electrophoretic buffer being composed of acetic acid and benzylamine. However, the factors influencing the selectivity of complexed cations were essentially studied by Weston and co-workers [42–44], the complexing agent being systematically HIBA. HIBA is the most commonly used complexing agent for the separation of cations, in association with indirect UV photometric detection [40–46]. However, Beck and Engelhardt [47] have recently developed an electrophoretic buffer containing imidazole, in association with indirect UV detection, for the separation of alkali and alkaline earth metal cations. Similarly, Riviello and Harold [48] recently proposed a new electrophoretic system for the determination of alkali and alkaline earth metal and ammonium cations in association with indirect UV detection. This new electrophoretic system was based on the utilization of Cu(II) as the primary component of the electrolyte, the system selectivity being adapted by addition of a crown ether, 18-crown-6, as complexing agent.

Several workers have proposed the use of laser-induced fluorimetric detection in the presence of complexing agents to determine alkali and alkaline earth metal cations. Gross and Yeung [49] were the first to use indirect fluorimetric detection to determine alkali and alkaline earth metal cations by CZE. They used quinine sulfate to generate the background signal. More recently, Bächmann et al. [50] used in the same way indirect fluorimetric detection in the separation of alkali and alkaline earth metal cations. They developed a new electrophoretic system based on the association of Ce(II) and 18-crown-6, the latter being the complexing agent allowing the electrophoretic system selectivity to be adjusted. Swaile and Sepaniak [51]

used the fluorescence of complexes of 8-hydroxyquinoline with a large number of metallic cations, the chelating agent not presenting native fluorescence, and visualized by direct fluorescence a mixture of Ca^{2+} , Mg^{2+} and Zn^{2+} cations.

As laser-induced fluorimetric detection is now commercially available, we considered the development of a new electrophoretic system allowing the use of indirect fluorimetric detection and compatible with the detector without modification of this latter. As this detector has excitation and emission wavelengths in the visible range, all the electrophoretic systems previously described for the indirect fluorimetric detection of inorganic cations are ineffectual, the detectors used by different workers being equipped with lasers operating in the UV range. Considering the characteristics of the laser associated with the commercial fluorimetric detector we utilized, we decided to examine the potential offered by the sodium salt of fluorescein as a fluorescent reagent generating the background signal, with a view to detecting alkali, alkaline earth and transition metal cations separated by CZE.

Moreover, as the fluorescence intensity of fluorescein sodium salt is a function of pH, and that it is essential to operate in a basic medium, we therefore studied the possibilities offered by ethylenediaminetetraacetic acid (EDTA) as chelating agent, because alkaline earth metal cations become insoluble or are adsorbed on the capillary wall at such pH values.

2. Experimental

2.1. Reagents

All solutions, electrolytes and standards were prepared using 18-M Ω water generated by an Alpha-Q laboratory water purification system (Millipore, Bedford, MA, USA). The alkali, alkaline earth and transition metal cation standards were prepared from salts (nitrate or chloride, purity $\geq 99.99\%$) obtained from Aldrich France (La Verpillière, France). Citric acid and its sodium salt and sodium tetraborate utilized

for the preparation of buffers and the disodium salt of EDTA used as a complexing agent (purity 99+%) were purchased from Aldrich France and used as received. Fluorescein sodium salt, providing the fluorescence background, was also purchased from Aldrich France and was used as received.

2.2. Preparation of electrolytes and standards

The electrolytes used were prepared from solutions made daily, containing the fluorescein sodium salt on the one hand and on the other hand the suitable buffer (citric acid and its sodium salt, sodium tetraborate or disodium salt of EDTA). In the case of EDTA, the pH was adjusted by addition of sodium hydroxide. After dilution, the electrolytes were systematically degassed for 20 min using sonication and their pH was measured before use, at the experimental temperature. It must also be mentioned that all the vessels used for the preparation of the analyte solutions and of the electrolytes were made of polypropylene (Polylabo-Block, Strasbourg, France). The disposable sample vials were made of siliconed polypropylene and were also purchased from Polylabo-Block. Dilutions were performed using Gilson (Villiers Le Bel, France) electronic automatic pipettes, equipped with disposable cones made of polypropylene.

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 ($\lambda_{\text{exc}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$) and monitored by a PS/2 computer (IBM, Greenock, UK) using P/ACE software or Gold software (Beckman). Data collection was performed using the same software. Samples were loaded by pressure injection [injection pressure 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa)] or electromigration (applied voltage 10 kV) into a fused-silica capillary. The capillary columns used were untreated with respect to any covalently bonded stationary phase. Before use, several solutions were flushed through the capillary in the follow-

ing order: 0.1 M NaOH, water, 0.1 M HCl, water, buffer solution. These capillaries were 57 cm \times 75 μ m I.D. Injections were made at the high-voltage anode and cations were eluted to the grounded cathode. Indirect fluorimetric detection was performed at 520 nm through the capillary 50 cm from the inlet. The pH values of the electrolytes were measured using a Beckman Model ϕ pH meter at the analysis temperature.

