

## Optimization of detection sensitivity in the capillary electrophoresis of inorganic anions

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### ABSTRACT

Published reports describing applications of various capillary electrophoresis methods to the analysis of low-molecular-weight anionic species are reviewed and evaluated. An attempt is made to summarize the parameters relevant to the optimization of detection sensitivity in capillary electrophoresis. Based on a comparison with the results obtained by other techniques, indirect photometry is shown to have a number of important advantages for the detection of anions. For a given carrier electrolyte, the range of linearity can be extended by increasing the concentration of the anion responsible for background UV absorption. With chromate-containing carrier electrolytes the linearity range could be extended over more than three orders of magnitude. Good correlation between the relative molar absorptivities and sensitivity was found for six aromatic carboxylic acids serving as carrier electrolytes. Using optimized conditions for electromigrative sample introduction, the achievable detection limits are found to be in the low nanomolar range. This represents more than a 100-fold increase in sensitivity over the results obtained by hydrodynamic sample introduction. An isotachophoretic steady state occurring during the electromigration is presented as an explanation for the trace enrichment of low levels of analytes during sample introduction by electromigration.

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### INTRODUCTION

Separations of inorganic anions by capillary electrophoresis (CE) [1–12] are characterized by their unusually high efficiency. Complex ionic mixtures can be separated in less than 10 min. This increased efficiency creates in turn new, challenging requirements for the applications of detection techniques in CE. A detection procedure is expected to be without any negative effects on the separation achieved. In most existing instruments, this is accomplished by placing the translucent separation capillaries directly in the light path of an optical detector. Such an arrangement avoids the necessity to design dead-volume-free connections between the CE capillary and the detection cell. Dictated by the geometry of the capillaries utilized, injection volumes in CE are typically three or four orders of magnitude smaller than those in liquid chromatography [e.g., 100  $\mu$ l in ion chromatography (IC) versus 10 nl in CE]. However, such a limitation in sample size does not necessarily make CE inherently less sensitive than, for example, ion chromatography. The original “handicap” of smaller injection volumes is at least partially compensated for by the considerably higher

separation efficiency. Peak heights can be expected to increase with the square root of the ratio  $N_{CE}/N_{IC}$ , where  $N_{CE}$  and  $N_{IC}$  are the respective separation efficiencies for CE and IC measured in plate counts [13]. Improved separation efficiencies thus frequently increase mass sensitivities 10–100-fold on going from IC ( $10^3$  theoretical plates) to CE ( $10^5$ – $10^6$  theoretical plates). Such an influence of improved separation efficiency on detectability helps to explain the relatively sensitive levels of CE detection reported in the literature.

#### REVIEW OF CE APPLICATIONS FOR INORGANIC ANIONS

The first separations of inorganic cations by capillary electrophoresis were reported in 1967 [14] and 1974 [15]. The first reports on applications of CE to inorganic anions began to appear between 1979 and 1990. A brief summary of the applications of CE to inorganic anions is presented in Table I. These accounts mirror the evolution of the CE technique between the end of the 1970s and today. The first two reports (1979, 1983) were generated using isotachophoretic instruments, the only electrokinetic equipment commercially available at that time. The capillaries utilized in the early work were made of PTFE or similar materials. Isotachophoretic potential gradient detectors were adapted to detect the migrating CE zones. Instrumental limitations notwithstanding, an important understanding of fundamental principles was gained and impressive separations were demonstrated by the early workers. Up to sixteen anionic species could be separated in less than 10 min. Over the years, the focus of reports has shifted from PTFE to fused-silica capillaries and from electrochemical (potential gradient, conductivity) to optical (photometric, fluorescence) detection. Detection limits were steadily improved during the same period from sensitivities essentially only in the millimolar range to the micromolar range in the most recent investigations.

The following parameters were shown in the literature to be of great importance to the optimization of detection sensitivity in CE:

- (1) Separation efficiency (see Introduction).
- (2) Matched ionic mobilities (Excessive peak spreading usually results from a mismatch between the respective mobilities of the analyte and carrier electrolyte anions).
- (3) Internal diameter of capillaries (Within a certain range, detection sensitivity increases with increase in diameter).
- (4) Method of sample introduction (Electromigration improves sensitivity).
- (5) Separation potential (Improved separation efficiency at higher voltages improves sensitivity. Overheating of electrolytes at too high potentials limits the range of available voltages).
- (6) Length of capillaries (Generally, the capillaries should be as short as possible).
- (7) Cooling (Intensive cooling reduces the thermal noise of baselines and allows higher voltages to be used for a separation).

Two of the latest reports [8,11] demonstrated sensitivity in the micromolar range by means of indirect photometric detection. As all of the state-of-the-art CE instruments are now equipped with photometric detectors, this approach to the CE detection of low-molecular-weight ionic species appears to be more universal than, for

TABLE I  
 BRIEF SUMMARY OF PUBLISHED CE APPLICATIONS FOR LOW-MOLECULAR-WEIGHT ANIONS  
 MES = 2-(N-Morpholino)ethanesulphonic acid; HEC = hydroxyethylcellulose; EOF = electroosmotic flow.

