

Study of quantitative analysis of traces in low-conductivity samples using capillary electrophoresis with electrokinetic injection

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Abstract

In this work we tried to clarify the relation between the signal detected by indirect UV mode and the concentration of the analyte introduced by electrokinetic (EK) injection from samples having small but different conductivities. A simple external calibration procedure was proposed for quantitative determination of traces that are present in low-conductivity samples. It was found that measuring the current through the capillary filled with the samples provides useful correction factor for the external calibration.

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1. Introduction

In capillary electrophoresis (CE) method there are two techniques to introduce a minute part of the sample into the separation capillary: hydrodynamic (HD, samples driven by a pressure) and electrokinetic (EK, samples driven by an electric field). In the majority of the published works in CE, hydrodynamic injection is preferred because its theoretical fundamentals are much more simple and more established, and it is more reliable and accurate for quantitative analysis. In EK injection the variations in conductivity of samples due to matrix effects result in differences in voltage drop and quantity loaded during the injection, therefore EK injection is generally not as reproducible as HD sample introduction. Despite of the known quantitative limitations, the practice of EK injection is very simple, it can provide much more sensitive determinations (field-amplified injection) than HD injection and its use in some fields (e.g. capillary gel electrophoresis, lab-on-a-chip-technology) cannot be substituted.

There are numerous works dealing with the theory of EK injection [1–9]. Some of them separate the occurring prob-

lems as EK injection of samples with conductivities similar and different to that of the background electrolyte (BGE). In case of EK injection the sample enters the capillary by a combined effect of electroosmosis and electrophoresis, therefore the amount of the analyte ion injected depends on its mobility (“mobility bias”) and a larger amount will be introduced if the analytes co-migrates with the electroosmotic flow (EOF). Therefore, when two different analytes are present in the sample of the same concentration, different amounts will be injected resulting different responses. These variances can be disregarded using external calibration only if the conductivities of the samples are equal, however the conductivities of real samples are almost always different. Since the injected analytes also depend on the relationship of the conductivities of the sample solution and the BGE, the bias between different samples with different matrix (“matrix bias”) caused by EK injection cannot be corrected by means of common calibration procedures.

In EK injection since the capillary is almost completely filled with the BGE, the EOF is practically not influenced by the conductivity of the sample solution, however, the electromigration of the analytes depends on the conductivities of each sample. The total amount (n_a) of the analyte a injected is given by:

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$$n_a = \frac{1}{4} \pi d^2 \left(\mu_{\text{EOF}} \pm \frac{k_{\text{BGE}}}{k_s} \mu_a \right) \frac{U_{\text{inj}}}{L} t_{\text{inj}} c_a \quad (1)$$

where the d is the inner diameter of the capillary, the μ_{EOF} is the mobility of the EOF, the μ_a is the electrophoretic mobility of the analyte, U_{inj} is the injection voltage, L is the total length of the capillary, t_{inj} is the injection time, c_a is the molar concentration of the analyte, k_{BGE} and k_s are the conductivities of the BGE and the sample solution, respectively.

The mobilities of analytes and the EOF can be determined experimentally on the basis of the migration times of the analyte (t_a) and a neutral, unretained compound (t_{EOF}), thus these mobilities can be expressed by:

$$\mu_{\text{EOF}} = \frac{L_{\text{eff}} L}{U t_{\text{EOF}}} \quad \text{and} \quad \mu_a = \frac{L_{\text{eff}} L}{U t_a} \quad (2)$$

where the U is the separation voltage, and L_{eff} is the effective length of the capillary.

If the mobilities in Eq. (1) are substituted for expressions of Eq. (2) then the amount of the analyte a injected will be:

$$n_a = \frac{1}{4} \pi d^2 \left(\frac{1}{t_{\text{EOF}}} \pm \frac{k_{\text{BGE}}}{k_s} \left(\frac{1}{t_a} - \frac{1}{t_{\text{EOF}}} \right) \right) \frac{U_{\text{inj}} L_{\text{eff}}}{U} t_{\text{inj}} c_a \quad (3)$$

from the Eq. (3) it is obvious that the injected amount of neutral components, (where t_{eof} and t_a are the same) does not depend on the matrix of the samples.

