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Systematic approach to the separation of mono- and hydroxycarboxylic acids in environmental samples by ion chromatography and capillary electrophoresis

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Abstract

Strategies for method development and optimization of conditions in ion chromatography (IC) and capillary electrophoresis (CE) are proposed. The approaches are based on well established theoretical models for analyte elution and rely on the inspection of effective capacity factor versus specific gradient ramp slope curves for IC and effective mobility versus pH curves for CE. Commonly used IC eluent systems, such as sodium hydroxide and sodium borate, and the CE electrolyte 3,5-dinitrobenzoic acid were investigated. A standard aqueous solution containing formate, acetate, α-hydroxyacetate (glycolate), propionate, α-hydroxypropionate (lactate), butyrate and β-hydroxybutyrate anions was used to test the analytical conditions indicated by the models. Among the IC eluent systems, borate presented a better separation performance than hydroxide. However, a complete resolution of all components in the standard mixture was not accomplished experimentally at the chosen gradient ramp slope of 0.05 m*M*/ml. The analyte pairs acetate/lactate and glycolate/butyrate, whose effective capacity factors differ by approximately 0.2 units, co-eluted at 0.17 and 0.24 resolution, respectively, and the retention time of the last eluting analyte was relatively long (20 min). Nevertheless, the IC method provided the best overall limit of detection (LOD; 0.016–0.082 mg/l). Under the optimized CE conditions, all seven components in the standard mixture were resolved satisfactorily in less than 7 min. The analyte pair β-hydroxybutyrate/butyrate presented the worst resolution, 0.45, and a difference in effective mobility of 1.6%. The CE methodology provided the best column efficiency, roughly a ten-fold improvement in terms of number of plates per meter over the IC method, but the limit of detection was comparatively poorer (0.050–0.36 mg/l). Both proposed IC and CE methodologies can be applied to the analysis of mono- and hydroxycarboxylic acids in samples of environmental interest, providing complementary information. The choice of the most appropriate method is a compromise between chemical composition of the sample and concentration level of the analytes under investigation. For instance, the classical co-elution of acetate/lactate that occurs in IC columns, can be solved by CE. But the CE method may lack sensitivity for these analytes, which compromises the analysis of certain real samples. The LOD for acetate and lactate as determined by IC is 0.036 and 0.082 mg/l, respectively, while for CE they increase to 0.11 and 0.20 mg/l. Under optimum conditions, the separation and identification of mono- and hydroxycarboxylic acids in an atmospheric particulate matter sample is illustrated by both techniques.

Keywords: Method development; Optimization; Environmental analysis; Air analysis; Carboxylic acids

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1. Introduction

To establish the deleterious effect of pollutants on global environmental processes it is necessary to pursue a detailed understanding of the chemistry in the atmosphere. In this context, the chemical nature and composition of atmospheric particulate matter and gaseous samples must be thoroughly characterized. The determination of pollutants in atmospheric air samples has been the subject of many studies [1,2], despite the inherent analytical difficulty that such a task presents, in handling low concentration levels of species in fairly complex matrices. Among important classes of pollutants, growing interest has been directed towards organic acids, ubiquitous chemical constituents of the atmosphere [3–6]. Although our knowledge of their sources, atmospheric formation and removal mechanisms is somehow limited, in polluted areas organic acids have been associated to vehicle exhausts as well as other anthropogenic activities [3,4]. Several photochemical reactions have been proposed to account for the gas-phase production of organic acids, particularly the reaction between atmospheric ozone and olefins [5,6]. Furthermore, organic acids have been recognized to be leading contributors to acid dry deposition [4]. Among the species identified in urban and non-urban air are the aliphatic mono-, di-, hydroxy- and ketocarboxylic acids as well as the aromatic carboxylic acids [7–9].

Several techniques have been employed to evaluate the organic acid contents in the atmosphere, including gas chromatography (GC) [10,11] and high-performance liquid chromatography (HPLC) [12,13]. Traditionally, ion chromatography (IC) at elevated pH values has been applied to the determination of anions deriving from strong and weak acids [14–16]. The availability of stationary phases with differing selectivity has provided a range of options for determining inorganic and organic anions from low-molecular-mass aliphatic carboxylic acids. Although IC is suitable for the simultaneous analysis of several anionic compounds, certain species interact similarly towards the resin sites and may not always be detected, misleading the interpretation of environmental data sets. Typical examples include the well known co-elution of acetate/lactate, sulfate/oxalate, and the quite often insufficient separation of acetate/glycolate [14].

Capillary electrophoresis (CE), a technique based on the differential migration of ionic analytes in an applied electric field, provides a distinct separation mechanism from IC and it has been considered an interesting alternative for anion analysis [17]. High efficiency (10^5–10^6 theoretical plates), high resolving power and short analysis time are among the most relevant aspects of CE [18]. In favorable conditions, the complete resolution of 30 to 40 anions can be achieved in less than 5 min [19].

In the past few years, the performance of IC and CE has been contrasted by several criteria, comprising practical aspects as well as analytical capabilities. Characteristics, such as cost per analysis, column endurance, sample handling, peak capacity and limit of detection have all been evaluated for the analysis of anions in real matrices [20–22]. However, quite often these studies are tied to specific conditions, or simply address few variables at a time. Moreover, optimization of experimental conditions is usually attempted by trial-and-error procedures. Therefore, a more systematic means to approach optimization and a better understanding of the variables controlling the separation process are highly desirable.

In this work, a criterious approach to optimize conditions has been proposed for separations conducted by both IC and CE techniques. The models are simple and easy to implement. In order to validate these strategies, analytes of environmental importance such as mono- and hydroxycarboxylic acids were selected. Once optimum conditions were determined, a comparative study of the two techniques was performed, contrasting analysis time, resolution and column efficiency as well as limit of detection. At the end, the IC and CE analysis of a particulate matter sample is contrasted.

