

Separation of Inorganic Anions by Capillary Zone Electrophoresis Employing Sulfonated Dyes as Probes for Indirect UV Detection

Petr Česla^{*}, Jana Váňová, Kateřina Hlavová, and Jan Fischer

*Department of Analytical Chemistry, Faculty of Chemical Technology, University
of Pardubice CZ-532 10 Pardubice, Czech Republic.*

Abstract: Using indirect photometric detection of anions in capillary zone electrophoresis, the composition of background electrolyte, i.e. buffering system, detection probe and electroosmotic flow velocity should be carefully optimized. In this work, the sulfonated dyes and dye intermediates were tested as the contrast highly absorbing compounds, which are displaced by non-absorbing analytes during the separation and detection. The citrate-based background electrolyte was applied with addition of up to 13 mmol·l⁻¹ of dyes. The best separation comparable to the separation of inorganic anions using chromate indirect detection probe was achieved using 1,3,5,7-naphthalene tetrasulfonic acid, which best matches the electrophoretic mobilities of the analyzed inorganic anions.

Keywords: Capillary zone electrophoresis; Inorganic anion determination; Indirect detection; Sulfonated dye intermediates; Sulfonated azo dyes; Electromigration dispersion.

*) Author to whom correspondence should be addressed. E-mail: Petr.Cesla@upce.cz

Introduction

In recent decades, capillary electrophoresis (CE) became separation technique widely used for the analysis of ionic compounds due to the high separation efficiency, short time of the analysis and low consumption of samples and compounds for preparation of background electrolytes. The most of the electrophoretic separation methods utilize photometric detection,

either to detect directly absorbing analytes, or to monitor the replacement of the strongly absorbing co-ion presented in the background electrolyte (which is the ion of the same charge sign and thus migrating in the same direction as the analyte) by non-absorbing compounds. The latter case of so called indirect photometric detection can be used for analytes, which do not contain suitable chromophores in their structures. When the non-absorbing analyte reaches the detector, the displacement of the visualizing co-ion in the analyte zone causes decrease of the absorbance and the negative peak is recorded. Although the indirect detection in ultraviolet or visible range of the electromagnetic spectra is likely to be less robust, more sensitive to the variations of working conditions and exhibits higher detection limits in comparison to the direct photometric detection, it is one of the most commonly used detection methods in CE [1].

The optimization of the background electrolyte system for indirect detection of ionic compounds is generally based on selection of suitable visualizing co-ion, often called the *probe*, selection of proper buffering system and adjustment of the electroosmotic flow (EOF) to achieve usually co-electroosmotic migration of analyzed ions. The migration characteristics of detection probe and its concentration are important parameters, that should be considered during optimization of the background electrolyte system composition for indirect detection. The effective mobility differences between the analytes and probe should be minimized as it can lead to the electromigration dispersion of the migrating zones and hinder the successful separation. Also the difference between the concentration of the probe and analytes should be maximized to obtain the best possible limit of detection (LOD) [2]. The spectral properties of the detection probe, i.e. the molar absorptivity at detection wavelength ε , is one of the key parameters affecting LOD, which is for non-absorbing analyte given by [3]:

$$c_{LOD} = \frac{c_p}{RD_r} = \frac{N_{BL}}{R\varepsilon l} \quad (1)$$

where c_{LOD} is the concentration of the limit of detection, c_p is the probe concentration, R is the number of the moles of the probe replaced by one mole of the analyte, D_r is the ratio of background absorbance to noise, N_{BL} is the baseline noise and l is the detection cell path length. According to the Eqn. (1), the simplest way to maximize the LOD can be utilized by using strongly absorbing probes, such as organic dyes, but the maximum concentration may be limited by the linearity range of the CE detector and by the electrical current in the separation capillary [4].

