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Long-term analyses in automated electrophoretic analyzer in hydrodynamically closed separation system

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ABSTRACT

Some potential problems that can occur during the analyses of complex samples by on-line combination of capillary isotachophoresis–capillary zone electrophoresis (cITP–CZE) in automated electrophoretic analyzer with the column-coupling configuration of the separation unit were studied in this work. The main focus was devoted on the reproducibility of important analytes' parameters (migration time, peak height and peak area) and also on the stability studies of selected low and high molecular mass analytes of inorganic/organic origins (bromate, vitamins, proteins) present at low concentration levels in different kinds of matrices (mineral water, human urine).

Such study was carried out for the first time for the electrophoretic analyzer operating in the hydrodynamically closed separation system provided with contact-less conductivity detectors and UV detector in CZE step. Hydrodynamic and electroosmotic flows of the buffer solutions were suppressed and therefore, only the electrophoretic transport of ions was significant. Obtained results showed the different stabilities of the analytes and samples depending on their origin. The focus in the long-term analyses should be paid on the storage of the samples and on the regular changing the contents of electrolyte vessels to keep the electrolyte composition and separation conditions as constant as possible.

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1. Introduction

Analytical chemistry has an irreplaceable role in control of the chemical and food production, health, agriculture, the control environment, and many other areas of human activity. The need of controlling of high-purity substances, analysis of large series of samples, samples with trace concentrations of analytes, complex samples, and small sample volumes, is very actual task.

The automated analyzers are solving mainly the problem of routine analysis of large series of samples. The main advantages of using automated analyzers include: (i) reducing the total analysis time (including data evaluation), (ii) the possibility of making continuous analyses, (iii) elimination of some subjective errors (excluding the effects of human factors), (iv) the minimization of the variation in results from one individual to another (increasing of reproducibility) and (v) very low consumption of samples, and reagents [1]. One of the disadvantages of performing automatic analyses is the fact that the whole series of the samples must be prepared in advance and therefore, the actual time when the first and the other samples are analyzed, is different. This may lead to severe problems when unstable samples are analyzed. In addition, different processes and changes (physical, chemical, biological) can take place in the samples, which can lead to the decomposition of the analyte and/or the whole sample and to the errors in determination of analyte [2]. Stability of the analyte in the complex matrix is an important factor which has to be taken into account in a long series of analyses when automated analyzer is used. One must also take into account the type of sample, i.e., whether the sample is of inorganic, organic or biological origin, as well as the concentration level(s) of the analyte(s) in the sample. Several studies have been published on this topic, which deal with the study of stability of various protein, drugs and organic compounds in biological matrices [e.g., 3–6] as well as the study of stability of biological matrices [7,8].

Capillary electrophoresis (CE) has become a well-established analytical method widely used for routine clinical and forensic analysis [9–11]. The facts that the sample preparation is often reduced to a minimum, separation usually takes place rapidly, and consumption of solutions is minimal, are the main reasons for its rapid development [12]. Other advantages of CE are its high resolution, sensitivity, compatibility with various detectors (conductivity, UV, LIF, etc.) [13], the possibility of miniaturization and automation

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[14]. Capillary zone electrophoresis (CZE), possibly in conjunction with a suitable preconcentration technique, has many advantages and is suitable for long-term analyses. During long-term analyses, some problems may arise that are not observable for short series. Therefore, attention must be paid to several, mostly simple details to guarantee the sufficient accuracy and reproducibility of analysis results. Several approaches how to improve precision in CE in hydrodynamically opened separation system are described in review of Meyer [15].

Hydrodynamically closed separation systems [16,17] are characterized by the use of separation capillary with a larger inside diameter (>200 μm) and therefore larger load capacity, which allows injection of larger volumes of samples, resulting in achieving lower concentration limits of detection. The strengths of hydrodynamically closed systems can be regarded as the use of column-coupling techniques, which allows online removing of interfering matrix components in the first separation column, thereby simplifying the complex matrix injected into the second separation stage, concentration of the analytes in the first separation step and thus reduction of risk of subsequent overlapping zones (peaks) in the second separation stage and achieving lower concentration limits of detection [18,19]. Electrophoretic separations performed in these systems require suppression of electroosmotic flow (EOF) and hydrodynamic flow (HDF), what ensures better reproducibility of the migration velocities of analytes. In electrophoretic separations carried out in closed systems, HDF is usually eliminated mechanically (using a semipermeable membrane) [20] and EOF is mostly eliminated by the addition of water-soluble high molecular mass polymers [21]. The analytical possibilities and limitations of both concepts used in the CE separation systems are summarized in work Kaniansky et al. [22].