2. Theory

2.1. Ion chromatography

In IC, anions can be generally separated during isocratic elution [23]. When the separation of a complex mixture of analytes having widely differing retention characteristics must be attempted, the use of gradient elution is recommended. The model proposed in this work is based on gradient elution.

However, since several terms and concepts within the theory of isocratic elution are needed, a brief discussion of isocratic chromatography is presented.

2.1.1. Isocratic elution

The distribution of analyte (An) and eluent ions (El) between the mobile phase (m) and the available sites (s) on the resin is represented by the ion-exchange reaction:

$$e\mathrm{An}_{\mathrm{m}}^{a} + a\mathrm{El}_{\mathrm{s}}^{e} \rightleftharpoons e\mathrm{An}_{\mathrm{s}}^{a} + a\mathrm{El}_{\mathrm{m}}^{e} \quad (1)$$

and the corresponding equilibrium coefficient is given by the expression:

$$K = \frac{[\mathrm{An}]_{\mathrm{s}}^{e}[\mathrm{El}]_{\mathrm{m}}^{a}}{[\mathrm{An}]_{\mathrm{m}}^{e}[\mathrm{El}]_{\mathrm{s}}^{a}} \quad (2)$$

where $[\mathrm{An}]_{\mathrm{m}}^{e}$, $[\mathrm{An}]_{\mathrm{s}}^{e}$ and $[\mathrm{El}]_{\mathrm{m}}^{a}$, $[\mathrm{El}]_{\mathrm{s}}^{a}$ are the concentrations of analyte and eluent ions in the mobile phase and the resin stationary phase, respectively, and a and e are the absolute values of the analyte and eluent ion charges.

The retention of an analyte is usually expressed by the capacity factor k', a dimensionless parameter defined by the ratio of the total mass of analyte in the stationary phase to the mobile phase:

$$k' = \frac{V_{\mathrm{R}} - V_{\mathrm{m}}}{V_{\mathrm{m}}} = \frac{V_{\mathrm{s}}[\mathrm{An}]_{\mathrm{s}}}{V_{\mathrm{m}}[\mathrm{An}]_{\mathrm{m}}} \quad (3)$$

where V_{R} is the retention volume of the analyte, V_{m} is the volume of the mobile phase or void volume, and V_{s} is the volume of the stationary phase. By combining Eqs. (2) and (3) the dependence of capacity factor on the eluent concentration is obtained:

$$k' = \frac{V_{\mathrm{s}}}{V_{\mathrm{m}}} K^{1/e} Q^{a/e} [\mathrm{El}]_{\mathrm{m}}^{-a/e} \quad (4)$$

where Q is the concentration of eluent in the resin stationary phase, also know as the resin ion-exchange capacity. For eluent concentrations lower than 100 m*M*, all constant terms in Eq. (4) can be combined into a single isocratic constant:

$$k' = C_{\mathrm{i}}[\mathrm{El}]^{-a/e} \quad (5)$$

2.1.2. Gradient elution

During gradient elution, the capacity factor varies gradually as the eluent concentration is changed. For a linear gradient starting in zero and increasing with time, the instantaneous eluent concentration at the column inlet is given by:

$$[\mathrm{El}]_{\mathrm{inst}} = RV_{\mathrm{inst}} \quad (6)$$

where R is the gradient ramp slope, calculated as the change in eluent concentration with time divided by the flow-rate and V_{inst} is the volume of eluent pumped since the beginning of the run. The instantaneous capacity factor, k'_{inst}, is calculated by substituting Eq. (6) into Eq. (5) to give:

$$k'_{\mathrm{inst}} = C_{\mathrm{i}}[RV]^{-a/e} \quad (7)$$

k'_{inst} is the capacity factor that would result if the instantaneous eluent concentration were held constant throughout the analysis (isocratic elution). To calculate the capacity factor for gradient elution, k'_{inst} must be integrated from injection to elution. Rocklin et al. [24] derived the following expression for the so-called effective capacity factor:

$$k'_{\mathrm{eff}} = \frac{(V_{\mathrm{R}} - V_{\mathrm{m}})}{V_{\mathrm{m}}} = \frac{(e)^{-e/(a+e)}}{(a+e)} V_{\mathrm{m}}^{-a/(a+e)} C_{\mathrm{i}}^{e/(a+e)} R^{-a/(a+e)} \quad (8)$$

The constant terms in Eq. (8) can be combined into a single gradient constant:

$$k'_{\mathrm{eff}} = \frac{(V_{\mathrm{R}} - V_{\mathrm{m}})}{V_{\mathrm{m}}} = C_{\mathrm{g}} R^{-a/(a+e)} \quad (9)$$

For singly-charged analyte and eluent systems, Eq. (9) predicts an inverse linear relationship between the effective capacity factor and the square root of R. For a doubly-charged eluent, k'_{eff} varies with the inverse of cubic root of R. Therefore, depending on the charge of the analyte and eluent a specific gradient ramp slope, $R^{-a/(a+e)}$ is defined.

2.2. Capillary electrophoresis

In free solution capillary electrophoresis, the migration time (t_{i}) of each zone to the detector position (L_{det}) is determined by the zone net rate of migration (v_{i}), which is a vectorial summation of the electroosmotic (v_{osm}) and electrophoretic (v_{ep}) velocities:

$$v_{\mathrm{i}} = \frac{L_{\mathrm{det}}}{t_{\mathrm{i}}} = v_{\mathrm{osm}} + v_{\mathrm{ep}} = (\mu_{\mathrm{osm}} + \mu_{\mathrm{ep}}) \frac{V_{\mathrm{app}}}{L_{\mathrm{tot}}} \quad (10)$$

where μ_{osm} and μ_{ep} are the electroosmotic and electrophoretic mobilities, respectively, V_{app} is the applied voltage and L_{tot} is the total capillary length.