The separation and detection of low molecular ions, both organic and inorganic, play important roles in the analysis of synthetic (mainly industrial) and naturally occurring samples of biological origin. One of the major field for determination of ionic species is the food analysis, where the ions play important role in several physiological activities [5]. The indirect detection is widely applied for the determination of anions in beverages [6-14], where the ionic composition of water is an important factor for both, alcoholic beverages in brewing processes, fermentation, and non-alcoholic beverages, as it also affects the taste.

Several methods for determination of inorganic and organic anions in non-alcoholic beverages have been presented in literature [6-9]. Most of the methods are based on the addition of high-mobility sodium chromate to the alkaline background electrolyte as detection probe. The detection wavelength was set to 214 and 254 nm, corresponding to the absorbance maxima in the UV spectrum [6,7,9]. The electroosmotic flow was reversed using adsorption of cetyltrimethylammonium bromide on the fused-silica capillary wall and the pH of the electrolyte was adjusted to the value higher than 8.0, as the lower pH can cause precipitation of the water-insoluble ion pair cetyltrimethylammonium-chromate [15]. Besides the chromate, the naphthol Yellow S and tartrazine dyes were used by Johns et al. in system buffered by isoelectric ampholyte histidine (pI 7.7) and hydroxypropylmethyl cellulose for the suppression of the electroosmotic flow [8].

The inorganic and organic anions in alcoholic beverages can be determined using background electrolytes buffered by histidine [11], detection probe itself [12], by counter-ion Tris (tris(hydroxymethyl) aminomethane) [13], or BIS-Tris (bis(2-hydroxyethyl)-imino-tris(hydroxymethyl)aminomethane) [14]. The advantage of using Tris electrolytes is that it do not absorb carbon dioxide, as other common alkaline solutions, which causes negative system peak of the carbonate. The co-electroosmotic migration was achieved using by addition of alkyltrimethylammonium bromide cationic surfactants or diethylenetetramine to the background electrolytes. In all presented electrolyte systems, the substituted carboxylic acids were used as co-migrating detection probes (p-aminobenzoic acid [11,14], 2,6-pyridincarboxylic acid [12] and benzene-1,2,4,5-tetracarboxylic acid [13]) with concentrations up to 7.5 mmol·l⁻¹.

The following work is focused on the application of sulfonated dyes and dye intermediates as the detection probes for development of the capillary zone electrophoresis separation of anions. We have tested four aromatic sulfonic acids for indirect detection and studied the effects of type and concentration of the probes on the separation of inorganic anions, potentially useful for analysis of beverages.

Experimental

Chemicals, Reagents, Stock and Standard Solutions

All chemicals used for the preparation of background electrolytes and stock solutions, i.e. citric acid, sodium chloride, sodium hydroxide, thiourea (all of analytical reagent grade) were obtained from Lachema (Brno, Czech Republic). Samples of anions and their mixtures were prepared from sodium chloride, sulfate, nitrate, phosphate and fluoride were also purchased from Lachema. Sulfonated dyes and dye intermediates used as the indirect detection probes were obtained from Synthesia (Pardubice, Czech Republic). The structures of the compounds are shown in Fig. 1.

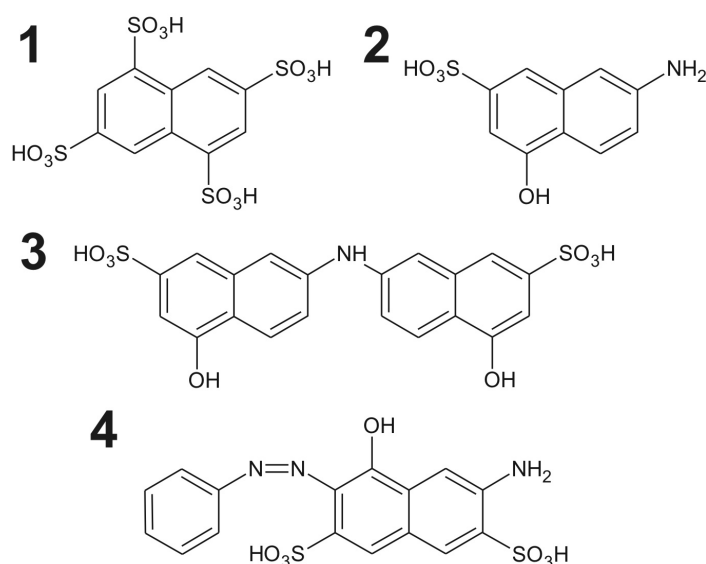


Fig. 1: Structures of the four dye intermediates and dyes used as the indirect detection probes.

