Focusing of alkali earth metals in ligand step gradient

Eliška Šišperová, Eliška Glovinová, Jana Budilová, Jan Pospíchal*

Department of Chemistry and Biochemistry, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

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ABSTRACT

A capillary electroseparation technique for focusing and selective pre-concentration of metal chelates with subsequent on-line isotachophoresis (ITP) analysis was developed and verified. The ions of alkali earth metals (Mg, Ca, Sr, and Ba) were pre-concentrated from the mixture and analyzed. The focusing of the metals was carried out in a ligand step gradient, which was created by the addition of a convenient ligand agent to the regular stationary pH step gradient. The analytical procedure consisted of three steps. During the first step, the metal ions were electrokinetically continuously dosed into the column where they were selectively trapped on the stationary ligand step gradient in the form of unmoving zones of chelate complexes with effectively zero charge. After a detectable amount of analyte was accumulated, the dosing was stopped. The accumulated zones were mobilized to the analytical column, where they were analyzed by the ITP method with conductivity or photometric detection. The proper electrolyte systems for dosing, mobilizing, and analyzing in isoelectric focusing (IEF), moving boundary electrophoresis (MBE), and ITP modes were consequently developed and put into practice. The trapping selectivity can be regulated by the choice of pH and convenient complexing agents. A mixture of alkali earth metals were used as model analytes. Using a 3000 s dosing time, the proposed method improved the detection limit by 5–29 times in comparison to analysis by ITP with classical injection.

1. Introduction

In electrophoresis, a single capillary technique, e.g., CZE or ITP with UV detection, is predominantly used to analyze simple, uncomplicated matrices, with the benefits of a short analysis time and a high separation efficiency for a small volume of the injected sample.

This is not the case for biological and environmental mixtures where matrices are mostly complex and present a very broad concentration range of analytes and often a very high concentration of inorganic salts. In such cases, a common UV-detection sensitivity in conjunction with a low-volume injected sample is mostly insufficient for a trace analysis, and detection limits have to be lowered.

In order to improve the detection limits and analytical responses, a sample with a complex matrix must be pre-concentrated and pre-cleaned prior to the analysis. Off-line pre-concentration and pre-cleaning methods, such as liquid extraction, adsorption, and SPE were mostly adopted from HPLC sample pretreatment [1]. They are effective, but laborious and time consuming, convenient only for large batch analysis. In terms of time and labor conservation, such procedures can be performed to advantage on-line, which has led to the development of more sophisticated or even abbreviated techniques.

The simplest techniques, mostly performed in a single capillary, e.g., field-amplified sample stacking, large volume sample stacking, transient ITP, dynamic pH junction, and sweeping, are used for pre-concentration mainly prior to CZE analysis [2]. These techniques can be easily performed with any type of apparatus, and are the first solution when a sample is more complicated. The concentration factor is usually 10–100. In sweeping, t-ITP, and electrokinetic supercharging, a concentration factor of 1000 was achieved [3–6]. For the ITP, the method called large volume sampling was developed, where using combination of hydrodynamic counter-flow of leading electrolyte from LE electrolyte chamber and hydrodynamic and electrokinetic co-flow of sample from injection point close to terminating chamber is possible nearly unlimited dosing sample to the column. Method works in the steady state, accumulated sample concentrations obey Kohlraush law and its volume is restricted by volume of the capillary [7].

Two-capillary techniques are more advanced and also more technically demanding, and are also mostly hyphenated, i.e., different electrolytes and electrophoretic methods can be used in each capillary. Combinations of ITP–CZE [8], IEF–CZE [9], carrier ampholyte free isoelectric focusing with capillary zone electrophoresis (CAF-IEF–CZE) and CAF-IEF–ITP [10] have been published. These techniques perform an efficient pre-concentration and pre-separation in one electrolyte in the first capillary. The sec-

* Corresponding author. Fax: +420 545 212 044.
  E-mail address: posp@mendelu.cz (J. Pospíchal).

ond analytical capillary filled with another analytical electrolyte serves for the detection. This provides advantages when subsequently performing the analysis. Two-capillary techniques provide greater flexibility when choosing the electrolyte; moreover, only a part of the pre-separated sample can be introduced to the analytical capillary, which increases the separability.

An achieved concentration factor of approximately 100 is usual when combining ITP–CZE [11], or IEF–CZE [12]; a combination of CAF-IEF–ITP with so-called continuous dosing can attain a factor of 10^6 [10]. This favorable concentration factor was reached because the separated substances were ampholytes that were dosed and focused on a stationary neutralization reaction boundary—NRB with a pH step gradient. In such an arrangement in a given electrolyte system, the amount of an accumulated substance is only a function of the dosing time, and can be increased if needed.