3. Results and discussion

As mentioned above, because of the characteristics of the laser-induced fluorimetric detector utilized, at both the excitation and emission wavelengths, there was only a limited choice of fluorescence agents that could be used to provide the background signal. Among the commercially available fluorescence agents, fluorescein sodium salt appeared as the most suitable, as it offers maximum absorption at 492 nm and a maximum re-emission at 518 nm, very close to the optical characteristics of the detector. This fluorescence agent has the drawback, however, of a pH-dependent fluorescence intensity. We searched for a pH range allowing an acceptable fluorescence intensity to be maintained and still to have the possibility of modulating the electroosmotic flow using this parameter. As shown in Fig. 1, the fluorescence intensity of fluorescein is con-

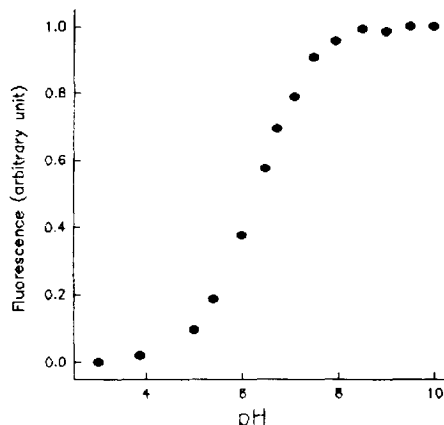


Fig. 1. Evolution of the fluorescence of fluorescein as a function of pH.

stant only in highly basic media, in a range where the electroosmotic flow velocity is independent of pH. However, in the pH range 7–9, this intensity is still hardly modified but the electroosmotic flow is noticeably changed.

We then optimized the fluorescein concentration in the mobile phase, as Kuhr and Yeung [52] showed that the limit concentration detectable is a function of this parameter in indirect detection.

3.1. Influence of the fluorophore concentration on the detection system sensitivity

In order to optimize the detection system sensitivity, we studied the influence of fluorescein sodium salt concentration on the signal-to-noise ratio. The electrolyte pH was settled at pH 9.2, i.e., where the fluorophore intensity is maximum. We used potassium *n*-butylbenzene sulfonate as the test sample because it has an electrophoretic mobility similar to that of the fluorophore. Foret et al. [53] proved that in indirect detection, the sensitivity obtained for a sample ion is greater if its mobility is similar to the mobility of the ion used to provide the background signal. As reported in Table 1, a range of fluorophore concentrations of 10^{-8} – 10^{-4} M was explored.

Table 1 shows that the maximum sensitivity is obtained with a 10^{-5} M fluorescein sodium salt concentration. Below this concentration, the background signal is not very stable and above it the difference between the initial background signal and the signal perturbed by the sample elution is no longer perceptible. Therefore, subsequent experiments were systematically performed using 10^{-5} M fluorescein sodium salt. Moreover, it is worth mentioning that increasing the photomultiplier gain resulted, as expected, in an enhancement of fluorescence but did not allow the signal-to-noise ratio to be improved.

We then still had to optimize the ionic strength of the electrophoretic medium and the injection parameters in order to attain the greatest sensitivity possible. As these two parameters are intimately related, because a concentration effect of the sample appears when low-conductivity samples are injected, consequently allowing

Table 1
Evolution of the signal-to-noise ratio as a function of the fluorescein sodium salt concentration

[<i>n</i> - BuC ₆ H ₄ SO ₃ K] (mM)	Fluorescein sodium salt concentration (M)				
	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
10	35	40	52	54	53
1	6	18	19	17	15
0.1	1.3	1.8	2	2.4	2.3

Electrolyte, 12.5 mM Na₂B₄O₇ (pH 9.2); temperature, 30°C; applied voltage, 30 kV; injection, hydrodynamic (injection time = 5 s).

larger injection volumes [54,55], we optimized these two parameters jointly. The two classical injection modes used in capillary electrophoresis, i.e., hydrodynamic and electrokinetic, were successively studied. The test sample was a mixture of lithium and potassium salts, both at 10⁻⁴ M concentration.

3.2. Influence of the ionic strength of the electrolyte on the system sensitivity. Comparison of electrokinetic and hydrodynamic injection

As demonstrated by Fig. 2, the signal-to-noise ratio is strongly dependent on the ionic strength of the electrolyte constituting the mobile phase in the case of hydrodynamic injection. Within the electrolyte concentration range explored (10–50 mM), the detection system sensitivity is maximum at a sodium borate concentration of 10 mM. Similar variations were observed in the electrokinetic injection mode but they were less important. Using this injection mode within the same concentration range, the sensitivity increased when the sodium tetraborate concentration decreased from 50 to 10 mM.

Therefore, we determined first the detection limit of lithium and potassium using an electrolyte with a sodium tetraborate concentration of 10 mM, using both the hydrodynamic and electrokinetic injection modes.

As the sample band injected has a very low conductivity, an important concentration effect is observed, allowing relatively large sample volumes to be injected, while keeping a constant

system efficiency and consequently a constant separation quality [54,55].

