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Review

Electrokinetic injection in capillary electrophoresis and its application to the analysis of inorganic compounds

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Abstract

In capillary electrophoresis, electrokinetic injection is a highly controversial sampling technique. It is a simple mode of sample introduction which is suitable for on-line preconcentration of the analytes, but its precision and accuracy are more strongly affected by experimental conditions compared to hydrodynamic injection. In the first part of this paper the features of electrokinetic and hydrodynamic injections are compared, followed by a detailed discussion on the different biases of electrokinetic injection and on how to reduce them. Finally, applications of the electrokinetic injection are reviewed with special emphasis on the analysis of inorganic compounds.

Keywords: Reviews; Electrokinetic injection; Injection methods; Capillary electrophoresis; Hydrodynamic injection; Inorganic anions; Inorganic cations

Contents

1. Introduction

In capillary electrophoresis (CE) the bottleneck of accurate and precise quantitative analysis is primarily the sample introduction. The sample is usually introduced into the capillary either by hydrodynamic (HD) or electrokinetic (EK) injection (often also called electromigration injection). In HD injection the sample is driven into the capillary by a pressure difference which can be established by applying either vacuum at the detector side or pressure at the

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injection side, or alternatively, by lifting the sample vial above the electrolyte level (hydrostatic injection). In EK injection the driving force is the electric field produced by the injection voltage applied to the electrode immersed in the sample solution. In the majority of the works in CE, HD injection is preferred because it is theoretically more established and less prone to measurement errors than EK injection. Nevertheless, because of its instrumental simplicity and applicability for on-line preconcentration of the analytes, EK injection has been gaining ground in CE since its introduction in the 1980s [1–11]. This paper is aimed to give a review on EK injection in the hope that it might contribute to clarifying the features, advantages, drawbacks and potential applications of EK injection in CE.

2. Comparison of electrokinetic and hydrodynamic sample introductions

The most important features of EK and HD injections are compared in Table 1. EK injection is definitely the simplest way of sample introduction. Unlike HD injection, there is no need for additional sampling apparatus, injection can be carried out with the same instrumental set-up used for the separation.

In EK injection the quantity of the analytes injected (n_a) can be expressed as follows [2,5]:

$$n_a = \frac{(\mu_a + \mu_{EOF})r^2\pi}{L} \cdot U_{inj} t_{inj} c_a \quad (1)$$

where μ_a is the electrophoretic mobility of the analyte, μ_{EOF} is the mobility of the electroosmotic flow (EOF), r is the inner radius of the capillary, L is the total length of the capillary, U_{inj} is the injection voltage, t_{inj} is the injection time, c_a is the molar concentration of the analyte.

Eq. (1) shows that the quantity of the analytes injected is linearly proportional to the sample concentration and can be controlled by the injection voltage and injection time. The injected volume of the sample (V_s) is determined by the EOF [12]:

$$V_s = \frac{\mu_{EOF} r^2 \pi}{L} \cdot U_{inj} t_{inj} \quad (2)$$

In HD injection V_s can be calculated by the Poiseuille 's law [5,13]:

$$V_s = \frac{r^4 \pi}{8\eta L} \cdot \Delta p_{inj} t_{inj} \quad (3)$$

where η is the dynamic viscosity of the sample, Δp_{inj} is the pressure difference used for injection.

The injected amount of the species can be obtained by multiplying V_s with the concentration of the species in the sample:

$$n_a = V_s c_a = \frac{r^4 \pi}{8\eta L} \cdot \Delta p_{inj} t_{inj} c_a \quad (4)$$

The comparison of Eqs. (1) and (4) reveals the fundamental difference between the two injection modes. In HD injection the only component dependent parameter is the concentration of the analyte, consequently sampling is non-discriminating and

Table 1
Comparison of electrokinetic and hydrodynamic injections

Parameter	Electrokinetic injection	Hydrodynamic injection
Instrumental requirements	No additional apparatus required	Additional apparatus to produce pressure difference is needed
Selectivity of injection	Selective for ions	Non-selective
Repeatability	0.2–2% for migration time 2–5% for peak area	0.1–0.5% for migration time 0.5–3% for peak area
Dynamic range	1–2 orders of magnitude	2–3 orders of magnitude
Sensitivity	Relatively high, but ion-dependent	Relatively low
Matrix effect	Significant	Negligible
Main application area(s)	Sample preconcentration, gel electrophoresis	Routine quantitative analysis

representative for the chemical composition of the sample. In EK injection, however, the quantity of the species injected is also a function of mobility and representative sample introduction can only be expected for neutral solutes. For ions EK injection is discriminative. Ions of high mobility are introduced into the capillary at higher rate than ions of low mobility and by selecting the electrode polarity either negative or positive ions can be entirely excluded from the column. This phenomenon is called mobility bias and will be discussed in detail in Section 3.1.

The relevance of Eqs. (1) and (4) was studied by several groups by recording the peak height or peak area as a function of the injection parameters. In HD injection peak area was unambiguously linear with the injection pressure and injection time [5,14,15]. In EK injection the results are more ambiguous. Rose and Jorgenson [5] found a linear relationship between n_a and U_{inj} or t_{inj} for a sample containing a single neutral component (mesityl oxide). The same conclusion was drawn by Robson et al. [16] for benzophenone in acetonitrile–water eluent using capillary electrochromatography. On the other hand, however, if inorganic and organic anions (chloride, nitrate, formate, acetate) were studied both linear and non-linear relationships were obtained [14]. If U_{inj} was -3 kV the quantities of all the ions injected increased linearly with injection time (1–30 s). If, however, U_{inj} was -6 or -9 kV linearity was no longer valid for formate and acetate. Furthermore, at constant injection time n_a was not linear with U_{inj} for any ion. The ratios of the injected quantities of the ions at $U_{inj} = -3$, -6 and -9 kV were (1:1.59:1.72), (1:1.45:1.50), (1:1.25:0.98) and (1:0.83:0.05) for chloride, nitrate, formate and acetate, respectively, instead of the theoretical ratio of (1:2:3). It can be concluded that the lower the mobility of the ion, the larger deviation from the theory can be observed, and for ions of low mobility n_a was even decreased by increasing injection voltage. The authors assumed that the reason for this unexpected results might be the depletion of the ions near the capillary tip due to the limiting diffusion transport from the bulk of the solution. This effect is most pronounced for ions of low diffusion velocity and/or if the injection voltage is high. Moring et al. [15] also observed deviations from Eq. (1) by studying proteins and obtained convex-shaped curves with increasing electrokinetic injection time (Fig. 1). Similar results were presented by Unger and Stöckigt [17] for alkaloids, like berberine and chelidonine. The phenomenon was attributed to the changing sample composition during injection. Furthermore, Lee and Yeung [18] pointed out that if the conductivity of the sample zone is nearly identical to that of the buffer, the injected amounts of the ions are proportional with the electric current. At constant injection voltage, however, electric current may significantly vary during the injection, especially at longer injection times.

The repeatability of the measurements is usually better using HD injection. Typical relative standard deviations (R.S.D.s) for migration time and peak area are 0.1–0.5% and 0.5–3%, respectively. With EK

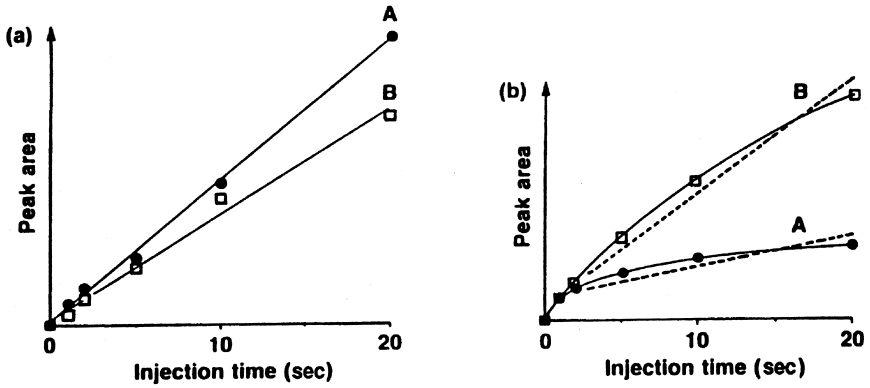

Fig. 1. Linearity of (a) vacuum and (b) electrokinetic injection as a function of injection time. (a) Injection, vacuum: 5.0 in. of Hg; run buffer: 20 m*M* bicine–TEA, pH 8.5; run voltage: 25 kV; current 4 μA. (b) Injection voltage: 5 kV; other conditions as in (a). Curves: A = β-Lactoglobulin; B = horse heart myoglobin. Samples were dissolved in water (100 μg/ml). Reprinted from Ref. [15].

injection these values are in most cases two- to three-times higher, especially if inorganic ions are analysed [19,20]. Migration times in EK injection are more uncertain, probably because the chemical composition of the sample zone changes continuously during injection affecting the migration velocity of the ions. As far as the injected quantity is concerned, repeatability in EK injection is worse than in HD injection despite the fact that the injection voltage can be controlled very accurately. Ermakov et al. [21] evaluated the accuracy of sample introduction on two commercially available instruments and found EK injection more accurate than HD injection. The explanation for these confusing results is that the driving force in EK injection is not the voltage, but the local electric field which may change significantly if the conditions of injection in the solution (e.g., local chemical composition of the sample, position of the electrode and the capillary in the sample vial) vary (see Section 3.3). In some cases, however, precision obtained by EK injection was found to be comparable with or even better than that obtained by HD injection. Smith et al. [10] reported about 1% precision analysing quaternary phosphonium salt mixture. In the quantitative determination of insulin [22] and in testing a mixture of dansyl amino acids [11] peak response was more repeatable using EK than HD injection. In one of the studies high precision was also achieved for cations, R.S.D. values were in the range of 0.032–0.046% and 0.95–3.17% for migration time and peak area, respectively [23]. By the application of an internal standard repeatability could be further improved for both HD [24–26] and EK injections [22,27–32]. It was also shown that higher precision can be obtained if the peak areas are corrected for the integrated electric current detected during injection [18]. In addition, it is worth noting that in the low concentration range the analytical precision of EK may be better than the precision obtained by HD injection. This is, however not due to the higher precision of EK injection, but the higher signal-to-noise ratio achieved by the preconcentration of the analytes during sample introduction.