We were interested in performing a sensitive metal analysis. Interesting possibilities for the transient pre-concentration of metals prior MEKC or CZE analysis were described lately in the [13]. The MEKC applicable method called sweeping uses the creation of the reaction boundary in between sample and background electrolyte for the increasing of metal concentration. Anionic metal complexes migrating from the hyrodynamically injected sample react on the boundary with oppositely migrating cationic micelles from BGE, depositing here zone of low mobility low charged metal-complex-micelles associates. The position of the reaction boundary is held in the column by the electroosmotic flow. The balanced velocities (de facto fluxes) of micelles/metal complexes keep the reaction deposit zone narrow and highly concentrated. This can be set up by proper concentration of the reaction component. The addition of organic solvents MeOH in BGE regulates the EOF and solubility of micelles. The pre-concentration achieved was 15–42-fold for Cu, Co, Zn, Mn, and Pb.

The similar pre-concentrating effect was used also for the CZE [14]. Here, analogously to the previous case, the reaction boundary is created in between sample and BGE, where cathodically migrating metals from the sample react with the anionically migrating chelating agents from the BGE. A deposit zone of low charged metal chelates is created on the reaction boundary. Again, the balanced velocities (de facto fluxes) of metal/complex agent keep the reaction deposit zone narrow and highly concentrated and are set up by proper concentration of the reaction component. The pre-concentration achieved was 140,000-fold in the case of Pb, Cu, Co, and Mn. For the metal separation, a use of moving chelating boundary was described lately in ITP [15].

Metal complexes were focused similarly to ampholytes by focusing electrochromatography [16,17] and/or by continuous electrophoresis with stepwise gradient of complexing agent [18], due to their dependence on the pH charge [19,20]. This implies that we can also explore the use of modified CAF-IEF for metal analysis.

The goal of our work is to develop method and electrolyte systems for efficient pre-concentration, focusing, and analyzing metal complexes. Here described method is based on principle of "iso-electric" focusing of metal complexes in stationary neutralization reaction boundary (NRB) with sharp pH step gradient, which is combined with stepwise concentration of the complexing agent. Such a combination was called ligand step gradient focusing (LSGF).

2. Theoretical considerations

2.1. Choice of the electrolyte system

The choice of the electrolyte system is a crucial point of all electromigration methods. The composition of electrolyte, i.e., solvent, concentrations, pH, presence of the complexing agents and or pseudo-stationary phase aims to differentiate mobility of analytes,

thus enabling separation. Mostly, a constant composition of electrolyte during the analysis is maintained. The electrolyte system is more complicated in the focusing methods, like IEF, where at least 2 mutually dependent electrolytes of different properties must be used.

A few rules must be fulfilled to reach conditionally steady state focusing with subsequent analysis.

The idea of focusing is based on the superposition of two counteracting driving forces, where one must be non-linear through the separation space.

The substance focuses in the place where these forces (influencing the substance velocity) are in equilibrium. Non-linear hydrodynamic flow fields, electric fields, and chemical field gradients were described in electromigration methods [21,22]. Extreme form of the non-linearity is a step change of chemical properties, which were published by Deman [23–26] on sharp moving reaction boundaries—MRB in zone electrophoresis. The most widely used and important chemical field is a pH gradient, principally introduced by Kolin [27] and practically realized using synthesized carrier ampholytes [28].

The simplest, smallest part of the pH gradient is an NRB where the pH gap, or difference in pH between two solutions, exists. A flux of solvolytic ions H^+ and OH^- enters the boundary from the opposite side, creating water and depleting ions from within the boundary. A simple conditional equality of solvolytic fluxes must be fulfilled to keep the boundary stationary (see Eq. (1), below), where $J_{H,H}$ denotes the flux of H^+ into the acid zone H, $c_{H,H}$ and $u_{H,H}$ indicates the concentration and mobility of H^+ in the zone, κ_H is conductivity, I is current density, and subscript OH denotes the same as OH^-:

$$J_{H,H} = J_{OH,OH} = c_{H,H} \cdot u_{H,H} \cdot I/\kappa_H = c_{OH,OH} \cdot u_{OH,OH} \cdot I/\kappa_{OH} \tag{1}$$

$$\nu = \frac{J_{H,H} + J_{OH,OH}}{c_{H,H} + c_{OH,OH}} \tag{2}$$

If the fluxes are not in equilibrium, the boundary starts to move in the direction of the prevailing flux with velocity ν (see Eq. (2)).