In general, the use of internal standards offers better precision than without standards, some one- and two-internal standards methods have been described [4,10]. Lee and Yeung improved the quantitative precision of CE with EK injection by monitoring the electrophoretic current during injection [4]. They showed that the effects of sample conductivities on the amounts of analytes introduced with EK injections could be nullified by proper corrections with the measured conductivities of sample and BGE together with the migration times of analyte and the EOF. They achieved 0–5% level of accuracy with the exception of low conductivity samples (smaller than 10 mM phosphate in sample) [4]. However, in a review on EK injection it is stated that “in high-purity water (matrix-free conditions) external calibration can also be used” [8]. Some authors found that standard addition can be well applicable for analysis of samples including large amount of matrix material [5], however thus the number of the measurements should be at least doubled or tripled. Most studies on the topic establish that it is important to further understand EK injection and to explore new ways to improve its quantitative capability [4,8].

The indirect UV detection is a popular and well-applicable detection mode in CE for analysis of inorganic anions, which are often UV non-active ones, e.g. [11]. It was observed that the slopes of calibration plots for alkali metal and alkaline earth metal cations are identical for the monovalent cations on one hand and for the divalent cations on the other hand, and the values of the slopes for the divalent analytes are different from those for the monovalent analytes by exactly a factor

of two [6]. The EK injection seems to have compensated the differences in transfer ratio between the higher- and lower mobility ions, that is the transfer ratio is the same for all analyte ions having the same valency [6].

Although the EK injection is generally not suggested for quantitative determinations with the statement that different conductivities of samples cause false results, there is a large number of application of EK injection trying to utilize the simply attainable high sensitivity. The aim of our work is to try to clarify the relation between the analytical response and the concentration of the analyte introduced by EK injection from samples having small but different conductivities. We propose a simple external calibration procedure for accurate quantitative determination of traces present in low-conductivity samples using EK injection. All our present work is restricted for the analysis of highly diluted (low-conductivity) samples.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument was a HP ^{3D}CE model (Agilent, Waldbronn, Germany). The sample solutions were introduced by EK injection (−2.5 kV for 15 s) at the cathodic end of the capillary. Separations were performed using fused-silica capillaries of 64.5 cm × 50 μm I.D. (effective length: 56 cm) (CS-Chromatographie, Langerwehe, Germany). The applied voltage was −25 kV (reversed polarity). The detection was carried out by on-column photometric measurement (detecting wavelength: 450 nm, reference wavelength: 275 nm). The electropherograms were recorded and processed by ChemStation computer program of 7.01 version (Agilent).

2.2. Chemicals

All reagents were of analytical grade. The sodium chromate and CTAB for preparing buffer electrolytes were purchased from Reanal (Hungary). The bromide, chloride, sulfate, nitrate and nitrite stock solutions (10 mM) were prepared from their sodium salts, the phosphate was obtained by dilution of phosphoric acid (all from Fluka).

The capillaries were preconditioned with the buffer electrolyte for 5 min prior each runs. The electrolyte vials were replenished with fresh electrolyte after each 10 runs. The sample and BGE solutions were degassed prior analysis.

2.3. Separation conditions

For the determination of the six inorganic anions a co-electroosmotic separation mode with reversed polarity was used. The electrolyte contained 5 mM chromate and 0.2 mM CTAB, the pH of the solution was 8.0. The separation was optimised by others [2].