Ref.	Year	Detection mode	Carrier electrolyte	Analyte anions	Estimated detection limits
1	1979	Potential gradient	MES-histidine pH 6, 0.1% HEC	Chloride, sulphate, chlorate, chromate, adipate, benzoate, malonate, glutamate, etc.	0.0001 M
2	1983	Potential gradient	Cadmium acetate, 0.004 M	Nitrate, sulphate, chloride	0.0001 M
3	1986	Conductivity	0.01 M Butyric acid and creatinine, 1% Triton X-100, pH 4.8	Chloride, sulphate, perchlorate, nitrate, fluoride, phosphate, formate, maleate, glycolate, etc.	0.00001 M
4	1988	Conductivity	MES-histidine pH 6, 0.1% HEC	Chloride, formate, acetate, propionate	0.00001 M
5	1989	Conductivity	MES-histidine pH 6, 0.05% HEC	Seven alkyl carboxylic acids (C <sub>1</sub> to C <sub>7</sub> )	0.00001 M
6	1989	Direct UV	20 mM Phosphate	Ferro- and ferricyanide, Zn and Cu CN complexes	0.00001 M
7	1987	Indirect UV	25 mM Sodium veronal pH 8.6	Bromide, cacodylate, four alkyl carboxylic acids (C <sub>1</sub> to C <sub>4</sub> )	0.0001 M
8	1989	Indirect UV	20 mM Benzoate adjusted to pH 6.2 by histidine	Chloride, chlorate, phosphate, various carboxylic acids	10-100 μM
9	1988	Laser-induced fluorescence	250 μM Salicylate pH 7	Bicarbonate, iodate	10-100 μM
10	1989	Laser-induced fluorescence	250 μM Salicylate pH 4.0	Chloride, nitrate, perchlorate, permanganate, chromate, iodate, phosphate	10-100 μM
11	1990	Indirect UV	5 mM Chromate, EOF modifier pH 8.1	53 Inorganic and low-molecular-weight organic anions	1-8 μM

example, the applications of laser-induced fluorescence [9,10], which have also been shown to be very promising. From the theory of indirect detection techniques [16], two additional factors are expected to play a role specifically in the indirect photometric detection applied to capillary electrophoresis:

(8) Molar absorptivity coefficient of the carrier electrolyte anion (Optimization of indirect detection is essentially an attempt to increase the dynamic reserve, defined as the ratio of background signal to background noise [16]).

(9) Energy of light source (A higher energy output of a detector lamp increases the dynamic reserve).

Optimization of detection sensitivity in CE frequently follows different rules to those in conventional liquid chromatography. In considerable contrast with ion chromatography (IC), the system peaks stemming from interactions of UV absorber molecules (creating the large background signal required for indirect photometric detection) with the chromatographic stationary phase [17] are completely absent in CE. Owing to considerably decreased or non-existing interactions of UV-absorbing molecules with the capillaries, indirect photometry can be expected to play a more important role in capillary electrophoresis than in IC. CE thus offers a possibility of choice from a much larger number of molecules giving a high UV background. Indirect photometric detection has been shown to give sensitivities in the micromolar range [8,11]. An important contribution to increased sensitivity, unique to capillary electrophoresis, is optimized electrokinetic sample introduction. An isotachophoretic preconcentration step by means of a specially designed precolumn was shown to enhance detectability in CE by several orders of magnitude [18]. As determined by the Kohlrausch regulation function [19], nanomolar concentrations of ionic species from a sample can be concentrated up to millimolar levels inside an optimally designed isotachophoretic device. This paper describes our attempts to combine the detection sensitivities achievable by indirect photometry with the preconcentration capabilities inherent in electromigrative sample introduction.

## EXPERIMENTAL

### *Chemicals*

All solutions (carrier electrolytes and standards) were prepared using 18-M $\Omega$  water generated by a Milli-Q laboratory water purification system (Millipore, Bedford, MA, U.S.A.). The chromate electrolytes utilized were prepared from two different concentrated stock solutions. The first of the two concentrates contained 100 mM analytical-reagent grade Na<sub>2</sub>CrO<sub>4</sub> (Mallinckrodt, Paris, KY, U.S.A.) and 0.34 mM Ultrex sulfuric acid (J. T. Baker, Phillipsburg, NJ, U.S.A.). The second concentrate was a 20 mM solution of OFM-BT electroosmotic flow modifier (proprietary cationic surfactant obtainable from Waters Chromatography Division, Millipore, Milford, MA, U.S.A.). All carrier electrolytes prepared for this investigation contained 0.5 mM of the electroosmotic flow modifier. Addition of this chemical reverses the electroosmotic flow, so that the resulting direction is toward the anode [12]. The concentration of chromate differed from experiment to experiment and is specified in the captions to the figures.