Some potential problems that can occur during the analyses of different samples by on-line combination of capillary isotachophoresis–capillary zone electrophoresis (cITP-CZE) in automated electrophoretic apparatus in hydrodynamically closed separation system were studied in this work together with their impact on some basic analytical parameters (precision, accuracy). The main focus was devoted on the stability studies of different types of analytes/matrices mainly in the cases when the analytes were present at low concentration levels. The following types of analytes and matrices were studied:

(i) bromate present in the mineral waters was studied as the example of matrix of inorganic origin with the analyte at trace concentration level;
(ii) three different forms of vitamin B6 (pyridoxamine, pyridoxine, pyridoxal) were studied as the example of low molecular mass analyte of organic origin;
(iii) human lysozyme and human serum albumin were studied as the example of high molecular mass analyte of organic origin;
(iv) urine, chosen as model matrix for the stability study of complex matrix of biological origin, represents complex matrix which composition is variable within the number of components as well as within very large concentration ranges of given compounds and the actual composition depends on many different objective and subjective factors.

2. Experimental

2.1. Instrumentation

The automated capillary electrophoresis analyzer EA 202A (Villa-Labeco, Spišská Nová Ves, Slovak Republic) assembled in the column-coupling configuration of the separation unit (schematic view is shown in [27]) and equipped by the autosampler Triathlon (Spark Holland, Emmen, The Netherlands) was used in this work. The tray of the autosampler was externally cooled to 4 °C. The samples were injected by a 30 μL internal sample loop of the injection valve of the analyzer. An ITP column was provided with an 800 μm I.D. capillary tube made of FEP (fluorinated ethylene–propylene copolymer) and its total length was 90 mm. A CZE column was provided with a 300 μm I.D. capillary tube made of fused silica of a 240 mm total length (180 mm to the detection cell). Both columns were equipped with the on-column contact-less conductivity detectors. High voltage power supply operating in constant current mode was delivering 200 μA and 100 μA currents on ITP and CZE column, respectively.

The automated electrophoresis analyzer EA 202A was provided with Knauer K2000 UV detector (Knauer, Berlin, Germany) connected to the UV detection cell, located on-line on the CZE column, via optical fibres and the detection wavelength could be set to 200, 220, 254 and 280 nm, respectively.

Win ACES software, ver. 1.4 (D. Kaniansky – Consulting, Bratislava, Slovak Republic) was used for a time-programmed automatic control of ITP–CZE runs, for data acquisition and for evaluation of the collected data. Data handling and long-term evaluation (construction of control diagrams) were performed using complementary module SPC for MS Excel, ver.3.0 (Business Process Improvement, Cypress, TX, U.S.A.) for programme Excel 2003 (Microsoft, Redmond, WA, USA).

Table 1
The composition of electrolyte systems.

	ES1	ES2	ES3
Leading ion	Chloride	Ammonium	Ammonium
Concentration (mmol/L)	10	10	10
Counterion	β-Alanine	Acetate	Acetate
Co-counterion	BTP		
Concentration (mmol/L)	5		
pH	5.2	4.9	4.9
EOF suppresor	mHEC	mHEC	mHEC
Concentration (%, w/V)	0.1	0.1	0.1
Terminating ion	Fluoride	Hydroxonium	Hydroxonium
Concentration (mmol/L)	20	0.42	0.42
Counterion	β-Alanine	Acetate	Acetate
EOF suppresor	mHEC	mHEC	mHEC
Concentration (%, w/V)	0.05	0.05	0.05
Carrier ion	Phosphate	Glycylglycine	Hydroxonium
Concentration (mmol/L)	50	200	2.5
Counterion	Glycine	Acetate	Acetate
Ph	1.9	2.6	2.6
EOF suppresor	mHEC	mHEC	mHEC
Concentration (%, w/V)	0.1	0.1	0.1

BTP, bis–tris propane; mHEC, methylhydroxyethylcelullose.

2.2. Chemicals

Chemicals used for the preparation of electrolyte solutions, solutions of the standards and model mixtures were obtained from Merck (Darmstadt, Germany) and Sigma–Aldrich (Steinheim, Germany). All chemicals were of analytical grade or additionally purified by the usual methods. Hydrochloric acid used for the preparations of leading electrolyte was purified by isothermic distillation. Water purified by a Labconco WaterPro PS water purification system (Labconco, Kansas City, MI, USA) and subsequently purified by Simplicity (Millipore, Molsheim, France) was used for the preparation of all solutions. The aqueous stock solution 1% (m/v) methylhydroxyethylcellulose 30 000 (Serva, Heidelberg, Germany), purified on a mixed-bed ion exchanger (Amberlite MB-1, Poole, UK), was added to the electrolytes to suppress an electroosmotic flow. The composition of electrolyte solutions is summarized in Table 1.pH values of electrolytes solutions were measured immediately after their preparation to ensure that their

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