The effective mobility is a parameter used to describe the migration of partially dissociated analytes consisting of several ionic and neutral species that interact by a dynamic acid–base equilibrium. Under the influence of an applied electric field, all of these analyte species migrate as a whole. According to this definition any substance (i), present in solution as a set of different species (j), has an effective mobility given by:

$$(\mu_{eff})_i = \sum(\alpha_j \mu_j) \quad (11)$$

where α_j represents the distribution function of each particular species and μ_j is the respective electrophoretic mobility. The distribution functions are derived from the dissociation constants of the system (K_a) and the pH [25].

3. Experimental

3.1. Instrumentation

The IC experiments were performed on an ion chromatography system (Model 4000I, Dionex, Sunnyvale, CA, USA), comprising a gradient pump, a chromatography module and a conductivity detector. An anion trap column (Model ATC-5A, Dionex) was installed ahead of the injection valve to minimize interference from contaminant anions. Separation was performed on a separator column (Model HPIC-AS5, Dionex). The eluent was suppressed with an anion micro-membrane suppressor (Model AMMS II, Dionex) and a 12.5 m*M* sulfuric acid solution was used as regenerant.

The CE experiments were performed in a capillary electrophoresis system (Model 270A-HT, Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA) equipped with a variable-wavelength UV–Vis detector, set at 254 nm, a temperature control device, maintained at 25.0°C and a data acquisition and treatment software (Turbochrom, Perkin-Elmer, PE-Nelson Division, Cupertino, CA, USA). A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with dimensions 72 cm total length×50 μm I.D.×375 μm O.D. was installed. A detection window of approximately 0.2 cm was created at 50 cm from the capillary inlet, by removing the polyimide coating. Samples were injected hydrodynamically, with 5 in. Hg for 10 s (1 in. Hg=3386.38 Pa). The electrophoresis system was operated under reversed polarity and constant voltage conditions of −15 kV.

3.2. Chemicals

All standards were prepared from reagent-grade chemicals and deionized water. Stock aqueous solutions of individual standards (formic, glycolic, acetic, lactic, propionic, β-hydroxybutyric and butyric acids, either in the acidic or in the sodium salt form) were prepared at 100 mg/l concentration and stored in refrigerator up to two months, with exception of the formic acid stock solution, which was prepared fresh for each experiment due to decomposition problems. Mixtures containing appropriate amounts of each analyte, in the range from 0.05 to 0.6 mg/ml, were prepared by dilution of the stocks with deionized water. Stock solutions of the IC eluents used in the gradient program were prepared from high-purity sodium hydroxide and sodium tetraborate at 25 m*M*. The CE electrolyte working solution consisted of 10 m*M* 3,5-dinitrobenzoic acid containing 0.1 m*M* cetyltrimethylammonium bromide (CTAB), adjusted to pH between 5 and 6 with 0.1 m*M* sodium hydroxide.

3.3. Sampling and extraction procedure

The airborne particulate matter sample was collected on a 47 mm diameter PTFE-coated quartz-fiber filter (TX40H120WW, Pallflex, Puttnam, CN, USA) at 10 l/min flow-rate for approximately 12 h. The material was extracted with 30 ml deionized water at room temperature and placed on a shaker for 90 min [26]. The extract was filtered through a 0.22 μm pore-size membrane filter (HAWP, Millipore, Bedford, MA, USA) and stocked in a freezer until analysis.

3.4. Analytical procedures

The IC column conditioning consisted of a 5 min

rinse with deionized water followed by the gradient program as described below:

Eluent	Time (min)	% A	% B
Borate	0	100	0
	25	95	5
	25.1	100	0
Hydroxide	0	100	0
	25	90	10
	25.1	100	0

A = deionized water; B = eluent (25 m*M*).

Other gradient programs were implemented throughout the work by varying the time and the percentage level of eluent B, always prepared at 25 m*M* concentration.

The CE electrolyte solutions were prepared fresh daily and filtered through a 0.22 μm membrane filter just prior to use. At the beginning of the day, the capillary was conditioned by a flush of 1 *M* NaOH solution (20 min), followed by a flush of deionized water (10 min) and a flush of the electrolyte solution (30 min). In between runs, the capillary was just replenished with fresh electrolyte solution (2 min flush).

4. Results and discussion

4.1. IC optimization strategy

The IC strategy is based on the inspection of effective capacity factor versus specific gradient ramp slope. According to Eq. (9), the calculation of the effective capacity factor relies on a priori knowledge of the gradient constant, C_g. The constant can be evaluated experimentally by the measurement of retention times at a known gradient ramp slope. Table 1 compiles the retention times of the analytes under investigation in the hydroxide and borate eluent systems at the gradient ramp slope of 1.0 m*M*/ml. The retention times were multiplied by the flow-rate to give the corresponding V_R, whereas the elution time of water was used to calculate V_m. The gradient constant was then determined by Eq. (9).

Table 1
Input parameters of the analytes under investigation for IC analysis

Peak label	Analyte	Hydroxide eluent		Borate eluent	
		t_R [a] (min)	C_g [b]	t_R [a] (min)	C_g [b]
1	β-Hydroxybutyric acid	7.10	5.45	6.23	4.67
2	Acetic acid	7.30	5.64	6.50	4.90
3	Lactic acid	7.58	5.89	6.58	4.98
4	Glycolic acid	7.65	5.95	6.85	5.23
5	Propionic acid	7.70	6.00	6.68	5.07
6	Butyric acid	7.94	6.22	6.92	5.29
7	Formic acid	8.43	6.66	7.43	5.76

[a] Flow-rate = 1.0 ml/min; V_m = 1.10 ml; R = 1.0 m*M*/ml.
[b] From Eq. (9).