Reproducibility over time is usually also better for HD injection. This is because any change of the condition of the capillary affects much more readily the EK injection. Churaev et al. [33] proved that during the lifetime of the capillary the surface charge density of the inner wall can change by hydrolysis and/or by adsorption of the ions. This surface modification may cause change in the electroosmotic mobility, and consequently, in the injected quantity. Sometimes this effect can occur on a relatively short time-scale as it can be seen in Fig. 2 [5]. This shift in the injected amount of the ions can influence the quantitative measurements daily, and to reduce this source of error frequent in-run calibration is required.

Separation efficiency for the two introduction methods was also compared [5]. If approximately the same amount of analytes were injected, about the same values for the number of theoretical plates were obtained. These values were in the range of 128 000–149 000, implying that peak broadening was probably determined by longitudinal diffusion and the effect of the sample injection on the separation efficiency was negligible. Theoretically, EK injection contributes to zone broadening to a lesser extent than HD injection, because the leading edge of the sample zone in EK injection has plug profile compared to the parabolic shape in HD injection. Furthermore, electrostacking effect (see Section 4) can also narrow the sample zones during the EK injection. These advantages of EK sampling can be exploited primarily for the separation of components of low diffusion coefficient, e.g., proteins, where the number of theoretical plates is in the order of millions [34].

Dynamic range of the methods is usually larger using HD injection. This was shown for example by Lee and Lin [20] by studying metal ions in the range of 0.01–1 m*M* (Fig. 3). For HD injection calibration curves were linear over the whole working concentration range. In EK injection linearity was restricted below the concentration of 0.1 m*M* and at higher concentrations the curves became hyperbolic. In most cases linear calibration curves can be obtained using EK injection if the concentration of the ions is in the ppb or at most in the low ppm range. Jimidar et al. [29] reported the extension of the dynamic range by the use of an internal standard. Linearity could also be extended to higher concentrations by a special ‘‘wire loop’’ injection technique (see in more detail in Section 3.3) [14].

Other drawbacks of EK injection are that the

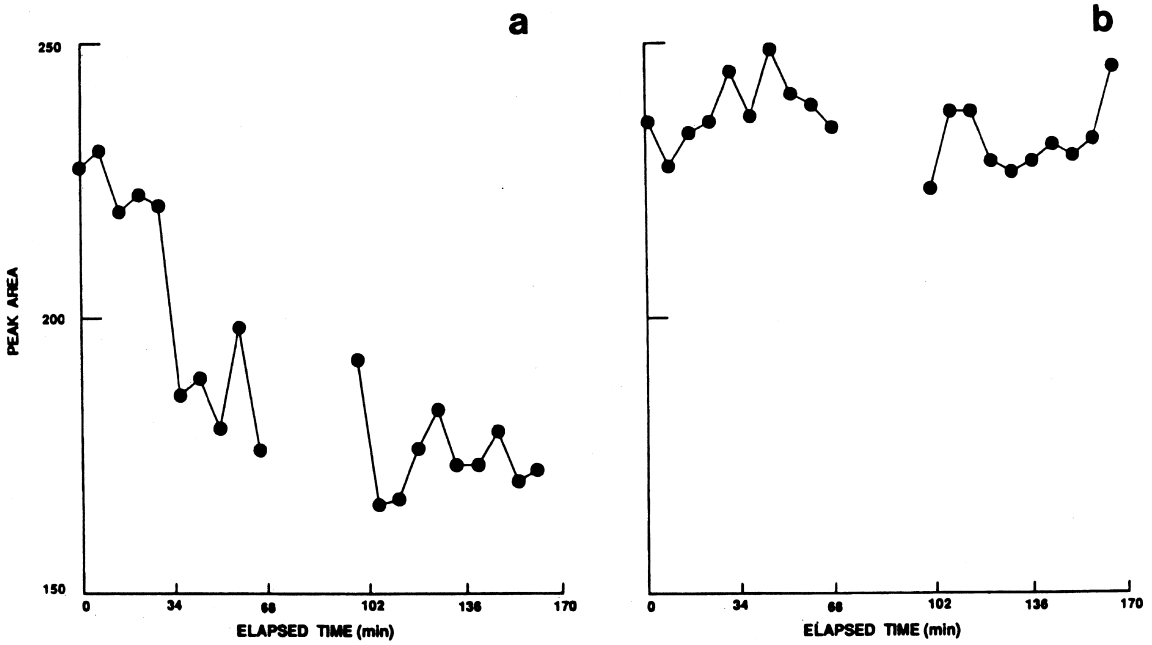

Fig. 2. Reproducibility of sample injection methods as a function of elapsed time: (a) two groups of 10 electromigration introductions (5 s at 10 kV), average peak area±standard deviation (20 runs) 190±22, 11.4% relative standard deviation; (b) two groups of 10 hydrodynamic flow introductions (10 s at 10 cm), average peak area±standard deviation (20 runs) 223±7, 2.8% relative standard deviation; operating buffer, 0.01 *M* phosphate, pH 7; sample, 0.1% (v/v) mesityl oxide; 75 μm I.D. capillary. Reprinted with permission from Ref. [5], ©(1988) American Chemical Society.

injected quantity of the analytes is matrix-dependent (see Section 3.2 for more details), the electric current flowing through the sample during injection can affect its chemical composition by electrochemical reactions and the Joule heating as a result of the high voltage may also cause some damage in the sample.

The main advantage of EK injection is that sensitivity of the methods can be by several orders of magnitude higher, and consequently, the limit of detection (LOD) correspondingly lower than using conventional HD injection. It must be emphasised, however, that sensitivity enhancement favours the ions of high mobility (mobility bias). Nevertheless, one of the most important field of the applications of EK injection is the on-line preconcentration of the analytes (see Sections 4 and 5), while for routine-like qualitative and quantitative analysis HD injection is used. It should be added for the sake of completeness that sensitivity of HD injection with sample stacking can be comparable with that of EK injection and in these cases HD injection is usually preferred to avoid disadvantages of EK injection [35].

3. Biases in electrokinetic injection

Based on Eq. (1) two different kinds of bias can be distinguished. The reason for these biases is the fact that the absolute amounts of the injected ions are the function of ion mobilities. This means that in any sample more ions of high mobility are introduced into the capillary than those of low mobility (mobility bias). The second bias can occur between samples of different ionic composition and conductivity. Since the mobility of both the ions and the electroosmotic flow can be strongly influenced by the pH and conductivity of the sample, even if the concentration of the ions is held constant the injected amount of the ions will vary according to the composition of the sample matrix (matrix bias). This

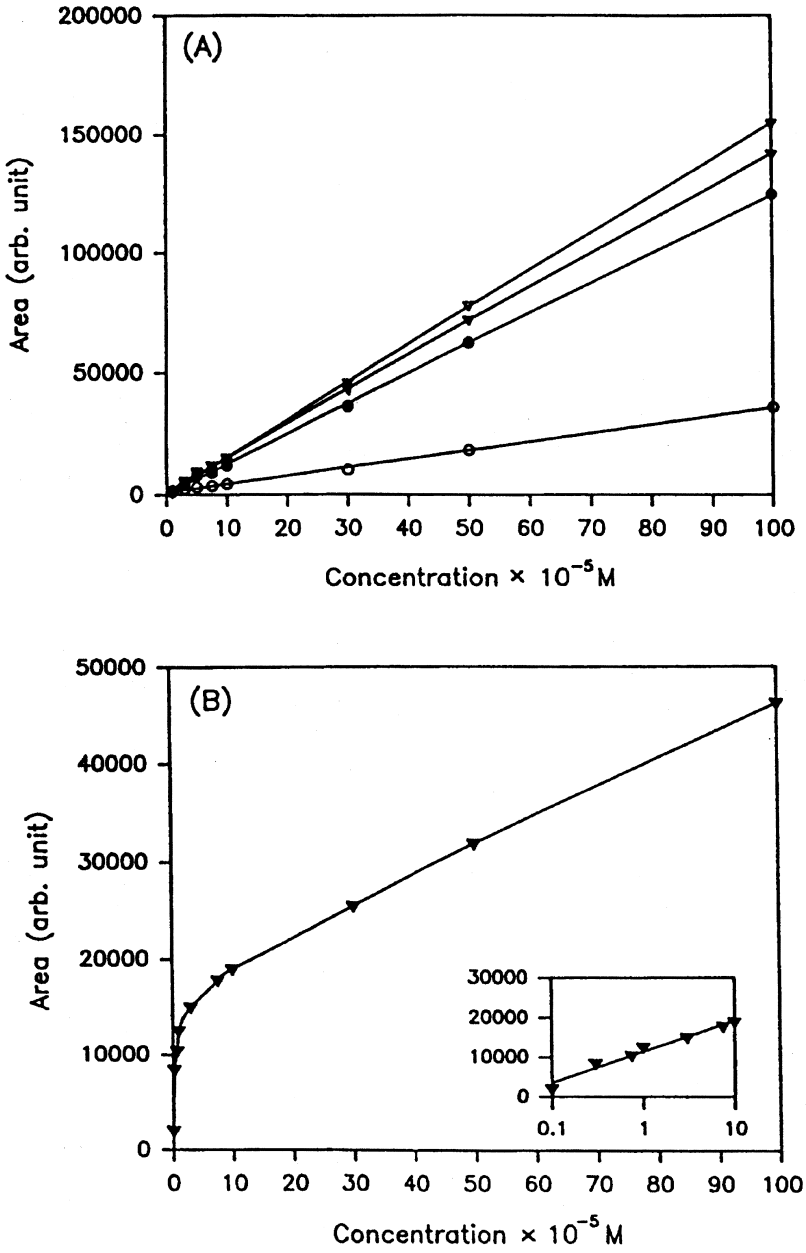

Fig. 3. Comparison of calibration graphs for selected metal ions in the (A) HD and (B) EK modes. ○=K; ●=Ca; ▽=Cd; and ▼=Zn. Only the Zn ion graph is shown in the EK mode. The inset is an enlargement for concentrations below 0.1 m*M*; operating buffer, 10 m*M* pyridine–12 m*M* glycolic acid (pH 4.0). Reprinted from Ref. [20] with permission.

means that the peak areas obtained for the same ions in samples of different matrices cannot be compared directly. There is a third group of biases which is related to the instrumentation of EK injection (instrumental biases).