1 – 1,3,5,7-naphthalene tetrasulfonic acid (1,3,5,7-NTeSA), **2** – I acid (6-amino-1-hydroxynaphthalene-3-sulfonic acid), **3** – di-I acid (7,7'-iminobis(4-hydroxynaphthalene-2-sulfonic acid), **4** – Azo Orseille R(A) (Colour Index Acid Red 17160).

Instruments

The Agilent ^{3D}CE (Agilent, Palo Alto, CA, USA) and Capel 105M (Lumex, St. Petersburg, Russia) capillary electrophoresis instruments were used for all electrophoretic separations. The 31 cm long (23 cm effective length) fused-silica capillaries with 50 µm inner diameter were obtained from Agilent.

New capillaries were conditioned by flushing with 0.5 mol/L NaOH for 10 min and deionized water purified by SG UltraClear apparatus (Siemens Water Technologies, Warrendale, PA, USA) for 10 min. Before each run, the capillary was rinsed with background electrolyte for 2 min. The background electrolytes were prepared from stock solution of 10 mmol/L citric acid with pH adjusted in the range of 3.5 – 7.2 using sodium hydroxide solution. The pH of the background electrolyte was measured using Metrohm 827 pH Lab meter with Metrohm PrimatrodeTM pH-measuring electrode (Metrohm AG, Switzerland).

Electrophoretic Measurements

The temperature of the separation capillary was maintained at 25°C. The UV detection was performed at 254 nm. Samples were injected hydrodynamically using 50 mBar overpressure at the inlet sample vial for 5 s. A voltage of 25 kV was applied during measurement of the electroosmotic flow mobility using thiourea as the marker of EOF and the voltage of -17 kV was applied on separation capillary during separation of ions in acidic background electrolyte.

Results and Discussion

Adjustment of the Electroosmotic Flow for Fast Separation of Anions

In typical separation conditions for capillary zone electrophoresis (CZE), a fused-silica capillary exhibit cathodic electroosmotic flow and the same direction of migration of cations, while the effective mobilities of anions are directed in opposite way towards the anode. To achieve fast separation of anions, reversal of the EOF is desirable, which can be achieved e.g. by adsorption of long-chain aliphatic quaternary ammonium surfactants. We have tested the possibility of addition of cetyltrimethylammonium bromide into the background electrolyte, however, by using sulfonated dyes and dye intermediates as the indirect detection probes (Fig. 1), precipitation of the dyes occurred at concentration level higher than 5 mmol/L. This phenomenon can be probably attributed to the interaction of sulfonated dyes and dye intermediates with cationic surfactant, leading to the formation of hydrophobic water-insoluble ion-pairs. As the EOF mobility in fused-silica capillary is strongly dependent on pH and decreases with suppressed ionization of the surface silanol groups, either by pH, or by increasing ionic strength of the electrolyte (Fig. 2), we have therefore decided to use acidic background electrolyte with negligible EOF for separation of anions by CZE.

To keep reasonable buffering capacity and conductivity of the background electrolyte as low as possible, we have used 10 mmol·l⁻¹ citrate buffer at pH 3.5 for further studies. Low pH of citrate electrolyte ensures, that negative system peak of citrate co-anion, which will appear in the electrophoregram with low effective mobility, will not interfere with the analyzed high mobility inorganic anions.