The curves of effective capacity factor for each analyte at various specific gradient ramp slopes for the eluent systems sodium hydroxide and borate are given in Fig. 1. As the eluent concentration increases, the effective capacity factor decreases and all curves approach zero. This effect is more pronounced for hydroxide than it is for borate, because hydroxide is a stronger eluent than borate. Also, the specific gradient ramp slope depends on the analyte and eluent charges. Therefore, for a similar specific gradient ramp slope interval, the behavior of a particular analyte will change more markedly if hydroxide instead of borate is employed. For a given eluent, the slope C_g reflects solely the strength of interaction between the analyte and the sites on the resin, given that other conditions are kept constant. C_g may be thought of as an intrinsic characteristic of the analyte for a given column, operated at a constant flow-rate, with a particular eluent system. Thus, different slopes are expected only when analytes behave distinctly towards that column.

As observed from Fig. 1A, despite of the fact that hydroxide is the most commonly used eluent system in IC, it is unlikely to distinguish experimentally several analytes, e.g., acetate, lactate and glycolate, which presented a fairly similar C_g. As observed from Fig. 1B, the column seems to be more selective for the separation of the chosen analytes when borate is used, provided that an appropriate gradient ramp slope is selected. The choice of a large value for

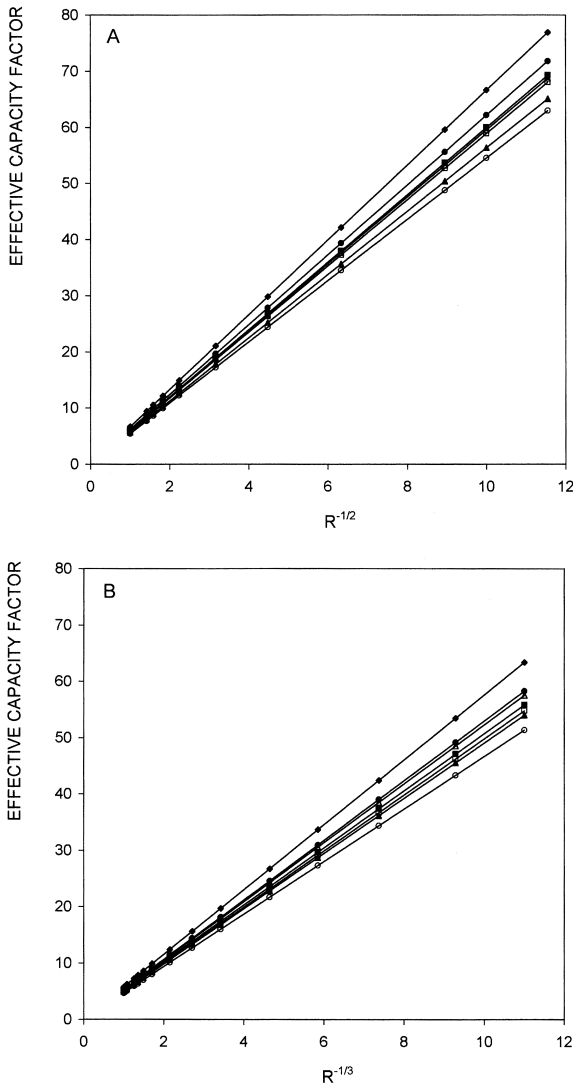

Fig. 1. IC theoretical curves of effective capacity factor versus specific gradient ramp slope for the analytes formic (♦), glycolic (△), acetic (▲), lactic (□), propionic (■), β-hydroxy-butyric (○) and butyric (●) acids in hydroxide (A) and borate (B) eluent systems.

$R^{-a/(a+e)}$ enhances the chance to achieve experimentally the complete separation of all analytes, at the expense of long time of analysis. Thus, to optimize a separation it is necessary to find the least value of $R^{-a/(a+e)}$ where the separation still occurs. For high efficiency columns, the gradient ramp slope can be diminished without compromising the separation quality.

Tables 2 and 3 show the differences in effective capacity factor ($\Delta k'_{\mathrm{eff}}$) for adjacent eluting analytes at several gradient ramp slopes for the hydroxide and borate eluent systems, respectively. In both cases, when the gradient ramp slope equals one, condition used to determine C_{g}, all analyte pairs co-eluted severely with exception of the last pair, which presented a resolution better than one (chromatograms not shown). These results indicate that, for a given pair of adjacent analytes be separated, a difference in effective capacity factor of approximately 0.45 must occur. If Tables 2 and 3 are inspected to find a value of R that provides a $\Delta k'_{\mathrm{eff}}$ of 0.45 for all analyte pairs, a gradient ramp of 0.0125 mM/ml for the hydroxide system and 0.005 mM/ml for the borate system would be found. However, these figures are beyond the experimental scope of IC. By decreasing the gradient ramp so drastically, a correspondent increase in band width and decrease in peak height, not anticipated by the model, would occur, compromising the separation performance, circumstance often referred as the general elution problem in chromatography. Therefore, a minimum gradient ramp slope of 0.1 mM/ml for the hydroxide system and 0.05 mM/ml for the borate system were found to be the best compromise between resolution, time of analysis and efficiency for the type of column employed and eluent systems studied. Within the above gradient ramp conditions, baseline resolution for all analyte pairs is not expected to occur, but given the similar behavior of the analytes under investigation, they represent the best conditions for analysis and they will be referred from now on as optimum conditions.