3. Results and discussion

While in case of hydrodynamic injection the response (peak area) depends linearly on the concentration of the analyte in a large concentration range (5–100 μM) (Fig. 1a), the graphs are hyperbola-shaped when EK injection is applied (Fig. 1b). After a very short initial linear part the graphs will be curved. The probable reason of this shape is that increasing the concentration of the standards (in pure water), the total ion content of the sample solution will be higher and more comparable to the conductivity of the BGE ($c_{\text{eq}} = 10.2 \text{ mM}$). The conductivity of sample containing the standards in 15 μM concentration is about 1% of that of the BGE. As the con-

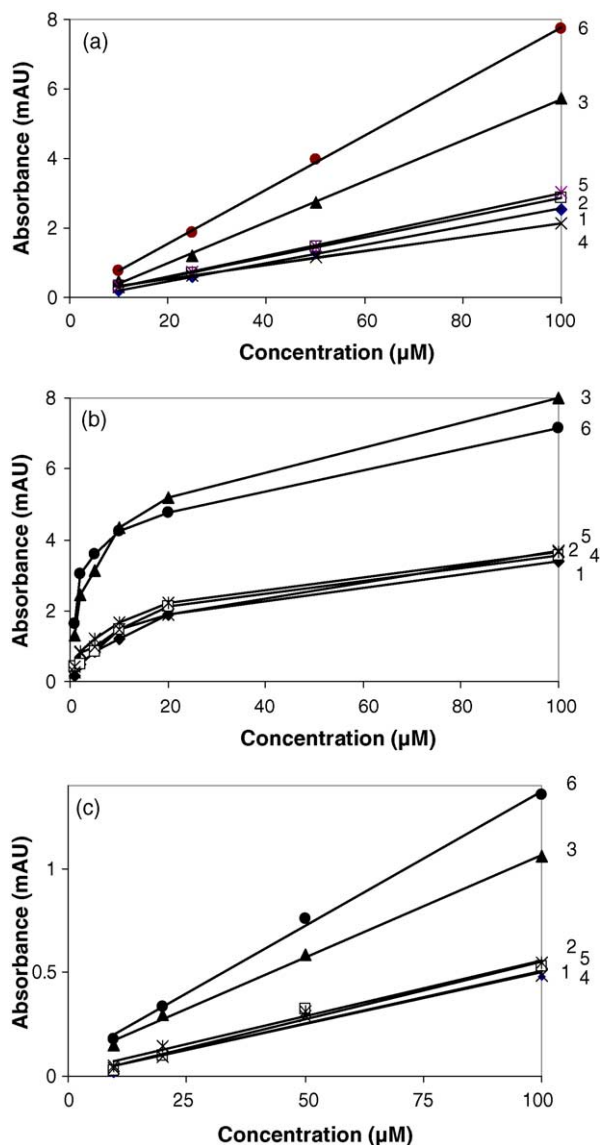


Fig. 1. Calibration graphs of: bromide (1), chloride (2); sulfate (3); nitrite (4); nitrate (5); and phosphate (6) using HD injection (a) and EK injection (b and c). (Condition of CZE determination: 64.5 cm \times 50 μm I.D., BGE: 5 mM chromate, 0.2 mM CTAB, pH: 8.0, voltage: -25 kV , $\lambda = 275 \text{ nm}$ (indirect UV), HD injection: 50 mbar 15 s. EK injection: -2.5 kV 15 s. Sample solutions were spiked with 2 mM sodium carbonate in case of (c).

ductivity of the sample solutions will be higher, smaller and smaller amount of analyte will be injected into the capillary (see Eq. (1)). It is interesting to note, that if the conductivity of the sample is much higher than that of the BGE and the EOF is high, the injected amount will be independent from the conductivities of the samples. It also should be noticed that the sample of maximum about 50 μM total ion content should be considered as an infinitely diluted solution which possesses special physico chemical characteristics. Although the increase of the conductivity of the sample solutions spiked with a constant large amount of salt (2 mM sodium carbonate) will result in calibration graphs with good linearity ($R^2 > 0.99$), parallel to this the sensitivity of the measurements will drop dramatically (Fig. 1c).