The solvolytic ion fluxes thus rule the behavior of the focusing electrolyte system. Maintaining the fluxes is crucial especially with CAF-IEF [29], where at the beginning only one NRB exists. Because the flux is the product of concentration and mobility, the magnitude of each flux can be influenced and regulated during the run only by decreasing the concentration through dilution with the proper co-ion, e.g., by electric regulation [30,31] and/or by changing the effective mobility using the appropriate buffering or non-buffering counter ion. The ionic mobility of the solvolytic ions in the non-buffered electrolytes is very high, and thermal fluctuations in mobility and/or minute changes in concentration without further additional electric regulation can prevent constant fluxes or hinder a stationary boundary.

Using buffered electrolytes is the only choice when working with commercial equipment that is not equipped with electric regulation. We use both cationic and anionic buffering. This ensures that H^+ and OH^- mobility is low, i.e., the boundary is stable and during the mobilization step cannot be faster than separated and accumulated ions.

If we add a charged, complex-forming ligand to the electrolyte with NRB, it will pass through the system and in dependence on pH will create two regions with different complex-forming strengths—a ligand step gradient (LSG).

The situation on the boundary is shown in Fig. 1. From the cathodic side, the boundary is entered by hydroxyl ions, which set up alkaline pH, acetate ions acting as a buffer for the acidic pH region, and ligand anions. Similarly, from the anodic side, the boundary is entered by hydronium ions, which set up acidic pH,
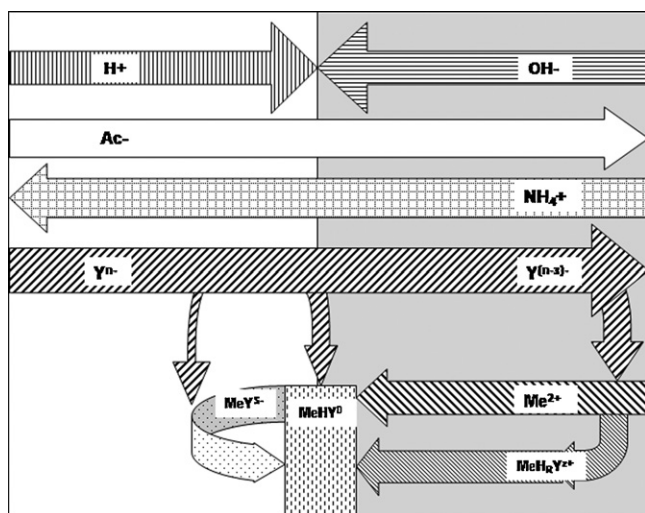
Fig. 1. The scheme of the flows in the ligand step gradient (LSG) boundary. Hydroxyl and hydronium ions are neutralized on the boundary, ammonium, acetate and ligand ions crosses the boundary and metal complexes are focused on the boundary.

ammonium cation acting as a buffer for the alkaline region, and metal cations, which are accumulated in the boundary.

By choosing of at least three-valent anion as a complex forming ligand, metal chelate complexes can be focused by reversing the charge on both side of the LSG. We observed that focused metal chelates create rectangular zones, which implies that the pH concentration in the zone is constant and follows the Kohlraush law. The length of the zone is proportional to the quantity of the metal focused, but it cannot be used for exact quantitative measurement because the Kohlraush omega function is not constant at the boundaries.

The strength and selectivity of LSG-chelating power can be regulated by the concentration of the ligand in the electrolyte system, by pH step on both sides of the boundary, and by choosing the nature of the chelating agents with convenient stability constants. The demands on the chelating agents are simple: in the alkaline part of the LSG, negatively charged anionic chelates must be created; in the acidic part of LSG, positively charged chelates or free metal cation must exist.

In practice, in the common concentrations used in electrophoresis, metal chelates with a stability constant pK_{MeHL} in the regions 6–11 were easily focused. The pH and the concentration of the ligand can be estimated from a calculated complex distribution diagram. Some convenient ligands can be found in the group of the metalochromic indicators. Their complexes are conditionally stable and are colored; moreover, the substances have properties of pH indicators. These facts enable the visual and photometrical observation of the movement of the LSG, focused complexes, and their sensitive photometric detection in a visible region. The solubility and dissociation in the acidic electrolyte should reasonably prevent the precipitation of the complex agent from the solution.