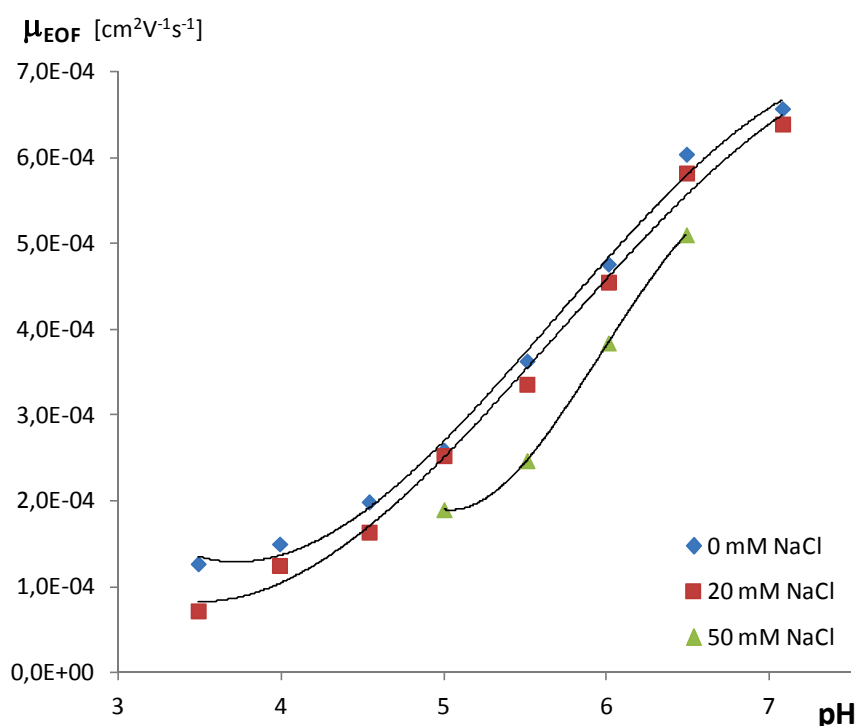


Fig. 2: Dependency of the electroosmotic flow mobility on the pH and ionic strength of the background electrolyte. The mobility was determined using thiourea as the non-ionized compound in 10 mmol·l⁻¹ citrate background electrolyte without and with addition of sodium chloride for adjustment of the ionic strength.

Selection of Probes for Indirect Detection of Anions

In many applications of CZE for separation of anions, the chromate is used as probe for indirect detection, especially for separation and determination of inorganic anions. Due to the inherent toxicity of hexavalent chromium salts, we have decided to test the possibility of replacement of the chromate by highly absorbing organic dyes and intermediates [16]. We have selected four compounds containing from one up to four sulfonic acid groups bonded on the naphthalene ring (Fig. 1).

These sulfonated dyes and intermediates exhibit effective mobilities ranging from $-3.2 \cdot 10^{-4}$ to $-6.9 \cdot 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ and absorption maxima in UV range with molar absorption coefficients up to the $65\,217 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (Table I), which is beneficial for decrease of LODs, according to the Eqn. (1).

Table I: Characteristics of the probes tested for indirect UV detection.

Indirect detection probe	λ_{max} [nm]	$\varepsilon (\lambda_{\text{max}})$ [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]	$\varepsilon (254 \text{ nm})$ [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]	μ_{eff} [$\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$]	
				0 mM NaCl	20 mM NaCl
1,3,5,7-NTeSA	233	65 217	3 261	$-6.87 \cdot 10^{-4}$	n.d.
I acid	226	33 961	22 968	$-3.19 \cdot 10^{-4}$	$-4.58 \cdot 10^{-4}$
di-I acid	359	37 720	13 040	$-4.15 \cdot 10^{-4}$	$-4.74 \cdot 10^{-4}$
C.I. Acid Red 17160	259	7 404	6 828	$-4.41 \cdot 10^{-4}$	$-5.23 \cdot 10^{-4}$

Abbreviations & symbols used: n.d. ... value not determined due to the instable current in fused-silica separation capillary, λ_{max} ... wavelength of the absorption maximum, $\varepsilon (\lambda_{\text{max}})$... molar absorptivity at λ_{max} , $\varepsilon (254 \text{ nm})$... molar absorptivity at 254 nm, μ_{eff} ... effective mobility in 10 $\text{mmol} \cdot \text{l}^{-1}$ citrate background electrolyte pH 7.0.