It should be also observed that the slopes of all graphs of Fig. 1b and c (where EK injection is applied) follow the valencies of the analytes: the slopes of bromide, chloride, nitrate, nitrite are within the experimental error, and the slope of the divalent sulfate is the double compared to monovalent. These results are in good agreement with others who analysed metal ions [4,6] or inorganic anions [12]. The fractional valency of phosphate is smaller than 2 because the second dissociation step is not complete at the pH 8 of the BGE ($\text{p}K_2$ of phosphate is 6.17×10^{-8}). However, in case of HD injection the slope of phosphate is larger than that of the divalent sulfate. This is probably caused by the distortion (tailing) of peak of phosphate (the other five investigated components have very similar mobility with only minimal distortion). In case of EK injection of low-conductivity sample smaller amount of phosphate will enter into the capillary due to its smaller mobility resulting smaller slope than using HD injection (Fig. 1b). At EK injection of high-conductivity samples larger amount of phosphate will be introduced into the capillary (Fig. 1c) because the contribution of the EOF in the driving force will be higher (see Eq. (1)).

As it was expected according to the data from literature an essential difference can be found between HD and EK injection if peak areas of the same amounts of analyte present in samples including different amount of matrix are compared. The Fig. 2 well illustrates the difference in influence of the sample matrix material (0–5 mM nitrate) on the signal of the analyte (0.05 mM chloride) caused by EK and HD injection. This figure indicates also the great possibility for highly sensitive determination of the low-conductivity samples using EK injection.

In present work we deal with analysis of trace amount of inorganic anions, which are present in low-conductivity samples. If the conductivity of the sample is much smaller than that of the running electrolyte (5 mM chromate, 0.2 mM CTAB), which is the subject of this work, the $k_{\text{BGE}}/k_{\text{S}}$ becomes very large and the first term of the Eq. (3) becomes negligible. In practice, this simplification can be valid, if the conductivity of the BGE is at least 100 times larger than the conductivity of the samples (Eq. (4)), therefore in our further investigation samples including maximum 100 μM ion

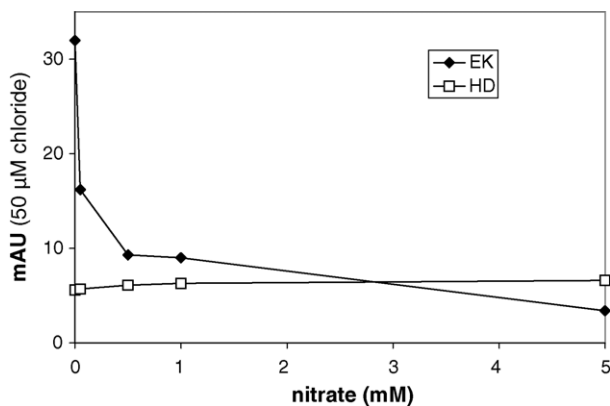


Fig. 2. Influence of the sample matrix (0–5 mM potassium nitrate) on the signal of the analyte (50 μM chloride) in case of EK and HD injections. (HD injection: 50 mbar; 15 s, EK injection: -2.5 kV 15 s. The condition of CZE determinations was described in Fig. 1.).

equivalent was studied (chromate possesses two electrical charges at pH 8 used in this study).

$$n_a = \frac{k_{\text{BGE}}}{k_{\text{S}}} \left(\frac{1}{t_a} - \frac{1}{t_{\text{EOF}}} \right) \frac{U_{\text{inj}} t_{\text{inj}} L_{\text{eff}} \pi d^2}{4U} c_a \quad (4)$$

Since n_a and the peak area A_a are proportional, the amounts of an analyte a injected from sample solutions 1 and 2 can be related as:

$$\frac{n_{a,S1}}{n_{a,S2}} = \frac{k_{S2} c_{a,S1}}{k_{S1} c_{a,S2}} \quad (5)$$