Analytical-reagent grade chemicals were obtained as follows: benzoic and trimesic acid from Sigma (St. Louis, MO, U.S.A.), phthalic acid from J. T. Baker,

*p*-anisic and 1,2,4,5-benzenetetracarboxylic acid from Aldrich (Milwaukee, WI, U.S.A.) and salicylic acid from Mallinckrodt. All aromatic carboxylate carrier electrolytes were prepared as 5 mM solutions containing 0.5 mM OFM-BT and adjusted to pH 6.0 by an addition of an appropriate volume of 0.1 M LiOH. Solutions of carboxylic acids (1 mM) for the evaluation of molar absorptivity coefficients were prepared without addition of OFM-BT.

All carrier electrolytes were prepared fresh daily and filtered and degassed using a Millipore solvent clarification kit with a 0.45- $\mu$ m cellulose acetate filter (Millipore HA) prior to use. All standard mixtures were prepared by diluting 1000 ppm stock solutions containing a single anion. Weighed amounts of salts rather than acids were used for the preparation of stock solutions. Conventional volumetric glassware was utilized for the ppm-level dilutions. However, only polypropylene containers (Nalge, Rochester, NY, U.S.A.) were found to be suitable for storage and handling of solutions at ppb levels.

### *Instrumentation*

A Waters Quanta 4000 CE system equipped with a negative high-voltage power supply was used to generate all electropherograms. Fused-silica capillaries, 75  $\mu$ m I.D. and length 52 cm from the point of sample introduction to the point where the polyimide coating was removed to permit detection, were obtained from Waters (AccuSep capillaries). In all instances indirect UV detection was accomplished with a mercury lamp and a 254-nm optical filter. Both sample introduction modes (hydrodynamic and electromigration) available with the Quanta 4000 CE system were evaluated. Standard 4-ml Waters Wisp vials made of borosilicate glass were used as containers for the carrier electrolyte and for standards at ppm levels. Only 2-ml polyethylene sample vials (Sun Brokers, Wilmington, NC, U.S.A.) were utilized for the standard solutions of anions at ppb ( $10^9$ ) levels.

All electropherograms were recorded and evaluated with the help of a Waters Model 840 data station and a Waters SIM interface. The subsequent data processing was done with either Cricket Graph (Cricket Software, Malvern, PA, U.S.A.) or Mathematica (Wolfram Research, Champaign, IL, U.S.A.) using a Macintosh SE personal computer (Apple Computers, Cupertino, CA, U.S.A.).

UV spectra were generated in a flow-injection analysis system consisting of a Waters Model M6000 pump, a Rheodyne (Cotati, CA, U.S.A.) Model 7010 injector and a Waters Model 990 photodiode-array detector. Water pumped at 1 ml/min carried 100- $\mu$ l samples from the injector to the detector, which was set to acquire UV spectra between 200 and 400 nm.

## RESULTS AND DISCUSSION

### *Indirect UV detection in carrier electrolytes containing chromate*

Chromate was chosen as a carrier electrolyte providing a suitable UV absorbance background in a wide range of wavelengths, while at the same time matching the ionic mobility of seven common inorganic anions (fluoride, carbonate, chloride, nitrite, bromide, nitrate, phosphate and sulfate) more closely than, for example, the benzoic acid used in previous investigations [11].

Fig. 1 shows some typical UV spectra of low-molecular-weight anions. All

substances were evaluated as 1 mM solutions. Broad UV absorption of the chromate anion in comparison with the narrow absorption by other anionic species is clearly demonstrated by this comparison. One of the principal requirements of the carrier electrolyte is to provide a maximum dynamic reserve for the largest possible number of anions, including those which are themselves UV absorbing. Significant UV absorption of an analyte anion at a wavelength chosen for indirect detection considerably decreases the dynamic reserve. Decreased sensitivity of detection and too narrow linear ranges of calibration are observed as a result of such a mismatch of UV-absorbing properties of analyte and electrolyte anions.

Vanadate is the only other inorganic anion in Fig. 1 having a broader range of UV absorption than the three aromatic carboxylic acids. However, the usefulness of this anion as a carrier electrolyte constituent is limited by its precipitation in 0.5 mM solutions of the electroosmotic flow modifier below pH 10.

Typical separation and detection limits for common inorganic anions are shown in Fig. 2. The sensitive detection limits demonstrated here can be seen as a direct result of optimization of the parameters discussed above. The mercury lamp chosen for detection exhibits a high energy output at 254 nm and the UV absorption by chromate sufficiently exceeds that by the analyte anions.