We have prepared the background electrolytes containing sulfonated dyes and intermediates as detection probes for indirect UV detection at concentrations up to the $4 \text{ mmol} \cdot \text{l}^{-1}$ for I acid, di-I acid and C.I. Acid Red 17160 and up to the $10 \text{ mmol} \cdot \text{l}^{-1}$ for 1,3,5,7-NTeSA. Background electrolytes containing sulfonated dyes and intermediates were applied for separation of inorganic anions by CZE.

Separation of Inorganic Anions using Sulfonated Dyes as Detection Probes

The separation of inorganic anions using CZE with indirect UV detection can be successfully achieved in background electrolyte containing chromate as detection probe (Fig. 3). Despite the short analysis time and high separation efficiency, the toxicity of hexavalent chromium salts makes such conditions adverse. The analysis of inorganic anions in background electrolytes containing sulfonated dye intermediates I acid, di-I acid and sulfonated dye C.I. Acid Red 17160 did not provide sufficient sensitivity of indirect UV detection. The zones of the analyzed anions were deformed by electromigration dispersion phenomenon and therefore could not be detected, because of the high differences in electrophoretic mobilities between inorganic anions and mono- and disulfonated probes.

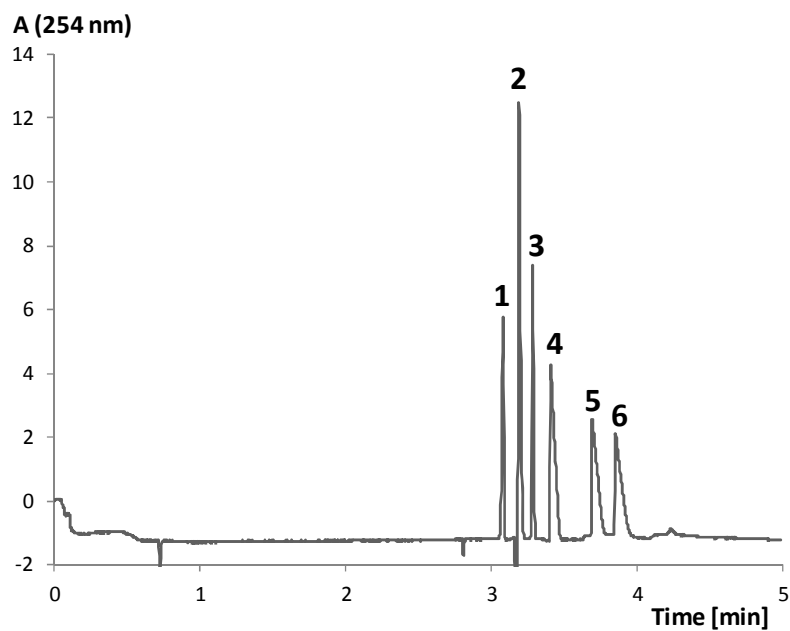


Fig. 3: Separation of the inorganic anions using chromate as detection probe. Background electrolyte containing $6 \text{ mmol}\cdot\text{l}^{-1}$ sodium chromate, $2.6 \text{ mmol}\cdot\text{l}^{-1}$ cetyltrimethylammonium bromide and 3.5 % (v/v) acetonitrile. Detection wavelength 254 nm, separation temperature 25°C . Anions: **1** – chloride, **2** – sulfate, **3** – nitrate, **4** – fluoride, **5** – phosphate, **6** – carbonate.

