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# Separations of inorganic anions based on their complexations with $\alpha$ -cyclodextrin by capillary zone electrophoresis with contactless conductivity detection

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## Abstract

Capillary zone electrophoresis (CZE) separations of inorganic anions based on their host–guest complex equilibria with  $\alpha$ -cyclodextrin ( $\alpha$ -CD) were investigated.  $\alpha$ -CD employed as a host was found to influence selectively the effective mobilities most of the studied anions (chloride, bromide, iodide, sulfate, nitrite, nitrate, fluoride, phosphate). Its complexing ability combined with a low pH of the carrier electrolyte solution provided working conditions suitable for rapid CZE separations of the anions. For example, iodide and chloride present in the injected sample in a concentration ratio of ca.  $1:2 \cdot 10^3$  could be separated in less than 100 s and seven of the above anions ( $\alpha$ -CD failed to resolve chloride and bromide) were baseline-resolved in less than 120 s. A high overall selectivity of the carrier electrolytes combining the host–guest equilibria with a low pH in the analysis of highly complex samples is illustrated by CZE separations of inorganic anions present in milk. Here, no interferences to the separations of inorganic anions due to co-migrations of organic acids present in milk were observed also when a 200 nl volume of a diluted (1:10) milk sample was loaded onto the column. A contactless conductivity detector used for the detection of anions in this work proved very reliable. Under our separating conditions it provided for the studied anions concentration limits of detection in the range of 0.5–1.4  $\mu\text{mol/l}$  (a 200-nl sample injection volume) when the separations were carried out in a 300  $\mu\text{m}$  I.D. capillary tube made of polytetrafluoroethylene. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Complexation; Host–guest complexation; Inorganic anions; Cyclodextrins

## 1. Introduction

Capillary zone electrophoresis (CZE) combined, especially, with indirect photometric detection becomes a subject of an increasing interest in the field of analysis of inorganic anions. From recent reviews dealing with the CZE separations of inorganic anions [1–4] it is apparent that the separations at a high pH (the separations according to ionic mobilities [5,6])

with electroosmotic flow (EOF) directed to the detector and chromate employed as the visualisation anion are preferred. Reasons for a preference towards this well elaborated approach are discussed in detail in the literature [3,7].

A proper choice of pH of the carrier electrolyte plays an important role in CZE separations of inorganic anions [8,9]. Its high value is not essential as all common inorganic anions can be separated at a low pH [8]. This, only seldom employed approach, offers an enhanced analytical selectivity, especially, in situations when the anions are to be determined in

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ionogenic, organic matrices [8]. Under such acid–base conditions the separations according to actual ionic mobilities (anions of strong acids) are combined with the ones according to differences in  $pK$  values (anions of moderately weak acids). Selectivities of the CZE separations can be further enhanced by implementing other separation mechanisms applicable to the electroseparations of inorganic anions. These include metal–ligand complex equilibria (by adding a suitable metal cation into the carrier electrolyte solution [10]), ion-pair formation combined with the electrophoretic and relaxation retardations [8], interactions of inorganic anions with electroneutral [8] or positively-charged polymers [11]. Some of these separation mechanisms were originally introduced into isotachopheresis (ITP) separations [12–15] and, subsequently, transferred into CZE. Although these basic electrophoretic techniques have some inherent methodological differences (see, e.g., Refs. [5,6]) such a transfer of the separation mechanism is straightforward [8,10].

The works by Tazaki et al. [16] and by Fukushi and Hiio [17] dealing with ITP separations of inorganic anions indicate that inclusion complexation of these anions with  $\alpha$ -cyclodextrin ( $\alpha$ -CD) offer an alternative separation mechanism also in CZE separations of these constituents. Despite the fact that both native and derivatized cyclodextrins (CDs) are widely used in CZE [18–21] to our knowledge only one work [22] paid attention to their use in the CZE separation of inorganic anions. This work was aimed at investigating influences of  $\alpha$ -CD on the effective mobilities and CZE separabilities of some inorganic anions (chloride, bromide, iodide, nitrate, nitrite, sulfate, fluoride and phosphate) which need to be determined in various samples of environmental and biological origins. The CZE separations were carried out in a hydrodynamically closed separation system with an enhanced sample loadability [23] in order to achieve the separation of the studied anions in samples containing them at very differing concentrations. A contactless conductivity detector as originally developed for ITP [24–26] was used in our experiments to monitor the separations. Although at present this detector is not as sensitive as the ones using contact conductivity sensors [27] its robustness is very favourable, for example, in the CZE analyses of samples contaminating surfaces of the contact

conductivity sensors (for a detailed treatment of the problems associated with the use of contact conductivity sensors in capillary electrophoresis see Ref. [5]).