Fig. 2 shows the chromatograms of the test standard mixture in hydroxide and borate eluent systems at a selected gradient ramp slope of 0.1 mM/ml and 0.05 mM/ml, respectively. As predicted by the model, in hydroxide, the co-elution of acetate, lactate and glycolate occurred. On the other hand, although the overall quality of the chromatogram obtained in borate is superior, a complete resolution of all analyte pairs was not accomplished experimentally. Nevertheless, the profile of the chromatogram

Table 2
Differential effective capacity factors for adjacent analytes as a function of gradient ramp slope in the hydroxide system

R	$R^{-1/2}$	$\Delta k'_{eff}$					
		β-Hydroxybutyrate/ acetate	Acetate/ lactate	Lactate/ glycolate	Glycolate/ propionate	Propionate/ butyrate	Butyrate/ formate
0.0125	8.94	1.63	2.28	0.57	0.41	1.95	3.98
0.025	6.32	1.15	1.61	0.40	0.29	1.38	2.82
0.05	4.45	0.81	1.14	0.28	0.20	0.98	2.00
0.10	3.16	0.57	0.80	0.20	0.14	0.69	1.41
0.50	1.41	0.26	0.36	0.090	0.064	0.31	0.63
1.00	1.00	0.18	0.25	0.064	0.045	0.22	0.45

R is the gradient ramp slope.
$R^{-1/2}$ is the specific gradient ramp slope.
$\Delta k'_{eff}$ is the difference in effective capacity factor between the specified analyte pair.

in terms of relative resolution between adjacent bands as well as the correct order of elution are readily obtained from the proposed model.

4.2. CE optimization strategy

A detailed description of the method development and optimization strategy for the analysis of anionic analytes by CE has been published in previous work [27]. A synopsis of the major steps is given below. A near optimum pH is chosen by inspection of the effective mobility versus pH curves of the sample components. The optimum pH lies in a region where the differences in the mobilities of all analytes are maximal. Next, an electrolyte system is selected by comparing the effective mobility of the electrolyte with the effective mobility of the primary components of the studied mixture. The appropriate electrolyte has a mobility as similar as possible to the mobility of the major components of the sample. An initial experiment is run with the selected electrolyte system adjusted to the chosen pH and input values for other instrumental variables and electrolyte characteristics. Depending on the degree of resolution obtained in the initial run, two important system variables, the applied voltage and the electrolyte concentration, are then manipulated to achieve separation.

The effective mobility versus pH curve constitutes a valuable tool for the preliminary assessment of an

Table 3
Differential effective capacity factors for adjacent analytes as a function of gradient ramp slope in the borate system

R	$R^{-1/3}$	$\Delta k'_{eff}$					
		β-Hydroxybutyrate/ acetate	Acetate/ lactate	Lactate/ propionate	Propionate/ glycolate	Glycolate/ butyrate	Butyrate/ formate
0.005	5.84	1.40	0.41	0.56	0.90	0.40	2.71
0.01	4.64	1.11	0.33	0.44	0.71	0.32	2.15
0.05	2.71	0.65	0.19	0.26	0.42	0.19	1.26
0.10	2.15	0.52	0.15	0.21	0.33	0.15	1.00
0.50	1.26	0.30	0.089	0.12	0.19	0.086	0.58
1.00	1.00	0.24	0.071	0.095	0.15	0.068	0.46

R is the gradient ramp slope.
$R^{-1/3}$ is the specific gradient ramp slope.
$\Delta k'_{eff}$ is the difference in effective capacity factor between the specified analyte pair.

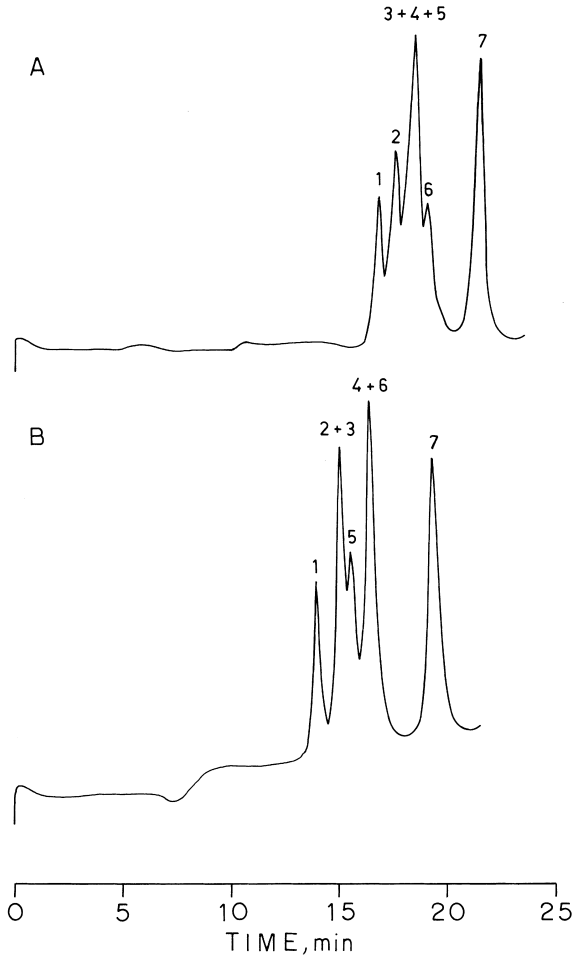

Fig. 2. Chromatograms of the standard mixture under optimum conditions in hydroxide (A) and borate (B) eluent systems. Peaks labeled as in Table 1.

electrophoretic separation. According to Eq. (11) the calculation of effective mobility relies on the knowledge of the dissociation constant and the intrinsic mobility of the species in equilibrium. Literature values of pK_a and μ_j for the substances of interest to the present work are reported in Table 4 [28]. Calculated values of effective mobility as a function of pH are displayed in Fig. 3.

As Fig. 3 illustrates, the separation of nearly all analytes is rather difficult. There are two narrow windows, one right above pH 5 and the other around pH 6, where the differences in the analytes mobility are maximized and the separation might be attempted. There is an important point to be emphasized here: the inspection of the mobility versus pH curve should not be taken rigorously but only as a preliminary assessment of the separation. Differences in the medium ionic strength, temperature and viscosity influence pK_a and the intrinsic mobility of the species. Therefore, in practice, a shift of the pH indicated by the curve is likely to occur.

Table 4
Input parameters of the analytes under investigation for CE analysis

Analyte	pK_a [a]	$\mu_j \cdot 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)[1]
Formic acid	3.752	−56.6
Acetic acid	4.756	−42.4
Glycolic acid	3.886	−42.4
Propionic acid	4.874	−37.1
Lactic acid	3.860	−36.5
β-Hydroxybutyric acid	4.519	−34.3
Butyric acid	4.820	−33.8

[a] Adapted from Ref. [28].