If a calibration graph is generated under the conditions given in Fig. 2 for six different concentrations in the range 2–50 ppm, correlation coefficients in excess of 0.995 are obtained. However, if a more rigorous linearity test, such as the evaluation of the slope of a log (signal) *versus* log (concentration) plot, is employed, the assumption of linearity is not confirmed. As seen for the calibration of sulfate anion in Fig. 3A in the concentration range 2–50 ppm, the slope of the logarithmic plots lies outside the specified linearity range of 0.98–1.02 [20]. It is only within a narrower range of

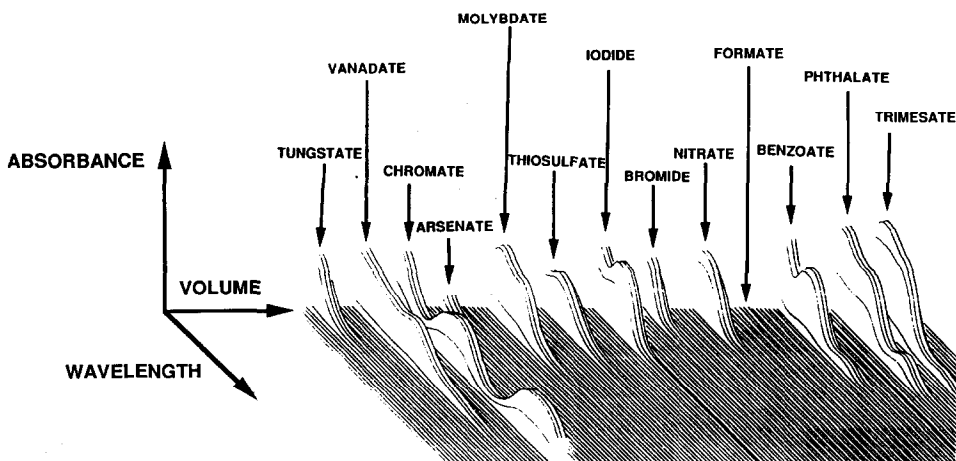


Fig. 1. Typical UV spectra of some inorganic and organic anions. The spectra were recorded between 200 and 400 nm. The range of sensitivity was set at 0–1.1 absorbance units. The volume between any two sample zones was determined by selected time intervals between two injections into the flow-injection analysis (FIA) apparatus and does not represent any separation effect. A more detailed description of the measurement is given under Experimental.

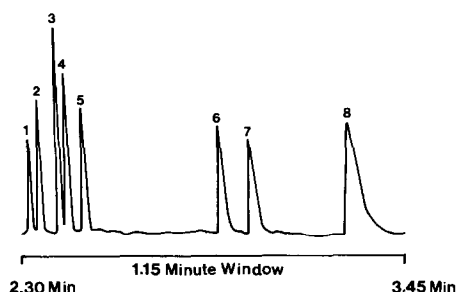


Fig. 2. Separation of inorganic anions using indirect photometric detection at 254 nm. The carrier electrolyte contained 5 mM chromate and 0.5 mM electroosmotic flow modifier and was adjusted to pH 8.1. A fused-silica capillary (75  $\mu\text{m}$  I.D., 52 cm from the point of sample introduction to the detector) was used for the separation. During the analysis the injection side was at  $-20$  kV. Hydrodynamic injection was carried out at a 10-cm height for 30 s. The peak identities, concentrations injected (ppm) and detection limits (three times the noise in  $\mu\text{M}$ ) were as follows: 1 = bromide, 2 ppm, 4.8  $\mu\text{M}$ ; 2 = chloride, 2 ppm, 4.2  $\mu\text{M}$ ; 3 = sulfate, 2 ppm, 1.8  $\mu\text{M}$ ; 4 = nitrite, 2 ppm, 7.2  $\mu\text{M}$ ; 5 = nitrate, 2 ppm, 5.6  $\mu\text{M}$ ; 6 = fluoride, 1 ppm, 5.2  $\mu\text{M}$ ; 7 = phosphate, 4 ppm, 4  $\mu\text{M}$ ; 8 = carbonate, 2 ppm, 2.2  $\mu\text{M}$ .

concentrations (2–30 or 2–15 ppm) where this calibration graph can be considered to be completely linear. According to our experimental results, the linearity range can be extended by increasing the concentration of the carrier electrolyte. In 7 mM chromate the slope of the logarithmic plots for 2–50 ppm standards becomes 0.99–1.00, indicating a reasonable linearity of calibration in that range.

Fig. 3B summarizes the results of another series of linearity tests. At a concentration of 5 mM the chromate electrolyte fails the linearity test in the range between 50 and 200 ppm (slope = 0.80). Even an increase in chromate concentration to 7 mM does not suffice to achieve linearity in this range of higher concentrations (slope = 0.90). If, however, the concentration of chromate is increased to 9 mM, good linearity of calibration can be observed not only between 50 and 200 ppm, but also over more than three orders of magnitude, between 0.5 and 200 ppm of sulfate.