## 2. Experimental

### 2.1. Instrumentation

A CS Isotachophoretic Analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in the single-column configuration of the separation unit. The separation unit consisted of the following modules: (i) a CZE injection valve with a 200-nl internal sample loop (Villa-Labeco); (ii) a column provided with a 300  $\mu\text{m}$  I.D.  $\times$  500  $\mu\text{m}$  O.D. capillary tube made of polytetrafluoroethylene (PTFE) (Villa-Labeco); total length of the capillary tube was 230 mm (130 mm to the detector); (iii) a counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment (Villa-Labeco).

The column was provided with an on-column contactless conductivity detector (Villa-Labeco). The detector was calibrated with a series of aqueous solutions of KCl (0.5–10 mmol/l) of known conductivities. The signal from the detector was led to a 486 DX computer via a Unilab data acquisition unit (Fitek, Šal'a, Slovak Republic). ITP Win software (version 2.31) obtained from Kascomp (Bratislava, Slovak Republic) was used for the acquisition of data and their processing.

### 2.2. Chemicals

Potassium and sodium salts of inorganic acids were bought from Fluka (Buchs, Switzerland), Lachema (Brno, Czech Republic), Serva (Heidelberg, Germany) and Sigma (St. Louis, MO, USA). Stock solutions of these salts at 10 mmol/l concentrations were used for preparations of model samples. The model samples were prepared fresh before the analysis by appropriately diluting the stock solutions with water purified by a Pro-PS water purification system (Labconco, Kansas City, KS, USA).

Chemicals used for the preparation of the carrier electrolyte solutions were obtained from Lachema,

Sigma and Merck (Darmstadt, Germany). Methylhydroxyethylcellulose 30 000 (m-HEC) obtained from Serva served as an EOF suppressor in the carrier electrolyte solutions [28]. The solutions of the carrier electrolytes were prepared in purified water (Labconco) and before the use they were filtered through disposable membrane filters (1.2- $\mu\text{m}$  pore size) bought from Sigma.

### 2.3. Samples

Iodide containing dietary salt (Solivary, Prešov, Slovak Republic) and milk (Farma fresh, Majcichov, Slovak Republic) were bought in a local supermarket. An aqueous stock solution of the dietary salt (containing chloride at a 100 mmol/l concentration) was filtered through a 1.2- $\mu\text{m}$  pore size membrane filter (Sigma). Milk was centrifuged at 6000 rpm and the supernatant was filtered through a 1.2- $\mu\text{m}$  pore size membrane filter (Sigma).

## 3. Results and discussion

### 3.1. Influence of host–guest complex equilibria on the effective mobilities of inorganic anions

The electropherogram in Fig. 1a obtained in the carrier electrolyte solution without  $\alpha$ -CD (Table 1) shows that under these separating conditions only the anions of weak acids (nitrite, fluoride, phosphate) could be completely resolved while the anions of strong acids (having close effective mobilities) migrated unresolved in two peaks. This was due to the fact that in this instance the acid–base equilibria participated in the separation and, in fact, two separation mechanisms were effective: (i) the anions of weak acids were separated (at least partially) according to differences in their  $pK$  values [5,6]; (ii) for the anions of strong acids differences in their actual ionic mobilities were essential.