Other information that can be withdrawn from the mobility curve is the choice of an appropriate electrolyte system to conduct the separation. Over the two pH regions investigated experimentally, the electrolyte 3,5-dinitrobenzoic acid seems to be a reasonable choice, since its mobility approaches the mobility of the majority of the analytes under investigation (mobility versus pH curve for 3,5-dinitrobenzoic acid is presented in Ref. [27]). As recommended in the Section 2.2, once the pH and

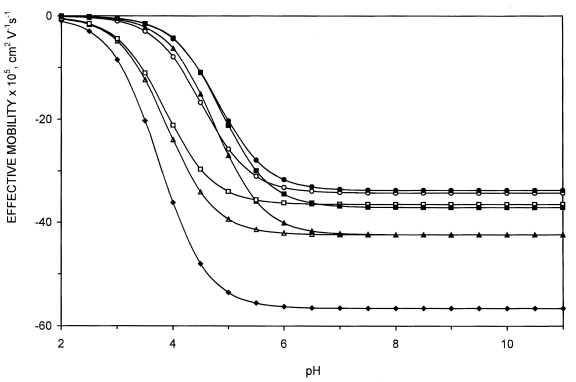

Fig. 3. CE theoretical curves of effective mobility versus pH for the analytes under investigation. Analyte curves labeled as in Fig. 1.

electrolyte are chosen, the manipulation of applied voltage and electrolyte concentration is performed. In this work, the values −15 kV and 10 m*M* were found to produce the best results.

Fig. 4 shows a sequence of electropherograms registered in the proximity of the optimum pH. At pH 5.9, the separation of nearly all analytes was accomplished satisfactorily. As it can be observed, however, a slight variation of pH can change dramatically the profile of the electropherograms, leading to peak co-elution. Thus, in practice, it is imperative to adjust carefully the electrolyte pH, and even during the analysis, the electrolyte vials must be kept closed to prevent absorption of CO_2 from ambient air, which could change the pH and affect the separation quality throughout repetitive runs.

4.3. IC versus CE performance comparison

For comparison purposes, the IC analysis was performed with the borate eluent system only. Sequential chromatograms of individual analytes under investigation in the concentration range from 0.010 to 0.6 mg/l were run in duplicate and the corresponding peak area and height were registered. Likewise, electropherograms were recorded in the concentration range from 0.050 to 0.6 mg/l. Calibration curves of concentration versus peak area or height were built. The statistical parameters of these curves for both IC and CE were compiled in Table 5 and the limit of detection (LOD) was calculated. The criterion used for the calculation of LOD was the sum of the intercept and three times the estimated standard deviation of the intercept [29]. As it can be observed, the slopes of the IC calibration curves were substantially higher than those obtained from the CE curves, indicating that the IC method offers a much larger concentration sensitivity for each analyte. The IC curves also provided an overall better fitting. Regarding the limit of detection, the IC curves indicated roughly an LOD of an order of magnitude smaller than those obtained by CE, probably as a consequence of the type of detection, direct conductivity detection for IC against indirect UV detection for CE.

Tables 6 and 7 compile the separation parameters calculated from the chromatogram and electropherogram obtained in the optimum conditions for

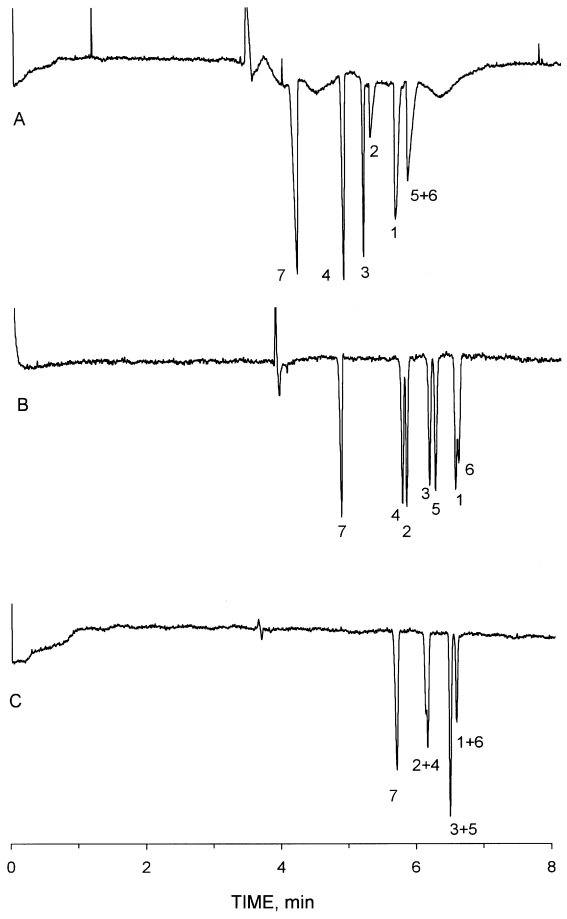

Fig. 4. Electropherograms of the standard mixture obtained in 10 m*M* 3,5-dinitrobenzoic acid containing 0.1 m*M* CTAB at pH 5.0 (A), pH 5.9 (B) and pH 6.2 (C). Peaks labeled as in Table 1.

both IC and CE. All parameters used there, such as resolution (R_s), number of plates per meter of column (N) and selectivity factor (α) are defined in a conventional manner. In terms of analysis time, both techniques performed reasonably, presenting a distinct order of elution. In IC, the total analysis time was 25 min, considering elution of all analytes and conditioning of the column. CE was favored by a much shorter analysis time with complete elution in less than 7 min and additional 2 min for column conditioning. The peaks in the electropherogram presented a smaller band width, which reflects directly the much higher efficiency of CE over IC. In the IC analysis, there are two analyte pairs completely co-eluted, i.e., acetate/lactacte and glycolate/