From Eqs. (4) and (5) it is obvious that even in low-conductivity samples the injected amount depends on the conductivities of the sample (there may be large differences between conductivities of low-conductivity samples), and this matrix bias is not negligible in contrast with statement of others, e.g. [8]. Eq. (5) shows also that *external calibration* is possible only with considering the conductivities of both the sample and the external standard solution. However, when we try to apply the Eq. (5) in practice measuring the conductivity of samples by means of conductometer, the quotient of peak areas ($A_{a,S1}/A_{a,S2}$) obtained at the electropherograms were largely different from the quotient coming from the conductivities measured by conductometry ($k_{S2}c_{a,S1}/k_{S1}c_{a,S2}$). Three sample solutions, containing 0; 0.5 mM and 5 mM nitrate and the same concentration 0.05 mM chloride, have conductivities 14, 78 and 480 μS , respectively, however, the peak areas of chloride do not follow the expectation derived from Eq. (5): the quotients of the peak areas are 2.2 and 3.0, respectively, while the quotients of conductivities are 5.5 and 6.2, respectively. Considering these data it is not understandable why the clear and simple deduction of Eq. (5) does not provide an applicable aid for quantification of analytical signals obtained in low-conductivity samples with EK injection. In order to understand this problem the conductivity measurements using conductometer had to be studied.

As it is known there is a linear relation between the concentration of the ions of the electrolyte and the conductivity in a wide range. However, the straight line will slightly incline to the abscissa in high (higher than 100 mM) con-

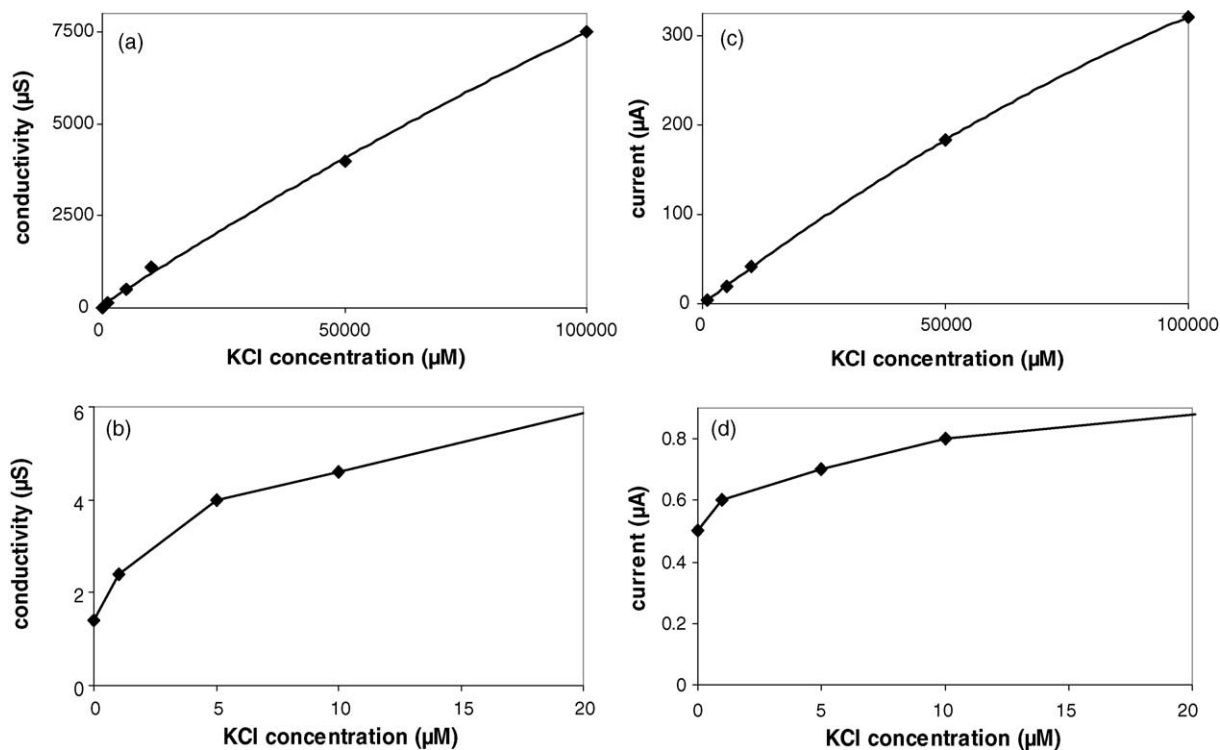


Fig. 3. Relationship between the electrolyte concentration and the conductivity (a and b) measured by conductometer and current (c and d) measured in CE instrument.