In experiments aimed at investigating influences of  $\alpha$ -CD on the effective mobilities of the anions, the complexing agent was present in the carrier electrolyte solutions at 25–90 mmol/l concentrations. These experiments, evaluated in relative mobility scales (taking one of the anions as a reference), revealed that  $\alpha$ -CD influences the effective mobili-

ties of the anions in a differentiating way. Its influence on the effective mobility of fluoride was the lowest and this anion was chosen as a reference constituent for a relative mobility scale in which changes in the effective mobilities of the anions due to their host–guest complexations were presented. The plots in this scale (Fig. 2) show relative contributions of the host–guest complexations to the effective mobilities of the studied anions. From the point of view of the CZE separations it is important that extents of these contributions were different so that the separations of the anions based on these differences could be performed (see, Fig. 1b and c). The plots in Fig. 2 and electropherograms in Fig. 1 show that chloride and bromide were the only exceptions and within the studied concentration range  $\alpha$ -CD failed to resolve them. This suggests that the two have not only close actual ionic mobilities [5,6] but also their stability constants with  $\alpha$ -CD are very close.

### 3.2. Some performance parameters

The separation efficiencies for the studied anions were evaluated in the carrier electrolyte solution containing  $\alpha$ -CD at a 75 mmol/l concentration. From the data obtained in this evaluation (Table 2) we can see that the separation efficiencies for chloride and sulfate are considerably lower than those for the rest of the anions. Our attempts to explain this somewhat unexpected results with the aid of computer simulations of the CZE separations [29] revealed that the time constant of the contactless conductivity detector (0.5 s) contributed significantly to the plate heights of these analytes. The simulations carried out for hypothetical anions of different effective mobilities and the working conditions approaching to those used in our experiments showed that the dispersive effect due to the time constant of the detector (for relevant relationships see Ref. [29]) was dramatic for the most mobile anions. However, its contribution to the total dispersion was found to drop very steeply within a narrow mobility interval. Similar experimental findings were reported very recently by Lucy et al. [30] in conjunction with the CZE separations of inorganic anions with indirect photometric detection operating at various time constants.