At this concentration and with a 57 cm × 75 μm I.D. capillary, it was possible to use injection times up to 30 s, resulting in a pronounced improvement in the detection sensitivity. Beyond this limit, the signal-to-noise ratio remains nearly constant, while a drift of the baseline is observed before the system peak corresponding to the electroosmotic flow (water

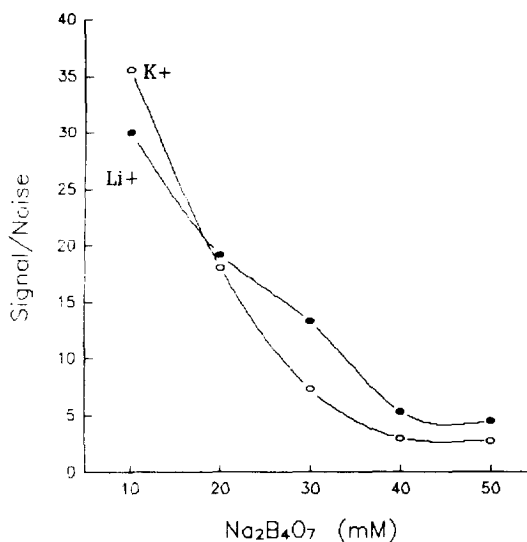


Fig. 2. Evolution of the signal-to-noise ratio, in the hydrodynamic injection mode, as a function of the ionic strength of the electrolyte in the mobile phase. Operating conditions: fused silica-capillary 57 cm × 75 μm I.D.; applied voltage, 10 kV; temperature, 30°C; sample, mixture of LiNO₃ (10⁻⁴ M) and KNO₃ (10⁻⁴ M); electrolyte, Na₂B₄O₇ (pH 9.2); fluorescein concentration, 10⁻⁵ M; detection, fluorimetric (λ_{exc.} = 488 nm, λ_{detect.} = 520 nm).

peak), accompanied by peak broadening resulting in a noticeable loss of efficiency.

The detection thresholds obtained under these conditions for lithium and potassium are reported in Table 2. Further, as the sensitivity of the detection system seems to increase when the mobile phase electrolyte becomes more diluted, we also determined the detection thresholds of these two cations in the case of a mobile phase containing 2.5 mM sodium tetraborate at pH 9.2 (Table 2). The following comments can be made based on these results. (i) With a sodium salt being used to provide the background signal, laser-induced fluorimetric indirect detection appears far more sensitive in the case of lithium than with potassium. Whatever the electrolyte concentration in the mobile phase and the injection mode used, the detection threshold of potassium is 3–6 times greater than that of lithium. (ii) Whatever the cation (lithium or potassium) and the electrolyte concentration in the mobile phase, electrokinetic injection results in a greater sensitivity than hydrodynamic injection. This has already been reported by Weston et al. [46] using indirect UV detection. (iii) This detection system appears to be more sensitive if the electrolyte concentration in the mobile phase is lower. For instance, lithium and potassium cations at a 10^{-6} M concentration are not detected if a 10 mM sodium tetraborate electrolyte is used, but they are detected without difficulty at this concentration if an electrolyte containing 2.5 mM sodium tetraborate is used.

As shown by Table 2, the sensitivity is improved tenfold if the sodium tetraborate concentration in the mobile phase is decreased from 10 to 2.5 mM. This last concentration is the lower limit of the electrolyte buffer effect.

The sensitivity thresholds obtained in indirect fluorimetric detection (39.5 and 3.6 ppb for potassium and 15 and 0.7 ppb for lithium in the hydrodynamic and electrokinetic injection modes, respectively) are comparable to the best detection limits reported in the literature for the same cations [50,51].

Considering these results, we then studied the potential offered by this new detection system for alkaline earth and transition metal cations.

3.3. Application to the analysis of mixtures of alkali, alkaline earth and transition metal cations

As the fluorophore used to provide the background signal, fluorescein, gives a noticeable fluorescence only in a basic medium, the determination of alkaline earth and transition metals appeared impossible in such a medium because these ions were adsorbed and/or precipitated on the negatively charged capillary wall at basic pH. Inhibition of adsorption could be achieved only by suppressing the cation charges or, better, by charging them negatively. Therefore, we considered the use of complexation. Such a strategy appeared necessary as Jandik et al. [43] has previously demonstrated that com-

Table 2
Detection thresholds obtained using laser-induced indirect fluorimetric detection, with fluorescein sodium salt as fluorophore

Cation	[Na ₂ B ₄ O ₇] (mM)	Detection limit (ppb) ^a	
		Hydrodynamic injection ^b	Electrokinetic injection ^c
K ⁺	10	390	44
	2.5	39.5	3.6
Li ⁺	10	119	8
	2.5	15	0.7

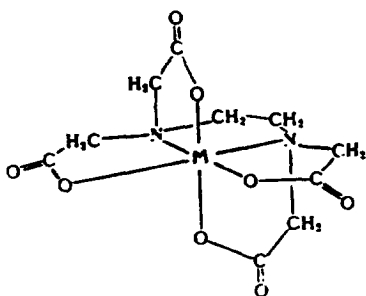
Fused-silica capillary, 57 cm × 75 μm I.D.; applied voltage, 10 kV; temperature, 30°C.

^a Detection limit = 3 × signal-to-noise ratio.

^b Injection pressure 0.5 p.s.i.; time 30 s.

^c Injection voltage 10 kV; time 30 s.

plexation is required to separate some transition and alkaline earth metal cations, even in acidic medium, because their individual electrophoretic mobilities are insufficient to allow a satisfactory resolution. Different complexing agents have been used, HIBA in acetic medium and indirect UV detection [40,41,44,45] and 8-hydroxy-quinolinesulfonic acid in basic medium and direct fluorimetric detection, the latter complexing agent giving fluorescent complexes with Ca(II), Mg(II) and Zn(II) [51]. As we are operating in a basic medium, and in the presence of a fluorophore providing a background signal, none of these approaches could be totally satisfactory. Consequently, we considered, developing a new complexing system compatible with the basic medium utilized and not leading to the formation of fluorescing complexes with metals. As the EDTA anion is known to give, in basic medium, octaedral complexes with a large number of cations, with the structure shown, and these complexes are more or less stable according to the nature of the cation (Table 3), we studied its potential for the determination by capillary electrophoresis of alkali, alkaline earth and transition metal cation mixtures.