As a model sample, we chose a mixture of alkali earth metals, for which it was convenient to use o-cresolphthalein complexone (OCP). OCP is a hexavalent triphenylmethane acid dye with a chelating ligand containing two pairs of iminodiacetic acid functional groups. OCP forms characteristic deep purple complexes (MHL^{3-}) with alkali earth metals (abs. max. at 575 nm). It is highly soluble in aqueous alkaline solutions. At pH 10.0–11.0, an OCP solution is slightly pink ($pK_{a4} = 7.8$, $pK_{a5} = 11.4$) due to impurities. The background absorbance of the electrolyte and the absorbance of the alkali earth complexes at a given electrolyte condition were so different in color and density that it was possible to observe the LSG boundary and distinct zones of analytes. OCP creates convenient

complexes only with alkali earth metals. Metals creating weaker complexes are not focused and pass through LSG; metals creating stronger anionic complexes at DE condition are not even dosed to the column.

3. Materials and methods

3.1. Apparatus

A commercially available isotachophoretic apparatus (CS Isotachophoretic Analyzer, Labeco, Slovak Republic) in the column-coupling configuration equipped with conductivity detection in the pre-separation and analytical column was used. The capillaries were made from Teflon; the upper pre-separation was 150 mm in length, i.d. 0.8 mm; the lower analytical capillary was 160 mm in length, i.d. 0.3 mm. The apparatus was equipped with a dosing valve volume of 30 µl.

The CSW data collection and evaluation software (Prague, Czech Republic), running on IBM PC with OS Microsoft Windows XP (Redmond, USA), was used for data acquisition.

3.2. Chemicals

Ostion KS 0809 strong acid ion exchanger based on styrene–divinylbenzene matrix was obtained from Spolchemie a.s. Ústí n. Labem, Czech Republic. All other chemicals – polyethyleneglycol (PEG 6000), Triton X-100, ammonium, calcium, magnesium, barium, strontium acetate, and barium sulfate – were of analytical grade, and were obtained from PLIVA-Lachema (Brno, Czech Republic). OCP was the only indicator-grade chemical.

Preliminary experiments showed that some chemicals – OCP and PEG 6000 – contained substantial amounts of disturbing metallic impurities, mainly calcium. These chemicals were cleaned by passage through a bed of strong acid ion exchanger in H^+ form. OCP was cleaned as a 0.2 M solution and diluted to a final 0.1 M concentration of stock solution, which was stored in a cool, dark place. The residual concentration of Ca^{2+} determined by ITP was 0.0028 M in 1 M of OCP. PEG 6000 was similarly cleaned as a 2% solution.

A saturated solution of barium sulfate was prepared by shaking surplus $BaSO_4$ with distilled water for 20 min. After 24 h, sedimentation and a clear supernatant were collected and used.

3.3. Electrophoretic procedure

We adopted a previously developed electrophoretic procedure that was used for the CAF-IEF of ampholytic dyes and amino acid [10,32]. The analysis was performed in three steps, which consisted of: continuous dosing for the accumulation of the analytes in the column (LSGF mode), mobilization of the accumulated zones for their transfer to the analytical column (ITP or MBE mode), and a final detection step (ITP mode). The whole procedure is shown in the schematic diagram in Fig. 2.

In Fig. 2, part A, a scheme of the column filled with electrolytes is shown. The lower analytical column and its electrolyte chamber is filled with the leading electrolyte (LE); the upper pre-separation column and its electrolyte chamber is filled with alkaline primary electrolyte (PE); the dosing valve is optionally filled with sample dissolved in the terminating electrolyte (TE), for shifting a boundary down to the column; the upper electrolyte chamber is filled with the dosing electrolyte (DE).

In the first step of analysis, depicted in Fig. 2, part B, a sharp stationary neutralization boundary with a ligand step gradient is created by the influence of the driving current in the upper pre-separation column in between the acidic dosing and the alkaline primary electrolyte. An optional terminating electrolyte is located
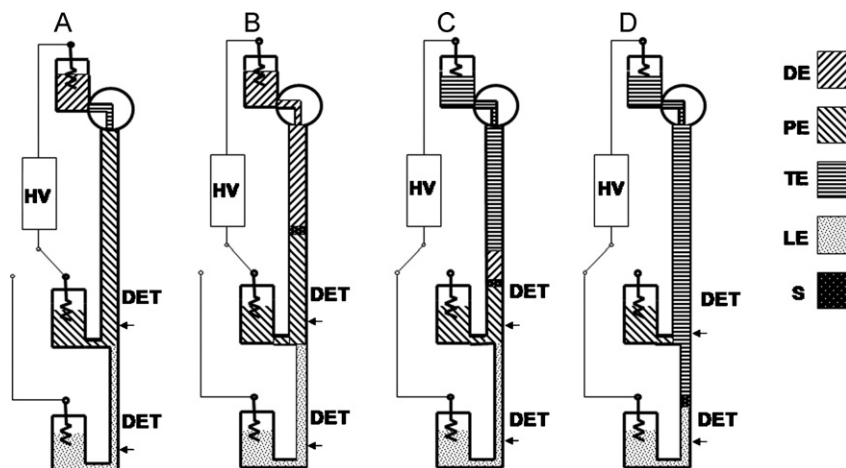
Fig. 2. Scheme of the analytical procedure. (A) Column prepared for the analysis, filled with proper electrolytes. (B) Continuous dosing procedure. (C) Mobilizing procedure. (D) Detection procedure.