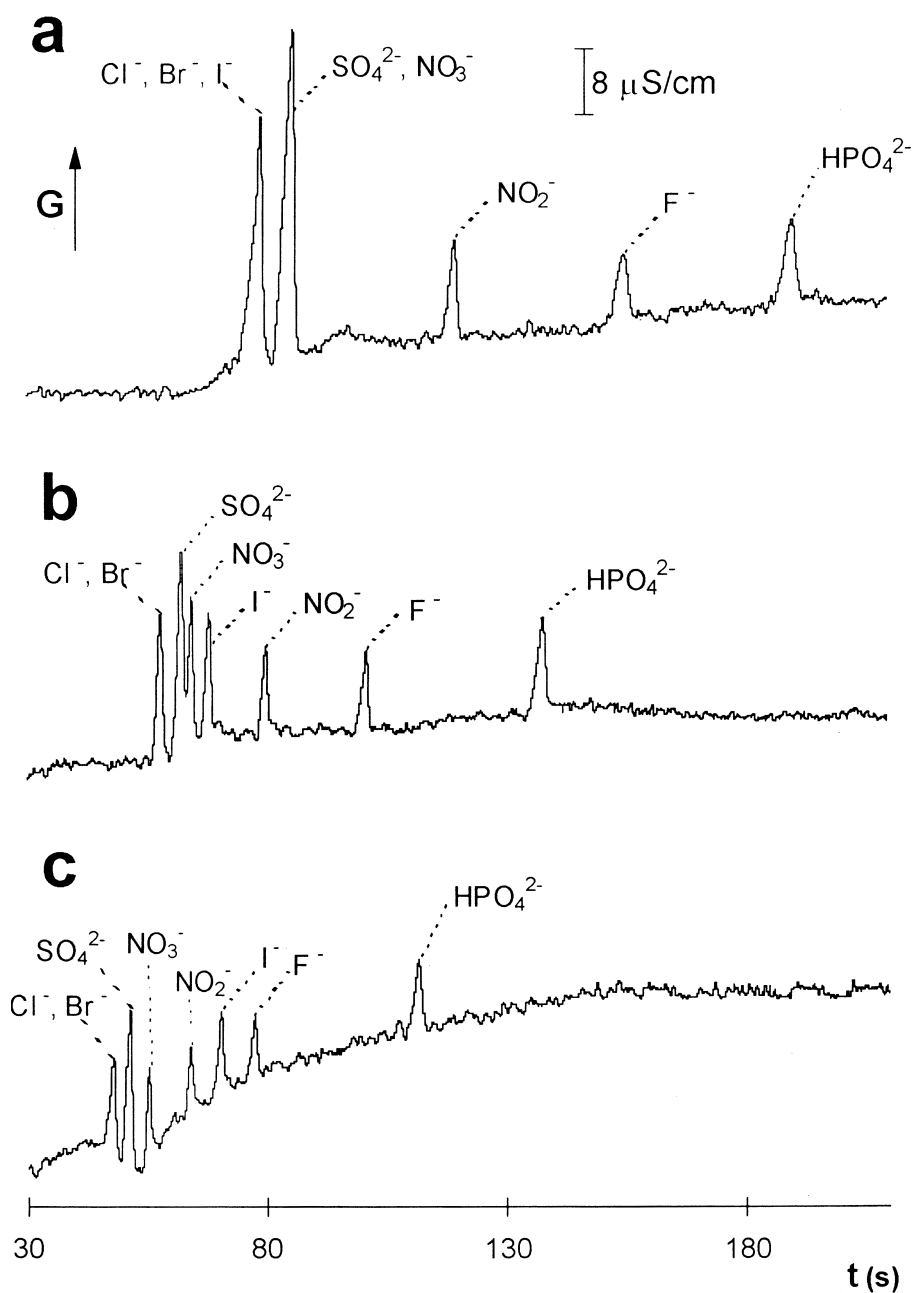


Fig. 1. Electropherograms from the separations of inorganic anions at various  $\alpha$ -CD concentrations in the carrier electrolyte. (a) Without  $\alpha$ -CD; (b) 25 mmol/l  $\alpha$ -CD; (c) 75 mmol/l  $\alpha$ -CD. The concentrations of the anions in the injected samples were 10  $\mu$ mol/l. The separations were carried out with the driving current stabilized at 30  $\mu$ A. The voltage between the driving electrodes was 5.5 kV in the carrier electrolyte solution without  $\alpha$ -CD. It was 11.0 kV when the carrier electrolyte solution contained  $\alpha$ -CD at a 75 mmol/l concentration (for other concentrations of  $\alpha$ -CD it was between 5.5–11 kV in a proportion to the concentration of  $\alpha$ -CD). For further details on the compositions of the carrier electrolyte solutions see Table 1. G=Increasing conductivity.

Table 1  
Electrolyte system

Parameter	
Solvent	Water
Carrier ion	Succinate
Concentration (mmol/l)	7
Counter-ion	BTP
Concentration (mmol/l)	0.5
pH	3.55
Additive	m-HEC
Concentration (% w/v)	0.2
Complexing additive	$\alpha$ -CD
Concentration (mmol/l)	0–90

BTP=1,3-Bis[tris(hydroxymethyl)methylamino]propane; m-HEC=methylhydroxyethylcellulose;  $\alpha$ -CD= $\alpha$ -cyclodextrin.

The concentration limits of detection (cLODs) for the anions were estimated in the way as proposed for elution chromatography by Foley and Dorsey [31]. From the cLOD data in Table 2 it is apparent that the contactless conductivity detection was less sensitive than its contact counterpart under similar working

conditions [8]. This appears a certain disadvantage of the present contactless detection technique. In this context we should note that developments of the contactless conductivity detectors for CZE have just started and further improvements of these key detection performance parameters can be expected. For example, a new detection principle described recently by Zemmann et al. [32] offers an alternative solution to the contactless conductivity detection while the one originating in the ITP works by Vacík and co-workers [24–26] can be probably modified to meet better requirements of CZE. However, from recent reviews dealing with the CZE analysis of inorganic anions [1–4] one can deduce that the cLOD values as given in Table 2 need not be limiting for many practical samples. In addition, an inherent robustness of the contactless detection favours its use, especially, in instances when sample matrices adversely affect performances of the contact conductivity detection sensors [5,33].