#### *Evaluation of aromatic carboxylic acids for use in carrier electrolytes*

The main usefulness of carboxylates as components of carrier electrolytes lies in the CE analysis of less mobile anions (fluoride, carboxylic acids, alkylsulfonates, etc.) producing broadly asymmetric peaks, if analyzed in the chromate electrolyte. On the other hand, because of their relatively low mobilities, aromatic carboxylic acids are not very suitable as electrolytes for the analysis of complex mixtures of highly mobile inorganic anions (bromide, chloride, sulfate, nitrite and nitrate). The requirement of closely matched mobilities between the respective anionic components of a carrier electrolyte and the analyte anions makes it necessary to develop a whole group of highly UV-absorbing carrier electrolytes, covering the range of ionic mobilities of all inorganic anions and other, low-molecular-weight, anionic species such as carboxylic acids, amino acids, carbohydrates and sulfonates. It is therefore of practical interest to compare the molar absorptivity coefficients of common water-soluble aromatic carboxylic acids.

Three aromatic carboxylates in Fig. 1 show sufficiently high levels of UV absorption to be considered useful as carrier electrolyte anions for indirect UV

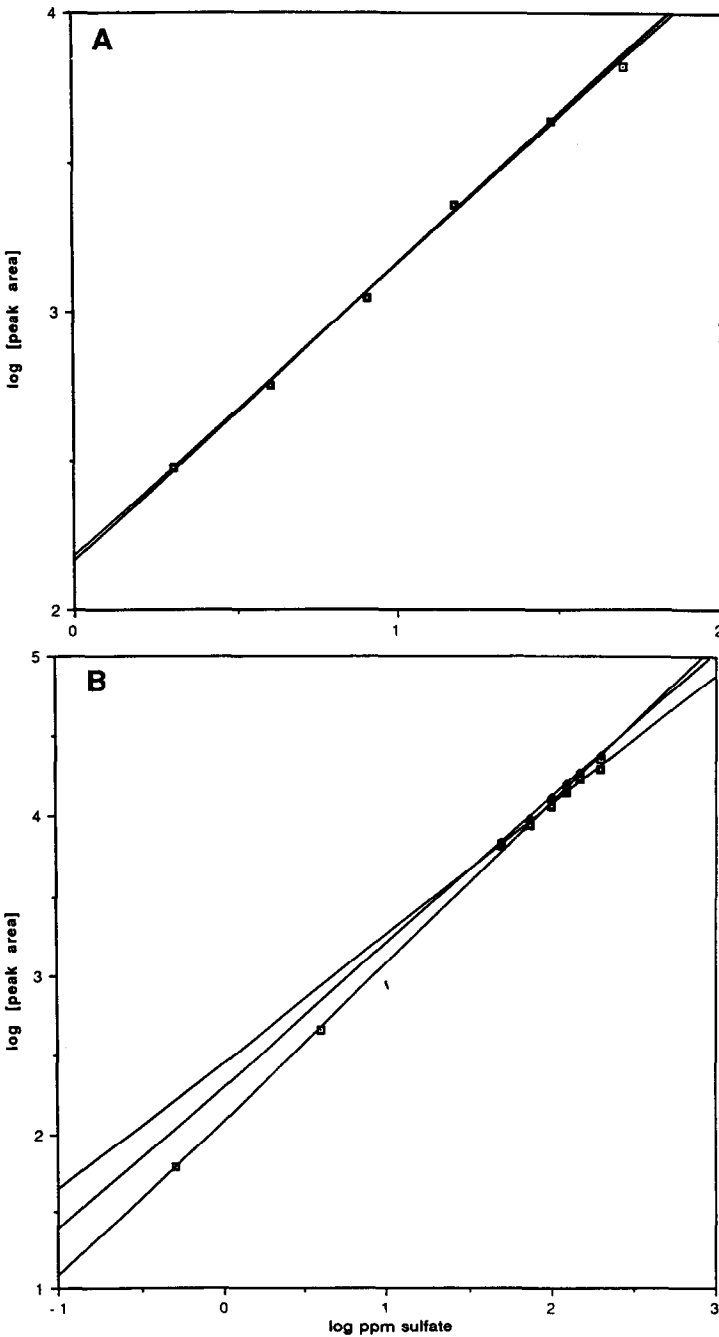


Fig. 3. Linearity tests using logarithmic plots [20]. Calibration graphs are considered linear only for slopes between 0.98 and 1.02. (A) Evaluation of linearity in 5 mM chromate electrolyte. Three calibration graphs for three different concentration ranges are overlaid. CE conditions were as specified in Fig. 2. (□) 2–50 ppm,  $y = 2.1790 + 0.97848x$ ; (●) 2–30 ppm,  $y = 2.1658 + 0.99799x$ ; (■) 2–15 ppm,  $y = 2.1648 + 0.99956x$ . (B) Linearity evaluation between 0.5 and 200 ppm using three different concentrations of chromate electrolyte. Linear regression was calculated only in the range between 50 and 200 ppm for 5 and 7 mM electrolytes. CE conditions as specified in Fig. 2, except for the changes in the concentrations of chromate. (□) 9 mM chromate,  $y = 2.0749 + 1.0013x$ ; (●) 7 mM chromate,  $y = 2.2923 + 0.90673x$ ; (■) 5 mM chromate,  $y = 2.4419 + 0.80981x$ .



detection. The molar absorptivity coefficients of these three carboxylates together with those of several others were evaluated in aqueous solutions of pH 6. The relative molar absorptivity coefficients correlated in Fig. 4 were obtained by dividing the absorbance of a given carboxylate by the absorbance of benzoate at the same wavelength and concentration (254 nm and 1 mM, respectively). The relative molar absorptivity of chromate on this scale equals 3.07. As shown, a very good correlation exists between the intensity of UV absorption by the carrier electrolyte molecule and the sensitivity of indirect photometric detection measured as peak area obtained for 10 ppm propionate.