in the dosing valve. Here, acetic acid neutralizes part of the primary electrolyte and cause a shift of the LSG boundary down to the column, where it can be visually observed. Metal cations and positively charged metal complexes are driven from the acidic dosing electrolyte to the LSG boundary, where the influence of pH and the increased ligand affinity are changed to complexes with an effective charge of zero. Here on the boundary, complexes accumulate and stay in the boundary until the second, mobilizing step. The amount of the accumulated substance is proportionally dependent on the dosing time, and thus can be properly regulated. A replacement dosing for the terminating electrolyte in a terminating chamber, as depicted in Fig. 2, part C, causes mobilization. The focused zones of metal complexes are acidified, gain a positive charge or break down, release free metal cations, and migrate to the second, analytical column, where they are analyzed in the leading electrolyte, and detected as seen in Fig. 2, part D.

4. Results and discussion

4.1. Electrolyte system

4.1.1. Leading electrolyte—LE

For a cationic analysis, an ammonia cation was used as a leading ion, mainly for its purity. Purified OCP was used as a buffering and complex forming counter-ion. Its buffering properties were not satisfactory: the effective mobility of the H^+ as a terminator was so high that the zone of magnesium disappeared during the analysis. A partial replacement of OCP by a well-buffering acetate anion lowered the effective mobility of the H^+, but the color density was then unsatisfactory. We doubled the total concentration of LE, so the final composition of LE was 0.02 M NH_4OH + 0.005 M OCP, 1% PEG 6000, 400 ppm Triton X-100, pH = 8.6. The electrolyte also contained a residual concentration of Ca^{2+} coming from OCP impurities; its concentration was determined as a 14 µM. This electrolyte was used for focusing in the first capillary and for analysis in the analytical capillary. Zones of alkali earth metals migrate as positively charged complexes in the enforced ITP mode (see Fig. 3). PEG 6000 was added to suppress violent electro-osmotic buoyancy on the NRB-LSG boundary due to the strong difference in pH and thus EOF. The suppression of EOF improves the disturbed shapes of the separated zones and the separation, which is almost impossible without added PEG. The used driving current was 300 µA/100 µA and separation time was 24 min/25 min in the pre-separation/analytical column.
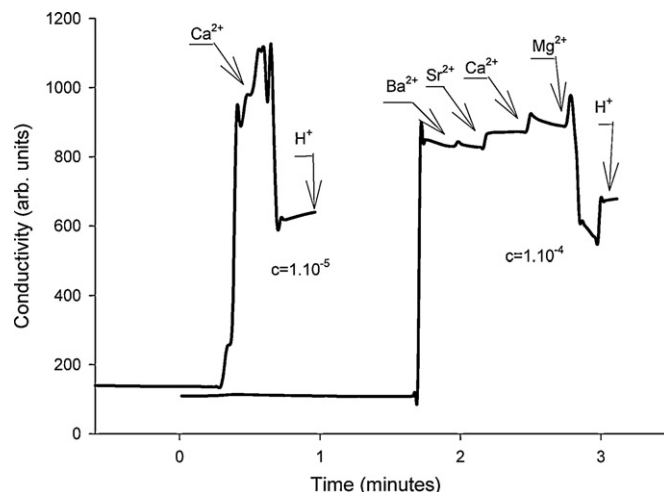

Fig. 3. Analytical record showing the dependence of the zone length on the concentration of analyzed ions—regular ITP–ITP analysis. Sample: 1×10^{-5} M and 1×10^{-4} M solution of Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}. LE = 0.02 M NH_4OH + 0.005 M OCP, 1% PEG 6000, 400 ppm Triton X-100, pH = 8.6. TE = 0.03 M HAc.