First we examined the influence of EDTA concentration on the electrophoretic behaviour of a test sample containing Ba^{2+} , Ca^{2+} , Mn^{2+} and Zn^{2+} cations at pH 9.2 and for a 10^{-5} M concentration of fluorescein sodium salt. As the pH changes as a function of EDTA concentration it was adjusted to 9.2 by addition of sodium hydroxide. The evolution of the effective electrophoretic mobilities of the different cations studied as a function of EDTA concentration is reported in Fig. 3.

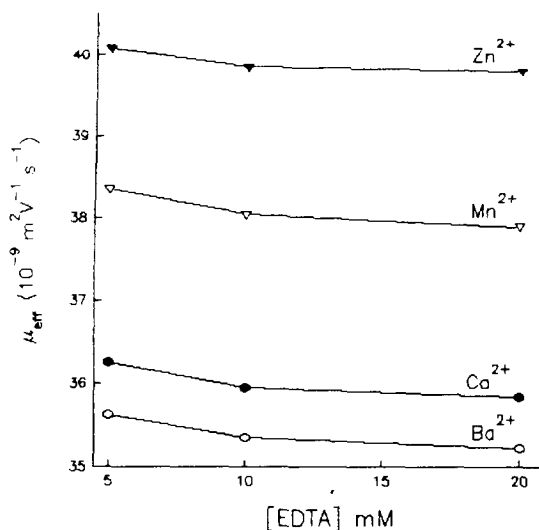


Fig. 3. Evolution of the electrophoretic behaviour of some alkaline earth and transition metal cations as a function of EDTA concentration. Operating conditions: fused-silica capillary, 57 cm \times 75 μm I.D.; applied voltage, 30 kV; temperature, 30°C; electrolyte, EDTA of variable concentration, pH adjusted to 9.2 by addition of sodium hydroxide; fluorescein concentration, 10^{-5} M; injection, hydrodynamic (injection time = 2 s); detection, fluorimetric ($\lambda_{\text{exc.}} = 488$ nm, $\lambda_{\text{detect}} = 520$ nm).

It is clear that the electrophoretic system selectivity does not change as a function of EDTA concentration for the cations studied, in spite of the fact that their electrophoretic mobilities increase as a function of the latter. Under these conditions, as the previous study of the signal-to-noise ratio as a function of the electrolyte ionic strength had shown that the sensitivity is enhanced at weak ionic strengths, we choose a 5 mM concentration of EDTA to complete this study.

Moreover, EDTA presents several acidities. The three weaker acidities are characterized, in pure water and at 0.1 M ionic strength, by pK_a values of 2.75, 6.25 and 10.38 [56]. Therefore, a study of the electrophoretic behaviour of transition and alkaline earth metal cations as a function of pH appeared necessary. We report this evolution in Fig. 4 for the above-mentioned cations.

As can be seen in Fig. 4a, there is no noticeable change in the selectivity as a function of pH.

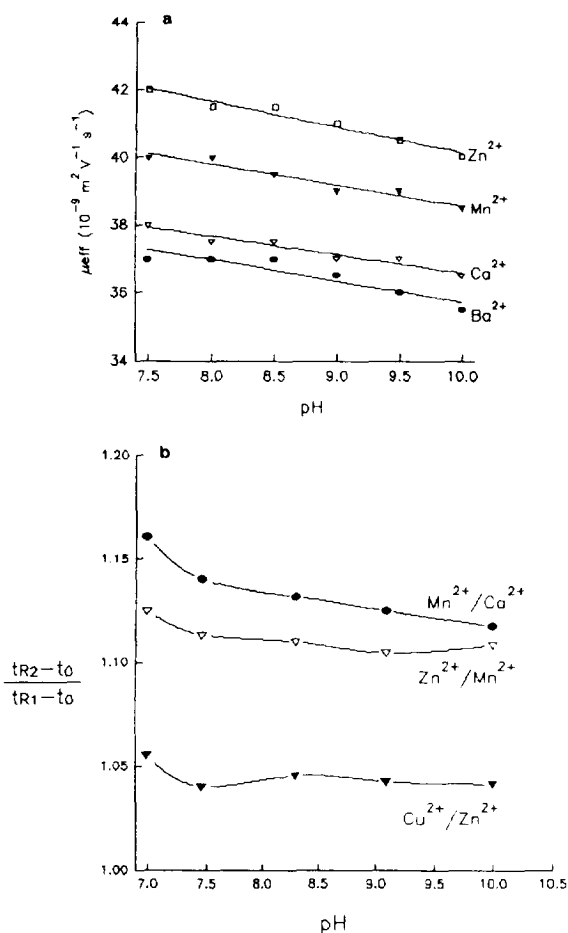


Fig. 4. Evolution of the electrophoretic behaviour of some alkaline earth and transition metal cations as a function of pH of the electrolyte: (a) electrophoretic mobilities; (b) ratio of corrected migration times ($t_{R2} - t_0$)/($t_{R1} - t_0$). Operating conditions: identical with those in Fig. 3, with the exception of the electrolyte, 5 mM EDTA, adjusted to a convenient pH by addition of sodium hydroxide.