Table 5
Statistical parameters of the calibration curves

Analyte	Slope		Intercept		R^2		LOD	
	IC[a] (μS l/mg)	CE[b] (mV l/mg)	IC (μS)	CE (mV)	IC	CE	IC (mg/l)	CE (mg/l)
β-Hydroxybutyric acid	70 740	2773	−7141	−98.9	0.991	0.994	0.067	0.050
Acetic acid	335 177	1012	8707	−44.6	0.996	0.986	0.036	0.11
Lactic acid	59 847	1758	−2355	−103	0.985	0.96	0.082	0.20
Glycolic acid	106 725	3006	−490	617	0.9992	0.93	0.016	0.32
Propionic acid	51 655	1209	−795	−63.8	0.997	0.91	0.035	0.36
Butyric acid	53 853	3666	−637	−30.8	0.997	0.97	0.032	0.12
Formic acid	98 977	5767	−952	−400	0.998	0.96	0.027	0.18

[a] Based on peak area.
[b] Based on peak height.

Table 6
Separation parameters for IC analysis

Analyte	t_R (min)	k'_{eff}	w_b (min)	N (plates/m)	R_s	α
β-Hydroxybutyric acid	13.86	11.60	0.74	31 182	–	–
Acetic acid	14.70	12.36	0.81	29 276	1.08	1.07
Lactic acid	14.32	12.48	0.73	36 685	0.17	1.01
Propionic acid	15.30	12.91	0.66	47 769	0.68	1.03
Glycolic acid	15.94	13.49	0.63	56 904	0.99	1.05
Butyric acid	16.10	13.64	0.72	44 446	0.24	1.01
Formic acid	19.20	16.45	0.90	40 454	3.83	1.21

Conditions: borate gradient in HPIC-AS5 column (18 cm), loop of 500 μl; 1.0 ml/min; $t_0 = 1.10$ min; conductivity detection.

butyrate, which presented resolution below 0.24 and a small selectivity coefficient (less than 1%). In CE, all analytes were resolved satisfactorily, except the pair β-hydroxybutyrate/butyrate, which presented a resolution of 0.45. In general, a resolution of 0.5 is considered appropriate for quantitative work. The selectivity in CE is a consequence of differences in the analyte mobility. As it can be observed, the

Table 7
Separation parameters for CE analysis

Analyte	t_i (min)	$\mu_{eff} \cdot 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	w_b (min)	N (plates/m)	R_s	α
Formic acid	4.79	52.4	0.09	90 643	–	–
Glycolic acid	5.68	39.3	0.08	161 312	10.5	1.33
Acetic acid	5.75	38.4	0.07	215 918	0.93	1.02
Lactic acid	6.08	34.7	0.09	146 040	4.1	1.11
Propionic acid	6.17	33.7	0.09	150 396	1.0	1.03
β-Hydroxybutyric acid	6.46	30.8	0.07	272 533	3.4	1.09
Butyric acid	6.51	30.3	0.15	60 274	0.45	1.02

Conditions: 10 m*M* 3,5-dinitrobenzoic acid, 0.1 m*M* CTAB (pH 5.9); 10 s injection (5 in. Hg); −15 kV; 72 cm capillary (50 cm effective length) − 50 μm; $t_{osm} = 12.85$ min; 254 nm, indirect UV detection.

variation of effective mobility for the analyte pair β-hydroxybutyrate/butyrate was about 1.6%, suggesting that, whenever a difference in effective mobility is higher than this value, the separation is viable to be pursued experimentally.

4.4. Applications

Figs. 5 and 6 show the separation of organic anionic analytes from an atmospheric particulate matter sample by both IC and CE techniques under the optimized conditions. Although these results represent the outcome of a single sample and may not be taken as representative of a class of environmental samples, some interesting features are disclosed. Analytes 1 (β-hydroxybutyrate) and 7 (formate) are readily indicated by both methods. As mentioned before, peaks 2 (acetate) and 3 (lactate) represent a classical case of co-elution in IC [14] and, indeed, they appear as a single peak in the chromatogram. If peak 2 in the chromatogram is erroneously identified as acetate and quantified as such, a relative error of 174% would result. In other words, the concentration of acetate would be overestimated by a factor of 3. Fortunately, CE analysis can differentiate unequivocally acetate from lactate.

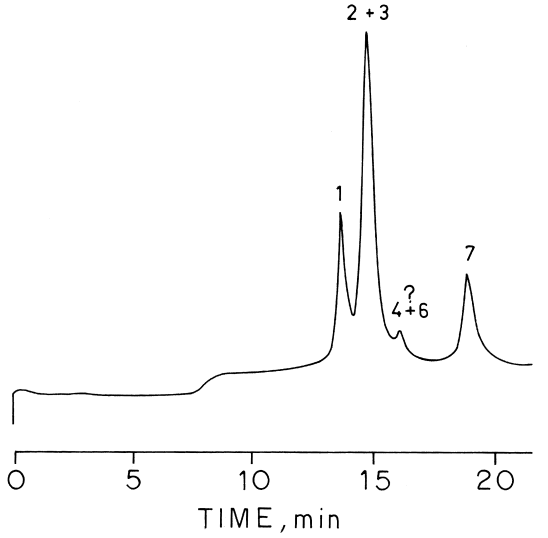

Fig. 5. Typical chromatogram of an atmospheric particulate matter sample in borate eluent system under the optimum conditions. Peaks labeled as in Table 1.