The discussed correlation is also of great practical usefulness in the optimization of detection sensitivity. As seen in Fig. 4, the sensitivity of indirect UV detection can be increased 20-fold on going from salicylate to *p*-anisate. Sensitivity improvements by at least one order of magnitude can be expected by changing from the currently very popular benzoate to other, more suitable UV-absorbing components of carrier electrolytes (*p*-anisate, *p*-hydroxybenzoate, etc.).

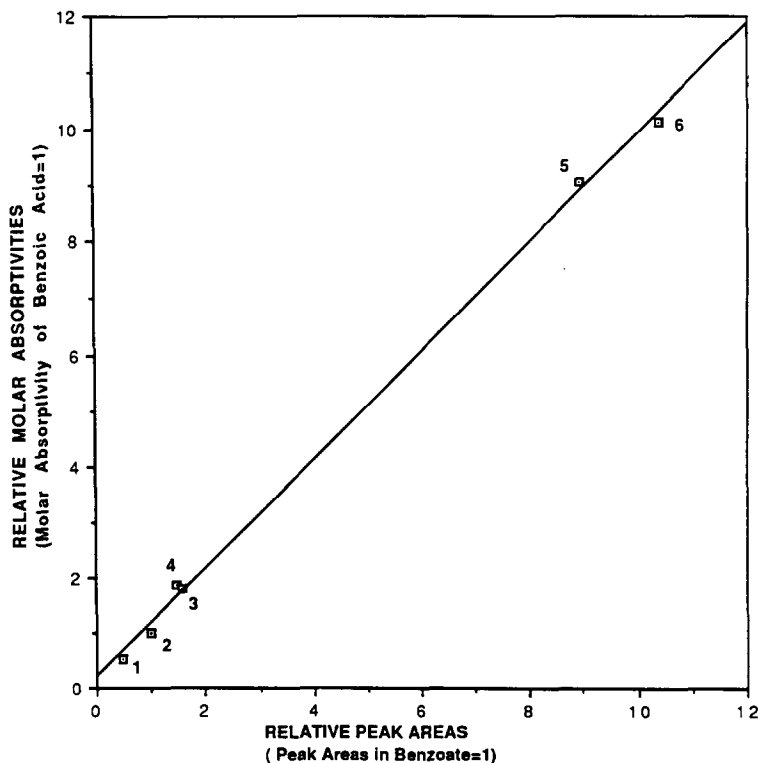


Fig. 4. Correlation of relative molar absorptivities and peak areas for 10 ppm propionic acid introduced by a 15-s hydrodynamic injection. The carrier electrolyte contained 5 mM of one of the six aromatic carboxylic acids in a mixture with 0.5 mM electroosmotic flow modifier adjusted to pH 6.0. The separations were carried out at -20 kV. 1 = Salicylate; 2 = benzoate; 3 = *o*-phthalate; 4 = trimesate; 5 = benzenetetra-carboxylate; 6 = *p*-anisate.  $y = 0.19349 + 0.97249x$ ;  $R^2 = 0.998$ .

### Improving sensitivity by optimized electromigrative sample introduction

Under optimum conditions, an electromigrative sample introduction in CE can be expected to yield an isotachophoretic distribution of ionic concentrations inside the separation capillary [8]. Such a distribution is governed by a relationship introduced by Kohlrausch [19] (Kohlrausch regulation function):

$$\frac{c_x}{c_1} = \frac{m_x}{m_x + m_c} \frac{m_1 + m_c}{m_1} \quad (1)$$

$c_x$  and  $c_1$  are the concentrations of the analyte anion and leading electrolyte anion in their respective isotachophoretic zones and  $m_x$ ,  $m_1$  and  $m_c$  are the ionic mobilities of the analyte anion, leading electrolyte anion and the common counter cation, respectively. The ionic mobilities are directly proportional to ionic equivalent conductances. It is therefore possible to generate plots depicting the dependence of the ratio  $c_x/c_1$  on the respective ionic equivalent conductances of a common cation and leading electrolyte anion. Fig. 5 was developed using the value of the ionic equivalent conductance of  $40 \text{ cm}^2 \text{ equiv}^{-1} \text{ ohm}^{-1}$  for the analyte anion.