As the electroosmotic flow velocity is strongly related to pH in the range 7–9, it also seemed worth, considering the above-mentioned results relative to selectivity, to study the evolution of the ratio of the corrected migration times of the different analyte species (Fig. 4b). Under these conditions, the differences in the electrophoretic behaviour should have been enhanced when the mobile phase pH decreased. This is effectively what we observed, although the phenomenon became noticeable only below pH 7.5 (Fig. 4b).

Moreover, the electrophoretic system efficiency is hardly dependent of pH variations in the range 7–9. For instance, for Mn^{2+} , the efficiency varies from 223 000 theoretical plates at pH 9.0 to 206 000 theoretical plates at pH 7.0. Therefore, the resolving power of the electrophoretic system is obviously greater at pH 7.0 than at pH 9.2, this better resolution being obtained to the detriment of the analysis time.

As we also intended to obtain the greatest possible detection sensitivity, a compromise had to be found for the value of pH because the intensity of induced fluorescence is strongly dependent on pH below pH 9 in the case of fluorescein. Facing this dilemma, the choice of an electrolyte containing 5 mM EDTA adjusted to pH 7.5 by addition of sodium hydroxide appeared to be an acceptable compromise between the resolution and the sensitivity of the electrophoretic system. At such a pH value, the resolving power is already fairly good and the system sensitivity is not too lowered. We report in Fig. 5 the analysis of a mixture of alkali, alkaline earth and transition metals performed under these conditions.

This separation shows that two system peaks appear in the electropherogram. The first, corresponding to water, allows the determination of the electroosmotic flow velocity under the operating conditions, while the second is attributed to one of the various ionic forms of EDTA existing at this pH. These two electrophoretic peaks, due to the displacement of fluorophore molecules, appear logically as negative signals. Their attribution was confirmed, without any doubt, by injecting, under the same operating conditions, pure water on the one hand and EDTA on the other. Further, the migration time of the electroosmotic flow having been determined, it appears that the analyte cations are not all complexed by EDTA. Lithium and potassium cations, which elute before the electroosmotic flow, still possess a positive charge under these operating conditions. This is not surprising, as can be seen from the literature data concerning the complexation of cations by EDTA [56] reported in Table 3.

These two cations having charges opposite to

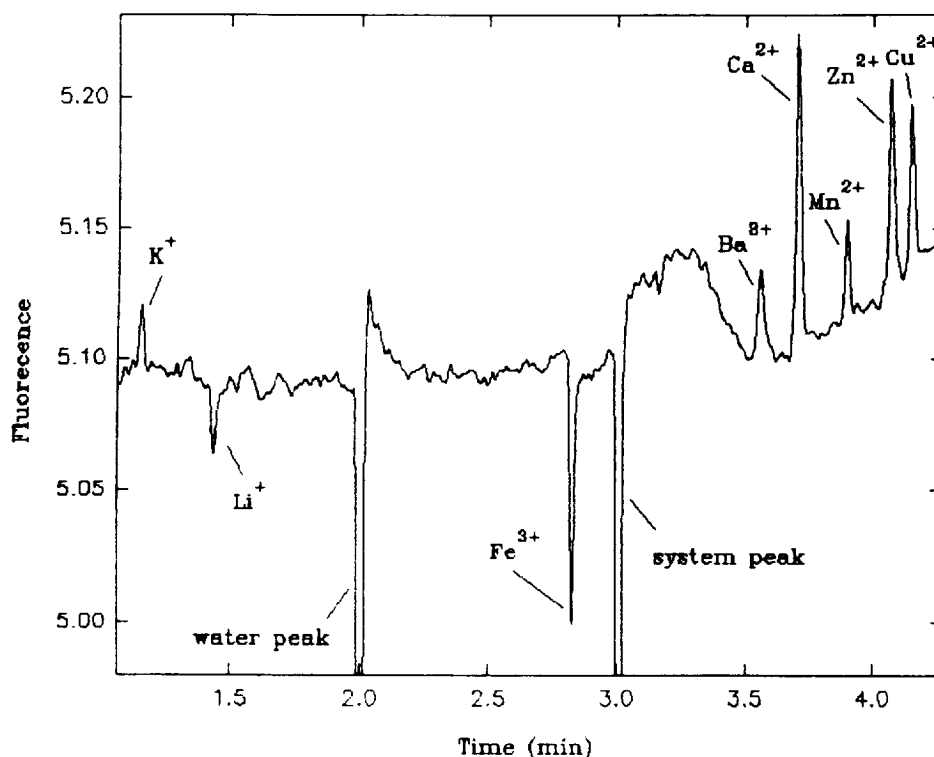


Fig. 5. Separation of alkali, alkaline earth and transition metal cations by CZE under the optimum conditions. Operating conditions: fused silica capillary, 57 cm \times 75 μ m I.D.; applied voltage, 30 kV; temperature, 30°C; electrolyte, 5 mM EDTA, adjusted to pH 7.5 by addition of sodium hydroxide; fluorescein concentration, 10^{-5} M; injection, hydrodynamic (injection time = 2 s); detection, fluorimetric ($\lambda_{\text{exc.}} = 488$ nm, $\lambda_{\text{detect.}} = 520$ nm).