3.1. Bias within one sample – the mobility bias

Although mobility bias was already mentioned in 1981 by Jorgenson and Lukacs [36] and later by Tsuda and co-workers [37,38], the first more detailed

discussion was given by Huang et al. in 1988 [6]. They showed that the ratio of the amounts of the species 1 and 2 $[n_a(1)/n_a(2)]$ injected electrokinetically can be read as follows:

$$\frac{n_a(1)}{n_a(2)} = \frac{\mu_a(1) + \mu_{EOF}}{\mu_a(2) + \mu_{EOF}} \cdot \frac{c_a(1)}{c_a(2)} = b \cdot \frac{c_a(1)}{c_a(2)} \quad (5)$$

where b was called the bias factor. If $b=1$ then $n_a(1)/n_a(2)$ is directly proportional to $c_a(1)/c_a(2)$ and the injection is free of mobility bias. In many cases the bias factor can be one or close to unity. One example is the injection of neutral compounds when the species are driven into the capillary only by the EOF. Another case is when the mobilities of the ions are very similar (e.g., proteins). For inorganic ions, however, the mobility bias can be quite high because the mobilities of these ions may vary in a wide range and usually predominate over the mobility of EOF.

Two different technical solutions were suggested to reduce the effect of mobility bias in EK. Wu et al. [39] isolated the injection end of the capillary from the electric field by an on-column Nafion joint to ensure that the sample is driven only by the EOF into the capillary. Dasgupta and Surowiec [14] used a wire loop as the high voltage electrode for injection. A thin film of the sample solution was formed on this wire loop and exhaustive EK injection of the ions were performed within 1 min, and mobility bias was practically negligible.

Other researchers looked for mathematical corrections. Huang et al. [6] showed that the bias factor is the ratio of the migration times of the analytes:

$$b = \frac{t_{mig,a}(2)}{t_{mig,a}(1)} \quad (6)$$

and by measuring migration times the results can be corrected for the mobility bias. According to the authors the requirement of the applicability of Eq. (6) is that the mobility of both the ions and the EOF are constant during the whole analysis including the injection and the separation. They pointed out that this condition can be met in practice if the concentration of the ions in the sample is low compared to that of the running buffer and if injection and separation voltages are the same. Correction of the mobility bias with the migration time was reconsidered by van der Moolen et al. [40]. Migration time was expressed as

$$t_{mig,a} = \frac{lL}{U_{sep}(\mu_a + \mu_{EOF})} \quad (7)$$

where l is the effective length of the capillary, U_{sep} is the separation voltage.

By multiplying Eq. (1) by Eq. (7) the following equation was obtained:

$$n_a t_{mig,a} = lr^2 \pi c_a t_{inj} \cdot \frac{U_{inj}}{U_{sep}} \quad (8)$$

In Eq. (8) the value of $n_a t_{mig,a}$, apart from the instrumental parameters, depends only on the concentration of the analyte. Based on Eq. (8) and in contradiction with Huang et al. [6] the authors claim in one hand, that correction is also possible if the injection and the separation voltages are different, and in other hand, that the sample should be dissolved in the electrolyte in order to keep the ion mobilities constant during the whole process. This is, however, not advantageous for the preconcentration of the ions.

Correction for the mobility bias is very simple if on-column UV detection is used, in which the magnitude of the peak area is influenced by the velocity of the sample zone [40,41]. The lower the mobility of the zone is to larger extent the peak area (A_a) is artificially increased:

$$A_a = \alpha \cdot \frac{\epsilon_a d}{\mu_a + \mu_{EOF}} \cdot \frac{n_a}{r^2 \pi} \quad (9)$$

where α is constant deriving from the integration of the signal over the capillary window length, ϵ_a is the molar absorption coefficient of the analyte, d is the optical path length.

This type of detection bias is eliminated by dividing the measured peak area with the migration time of the analyte. However, if Eqs. (1) and (9) are combined then the equation

$$A_a = \alpha d t_{inj} \frac{U_{inj}}{L} \cdot \epsilon_a c_a \quad (10)$$

is obtained indicating that mobility bias and UV detection bias counteract each other and mobility bias is eliminated if the peak area obtained by UV detection is not corrected for the velocity of the sample zone. However, it should be emphasised

again that this is true only if mobilities are the same during the injection and separation. This condition might not hold in practice. We think that mobility bias should be considered as a special feature of EK injection and should not or cannot be corrected in all cases. If EK injection is used for quantitative analysis the mobility bias can be taken into account in the calibration.

3.2. Bias between samples – the matrix bias

If the ionic strength of the samples is different the mobility of the ions and the EOF will also be different and the relative amounts of the same species introduced into the capillary will depend not only on the relative concentrations, but also on the relative mobilities [6]:

$$\frac{n_a(S_1)}{n_a(S_2)} = \frac{\mu_a(S_1) + \mu_{EOF}}{\mu_a(S_2) + \mu_{EOF}} \cdot \frac{c_a(S_1)}{c_a(S_2)} \tag{11}$$

where S_1 and S_2 denote sample 1 and 2, respectively. This bias was studied first by dissolving Li^+ and K^+ in a buffer of different concentrations. The peak area of the ions was found to be inversely proportional with the sample conductivity (Fig. 4). Carnerio et al. [42] observed similar relationship during the study of herbicides.

Later Lee and Yeung [18] showed that

$$\frac{n_a(S_1)}{n_a(S_2)} = \left[\frac{1/t_{EOF} + \kappa_{el}/\kappa(S_2)(1/t_{mig,a} - 1/t_{EOF})}{1/t_{EOF} + \kappa_{el}/\kappa(S_1)(1/t_{mig,a} - 1/t_{EOF})} \right] \cdot \frac{c_a(S_1)}{c_a(S_2)} \tag{12}$$

where t_{EOF} is the migration time related to the EOF, $t_{mig,a}$ is the migration time of the analyte, κ_{el} is the conductivity of the electrolyte, $\kappa(S_1)$ and $\kappa(S_2)$ are the conductivities of sample S_1 and S_2, respectively.

Eq. (12) implies that if the conductivities of the electrolyte and the samples are known matrix bias can be corrected mathematically. The method was found to be applicable if sample introduction is determined by electromigration. In this case an accuracy $<5\%$ was achieved.

Leube and Roeckel [43] proposed the use of a matrix factor which was defined as the ratio of the peak areas of the analyte in the sample matrix and in

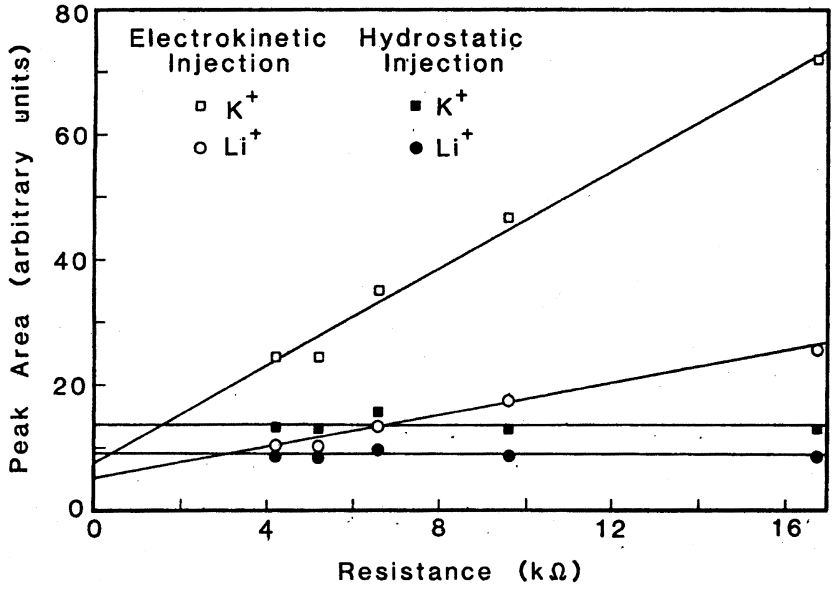

Fig. 4. Plot of K^+ and Li^+ peak areas as a function of sample solution resistance for both electrokinetic and hydrostatic injection. Electrokinetic injection causes a bias linear in sample solution resistance (which is inversely proportional to electrolyte concentration). Reprinted with permission from Ref. [6], ©(1988) American Chemical Society.

the calibration solution. Provided that the matrix factor is known it can be used to calculate the matrix-corrected peak area from the matrix-affected peak area of the same analyte. The method was applied for biological samples and accuracy was found to be <5%. The theory behind this procedure is very simple and straightforward, but its application in practice might be limited. First, the composition of the sample matrix has to be determined in order to prepare standard samples with the same matrix for the determination of the matrix factor. Furthermore, change of the matrix factor as a function of the matrix composition has to be evaluated. Unfortunately, matrix factor can significantly vary with pH and conductivity of the sample (see Figs. 5 and 6, respectively). This means that the use of the matrix factor is feasible only if the composition of the matrix is expected to be the same in all samples. Anyway, matrix factor should be determined one by one for each sample that would make the analysis very time-consuming and labour-intensive.