For chromate and other carrier electrolytes fulfilling the requirements placed on leading electrolytes (*i.e.*, higher ionic mobility than that of the analyte anion), it is reasonable to expect the ratio  $c_x/c_1$  to lie within the range *ca.* 0.2–1. Even if this applies only to a very narrow zone created during an optimized electromigrative sample introduction, a high enrichment factor can be accomplished, especially in situations where the sample concentration is in the nanomolar range and the concentration of the leading electrolyte anion is adjusted to 5–20 mM.

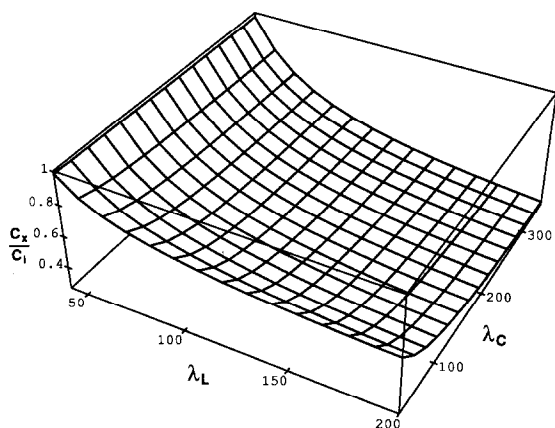


Fig. 5. Kohlrausch regulation function. As direct proportionality exists between the ionic equivalent conductance and ionic mobilities, equivalent conductances are taken directly for calculations of the ratio  $c_x/c_1$  of analyte to carrier anion concentrations according to eqn. 1. The range of leading electrolyte equivalent conductances ( $\lambda_L$ ) was chosen between 41 and 200  $\text{cm}^2 \text{ equiv.}^{-1} \Omega^{-1}$  to cover a large number of possible carrier electrolyte anions, from carboxylates (*ca.* 40) to hydroxide (198). Common cation equivalent conductances ( $\lambda_C$ ) are considered in the range 35–350  $\text{cm}^2 \text{ equiv.}^{-1} \Omega^{-1}$  to represent the largest possible selection of cations between alkylammonium (*ca.* 30) and hydronium (349.7) ions.

Another important requirement for such a preconcentration effect is the presence of an anion acting as a terminating electrolyte. Its ionic mobility must be smaller than that of any analyte anion. Such terminating electrolyte anions may be added on purpose. In solutions containing total ionic concentrations in the nanomolar range, the sample conductivity becomes too low and has to be adjusted by a suitable additive to permit a sufficient electric charge throughput for ionic transfer from the bulk of the sample solution into the capillary. Four possible stages of such optimized sample introduction by electromigration are represented in Fig. 6.

Chromate, citrate, carbonate and octanesulfonate solutions were evaluated as electromigration additives. In agreement with the rules discussed above, additions of a carrier electrolyte anion to the sample do not lead to the establishment of an isotachophoretic steady state and in consequence do not produce any useful enrichment of low-level analytes by electromigration. This was confirmed in an experimental series with chromate as a carrier electrolyte and chloride as an analyte. Citrate, carbonate and octanesulfonate, on the other hand, do exhibit lower ionic mobilities in comparison with chromate and can thus be used as additives for electromigrative trace enrichment with chromate-containing carrier electrolytes.

Of the remaining three anions, the best results were obtained with sodium octanesulfonate adjusted within the range 15–40  $\mu\text{M}$  in the low ionic content samples. Simultaneously with the preconcentration of analyte anions, it is also possible to observe the enrichment of anions acting as an isotachophoretic terminating electrolyte (see Fig. 6C). In contrast to carbonate and citrate with short CE migration times, addition of relatively excessive concentrations of octanesulfonate does not lead to an interfering comigration with any of the over 50 anionic species analyzable by the CE method under discussion. An additional benefit is that unlike, *e.g.*, citrate, sodium octanesulfonate can be obtained free from common ionic impurities that disturb the determination of common anions such as sulfate and chloride in unknown samples. An example of the determination of common inorganic anions at low ppb levels is shown in Fig. 7. As indicated, the detection limits (calculated as three times the noise in concentration units) are in the low nanomolar range for this separation. This represents approximately a 400-fold increase in sensitivity in comparison with the results achievable in the same carrier electrolyte by hydrodynamic sample introduction.

## CONCLUSIONS

Linearity of calibration graphs in CE with indirect photometric detection depends on the total concentration of UV-absorbing molecules in the carrier electrolyte. Linearity across three to four orders of magnitude is possible in chromate electrolytes.

The sensitivity of indirect photometric detection was found to be directly proportional to molar absorptivities for the series of six aromatic carboxylic acids. Application of, *e.g.*, *p*-anisate instead of benzoate or salicylate results in 10–20-fold improvements in sensitivity.