Table 3

Complexation constants with EDTA in aqueous solution at 25°C [56] and equivalent ionic conductivity [57] of some alkali, alkaline earth and transition metal cations

Cation	Log K	Λ_0 (10^{-4} m ² S mol ⁻¹)
Li ⁺	2.8	38.66
Ba ²⁺	7.8	63.6
Sn ²⁺	8.6	—
Mg ²⁺	8.7	53.0
Ca ²⁺	10.7	59.47
Mn ²⁺	14.0	53.5
Co ²⁺	16.3	55
Cd ²⁺	16.5	54
Zn ²⁺	16.5	—
Ni ²⁺	18.6	50
Cu ²⁺	18.8	53.6
Fe ³⁺	25.1	—
K ⁺	—	73.48
Na ⁺	1.7	50.08

the fluorophore charge, as fluorescein is negatively charged at pH 7.5, according to Kuhr and Yeung [52] an enhancement of fluorescence due to electrostatic attraction should be observed, resulting in positive peaks. This is in fact observed with the potassium cation. In contrast, lithium is visualized as a negative peak. This seemingly anomalous response results from the differences in the solvation of these cations, which are evidenced by their different equivalent ionic conductances Λ [57] and by their different electrophoretic mobilities, which are directly proportional to their equivalent ionic conductances [42].

The apparently opposite fluorescence behaviours of Li⁺ and K⁺ cations can be rationalized if the importance of the perturbation of the background fluorescence due to the electrolyte during the elution of a sample (here a cation) possessing

a charge opposite to the fluorophore charge, here the sodium salt of fluorescein, is evaluated. This perturbation of fluorescence can be calculated with the help of the treatment developed by Ackermans et al. [58] in the case of the indirect UV detection of a sample possessing the same charge as the chromophoric ion. In the present case, the intensity of fluorescence of a binary electrolyte AB, in the absence of any sample ion, is given by the equation

$$I_F^E = I_0(\phi_A \varepsilon_A + \phi_B \varepsilon_B) l c_A^E$$

where ε_A and ε_B are the molecular absorptivities of the electrolyte ions, ϕ_A and ϕ_B are the fluorescence efficiencies of the electrolyte ions, c_A^E and c_B^E are the electrolyte ion concentrations, with $c_A^E = c_B^E$, l is the optical path length and I_0 is the incident light intensity. In the presence of a sample ion, the fluorescence intensity becomes

$$I_F^S = I_0(\phi_X \varepsilon_X + \phi_B \varepsilon_B) l c_X^S + I_0(\phi_A \varepsilon_A + \phi_B \varepsilon_B) l c_A^S$$

where ε_X , ε_B and ϕ_X , ϕ_B are the molecular absorptivities and the fluorescence efficiencies of the sample ion and the counter ion, respectively, c_X^S is the sample ion concentration in the mobile phase and c_A^S and c_B^S are the concentrations of the ions constituting the electrolyte in the presence of the sample ion, with $c_A^S = c_B^S$.

The perturbation of the background fluorescence intensity in the presence of a sample is

$$\Delta I_F = I_F^S - I_F^E$$

or

$$\Delta I_F = I_0(\phi_X \varepsilon_X + \phi_B \varepsilon_B) l c_X^S + I_0(\phi_A \varepsilon_A + \phi_B \varepsilon_B) l c_A^S - I_0(\phi_A \varepsilon_A + \phi_B \varepsilon_B) l c_A^E$$

If the ion A is the fluorophore ($\phi_A \neq 0$) and if the other ions present in the mobile phase are not fluorescent (sample ion X and counter ions B), i.e., $\phi_X = \phi_B = 0$, the relationship giving the fluorescence intensity perturbation becomes:

$$\Delta I_F = I_0 \phi_A \varepsilon_A l c_A^S - I_0 \phi_A \varepsilon_A l c_A^E \quad (1)$$

Under these conditions, the Kohlrausch regulation equation [59] is, in agreement with Dismukes and Alberty [60],

$$\frac{c_A^E}{m_A^0} + \frac{c_B^E}{m_B^0} = \frac{c_A^S}{m_A^0} + \frac{c_B^S}{m_B^0} + \frac{c_X^S}{m_X^0} \quad (2)$$

where m_A^0 , m_B^0 , and m_X^0 are the absolute mobilities of the ions A and B in the electrolyte and of the sample ion X, respectively.

In the case of a fluorophoric ion A charged oppositely to the X sample ion, taking in account the electroneutrality equations, on the one hand in the electrolyte $c_A^E = c_B^E$ and on the other in the electrolyte containing the sample ion X $c_A^S = c_X^S + c_B^S$, the Kohlrausch regulation, Eq. 2, becomes

$$\frac{c_A^E}{m_A^0} + \frac{c_A^E}{m_B^0} = \frac{c_A^S}{m_A^0} + \frac{c_A^S - c_X^S}{m_B^0} + \frac{c_X^S}{m_X^0}$$

$$c_A^E = c_A^S + \frac{m_A^0(m_B^0 - m_X^0)}{m_X^0(m_B^0 + m_A^0)} \cdot c_X^S \quad (3)$$

or

$$c_A^E = c_A^S + \lambda_X c_X^S$$

with

$$\lambda_X = \frac{m_A^0(m_B^0 - m_X^0)}{m_X^0(m_B^0 + m_A^0)}$$

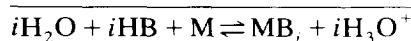
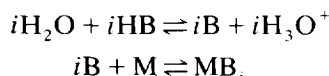
Taking in account Eq. 3, the perturbation of the fluorescence intensity in the presence of a sample ion becomes