The reproducibilities of the determination of the

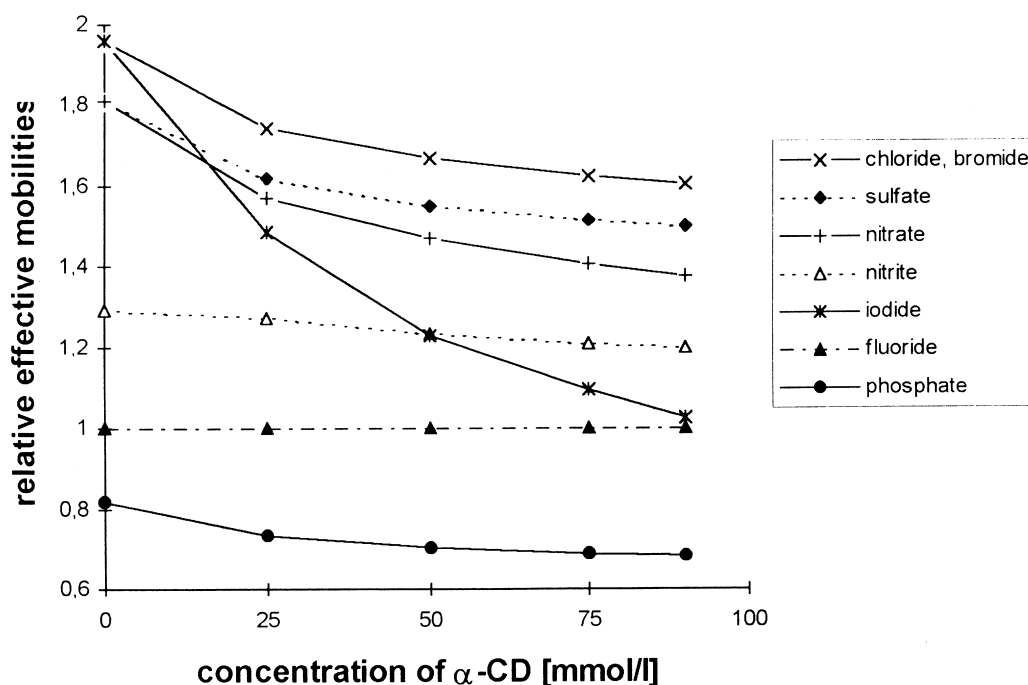


Fig. 2. Dependences of relative effective mobilities of the inorganic anions on the concentration of  $\alpha$ -CD in the carrier electrolyte solution. The effective mobility of fluoride served as a reference and the relative effective mobilities of the anions were calculated from their migration times and the migration time of fluoride at a given concentration of  $\alpha$ -CD. The migration data for the anions were obtained with the driving current stabilized at 30  $\mu$ A. For further details on the compositions of the carrier electrolyte solutions see Table 1.

Table 2  
Separation performance parameters for the anions

Anion	Migration time		Separation efficiency ( <i>N/m</i> )	cLOD ( $\mu\text{mol/l}$ )	R.S.D. of the peak area (%)
	Mean (s)	R.S.D. (%)			
Chloride	45.1	0.30	60 000	0.99	14.0
Sulfate	49.4	0.15	64 000	0.46	3.0
Nitrate	53.5	0.04	135 000	0.87	1.9
Nitrite	62.2	0.10	131 000	1.11	5.4
Iodide	69.4	0.20	131 000	0.97	1.6
Fluoride	75.1	0.15	163 000	0.89	2.1
Phosphate	109.3	0.06	180 000	1.40	1.2

R.S.D.=Relative standard deviation for five parallel determinations ( $10 \mu\text{mol/l}$  concentration of the anions); *N/m*=the number of theoretical plates per metre; cLOD=the concentration limit of detection. The separations were carried out in an electrolyte system with  $75 \text{ mmol/l}$   $\alpha$ -CD. The driving current was  $30 \mu\text{A}$ .

studied anions were evaluated for their  $10 \mu\text{mol/l}$  concentrations in the injected sample. From the relevant data in Table 2 we can see that with the exception of chloride very reproducible CZE determinations of the anions were possible at the concentrations approaching those corresponding to the limits of quantitations [34]. A significantly higher R.S.D. value in the quantitation of chloride on this concentration level was probably linked with fluctuations in the concentration of this anion in water used for dilutions ( $0.1$ – $1 \mu\text{mol/l}$  concentrations of chloride as determined by CZE) of the stock solutions of the model samples.