Another way of the correction of the matrix bias is the application of two internal standards proposed by Dose and Guiochon [27], but tested for this purpose by Lee and Yeung [18]. The method is based on the fact that the injected amounts of the analytes are in

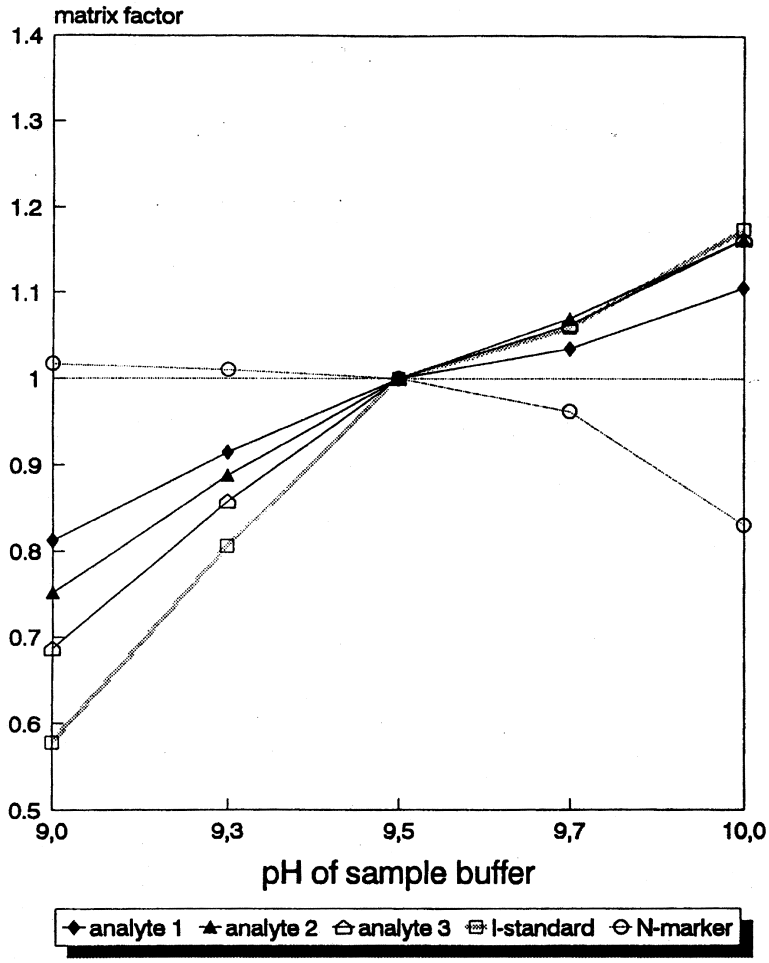

Fig. 5. Matrix factors F_{Mxi} of analytes 1, 2 and 3, internal standard (I, standard), and neutral marker (N marker) as a function of sample buffer pH. Reprinted with permission from Ref. [43], ©(1994) American Chemical Society.

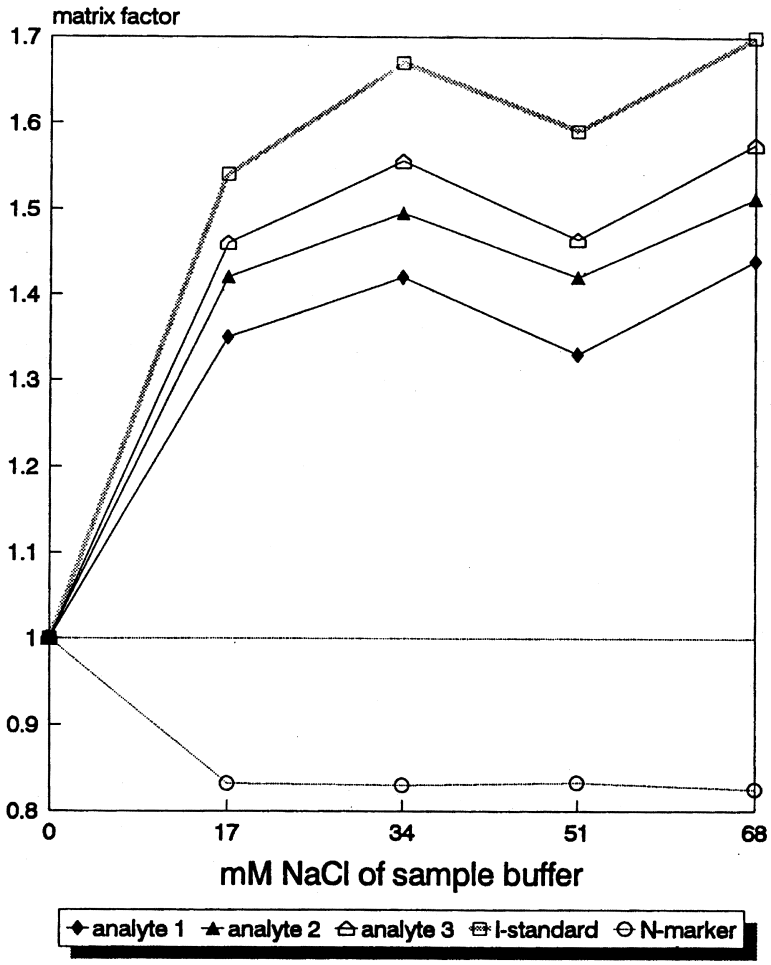

Fig. 6. Matrix factors F_{Mxi} of analytes 1, 2 and 3, internal standard (I, standard), and neutral marker (N marker) as a function of sample buffer conductivity (molar content NaCl). Reprinted with permission from Ref. [43], ©(1994) American Chemical Society.

linear relationship with their mobilities, and by selection of two internal standards, one of which mobility is lower (standard A) and one of which mobility is higher (standard B) than that of the analyte, an interpolation constant (F_a) can be defined as

$$F_a = \frac{\mu_a - \mu_A}{\mu_B - \mu_A} = \frac{t_{mig,A} - t_{mig,a}}{t_{mig,A} - t_{mig,B}} \cdot \frac{t_{mig,B}}{t_{mig,a}} \quad (13)$$

where $t_{mig,A}$ and $t_{mig,B}$ are the migration times of standard A and B, respectively.

By means of the interpolation constant the concentration of the analyte (c_a) can be calculated as follows:

$$c_a = \frac{A_a}{R_a t_{mig,a} \cdot \left[F_a \cdot \frac{A_A}{R_A c_A t_{mig,A}} + (1 - F_a) \cdot \frac{A_B}{R_B c_B t_{mig,B}} \right]} \quad (14)$$

where A_a, A_A and A_B are the peak areas of the analyte, the standard A and B, respectively, R_a, R_A and R_B are the response factors for the same order, c_A and c_B are the concentrations of the standard A and B, respectively.

This method was compared to the matrix factor method and was found to be useful also in cases in

which the matrix factor method failed. The accuracy of the two methods were about the same (<5%).

The ''most historical'' and perhaps the most practical way for the correction of the matrix bias is the standard addition method. A good example of that is the work of Jackson and Haddad [28] who determined fluoride in bore water containing chloride at high concentration. Although fluoride response was considerably affected by chloride concentration (Fig. 7, upper trace) standard addition curve for

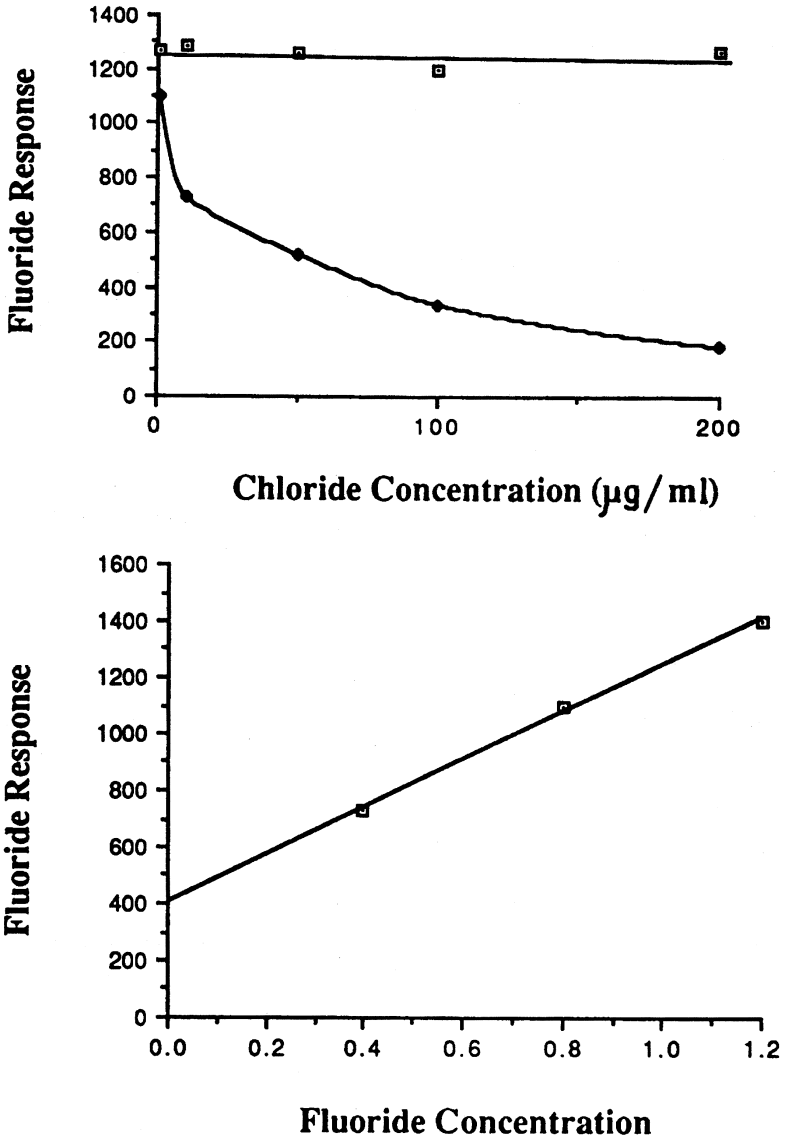

Fig. 7. Upper trace: Effect of chloride concentration on fluoride response using hydrostatic and electromigration injection. Conditions: capillary, 60 cm×75 μm I.D. fused-silica; power supply, negative at 20 kV; electrolyte, 5 m*M* chromate with 0.5 m*M* CIA-Pak OFM Anion-BT at pH=8; injection, hydrostatic for 30 s, electromigration, 3 kV for 10 s; detection, indirect UV at 254 nm. Solutes: fluoride (1 μg/ml); chloride as indicated. Lower trace: Standard addition calibration plot for fluoride in bore water using electromigration injection. Solutes: Fluoride spiked at 0.4, 0.8 and 1.2 μg/ml; original fluoride sample concentration calculated to be 0.47 μg/ml. Reprinted from Ref. [28] with permission.

fluoride was linear (Fig. 7, lower trace), and the results were in a good agreement with those obtained by ion chromatography. The main disadvantage of this method is that the number of the measurements are at least doubled.

3.3. Instrumental biases

It was demonstrated by some authors [42,44] that the peak area using EK injection is also influenced by the volume of the sample. The effect is more pronounced if the sample volume is small (less than 200 μl) (Fig. 8). The observation was explained in one hand, by the variation of the liquid height above the capillary, and in the other hand, by changing the magnitude of the electric field over the sample solution with the changing volume. In addition, de Boer and Ensing [44] emphasised the importance of the relative position of the high-voltage electrode and the tip of the capillary during the injection.