The experimental findings confirm the role of isotachophoretic conditions during the electromigrative sample introduction prior to a separation by capillary electrophoresis. With common inorganic anions, preconcentration can be accomplished

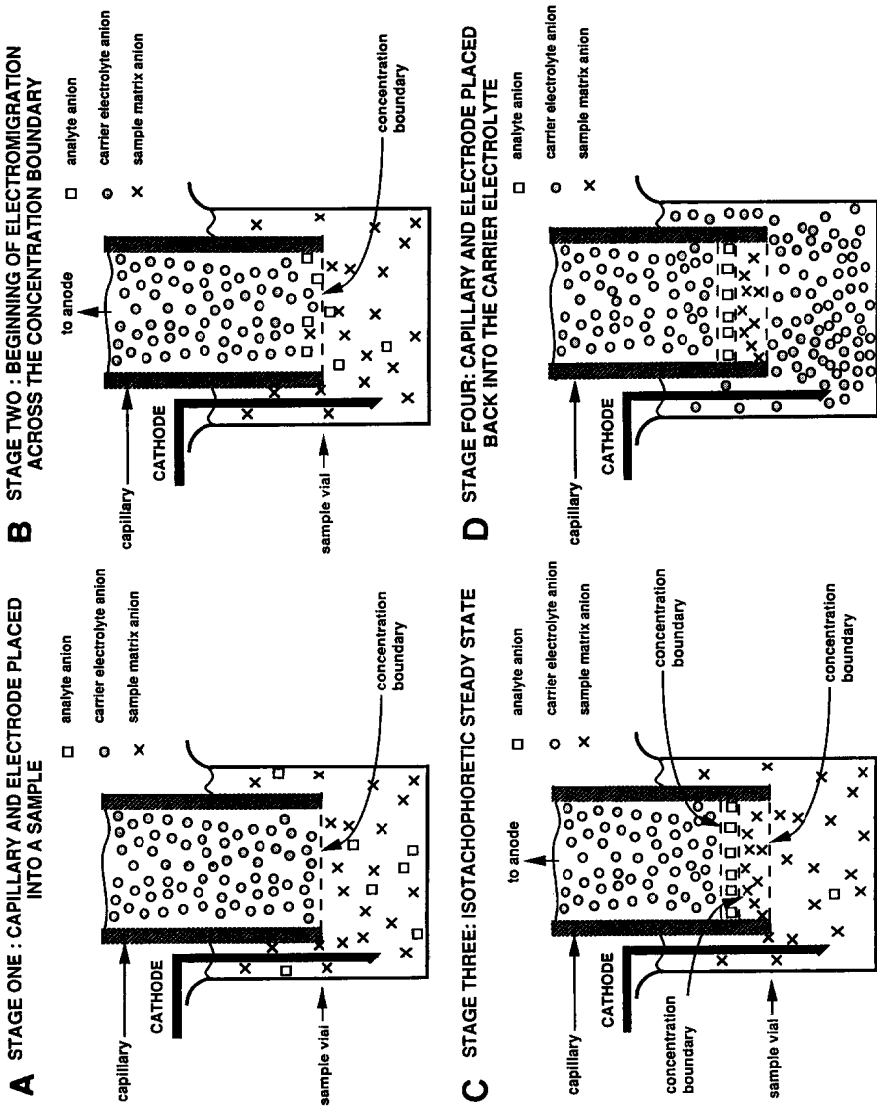


Fig. 6. Four stages of sample introduction by electromigration. Another possible way to describe stage three is as "frontal electrophoresis" [15].

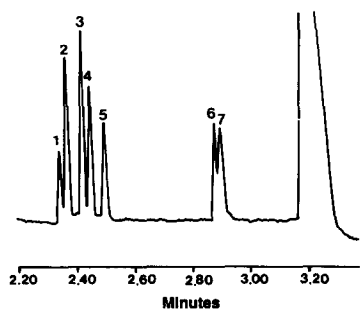


Fig. 7. CE separation after trace enrichment by electromigration. The capillary, carrier electrolyte, separation voltage and detector settings were identical with those in Fig. 2. The electromigrative sample introduction was carried out at 5 kV for 45 s. Sample conductivity was adjusted by addition of octanesulfonate at 18  $\mu$ M in the sample. The peak identities, concentrations (ppb) and detection limits (nM) (three times the noise) were as follows: 1 = bromide, 4 ppb, 13.6 nM; 2 = chloride, 4 ppb, 13 nM; 3 = sulfate, 4 ppb, 8.4 nM; 4 = nitrite, 4 ppb, 25.4 nM; 5 = nitrate, 4 ppb, 24 nM; 6 = fluoride, 2 ppb, 19.8 nM; 7 = phosphate, 8 ppb, 17.8 nM. The large peak at ca. 3.2 min is carbonate. The levels of carbonate were not controlled under the conditions of our experiments.

directly in the separation capillary immersed in a sample containing very low concentrations of analyte anions. One of the requirements for the preconcentration of anions present at very low levels is the addition of a suitable anion capable of acting as an isotachophoretic terminating electrolyte.

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