### 3.3. CZE separations of inorganic anions present in practical samples

A large difference in the effective mobilities of chloride (bromide) and iodide due to a stronger complexation of the latter with  $\alpha$ -CD is favourable for the CZE determinations of iodide in samples containing in large excesses the former halide anions. Experiments with a dietary salt sample, containing iodide at  $1$ – $2 \cdot 10^5$ -times lower concentrations than chloride, were aimed at determining a maximum concentration ratio of the two which still allows the quantitation of iodide. Illustrative electropherograms from these experiments are given in Fig. 3. When we compare these electropherograms with the ones obtained for model samples containing the anions at  $10 \mu\text{mol/l}$  concentrations (Fig. 1) it is apparent that a transient ITP migration of the sample constituents induced by chloride present in the

injected sample (a transient leading anion) stacked iodide with chloride. Here, succinate from the carrier electrolyte acted as a terminating anion in the initial phase of the separation (for details on the transient ITP in the CZE separations see, e.g., Refs. [35–37]). This transient phase of the separation, in fact, set a limit for a maximum concentration ratio of these anions loadable onto the column once iodide was present in the injected sample at a detectable concentration. Consequently, under our working conditions the resolution of chloride and iodide was lost when these anions were present in the injected samples at the chloride/iodide molar concentration ratio higher than 1700:1 (see Fig. 3b). In spite of the fact that these anions probably cannot be resolved at such a concentration ratio in currently used CZE separation systems it was still small to make the detection of iodide present in the dietary salt sample with the present conductivity detector possible (Fig. 3a).

A series of electropherograms in Fig. 4 was obtained in our experiments aimed at separating inorganic anions to be determined in milk [38] in a combination with a simple sample pretreatment procedure as described in Section 2.3. This sample was chosen to test a potential applicability of the electrolyte systems studied in this work to the CZE separations of inorganic anions present in highly complex matrices. From one of the electropherograms obtained in these tests for a highly diluted milk sample (Fig. 4a) it is apparent that our approach offers a possibility for a rapid CZE separation of anionic macroconstituents present in milk (chloride,