Guttmann and Schwartz [45] observed continuous decrease of the analytical signal when performing consecutive runs from the same, low-volume (50 μl) sample in capillary gel electrophoresis of DNA (Fig. 9). The phenomenon was explained by the electrochemical processes taking place during the injection and the artifact was partially eliminated by performing water injection between runs (see Fig. 9). The same problem was studied theoretically by Effenhauser [46] who pointed out the particular impor-

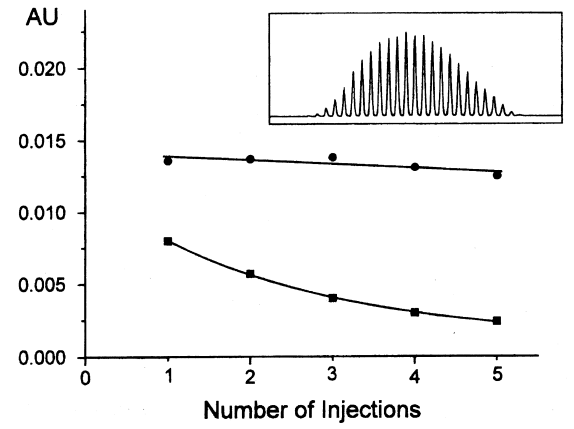

Fig. 9. Peak height of the $p(dA)_{50}$ (absorbance unit) versus numbers of consecutive injections. Inset: Capillary gel electrophoresis separation of the test mixture of $p(dA)_{40-60}$; time frame, 20–27 min. Conditions: eCAP-ssDNA-100 gel-filled capillary column; $E=300$ V/cm; $t=20$°C; sample, 7.4 μg/ml total DNA in water; injection, (■) 1.5 s, 7.5 kV electrokinetic injection from sample vial (''regular injection''), and (●) 1.5 s, 7.5 kV electrokinetic injection from sample vial preceded by a 1.5 s, 7.5 kV electrokinetic injection from water (''water preinjection''). Reprinted with permission from Ref. [45], © (1995) American Chemical Society.

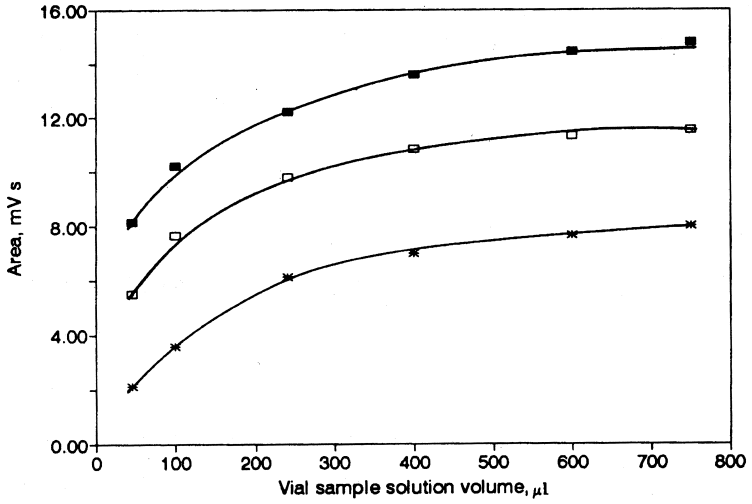

Fig. 8. Effect of vial sample solution volume on peak area. Standard aqueous solution: paraquat (78.0 μg/l), diquat (91.8 μg/l) and difenzoquat (102.2 μg/l). Electrokinetic injection, 10 s. □=Paraquat; ■=diquat; ★=difenzoquat. Reprinted from Ref. [42] with permission.

tance of this bias in relation to microvials and micromachined CE devices.

4. General applications of electrokinetic injection

In one part of the applications EK injection is used for instrumental reasons. In capillary gel electrophoresis where the capillary is filled with a non-convective media (e.g., polyacrylamide gel), usually this type of sample introduction is applied, because pressure injection is either impractical or damaging to the gel in the column [47–52]. Padarauskas et al. [53] built a CE system for the simultaneous separation of inorganic cations and anions. The samples are introduced by EK injection at both ends of the capillary at the same time and the detection window is formed at the middle of the capillary. In this special set-up HD injection cannot be applied. EK injection is also preferred in miniaturised CE systems formed on a chip [54,55].

Recently, CE is becoming part of hyphenated techniques. Since one of the most important requirements for these hyphenated methods is easy automation, EK injection is used in most cases, especially if CE is not the first unit in the system. CE system with EK injection was linked to reversed-phase high-performance liquid chromatography (HPLC) [56] and size-exclusion chromatography (SEC) [57] for the two-dimensional separation of proteins and peptides. In another case a microreactor–SEC–CE on-line coupling was accomplished for DNA sequencing [58]. Busher et al. [59] developed an on-line electrodialysis–CE system where electromigration takes place through a membrane and acts as a separator between the small anions and the other compounds. Other pre-treatment procedures, like dialysis and gas diffusion were also connected to CE instruments [60,61]. Compounds are transported continuously to the capillary by a flow injection analysis (FIA) system for EK injection to CE. The procedures are fully automated and used for the determination of small anions in water, soft drinks, vinegar and wine. Nowadays, the most developing area in this field is perhaps the CE–mass spectrometry (MS) coupling [3,10,62,63]. However, Valaskovic and McLafferty [63] reported two sources of systematic error using EK injection with CE–electrospray interface. One of the errors is due to the hydrodynamic flow induced in the capillary by the evaporation of the buffer solvent at the tip of the interface. The second error is also caused by the hydrodynamic flow resulted from the electrostatic influence of the electrospray process on the separation buffer. The first bias can be eliminated by inserting the tip of the interface in the buffer solution during injection, while for the second bias no exact solution is known yet. Ion-selective microelectrodes were also coupled to CE using EK injection for the potentiometric detection of metal ions [64].

In most of the works the purpose of using EK injection is the on-line preconcentration of the analytes. If the velocity of the EOF is low or negligible ions can be introduced into the capillary in a smaller volume of solvent. Moreover, if the sample is dissolved in pure water or in a solvent of lower conductivity than that of the separation buffer the electric field at the injection point will be higher than in the capillary and ions can be concentrated in narrow zones (sample stacking or field amplified CE). Sample stacking can be applied both to HD and EK injection (the latter is often called electrostacking) [65].

Electrostacking was already mentioned by Hjertén et al. in 1965 [66], but was more thoroughly studied by Chien and Burghi much later [67,68]. Under general conditions either cations or anions can be concentrated by choosing the proper electrode polarity. In the presence of EOF only ions migrating in the same direction as the EOF can be stacked. Moreover, the enrichment factor was found to be smaller than expected when the capillary was switched directly between the separation buffer and the sample solution. The efficiency of electrostacking can be improved by injecting a short plug of water or low conductivity buffer hydrodynamically before the sample introduction. In this case both cations and anions can be concentrated. The steps of this form of electrostacking are shown in Fig. 10. The beneficial influence of electrostacking on the peak intensities and peak shapes of the compounds can be seen in Fig. 11. In practice, electrostacking occurs automatically when ions are analysed in a sample of low conductivity (e.g., high purity water).

Another and perhaps the most effective way of

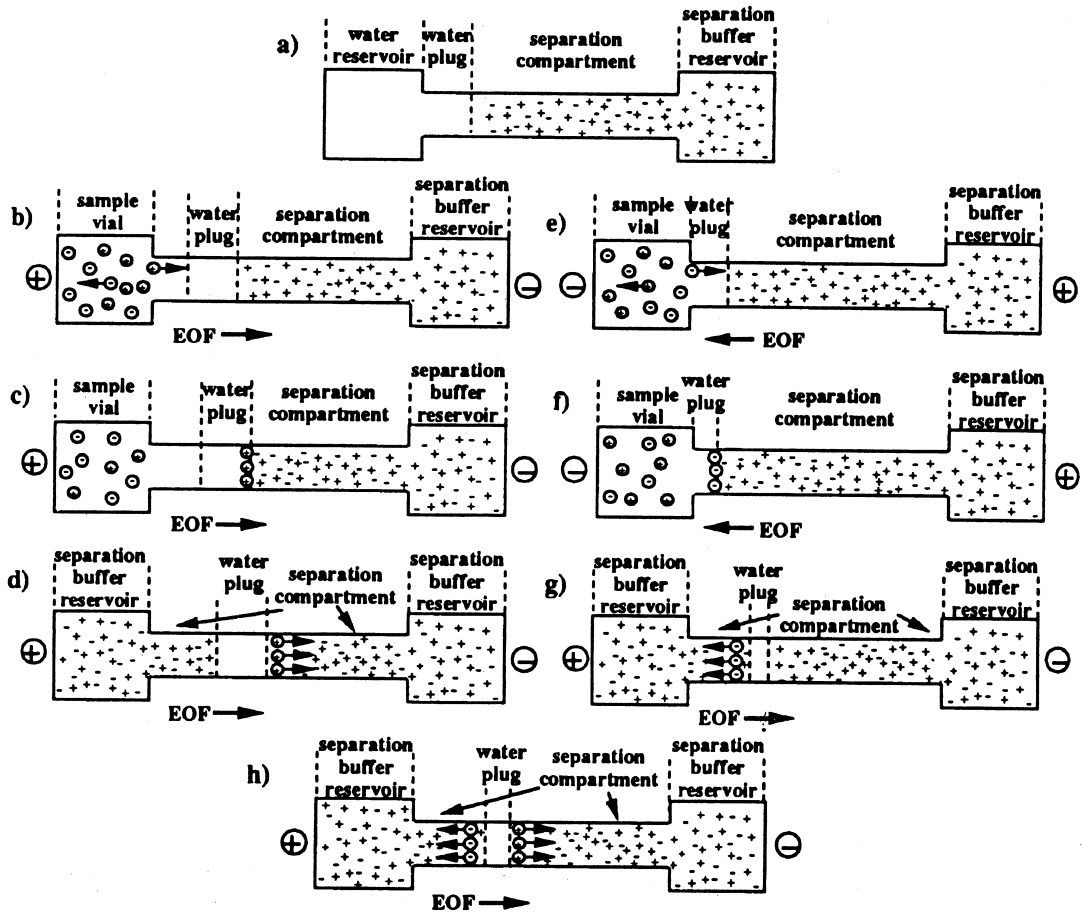

Fig. 10. Sample stacking after electrokinetic injection of positively (b–d) and negatively (e–g) charged analytes, respectively. For the sake of simplicity the counter charges are not shown in the water plug region. (a) Hydrodynamic injection of a sort plug of water or low-conductivity buffer. (b, e) Electrokinetic injection of the sample by choosing the proper polarity. (c, f) Stacking of the analytes at the front boundary of the water plug. (d, g) Migration of the analytes out of the water plug and start of the separation. Before leaving the water plug region negatively charged analytes migrate to the rear boundary of the water plug when the polarity of the arrangement is switched (not shown). (h) Start of the separation after polarity-switching electrokinetically injection for the simultaneous analysis of cations and anions. Reprinted with permission from Ref. [12], ©1993 Springer Verlag.