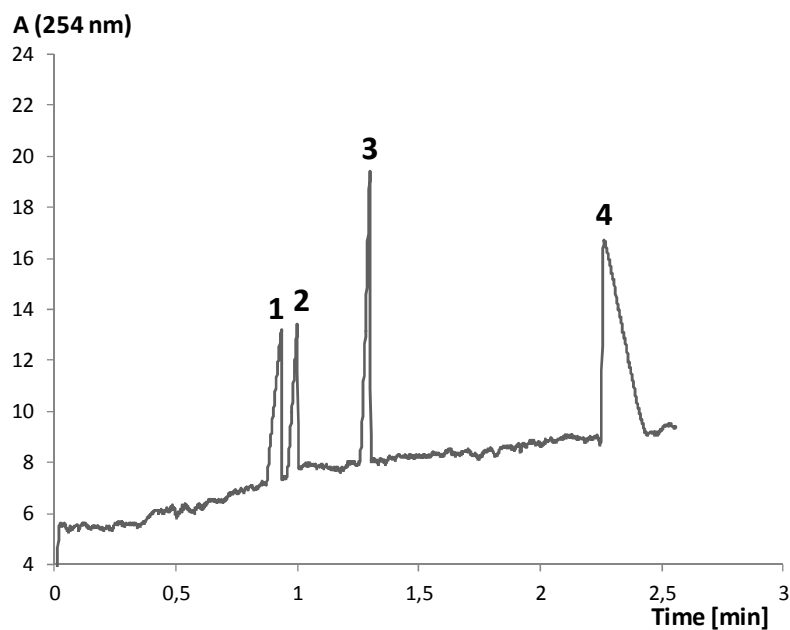


Fig. 4: Separation of the inorganic anions using 1,3,5,7-NTeSA as detection probe. Background electrolyte containing $10 \text{ mmol}\cdot\text{l}^{-1}$ citrate with pH adjusted to 3.5 and $10 \text{ mmol}\cdot\text{l}^{-1}$ 1,3,5,7-NTeSA. Detection wavelength 254 nm, separation temperature 25°C . Anions: **1** – chloride, **2** – sulfate, **3** – nitrate, **4** – phosphate.

In the electrolytes with addition of 1,3,5,7-NTeSA, the higher electrophoretic mobility of the probe, due to the presence of four sulfonic acid groups, that matches the mobilities of the most of inorganic anions led to the successful detection of anions by displacement mechanism of indirect UV detection (see Fig. 4 above).

Using citrate electrolyte with tetrasulfonic acid, the anions are separated in 2.5 min analysis time. The negative system peak caused by the presence of citrate in the background electrolyte appears after the peak of the phosphate and therefore do not interfere with the analyzed anions. The resolution of the sulfate and nitrate anions and the time of the analysis increases with increasing concentration of 1,3,5,7-NTeSA from $1 \text{ mmol}\cdot\text{l}^{-1}$ to $13 \text{ mmol}\cdot\text{l}^{-1}$, however, the phosphate and fluoride anions could not be detected at concentration of 1,3,5,7-NTeSA lower than $5 \text{ mmol}\cdot\text{l}^{-1}$. For the separation and detection of middle to low mobility organic anions like adipate, oxalate or acetate, it would be most likely possible to use the mixture of sulfonated dyes and dye intermediates to cover higher range of electrophoretic mobilities of analyte anions. The research is continuing in this direction.

Conclusions

In this article, the possibilities of using sulfonated dyes and dye intermediates as the probes for indirect UV detection in capillary zone electrophoresis separation of anions are tested. Four dyes and dye intermediates with one- to four- sulfonic acid groups were applied in citrate background electrolyte for separation of mixture of inorganic anions.

The electrophoretic mobilities of probes matching mobilities of analyzed anions are necessary to avoid the electromigration dispersion of the analyzed sample zones. The best conditions were found using 1,3,5,7-naphthalene tetrasulfonic acid as detection probe at concentration $10 \text{ mmol}\cdot\text{l}^{-1}$.