4.1.2. Dosing electrolyte—DE

The DE has two important roles. First, it must use its H^+ flux to balance the incoming OH^- flux from the LE. Second, it serves in the electromigration dosing of metals to the column. Establishing the concentration ratio of H^+/NH_4^+ cations regulates the magnitude of the H^+ flux. The proper ratio was evaluated experimentally by plotting the speed of the LSG boundary against the composition of the DE with different ratios of H^+/NH_4^+ whilst maintaining the constant composition of primary electrolyte. The measured dependence of relative velocity of the PE–DE boundary on the molar fraction of mixture HAc/NH_4OH is shown in Fig. 4. The relative velocity of the boundary was referenced to ITP velocity PE–TE at a given primary electrolyte, which was chosen as a unity. A composition of the electrolyte giving zero relative velocity was find out by extrapolation from the plot in Fig. 4. The molar fraction 0.8 of the HAc/NH_4OH must be kept in the dosing electrolyte for a given composition of primary electrolyte. The actual concentrations depend on the buffering properties of the sample and on conductivity, which is limited by column construction (Joule heat production).

The final usable composition was 0.04 M HAc + 0.01 M NH_4OH + sample, pH = 3.83, which was used in all experiments with continuous dosing.
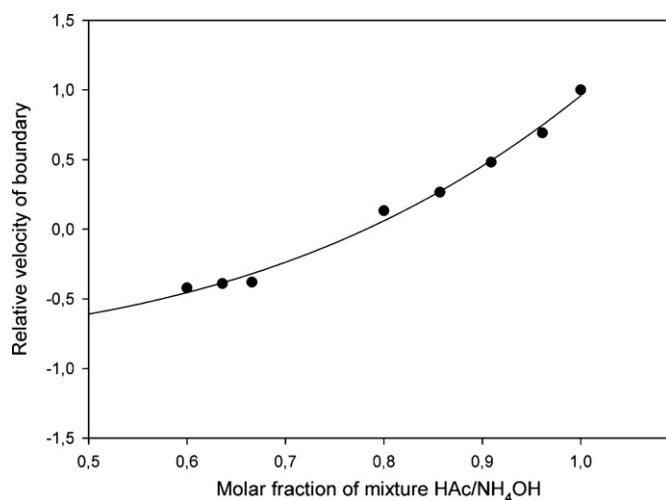
Fig. 4. Measured dependence of the relative velocity of the neutralization reaction boundary (NRB) on the composition of the adjacent electrolytes. Relative velocity is referenced to ITP velocity PE–TE at a given primary electrolyte, which is chosen as a unity.

Fig. 5. Dependence of the zone length Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+} on the dosing time for the combination of ligand step gradient focusing with ITP (LSGF–ITP–ITP) combination, at a constant concentration of metals $c = 1 \times 10^{-5}$ M/. LE = 0.02 M NH_4OH + 0.005 M OCP, 1% PEG 6000, 400 ppm Triton X-100, pH = 8.6. DE = 0.04 M HAc + 0.01 M NH_4OH + sample, pH = 3.83. TE = 0.03 M HAc.

The dosing speed of the DE sample is affected by the sample mobility and concentration, by the driving current, and by the concentration-conductivity of the DE. An increase of driving current and decrease of conductivity can produce an increase in dosing speed of more than 20 times. This has been shown [23], and is not the subject of our investigation. The used driving current for the continuous dosing—focusing was 300 μA.

4.1.3. Terminating electrolyte—TE
A classical 0.03 M HAc, served as the TE.

4.2. Estimation of the detection limits for classical ITP–ITP mode

To estimate the degradability of the complexes and detection limits in the given electrolytes, the calibration curves of the alkali earth metal were measured. A sample of a different concentration was introduced via a 30 μl injection valve. It was pre-separated in the pre-separation column working in the ITP mode and transferred into the analytical column. Metals and cationic metal complexes were detected in the ITP detection mode using the same leading electrolyte as in the pre-separation column. The length of the ITP zone was evaluated from the conductivity record.

The calibration curves of the Ca (intercept 1.4673e−3; slope 2230.5; $r = 0.9933$), Mg (intercept 2.2449e−3; slope 2416.4; $r = 0.9981$), Ba (intercept −1.4234e−3; slope 2360.8; $r = 0.9930$), and Sr (intercept −5.3079e−4; slope 1722.5; $r = 0.9973$) are fairly linear. Low intercepts prove that during the analysis no metal ions are bound to the form of kinetically stable/non-degradable complexes.

The zone length was plotted against the amount of the injected sample, ranging from 20 mmol/l to 10 μmol/l. The estimated detection limit of metals was 10 μmol/l in the concentration terms for a 30 μl injection.