preconcentration is EK injection supported by isotachophoretic effect. In this configuration the mobility of the electrolyte ion should be higher than those of the ions in the sample in order to act as a leading electrolyte, and terminating ion must be added on purpose to the sample. Isotachophoretic preconcentration was primarily used for inorganic anions with the high-mobility chromate electrolyte [69–72]. The magnitude of the enrichment factor is a function of the quality and the concentration of the leading and the terminating electrolytes, and also depends on the sample concentration. About 400-fold increase in the sensitivity for inorganic anions was achieved if the concentration of chromate was 5–20 m*M*, the terminating ion was octanesulfonate at a concentration of 40 μ*M* and the concentration of the analytes was in the nanomolar range. There are additional benefits of adding terminating ion to the sample. If the total ionic concentration of the sample is very low (it actually is in the nanomolar range) terminating ion supports the ionic transfer from the bulk to the capillary by increasing the sample conductivity. At

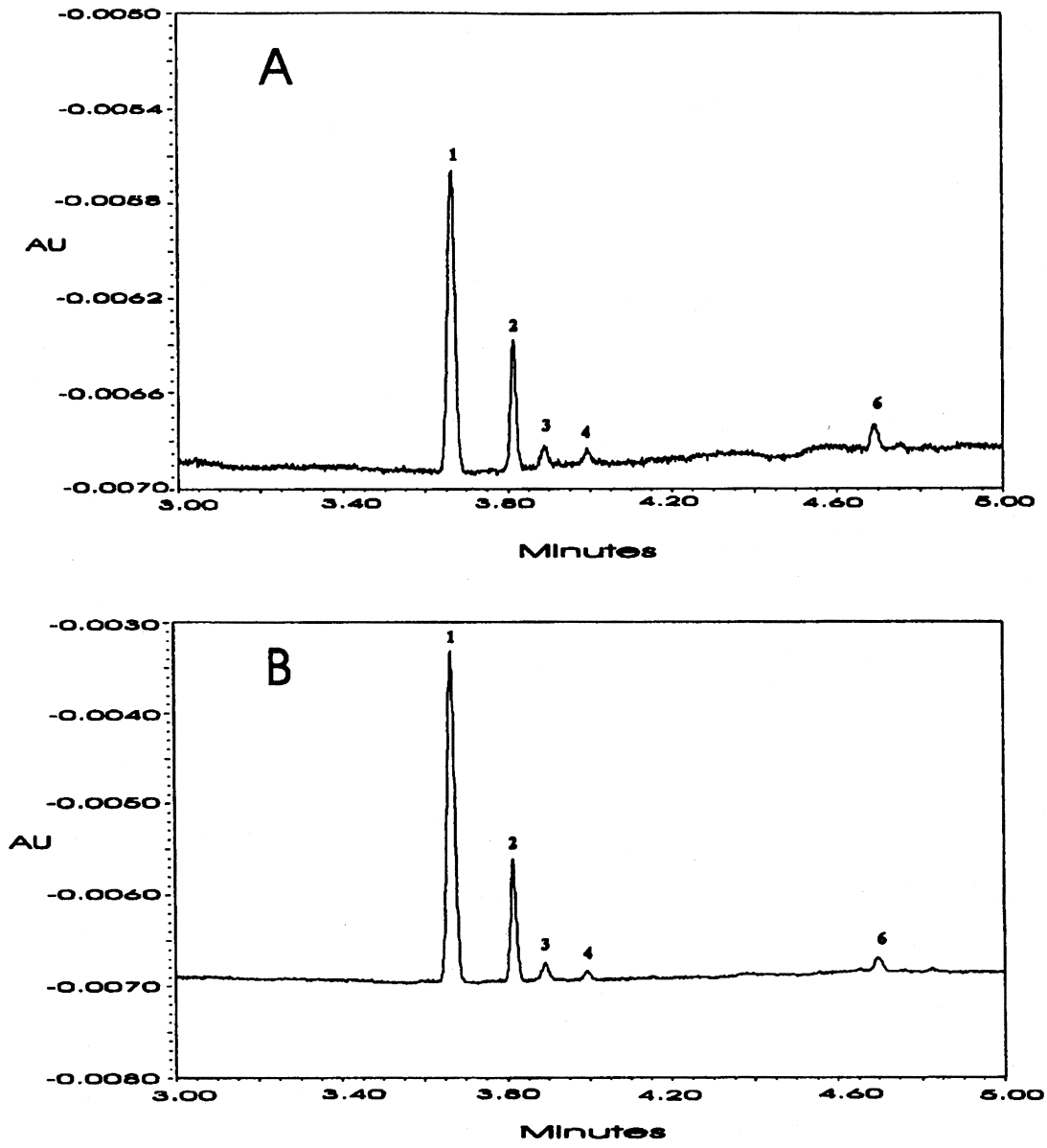

Fig. 11. Dependence of protein separation on injection technique. Conditions: capillary, polyacrylamide–vinyltrichlorosilane, 20/27 cm×75 μm I.D.; buffer, 50 m*M* phosphate (pH 3); field strength, 260 V/cm; samples, 1=cytochrome *c*, 2=lysozyme, 3=ribonuclease A, 4=chymotrypsinogen; injection, (A1) pressure [$3.45 \cdot 10^3$ Pa (0.5 p.s.i.)], 1 s, samples in water; (A2) pressure (0.5 p.s.i.), 1 s, samples in buffer; (B1) 1 kV, 5 s, samples in water; (B2) 1 kV, 5 s, samples in buffer. Reprinted from Ref. [34], with permission.

the same time, if sample conductivity is controlled by the terminating ion matrix bias can also be controlled. On the other hand, however, concentration of the terminating electrolyte can only be moderate, because by increasing the conductivity of the sample electrostacking effect will be reduced. Electrostacking was applied for the concentration of nucleotides in capillary gel electrophoresis, too [73]. Zone sharpening of neutral solutes (alkylphenons) in micellar electrokinetic chromatography (MEKC) by

Table 2
Application of electrokinetic injection for the preconcentration of organic solutes

Component(s)	Matrix	Comment(s)	Ref.
Alkaloids	Crude plant extracts	Preconcentration factor is ca. 1000 with short plug of 70% (v/v) methanol injection	[17]
Industrial dyes	Standard samples	For one part of the components EK injection, while for others HD injection with stacking provided lower LOD	[77]
LSD	Blood	LOD = 0.1–0.2 μg/l using bubble cell and laser-induced fluorescent detection	[78]
Alditols and alcohols	Beverages	Absolut LOD is 0.1 fmol	[79]
Drugs	Urine	LOD is about 10 μg/l	[80]
Synthetic food colorants	Food additives	–	[81]
Fiber-reactive textile dyes	Standard samples	Preconcentration factor is about 100	[82]
Antimalarial drugs	Urine	Preconcentration is improved by injection with organic solvent	[83]
Herbicides	Crop water	Analytes are dissolved in water for efficient preconcentration	[42]
Carbonyls	Standard samples	100-fold preconcentration with polarity switch between injection and separation and with pressure shift to oppose EOF	[84]
Sulfonates and sulfate surfactants	Standard samples	Absolute LOD is 0.05–0.6 pmol	[85]
Anthracyclines	Human plasma	Preconcentration factor is 20–30 if sample conductivity is low	[86]

EK injection was carried out by Nielsen and Foley [74] by means of cationic mixed micelles added to the sample. Last but not least, multiple EK injection combined with correlation technique can be another way of sensitivity enhancement [75,76].

The preconcentration procedures are applied for the determination of both organic molecules and inorganic compounds. Applications in organic analysis are summarised in Table 2, while applications for inorganics will be discussed separately in Section 5.

5. Application of electrokinetic injection for the analysis of inorganic ions

One of the most common applications of EK injection is the analysis of inorganic species at low level (ppb and even ppt) concentrations. The methods used for the study of cations and anions are summarised in Tables 3 and 4, respectively. In each case EK injection was applied to improve the analytical sensitivity and to lower the LOD by on-column preconcentration of the ions. LOD is usually in the low-ppb or even in the ppt-range which makes the methods useful for the determination of trace inorganic ions in samples of high purity. The methods can be used e.g., for quality control of high purity water, chemicals and different electronic elements, for the determination of highly toxic ions (e.g., nitrite, arsenite, arsenate) in drinking water etc. (for examples see Figs. 12 and 13).