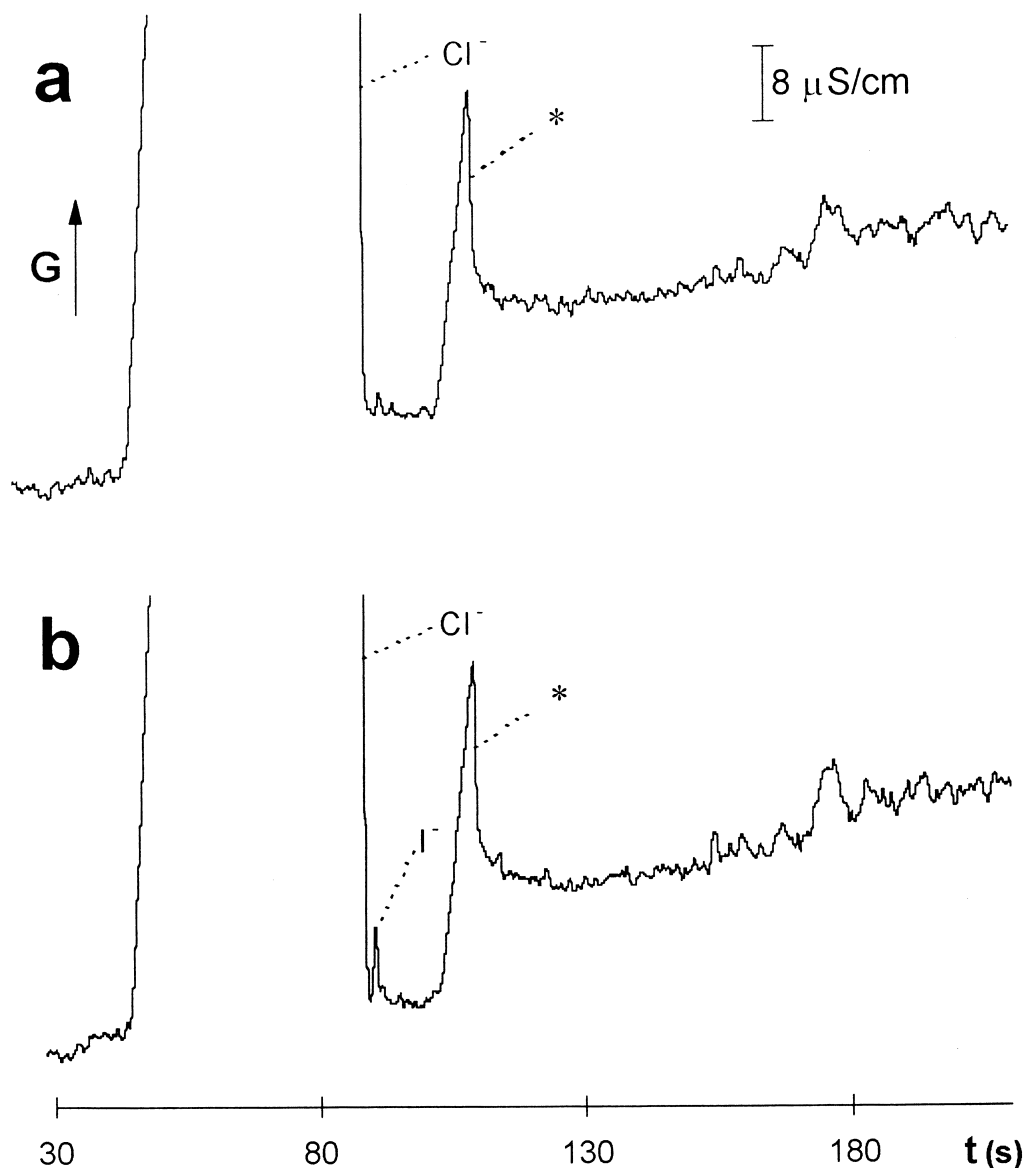


Fig. 3. CZE of a dietary salt sample in the carrier electrolyte solution containing  $\alpha$ -CD at a 75 mmol/l concentration. (a) 8 mmol/l concentration of the salt sample (calculated as NaCl); (b) the same as (a) only the sample was spiked with iodide at a 5  $\mu$ mol/l concentration. The peak marked with an asterisk was not identified. The driving current was stabilized at 30  $\mu$ A. For further details on the compositions of the carrier electrolyte solutions see Table 1. G=Increasing conductivity.

sulfate and phosphate). The rest of current inorganic anions (e.g., iodide, fluoride, nitrate) can be expected to be present in the injected sample at concentrations [38] below the cLOD values (see Table 2) for such a sample dilution. An electropherogram in Fig. 4b obtained for a 1:10 diluted milk sample shows that

they were not detected by the conductivity detector even at a 50-times increased sample load. The cLOD values (Table 2) indicate that iodide, fluoride, nitrate were present in this sample at concentrations below 5–10  $\mu$ mol/l. However, in this context it should be noted that for such a sample load the presence of