References

1. Z. Malá, R. Vespalec, P. Boček: "Capillary zone electrophoresis with indirect photometric detection in the visible range". *Electrophoresis* **15**(12) (1994) 1526-1530.
2. M. Macka, C. Johns, P. Doble, P.R. Haddad: "Indirect Photometric Detection in CE Using Buffered Electrolytes – Part I, Principles". *LC GC North America* **19**(1) (2001) 38-47.

3. M. Macka, P.R. Haddad: "Determination of metal ions by capillary electrophoresis". *Electrophoresis* **18**(12-13) (1997) 2482-2501.
4. C. Johns, M. Macka, P.R. Haddad: "Optimisation of probe concentration in indirect photometric detection in capillary electrophoresis using highly absorbing dyes". *Electrophoresis* **23**(1) (2002) 43-48.
5. M. Castro-Puyana, V. Garcia-Canas, C. Simo, A. Cifuentes: "Recent advances in the application of capillary electromigration methods for food analysis and Foodomics". *Electrophoresis* **33**(1) (2012) 147-167.
6. K.W. Swallow, N.H. Low: "Capillary zone electrophoretic analysis of the minor anions present in orange juice and orange pulpwash". *Journal of Agricultural and Food Chemistry* **42**(12) (1994) 2808-2811.
7. W.P. Yang, Z.J. Zhang: "Fast and direct determination of small anions in proteinaceous samples using low voltage-short tube capillary zone electrophoresis system". *Analytical Letters* **36**(1) (2003) 465-477.
8. C. Johns, M. Macka, P.R. Haddad: "Indirect photometric detection of anions in capillary electrophoresis using dyes as probes and electrolytes buffered with an isoelectric ampholyte". *Electrophoresis* **21**(7) (2000) 1312-1319.
9. W.P. Yang, B. O'Flaherty, A.L. Cholli: „Fast analysis of water samples for detection of anions by capillary zone electrophoresis“. *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering* **36**(7) (2001) 1271-1285.
10. G.A.B. Heras, M.C. Breadmore, C. Johns, J.P. Hutchinson, E.F. Hilder, P. Lopez-Mahia, P.R. Haddad: "Indirect photometric detection of anions in nonaqueous capillary electrophoresis employing Orange G as probe and a light-emitting diode-based detector". *Electrophoresis* **29**(14) (2008) 3032-3037.
11. C.W. Klampfl: "Analysis of organic acids and inorganic anions in different types of beer using capillary zone electrophoresis". *Journal of Agricultural and Food Chemistry* **47**(3) (1999) 987-990.
12. T. Soga, G.A. Ross: "Capillary electrophoretic determination of inorganic and organic anions using 2,6-pyridinedicarboxylic acid: Effect of electrolyte's complexing ability". *Journal of Chromatography A* **767**(1-2) (1997) 223-230.
13. M. Arellano, J. Andrianary, F. Dedieu, F. Couderc, P. Puig: "Method development and validation for the simultaneous determination of organic and inorganic acids by capillary zone electrophoresis". *Journal of Chromatography A* **765**(2) (1997) 321-328.
14. C.W. Klampfl, M.U. Katzmayer, W. Buchberger: "Separation of inorganic and organic anions by capillary zone electrophoresis with simultaneous indirect UV and conductivity detection". *Electrophoresis* **19**(14) (1998) 2459-2464.
15. M. Macka, C. Johns, P. Doble, P.R. Haddad: "Indirect Photometric Detection in CE Using Buffered Electrolytes – Part II, Practical Rules". *LC GC North America* **19**(2) (2001) 178-188.
16. K. Hlavová: "Sulphonated azo dyes as contrast compounds in the analysis of anions in beverages using capillary electrophoresis with indirect UV detection". *Bachelor Thesis* (2012) University of Pardubice.