centration of electrolyte (Fig. 3a), on the other hand in very small electrolyte concentration there is an initial hyperbolic period of the curve because of the special effects appearing in very (infinitively) diluted samples (Fig. 3b). The conductivity of solutions is measured by conductometer using alternating current in order to avoid electrolysis and polarising of the electrodes. Then we investigated whether the conductivity could be determined through measuring the current at constant voltage in the CE capillary filled with the sample electrolyte (both ends of the capillary immerse in the sample vials). Due to the special geometry of the leading system (very high ratio of length/cross-section of the capillary, very small flow of ions compared to the surface of the electrodes and the volumes of the vials at the electrodes) the effect of the polarization and electrolysis on the measured current is negligible. The composition of the solution over the length of the capillary (that is the resistance R_a of the filled capillary) can be considered constant during a short period (30–40 s) of applying voltage. During this period (except the first 1 second) the current I_a was constant. The measured current plotting against the electrolyte concentration gave very similar curve (Fig. 3c and d) than that was obtained for measuring conductivities proving the expectation that there should be a close relation between k_a and I_a ($I_a = U/R_a$). In Fig. 3b and d it can be observed that the curves start not from zero conductivity or current values due to the amount of hydroxide and hydroxonium ions defined by K_{aq} (the amount of these extremely mobile ions will be comparable to the sub or low-micromole of analytes). It should be also noted that even the dry surface of fused silica capillary has a small conductivity, which can be estimated as a constant contribution to the conductivity of the capillary filled with different electrolytes.

The current curve had also an initial hyperbolic period like in conductivity curve, but here was a much smaller change of the response with change of the concentration. It was noticed that if the quotients of proper current values are used in Eq. (5) in place of the quotients of conductivities measured by conductometer (Eq. (6)), the calculated values ($n_{a,S1}/n_{a,S2}$) agreed well with the ratio of peak areas ($A_{a,S1}/A_{a,S2}$) obtained from the electropherograms.

$$\frac{n_{a,S1}}{n_{a,S2}} = \frac{I_{S2} c_{a,S1}}{I_{S1} c_{a,S2}} \quad (6)$$

We cannot give a complete explanation for this observation, but it is probable that the ratio of current values measured in the CE capillary better describes the difference in the characters on conductivities of the sample solutions being in CE capillary than the ratio of conductivities of samples measured with conductometer in an other system. The exact relation between the conductivity and the current measured by the described manner should be studied in future. The determination of the currents are very simple with the most of CE instruments because the sample of some μL volume previously analysed by CE can also be used, and also in an automatic sequence analysing mode of the instrument (there is no need additional sample, sample pretreatment or any

work of the analyst). An advantage is also that there is no need for a conductometer.

In order to study the applicability of external calibration for low-conductivity samples five sample solutions were prepared containing the same amount of analytes (1: bromide; 2: chloride; 3: sulfate; 4: nitrite; 5: nitrate; each in $5 \mu\text{M}$), these samples contains also phosphate as matrix material in 0, 10, 25, 50 and $100 \mu\text{M}$ concentration, respectively. The sample without phosphate was regarded as external refer-