Injection voltages and injection times used in the different methods vary by more than one order of magnitude. Injection voltage is in the range of 2.5–30 kV, while injection time is in the range of 1–90 s. However, in most cases the reasons for selecting the given injection parameters are not reported. One exception is the work of Ehmann et al. [32] who optimised EK injection for the determination of anions on silicon wafer surfaces. They used EK

Table 3
CE methods for the analysis of inorganic cations using electrokinetic injection

Components	Matrix(ces)	Injection parameters	Separation parameters	Calibration method	Detection limits	Ref.
Ammonium, potassium, calcium, sodium, magnesium	Fermentation broth Soil extract	30 kV for 3 s	10 m*M* creatinine–5–7.5% PEG200 indirect UV detection at 214 nm	No quantitative analysis performed	No data	[87]
Cobalt, copper, iron, nickel, zinc, manganese, potassium, barium, calcium, magnesium, sodium, cadmium	Standard solutions	(a) and (b) 10 kV for 10 s (c) and (d) 5 kV for 2 s	(a) Fused-silica capillary and (b) CONHOH-coated capillary, 5 m*M* imidazole–8 m*M* HIBA (pH=4.0), indirect UV detection at 214 nm (c) NH_2-coated capillary, 15 m*M* NH_3–NH_4Cl (pH=9.0)–1.5 m*M* PAR, UV detection at 520 nm (d) COOH-coated capillary, 20 m*M* NH_3–NH_4Cl (pH=8.5)–0.3 m*M* PAR, UV detection at 520 nm	External calibration	(b) 5.83–8.08 nmol (d) 4.5–24.4 pmol	[23]
Ammonium, potassium, sodium, magnesium, calcium, manganese, lithium, stroncium, chromium, zinc, barium, nickel, copper	Chinese tea infusion	30 kV for 10 s	5 m*M* imidazole–6.5 m*M* HIBA–20% (v/v) methanol–0.53 m*M* 18-crown-6 (pH=4.5), indirect UV detection at 214 nm	Only for qualitative analysis	0.4–1000 μg/l	[88]
Sodium, potassium, calcium, magnesium	Ocular lenses	15 kV for 10 s	20 m*M* imidazole–0.1% HMC, indirect UV detection at 214 nm	Standard addition	In the fmol range (no exact data presented)	[89]
Lithium, sodium, potassium, cesium, magnesium, calcium, stroncium, barium, lead, chromium, mangane, iron, cobalt, nickel, copper, zinc, cadmium, silver, aluminium	Standard solutions	10 kV for 5 s	10 m*M* pyridine–12 m*M* glycolic acid (pH=4.0), indirect UV detection at 210 nm	External calibration	0.02–208 μg/l	[20]
Ammonium, potassium, sodium, calcium, magnesium, strontium, lithium, barium	Standard solutions	5 kV for 90 s+addition of 0.05 m*M* TBAOH as terminating ion	5 m*M* DDPOH–4 m*M* 18-crown-6–5 m*M* MES (pH=6.0), indirect UV detection at 215 nm	–	1–8 μg/l	[90]
Lanthanum, cerium, gadolinium, terbium	Standard solutions	30 kV for 20 s	10 m*M* creatinine–acetate buffer–2 m*M* HIBA (pH=4.1), indirect UV detection at 214 nm	External calibration and application of internal standard (cerium)	ca. 40 μg/l	[29]
Potassium, ammonium, sodium, calcium, magnesium, barium, strontium, zinc manganese, lithium, nickel	Hydrogen peroxide (semiconductor grade)	10 kV for 30 s	5 m*M* UV Cat 1 or 1.2 m*M* UV Cat 2–6.5 m*M* HIBA (pH=4.4), indirect UV detection at 185 nm	External calibration	0.5–3.3 μg/l	[91]
Potassium, barium, strontium, calcium, sodium, magnesium, lithium	Standard solutions	10 kV for 10 s	UV Cat 2–tropolone electrolyte (no concentrations added, pH=4.4), indirect UV detection at 185 nm	–	30–267 ng/l using extended path length capillary (300 μm)	[92]
Potassium, barium, strontium, calcium, sodium, magnesium, lithium	Deionized water	10 kV for 10 s	UV Cat 2–tropolone electrolyte (no concentrations added, pH=4.4), indirect UV detection at 185 nm	–	30–267 ng/l using extended path length capillary (300 μm)	[72]

Abbreviations: PEG=polyethylene glycol, HIBA=α-hydroxyisobutyric acid, PAR=4-(2-pyridylazo)resorcinol, HMC=hydroxypropyl methyl cellulose, TBAOH=tetrabutylammonium hydroxide, DDPOH=dimethyl-diphenylphosphonium hydroxide.

Table 4
CE methods for the analysis of inorganic anions using electrokinetic injection

Components	Matrix(ces)	Injection parameters	Separation parameters	Calibration method	Detection limit(s)	Ref.
Chloride, sulphate, nitrate, fluoride, (acetate)	Rainwater	3 kV for 20 s	5 m*M* molybdate–0.15 m*M* CTAH 0.01% PVA–5 m*M* Tris	Internal standard (chlorate)	2–20 μg/l	[93]
Bromide, chloride, sulphate, nitrite, nitrate, fluoride, phosphate (oxalate, formate)	Silicon wafer surface	10 kV for 10 s, addition of 25 μ*M* acetate to the sample 4.5 nl plug of sodium hydroxide before sample injection	2.25 m*M* pyromellitic acid, 6.5 m*M* sodium hydroxide, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethonium hydroxide (pH=7.7), indirect UV detection at 250, 350 nm	Internal standard (chlorate)	0.2–1 μg/l	[32]
Chloride, sulphate, nitrate, fluoride, phosphate, (oxalate)	Water extracts of hard disk drive heads	5 kV for 30 s	7–10 m*M* sodium chromate, 0.5–1.5 m*M* NICE Pak OFM Anion-BT (pH=8.0–11.0), indirect UV detection at 254 nm	External calibration	0.2–0.65 μg/l	[94]
Chloride, sulphate, nitrate, azide, phosphate	High-purity chemicals	–	5 m*M* sodium chromate (pH=8), indirect UV detection at 254 nm	Standard addition or application of internal standard	–	[31]
Bromide, chloride, sulphate, nitrate, fluoride, phosphate, chlorate, (oxalate)	Pure boric acid	3 kV for 10 s	5 m*M* sodium chromate, 0.01 m*M* TTAB (pH=8.1), indirect UV detection at 254 nm	Standard addition	0.2–0.7 μmol/l	[35]
Chloride, sulphate, nitrate, fluoride, (oxalate, formate, acetate)	Deionized water	5 kV for 45 s addition of 75 μ*M* octanesulphonate to the sample	10 m*M* sodium chromate 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), indirect UV detection at 254 nm	External calibration	0.3–0.8 μg/l	[72]
Chloride, sulphate, nitrite, nitrate, molybdate, azide, fluoride, phosphate, (formate)	Standard solution	2.5 kV for 45 s addition of 50 μ*M* octanesulphonate to the sample	2.25 m*M* pyromellitic acid, 6.5 m*M* sodium hydroxide, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethonium hydroxide (pH=7.7)	–	0.5–2 μg/l	[90]
Chloride, sulphate, nitrate	Power plant water	5 kV for 45 s	7 m*M* sodium chromate, 0.7 m*M* CIA-Pak OFM Anion BT, OH form, indirect UV detection at 254 nm	External calibration	Not presented	[95]
Chloride, sulphate, nitrate, fluoride, phosphate (oxalate, formate, acetate, propionate)	Power plant water	5 kV for 45 s, addition of 75 μ*M* octanesulphonate to the sample	10 m*M* sodium chromate, 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), indirect UV detection at 254 nm	External calibration	0.3–0.8 μg/l	[70]
Chloride, sulphate, fluoride, phosphate	Estuarine water, bore water soil extract	3 kV for 30 s	5 m*M* sodium chromate, 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), indirect UV detection at 254 nm	Standard addition, application of internal standard	7.1–18.9 μg/l	[28]

Bromide, chloride, sulphate, nitrite, nitrate, fluoride, phosphate	Standard solution	5 kV for 45 s, addition of 18 μ*M* octanesulphonate to the sample	5 m*M* sodium chromate, 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), indirect UV detection at 254 nm	–	8.4–25.4 nmol/l	[69]
Nitrite, nitrate	Tap and river water	7.5 kV for 5 s	20 m*M* sodium tetraborate, 1.1 m*M* CTAC (pH=8.94), UV detection at 200 nm	External calibration with internal standard (bromide)	1 μg/l	[30]
Nitrite, nitrate	Vegetables	10 kV for 10 s	10 m*M* sodium chromate, 2.3 m*M* CTAB (pH=11.5), indirect UV detection at 254 nm	External calibration weighted regression	34–37 μg/l	[96]
Nitrite, nitrate	Estuarine water	3 kV for 30 s	10 m*M* sulphate, 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), UV detection at 214 nm	–	1.6–2.5 μg/l	[28]
Nitrite, nitrate	Standard solution	5 kV for 45 s, addition of 40 μ*M* octanesulphonate to the sample	25 m*M* sodium chloride, 0.5 m*M* NICE Pak OFM Anion-BT, Cl form (pH=8.0), UV detection at 214 nm	No calibration performed	0.19 μg/l	[71]
Nitrate, fluoride	Water	5 kV for 45 s	9 m*M* sodium chromate, 0.5 m*M* NICE Pak OFM Anion-BT (pH=8.0), indirect UV detection at 254 nm	Standard addition	No data	[97]
Selenite, selenate	Bacterial suspension	18 kV for 1 s	20 m*M* sodium borate–1.0 m*M* TTAB (pH=9.5), UV detection at 200 nm	External calibration	0.4–2 mg/l	[98]
Selenite, selenate (selenocystine, selenomethionine)	Thermal water	10 kV for 20 s	5 m*M* sodium chromate, 0.5 m*M* TTAOH (pH=10.5), indirect UV detection at 254 nm	External calibration	5.7–16.4 μg/l	[19]
Arsenite, arsenate (monomethylarsonic and dimethylarsinic acids)	Drinking water	22 kV for 20–240 s	20 m*M* potassium hydrogen phthalate 20 m*M* boric acid (pH=9.03), ICP-MS detection	Standard addition	6–25 ng/l (9–58 ng/l)	[99]

Abbreviations: CTAH=cetyltrimethylammonium hydroxide, PVA=polyvinyl alcohol, Tris=tris(hydroxyl)aminomethane, TTAB=tetradecyltrimethylammonium bromide, CTAC=cetyltrimethylammonium chloride, CTAB=cetyltrimethylammonium bromide, TTAOH=trimethyltetradecylammonium hydroxide.