$$\Delta I_F = -I_0 \phi_A \varepsilon_A l \lambda_X c_X^S \quad (4)$$

Consequently, the presence of a sample X possessing a charge opposite to the charge of the fluorophore A used to generate the background signal results in a decrease in the fluorescence intensity, giving a negative peak, if $\lambda_X > 0$ ($m_B^0 > m_X^0$), i.e., if the absolute mobility of the analyte sample is smaller than the mobility of the ion associated with the fluorophore. This is the case with the Li^+ cation which has an absolute mobility smaller than that of Na^+ (see Table 3), which is the counter ion of the fluorophore (sodium salt of fluorescein). In addition, it results in the enhancement of the fluorescence, giving a positive peak, if $\lambda_X < 0$ ($m_B^0 < m_X^0$), i.e., if the absolute mobility of the analyte ion is greater

than that of the counter ion associated with the fluorophore. This is the case with the K^+ cation, which has an absolute mobility greater than that of Na^+ (see Table 3) associated with the fluorophore. Finally, it results in the total disappearance of the signal if the absolute mobility of the analyzed ion is exactly the same as the absolute mobility of the ion associated with the fluorophore.

The situation is far more complex in the case of the cations effectively complexed by EDTA. The metallic ions (M) complexing the EDTA base (B) increase the apparent strength of the EDTA conjugate (HB) of this base, owing to the displacement of the dissociation equilibrium [61,62]



Consequently, if lithium and potassium cations, which give poorly stable complexes with the EDTA anion, have little influence on the EDTA acidity and therefore on pK_a values, it is different in the case of metallic cations giving stable complexes with EDTA anion. Important displacements of the dissociation equilibrium of EDTA have been reported [61,62] in the case of these cations, resulting in a considerably decreased value of the final acidity constant pK_a . In such conditions, the apparent electrophoretic mobility of the analyte cations is the result of the electrophoretic mobilities of both the non-complexed cation (μ_{cat}) and its EDTA complex (μ_{compl}):

$$\mu_{app} = (1 - x)\mu_{cat} + x\mu_{compl}$$

where x is the molar fraction of the cations complexed by EDTA.

Obviously, the molar fraction is directly dependent on the complexation constant. The experimental electrophoretic mobilities must be directly related to the complexation constants of the different ions. This is effectively observed in the case of divalent cations, as evidenced by the comparison of the elution order and complexation constants reported in Table 3.

In the same way, the electrophoretic behaviour of the only trivalent cation analyzed, Fe^{3+} , can be explained by the formation of a very stable complex with EDTA (Table 3), conferring a total charge of about one electron to this cation and resulting in an experimental electrophoretic mobility smaller than the values obtained with divalent cations. Thus the analyte species, i.e., the complex Fe^{3+} -EDTA presenting a negative charge, is visualized, in agreement with Kuhr and Yeung [52], as a negative peak, due to the electrostatic repulsion with the negatively charged fluorophore at this pH.

As with the alkali metal cations K^+ and Li^+ , the decrease of the fluorescence intensity during the elution of the Fe^{3+} cation, i.e., during the elution of the negatively charged Fe^{3+} -EDTA complex, can be rationalized by using a treatment directly transposed from that developed by Ackermans et al. [58]. The fluorescence perturbation is the given by the following relationship:

$$\Delta I_F = -I_0 \phi_A \varepsilon_A l \lambda'_X c_X^S \quad (5)$$

with

$$\lambda'_X = \frac{m_A^0(m_B^0 + m_X^0)}{m_X^0(m_B^0 + m_A^0)}$$

This equation shows that in the case of a sample ion X with the same charge as the fluorophore ion A, which is the case with the Fe^{3+} -EDTA complex in the presence of fluorescein sodium salt, its elution will result in a negative peak owing to a systematic decrease in fluorescence, whatever the value of its absolute mobility, because λ'_X is systematically positive.

In the case of divalent cations, which are partially complexed by EDTA anion, their visualization can be explained by a mixed mechanism, represented by the Eqs. 4 and 5. As their absolute mobility is systematically greater than the mobility of the sodium cation associated with the fluorophore, they give (in agreement with Eq. 4) an increase in the fluorescence in their non-complexed form. In contrast, their complexation by EDTA results in a decrease in the fluorescence intensity (Eq. 5). Consequently, the intensity of the peaks must decrease as a func-