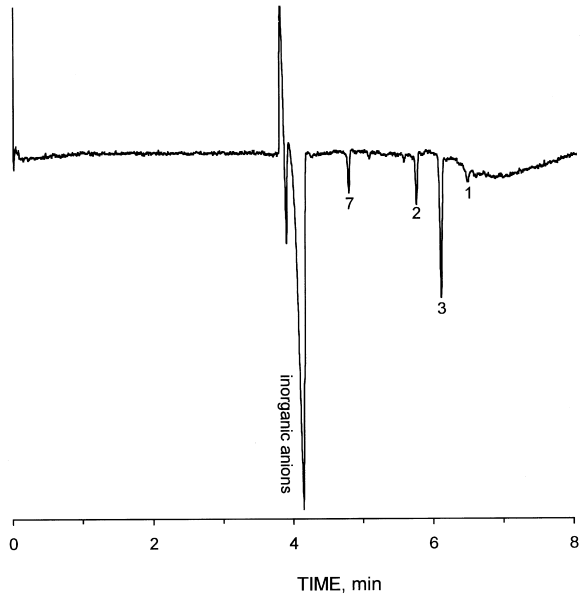

Fig. 6. Typical electropherogram of an atmospheric particulate matter sample in 3,5-dinitrobenzoic acid–CTAB electrolyte system under the optimum conditions. Peaks labeled as in Table 1.

Glycolate and butyrate co-elute under IC analysis conditions (peak labeled as 4+6? in the sample chromatogram). Since these analytes went undetected by CE, due to the method comparatively poorer sensitivity, their presence or absence in this particular sample cannot be confirmed. Even if the hydroxide eluent system is selected, only the presence of butyrate could eventually be proved, glycolate would remain co-eluted with lactate and/or propionate.

It is interesting to observe that the electropherogram presented a large initial peak. This peak corresponds to the co-migration of fast inorganic anions, such as chloride, sulfate, nitrite and nitrate, among others, also presented in the sample. In order to analyze inorganic anions, an appropriate electrolyte system of higher mobility such as chromate must be used [27].

5. Conclusions

In this work, a strategic approach to the development of methodology by IC and CE for the analysis

of anions derived from low-molecular-mass aliphatic carboxylic acids was provided, leading to near optimum conditions in a few-step procedures. Both methodologies can be applied to the analysis of environmental samples, and in this sense, they offer complementary information. The choice of the most appropriate analytical method is strictly dependent on the sample composition and concentration level of the sample components. A complete characterization of all analytes investigated in this work may not be achieved by IC analysis due to co-elution of certain analytes. On the other hand, in CE, it is possible to discriminate all analytes under investigation, but the method may lack concentration sensitivity.

The concepts presented in the proposed IC and CE models can be extended to consider other classes of analytes and a diversity of samples. One major contribution of this study is certainly within the environmental domain, where the unequivocal identification and reliable quantification of important analytes imposes a strong demand on the analytical methodology.

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References

[1] E.D. Zlotorzynska, J.F. Dlouhy, J. Chromatogr. A 671 (1994) 389.
[2] E.D. Zlotorzynska, J.F. Dlouhy, J. Chromatogr. 640 (1993) 217.
[3] K. Kawamura, H. Kasukabe, L.A. Barrie, Atmos. Environ. 30 (1996) 1709.
[4] D. Grosjean, Atmos. Environ. 26 (1992) 3279.
[5] G. Helas, H. Bingemer, M.O. Andreae, J. Geophys. Res. 97 (1992) 6187.
[6] H. Satsumabayashi, H. Kurita, Y. Yokouchi, H. Ueda, Atmos. Environ. 24 (1990) 1443.
[7] H.A. Khwaja, Atmos. Environ. 29 (1995) 127.
[8] N. Kumar, U.C. Kulshrestha, P. Khare, A. Saxena, K.M. Kumari, S. Srivastava, Atmos. Environ. 30 (1996) 3545.
[9] R. Sempéré, K. Kawamura, Atmos. Environ. 30 (1996) 1609.
[10] B.D. Barkenbus, C.S. MacDougall, W.H. Griest, J.E. Caton, Atmos. Environ. 17 (1983) 1537.
[11] S. Sollinger, K. Levsen, M. Emmrich, J. Chromatogr. 609 (1992) 297.
[12] D. Brocco, R. Tappa, J. Chromatogr. 367 (1986) 240.
[13] R. Shwarzenbach, J. Chromatogr. 251 (1982) 339.
[14] A. Ammann, T.B. Rüttimann, J. Chromatogr. A 706 (1995) 259.
[15] S. Peldszuz, P.M. Huck, S.A. Andrews, J. Chromatogr. A 723 (1996) 27.
[16] H. Miwa, M. Yamamoto, J. Chromatogr. A 721 (1996) 261.
[17] P. Jandik, G. Bonn, Capillary Electrophoresis of Small Molecules and Ions, VCH, New York, 1993.
[18] C.A. Monnig, R.T. Kennedy, Anal. Chem. 66 (1994) 280R.
[19] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[20] R. Stahl, J. Chromatogr. A 686 (1994) 143.
[21] M.A.G.T. Van Den Hoop, R.F.M.J. Cleven, J.J. Van Staden, J. Neele, J. Chromatogr. A 739 (1996) 259.
[22] P.E. Jackson, J. Chromatogr. A 693 (1995) 155.
[23] P.R. Haddad, P.E. Jackson, Ion Chromatography—Principles and Applications (Journal of Chromatography Library, Vol. 46), Elsevier, Amsterdam, 1990.
[24] R.D. Rocklin, C.A. Pohl, J.A. Shibler, J. Chromatogr. 411 (1987) 107.
[25] H.A. Laitinen, W.E. Harris, Chemical Analysis, McGraw-Hill, New York, 1975.
[26] L.R.F. Carvalho, S.R. Souza, B.S. Martinis, M. Korn, Anal. Chim. Acta 317 (1995) 171.
[27] M.F.M. Tavares, R. Colombara, S. Massaro, J.C. Rubim, J. Cap. Electrophoresis 3 (1996) 147.
[28] T. Hirokawa, M. Nishino, N. Aoki et al., J. Chromatogr. 271 (1983) D1.
[29] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, New York, 3rd ed., 1993.