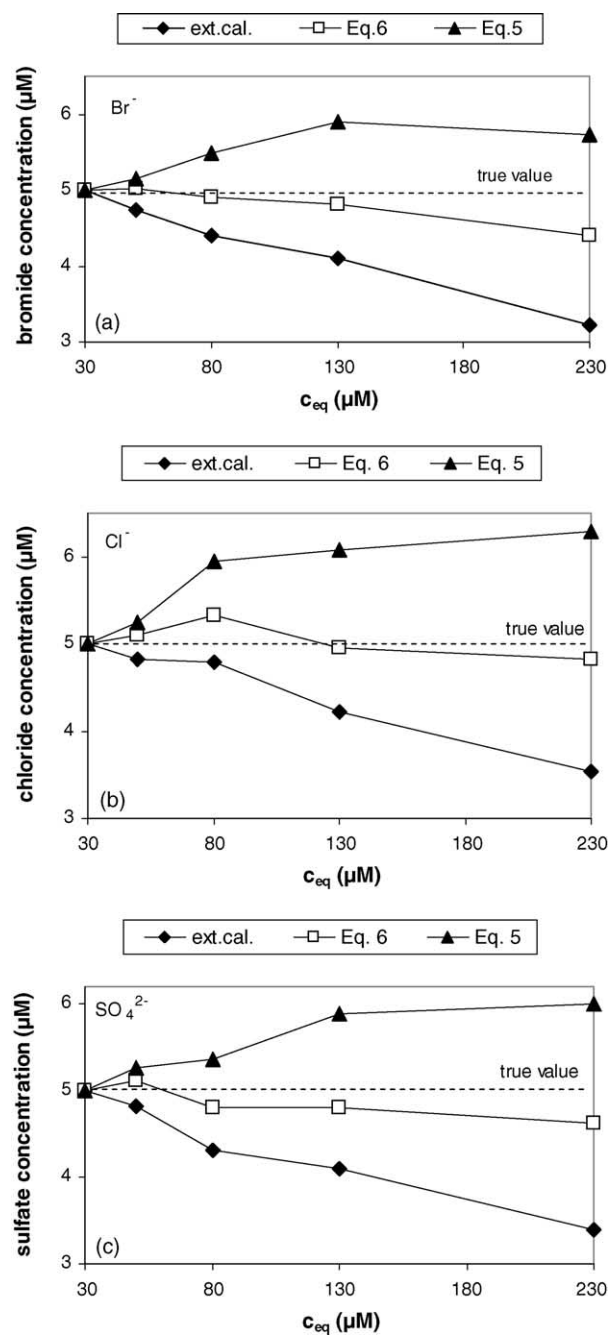


Fig. 4. Variations of the concentration values calculated by the different external calibration procedures for three different analytes (a: Br^- ; b: Cl^- ; c: SO_4^{2-}).

ence solution. For the calculations the peak area corrected with migration times were used to compensate the small difference of velocities of analytes. As it was expected from our earlier observation the ratio of current values measured in the CE capillary and the ratio of conductivities of samples were deviated.

Comparising the concentration data obtained using the three external calibration procedures the data calculated on the basis of the ratio of the current were proved to be the most close to the true values. A larger bias could be found in total ionic concentration higher than 150 μM , where the $k_{\text{BGE}}/k_{\text{S}}$ becomes not enough large to make negligible the first term of Eq. (3). In Fig. 4 the variations of the concentration values calculated by the different external calibration procedures are illustrated for three different analytes. Up to the 130 μM total ion content the precision of the concentration data calculated on the basis of the ratio of the current were within 3–4%, which is acceptable considering the low concentrations and the usual precision data in CE (e.g. in case of HD injection).

4. Conclusion

The main advantage of the use of EK injection is that the attainable sensitivity can be much better than using HD injection, however EK suffers from the poor reliability in quantification. In our work it was proved that the combination of EK injection of larger amount of sample (field-amplified stacking) can provide a very simple, cheap, rapid and effective tool for reliable quantitative analysis of traces of inorganic anions in low conductivity samples using the proposed external calibration procedure. Also, it was investigated whether

the conductivity could be determined through measuring the current at constant voltage in the CE capillary filled with the sample electrolyte. It was found that measuring the current through the capillary filled with the samples provides a useful correction factor for the external calibration.

The application of internal calibration for the quantitative analysis of low-conductivity samples with EK injection may be also possible, as it is focussed in our future work.

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