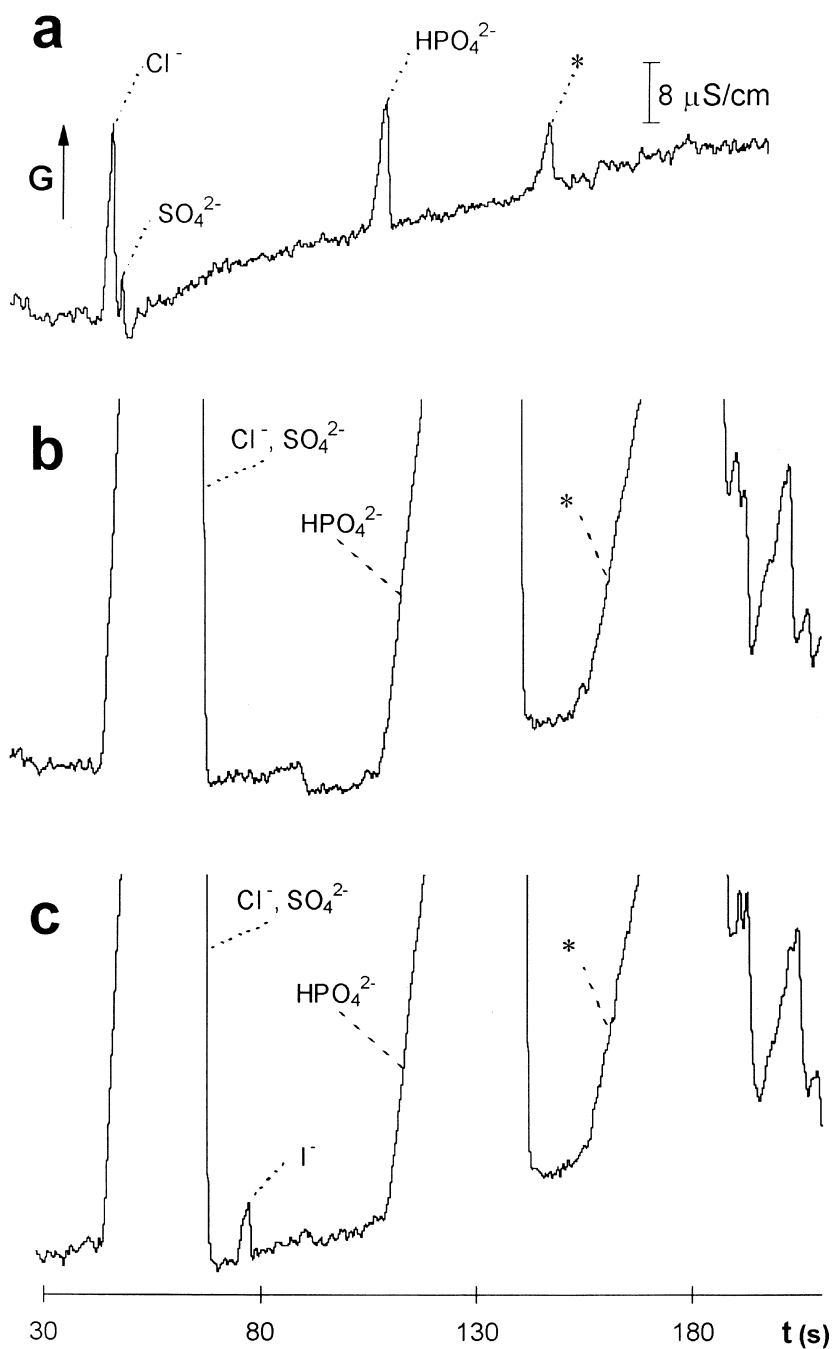


Fig. 4. Electropherograms from the separations of anions present in a milk sample. (a) Milk sample diluted with water in the ratio of 1:500; (b) the same milk sample as in (a) only the dilution was 1:10; (c) the same milk sample as in (b) spiked (after the sample dilution) with iodide at a  $10 \mu\text{mol}/\text{l}$  concentration. The CZE separations were carried out in the carrier electrolyte (Table 1) with  $\alpha\text{-CD}$  at a  $75 \text{ mmol}/\text{l}$  concentration. The driving current was stabilized at  $30 \mu\text{A}$ . The peaks in the electropherograms marked with an asterisk are unidentified organic anions. G=Increasing conductivity.



nitrate in the ITP stack [35–37] with chloride and sulfate could not be excluded.

Milk contains moderately weak organic acids at concentrations comparable to those of inorganic anions [39]. These acids can be considered to be main (potential) sources of interferences in the CZE analysis of inorganic anions at higher pH values (close effective mobilities of both groups of ionogenic constituents) as currently preferred in CZE [1–4,6,7]. A lower pH value of the electrolyte system as used in this work (Table 1) offers an effective way for minimizing such interferences by reducing the effective mobilities of organic acids via a well known separation mechanism (see, e.g., Refs. [5,6]). Electropherograms in Fig. 4 show that in the migration window of the inorganic anions (demarcated from the side of lower effective mobilities by the phosphate peak) none of the organic acid present in the milk sample was detected. Apparently, this is in an agreement with the results obtained for model samples of organic and inorganic anions under similar acid–base conditions [8]. Such a selectivity of the separating conditions for the inorganic microconstituents (iodide, fluoride, nitrate), however, was not accompanied by an adequate sensitivity of the detector. In this context we should note that the present electrolyte system is compatible with the direct UV photometric detection at the wavelengths suitable for the detection of microconstituents (for the absorptivities see, e.g., Ref. [40]). This suggests that a combination of the conductivity and UV photometric detectors as used in ITP [5] offers a convenient detection alternative for simultaneous determinations of both the macro- and microconstituents.

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