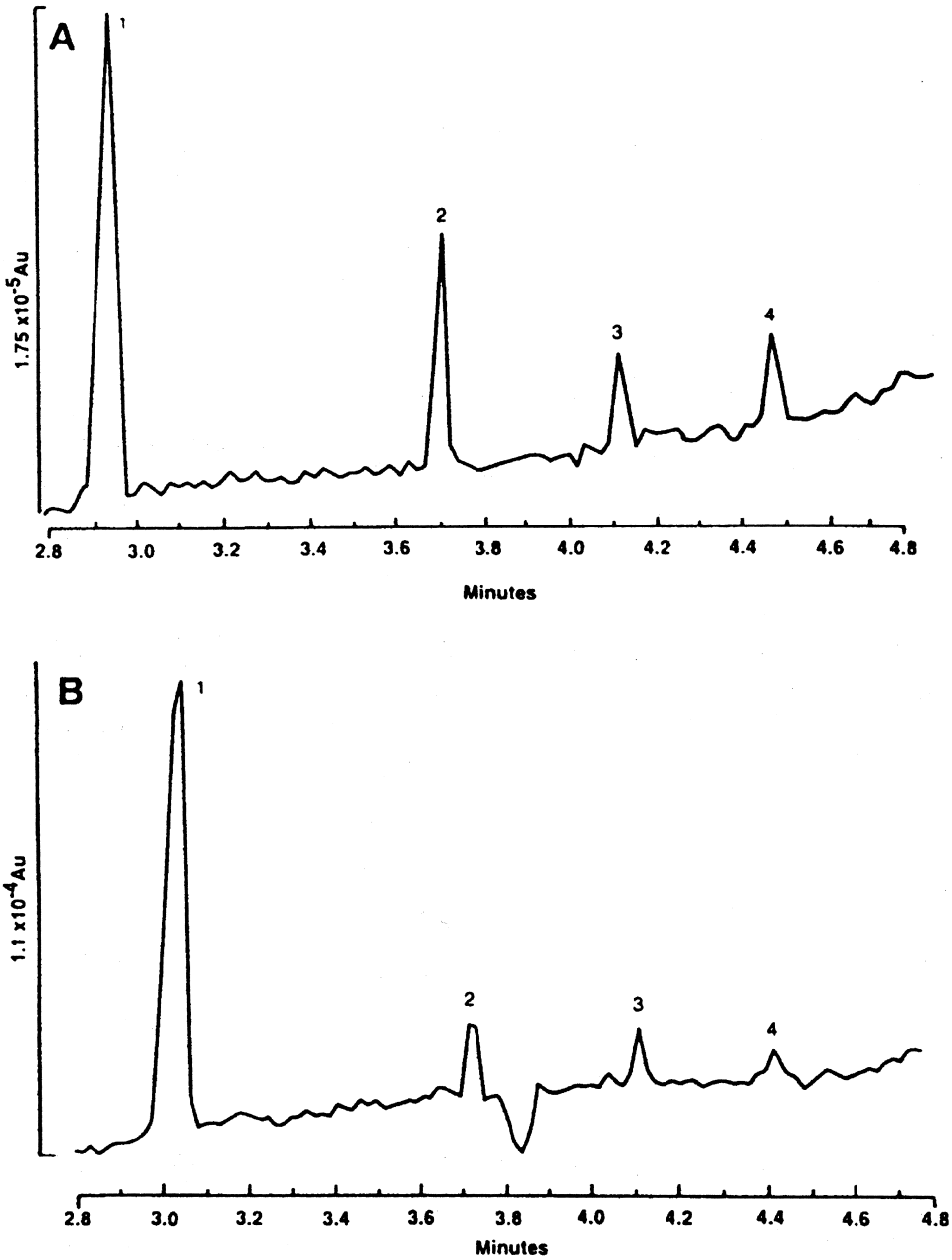

Fig. 12. Electropherogram of source A semiconductor grade hydrogen peroxide (30% undecomposed, unstabilized) after catalytic decomposition, using 5 m*M* UV Cat 1–6.5 m*M* HIBA (pH 4.4). Fused-silica capillary dimensions 60/52 cm×75 μm I.D.; separation voltage +20 kV; sampling for 30 s at 10 kV, indirect UV detection at 185 nm. Detected species: 1=potassium/ammonium; 2=calcium, 3=sodium; 4=magnesium. (B) Electropherogram of source B semiconductor grade hydrogen peroxide obtained using same conditions as in Fig. 13A. Detected species: 1=potassium/ammonium; 2=calcium, 3=sodium; 4=magnesium. Reprinted from Ref. [91], with permission.

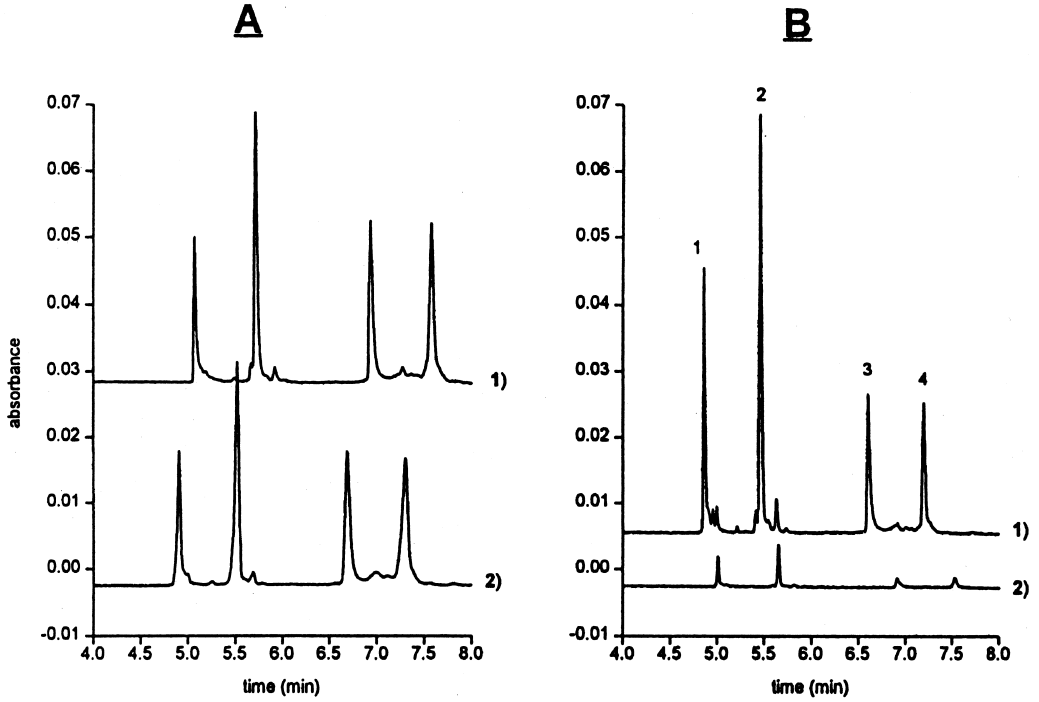

Fig. 13. Electropherogram of a slider sample (A) and the assembly arm that it was attached to (B). Conditions: fused-silica capillary, 60 cm×75 μm I.D.; voltage, 15 kV (negative); electrolyte, 7.0 m*M* chromate–0.5 m*M* OFM, pH=8.0; Indirect UV detection at 254 nm, electromigrative injection, −5 kV for 30 s. Peaks: 1=Chloride (9.4 μg/l); 2=sulfate (3.2 μg/l); 3=nitrate (0.9 μg/l); 4=oxalate (0.7 μg/l) and 6=formate (not quantitated). (B) Peaks: 1=Chloride (25.9 μg/l); 2=sulfate (9.7 μg/l); 3=nitrate (1.9 μg/l); 4=oxalate (0.8 μg/l) and 6=formate (not quantitated). Reprinted from Ref. [94], with permission.

injection combined with isotachophoretic preconcentration and optimised the method by Taguchi's experimental design [100] for the different analytical parameters, like peak area, peak asymmetry, efficiency, resolution, repeatability (the optimum conditions found can be seen in Table 4). The increase of the injection voltage or the injection time is limited by the deterioration of the separation efficiency and loss of resolution. Another point is that by increasing the injection voltage or injection time calibration curves can become non-linear.

In most cases repeatability of the measurements were good even without internal standard, the R.S.D.s for migration time and peak area were $<2\%$ and $<5\%$, respectively. By using internal standard precision could be improved further. Sensitivity enhancement compared to conventional HD injection varied from 10 to 500, the highest enrichment factors were achieved by using isotachophoretic effect. Effect of the mobility bias on the pre-concentration of the ions was clearly described by Yang et al. [88] who investigated metal ions and observed about the same LOD for ions of low mobility (e.g., Cu^{2+}) when using HD injection. LOD of Cu^{2+} was 1000 ppb, whereas LODs of the most mobile ions (e.g., Li^{+}, Mg^{2+}, NH_4^{+} and Ca^{2+}) were lower by three-orders of magnitude.

As regards the calibration in very high-purity matrices (usually in high-purity water) external calibration may be used for quantitative analysis because in these determinations matrix bias is negligible. In other examples, where the matrix effect on the EK injection was not negligible standard addition and/or internal standardisation was applied.

6. Conclusions

In CE, HD injection is definitely the preferred mode of sample introduction. EK injection can be, however, an alternative choice if the sensitivity of

HD injection is low and/or HD injection cannot be applied. The main field of the application of EK injection is the determination of inorganic ions in high-purity matrices at the low-ppb level. The highest enrichment factor can be achieved by electrostacking supported by isotachophoretic effect. Based on the high sample throughput and high separation efficiency of CE it can be concluded that CE using EK injection can be a good alternative technique to ion chromatography.

The basic equation of EK injection (Eq. (1)) seems to be valid only under limited conditions. These conditions may include the injection of neutral substances and injection of ions at a low voltage for a short time. In the injection of ions at higher voltages and/or for longer time considerable deviations from Eq. (1) were observed. Further theoretical considerations and experimental studies are required to evaluate the physico–chemical reasons for the deviation and to establish the boundary conditions for Eq. (1).

The methods for the correction of matrix bias should be tested further and compared for more compounds and matrices in order to define their validity.

For accurate and precise quantitative analysis using EK injection the following requirements should be fulfilled: (i) the injection is performed with accurate control of injection voltage and injection time, (ii) the sample volume is controlled and possibly large (1–2 ml), (iii) only one measurement is carried out from one vial, especially if the sample volume is low (less than 0.5 ml), (iv) frequent in-run calibration is carried out, (v) if necessary precision is improved by using internal standard, (vi) if necessary matrix bias is corrected by the most appropriate correction procedure. In the present state of our knowledge the best solution to improve both the accuracy and the precision might be the application of standard addition with one internal standard. In high-purity water (matrix-free conditions), however, external calibration can also be used.

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