4.3. Continuous dosing using combination LSGF–ITP–ITP

A time dependence of zone length on the dosing time was measured for the experimental verification of the use of LSGF for the continuous dosing. A constant concentration of Ca, Mg, Ba, Sr—10 μmol/l (i.e., concentration detection limit) was used in the dosing electrolyte described above. The resulting increase of the

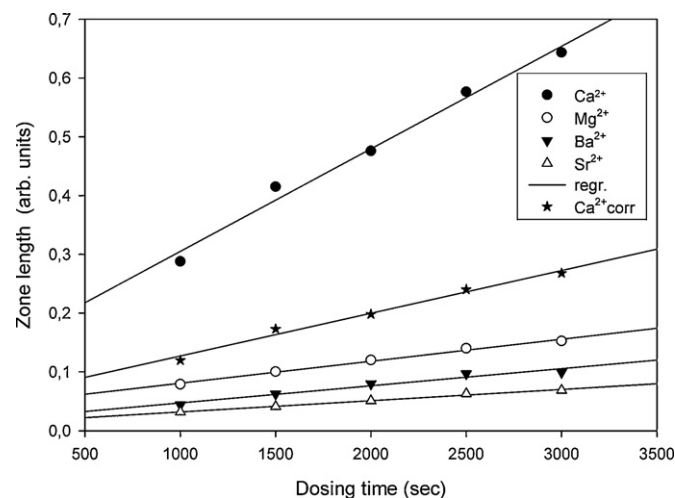metal zone length on dosing time at the dosing current of 300 μA is given in Fig. 5.

The dependence is fairly linear for Ca^{2+} (intercept 0.1305; slope 1.7453e−4; $r = 0.9865$) full circles, Mg^{2+} (intercept 0.0434; slope 3.7400e−5; $r = 0.9918$) empty circles, Ba^{2+} (intercept 0.0180; slope 2.9186e−5; $r = 0.9555$) full triangles, and Sr^{2+} (intercept 0.0126; slope 1.9200e−5; $r = 0.9910$) empty triangles. In the case of Ca^{2+} line, the curve was influenced by the presence of Ca^{2+} impurities in the OCP, which were also pre-concentrated. The corrected curve for Ca^{2+} (intercept 0.0543; slope 7.272e−5; $r = 0.9865$) is given with full stars.

For clarity, in these given conditions (current and DE concentration), measured dosing speed enables an increased amount of accumulated substance once in 314 s for the Ca^{2+} (taking into account Ca impurities presented in LE) 695 s for the Mg^{2+}, 756 s for Ba^{2+}, and 833 s for Sr^{2+}, which is equivalent to an injected volume of 30 μl of DE in a classical dosing procedure (for a given time).

To demonstrate the analytical possibilities of the method, continuous dosing of BaSO$_4$ supernatant as a model sample was performed, choosing the dosing time constant at a reasonably practical value of 2000 s.

The analytical records are shown in Fig. 6. Curve A is the record of supernatant using classical ITP–ITP mode without continuous dosing with a 30 μl injection; curve B is the same sample analyzed by LSGF–ITP–ITP using 2000 s of continuous dosing.

It is clear that during the selected time, a 9× improvement in the c-LOD can be easily reached.

This is not too much in comparison with previous work [10] where we attained a concentration factor 10^6. The one limiting factor of the method is the continuous electrokinetic dosing, where the amount of dosed analyte depends on its transference number in the dosing electrolyte. The transference number depends on the concentration and mobility of all of the ions presented in an electrolyte. In the previous work, we optimized the composition of the electrolyte. We used a 50% lower concentration of background buffering co-ions. Moreover, despite DE acidity, a positively charged complex of metals with lower mobility are created, which causes a lower dosing speed and also different dosing speeds for different metals. Further improvements can be made by optimizing the composition and pH of DE and by choosing the proper complex agents.
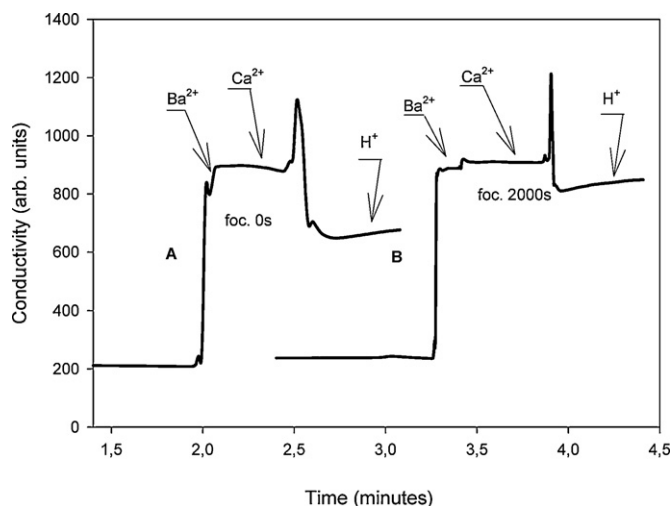
Fig. 6. Comparison of analytical record of routine ITP–ITP analysis of BaSO$_4$ supernatant panel (A), with method LSGF-ITP with 2000 s pre-concentration panel (B). LE = 0.02 M NH$_4$OH + 0.005 M OCP, 1% PEG 6000, 400 ppm Triton X-100, pH = 8.6. DE = 0.04 M HAc + 0.01 M NH$_4$OH + sample, pH = 3.83. TE = 0.03 M HAc.