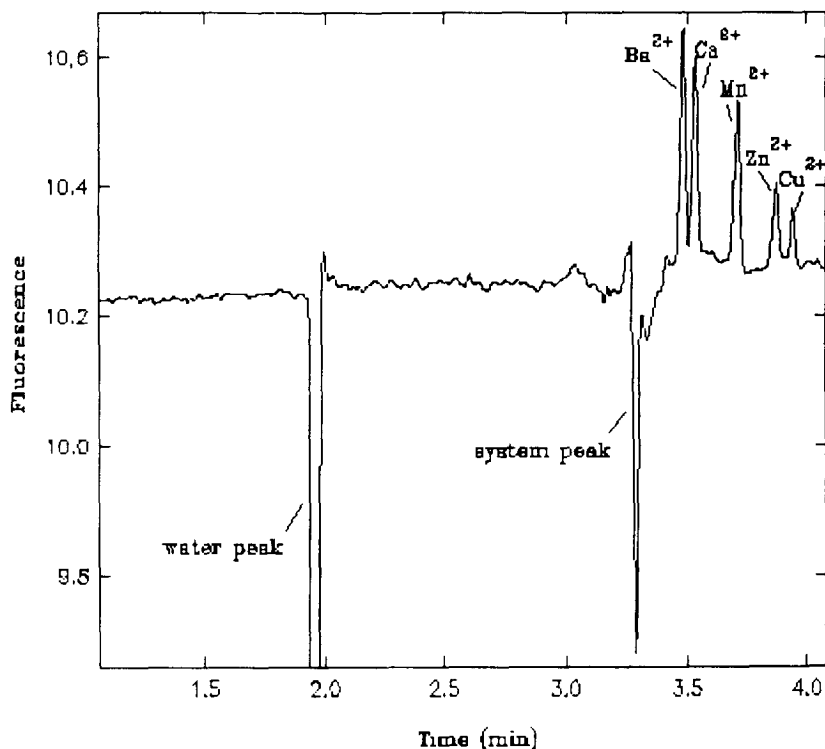


Fig. 6. Separation by CZE of five alkaline earth and transition metals cations injected in equal amounts using hydrodynamic injection (injection time = 2 s). Concentration of each cation, 10^{-4} M. Operating conditions as in Fig. 5.

tion of the importance of the complexation of divalent cations by EDTA. This is effectively observed in Fig. 6, and the results are those expected from divalent cation–EDTA–fluorescein mixed complexes [56,63].

Finally, other divalent alkaline earth and transition metal cations can be separated using this

electrophoretic system (Table 4). Unfortunately, the selectivity and the efficiency of the electrophoretic system utilized are not sufficient to allow their separation in the presence of the five divalent cations previously determined. Because of their too similar effective electrophoretic mobilities (Table 4), Ni^{2+} co-elutes with Zn^{2+} ,

Table 4

Effective electrophoretic mobilities of alkali, alkaline earth and transition metal cations determined in the presence of 5 mM EDTA at pH 7.5

Cation	μ_{eff} (10^{-9} m ² V ⁻¹ s ⁻¹)	Cation	μ_{eff} (10^{-9} m ² V ⁻¹ s ⁻¹)
Ba ²⁺	37	Co ²⁺	41
Ca ²⁺	38	Zn ²⁺	42
Mg ²⁺	40	Ni ²⁺	42
Mn ²⁺	40	Cu ²⁺	42.5
Cd ²⁺	40		

and similarly Mg^{2+} and Cd^{2+} co-elute with Mn^{2+} .

We completed this study by the determination of detection thresholds in the presence of EDTA. The detection limits of the various alkaline earth and transition metals cations are reported in Table 5.

The comparison of the detection thresholds, in the hydrodynamic injection mode, in the present case (i.e., in the presence of EDTA and at pH 7.5), for the lithium and potassium cations, with those obtained previously with the same fluorophore, fluorescein sodium salt, for the same cations without EDTA and at pH 9.2 (Table 2) indicates a noticeable loss of sensitivity. The system developed in the presence of EDTA (required for the determination in a basic medium of alkaline earth metal cations) is overall ten times less sensitive. This sensitivity loss is probably due to the pH of the electrolyte used, which is near neutral. Such operating conditions necessarily result in a decrease in the fluorescence emitted by the fluorescein utilized to provide the background signal. Under these conditions, the sensitivity limits obtained with lithium and potassium cations are only similar to those reached in indirect UV detection with a comparable optical path.

The detection limits of the alkaline earth cations and of the transition metals appear to be very satisfactory. In fact, the proposed electrophoretic system, which combines the fluorescein sodium salt (as fluorophore) and EDTA (as complexing agent), leads to slightly improved sensitivities compared with those obtained previ-

ously for this type of cation, the detection limits obtained varying between tens and hundreds of ppb.

4. Conclusions

From this study, it appears that the use of the fluorescein sodium salt at pH 9 leads to a well adapted electrophoretic system for the detection and determination of lithium and potassium cations. In fact, very high sensitivities can be obtained for these two cations (in the ppb range) if the injection is performed in the electrokinetic mode. With hydrodynamic injection, which is better adapted in a quantification context, the detection limits, even if slightly higher, appear to be acceptable, in the tens of ppb range. Concerning the determination of alkaline earth and transition metal cations, as the separation could not be performed with the fluorescein sodium salt alone, a noticeable decrease in the detection sensitivity was observed for the Li^+ and K^+ cations because of the electrolytic system used (fluorescein sodium salt–EDTA, pH 7.5). Nevertheless, this electrolyte, which combines fluorescein and EDTA, appears to be of great interest for the determination of alkaline earth and transition metal cations as it leads to low detection limits for these ions. Taking into account the satisfactory results obtained in this study, we shall study in the near future the potential of this electrophoretic system for the determination of organic and inorganic anions and also organic cations.

Table 5

Detection limits obtained in laser-induced indirect fluorimetric detection using hydrodynamic injection and the fluorescein sodium salt as fluorophore in the presence of EDTA

Cation	Detection limit ^a (ppb)	Cation	Detection limit ^a (ppb)
Li^+	74	Mn^{2+}	28
K^+	300	Zn^{2+}	22
Ba^{2+}	80	Cu^{2+}	18
Ca^{2+}	20	Fe^{3+}	100

Fused-silica capillary, 57 cm \times 75 μm I.D.; applied voltage, 30 kV; temperature, 30°C.

^a Detection limit = 3 \times signal-to-noise ratio.

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