5. Conclusion

We proved that the suggested combination of LSGF and ITP is a convenient method for pre-concentrating metal ions from diluted samples. Metals, electromigrationally dosed to the column with stationary LSG, were focused in the form of uncharged chelates, which were then mobilized and detected. The amount of substance that can be accumulated using LSGF is a matter of time, and can be established on demand.

The selectivity of LSG varies, and can be regulated by concentration and by the nature of the complex ligand used. Using OCP, a selective focusing and pre-concentration of alkali earth metal was performed.

Increases of up to 29× in the amount of focused model mixture of alkali metal ions in a column was achieved under reasonable working conditions in less than 150 min.

The BaSO$_4$ supernatant was analyzed with a 9× sample enrichment, which is impossible to achieve using the common ITP–ITP combination. The developed buffered electrolyte system also enables the use of commercially available equipment.

Acknowledgements

References

[1] W.G. Kuhr, C.A. Monnig, Anal. Chem. 64 (1992) 389.
[2] M.C. Breadmore, J.R.E. Thabano, M. Dawod, A.A. Kazarian, J.P. Quirino, R.M. Guijt, Electrophoresis 30 (2009) 230.
[3] T. Stroink, E. Paarlberg, J.C.M. Waterval, et al., Electrophoresis 22 (2001) 2375.
[4] L.A. Kartsova, E.A. Bessonova, J. Anal. Chem. 64 (2009) 326.
[5] A.T. Aranas, A.M. Guidote, J.P. Quirino, Anal. Bioanal. Chem. 394 (2009) 175.
[6] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[7] Z. Ryšlavý, P. Boček, M. Deml, J. Janák, J. Chromatogr. A 147 (1978) 369.
[8] D. Kaniansky, J. Marak, J. Chromatogr. 498 (1990) 191.
[9] L.H. Zhang, C.J. Zhang, X.Z. Wu, Anal. Sci. 22 (2006) 1039.
[10] J. Pospichal, E. Glovinova, J. Chromatogr. A 918 (2001) 195.
[11] D. Kaniansky, J. Marak, V. Madajova, E. Simunicova, J. Chromatogr. 638 (1993) 137.
[12] X.Z. Wu, L.H. Zhang, K. Onoda, Electrophoresis 26 (2005) 563.
[13] K. Isoo, S. Terabe, Anal. Sci. 21 (2005) 43.
[14] K. Isoo, S. Terabe, Anal. Chem. 75 (2003) 6789.
[15] W. Zhang, J.F. Chen, L.Y. Fan, C.X. Cao, J.C. Ren, S. Li, J. Shao, Analyst 135 (2010) 140.
[16] W. Friedli, E. Schumacher, Helv. Chim. Acta 43 (1960) 1706.
[17] E. Schumacher, Zeitschrift fur Elektrochemie 64 (1960) 1089.
[18] Zhao Shishan, Zeng Yune, H. Wagner, J. Chromatogr. A 291 (1984) 43.
[19] V. Jokl, J. Chromatogr. 14 (1964) 71.
[20] V. Jokl, J. Chromatogr. 13 (1964) 451.
[21] R.T. Kelly, A.T. Woolley, J. Sep. Sci. 28 (2005) 1985.
[22] X.C.F. Ivory, Sep. Sci. Technol. 35 (2000) 1777.
[23] J. Deman, W. Rigole, J. Phys. Chem. 74 (1970) 1122.
[24] J. Deman, Anal. Chem. 42 (1970) 321.
[25] J. Deman, Anal. Chem. 42 (1970) 1699.
[26] J. Deman, Anal. Chem. 42 (1970) 983.
[27] A. Kolin, J. Chem. Phys. 22 (1954) 1628.
[28] H. Svensson, Acta Chem. Scand. 15 (1961) 325.
[29] M. Deml, J. Pospíchal, J. Chmelík, J. Chromatogr. A 709 (1995) 39.
[30] J. Pospíchal, M. Deml, P. Boček, J. Chromatogr. 638 (1993) 179.
[31] J. Pospíchal, J. Chmelík, M. Deml, J. Microcolumn Sep. 7 (1995) 213.
[32] B. Procházková, E. Glovinová, J. Pospichal, Electrophoresis 28 (2007) 2168.