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Journal of Chromatography A, 916 (2001) 131–142

JOURNAL OF CHROMATOGRAPHY A

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New configuration in capillary isotachophoresis–capillary zone electrophoresis coupling

František Kvasnička[a,*], Michal Jaroš[b], Bohuslav Gaš[b]

[a]*Department of Food Preservation and Meat Technology, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic*

[b]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic*

Abstract

The paper surveys possible configurations of a coupling capillary column operating in various electromigration modes. Special attention is given to capillary isotachophoresis–capillary zone electrophoresis (cITP–CZE) coupling and its description from the theoretical point of view. Computer simulations of separation are presented and compared with experiments. Further, we propose a new configuration of electrolyte systems in cITP–CZE coupling, which offers a possibility to perform complex analyses of micro- and macro-constituents in one run. The electrolyte system is verified by practical experiments for both anionic and cationic modes of analysis. The advantages and disadvantages of the new combination are discussed.

Keywords: Isotachophoresis–capillary zone electrophoresis; Fruit juices; Food analysis; Computer simulation; Organic acids; Methylhistidine; Creatinine

1. Introduction

Sensitivity is still the main drawback of capillary electrophoresis (CE) when being applied to the analysis of complex matrix samples such as foodstuffs or other biological samples. There are several ways how to improve the detection limit [1]. In our opinion on-line coupled capillary isotachophoresis (cITP) with capillary zone electrophoresis (CZE) using a two-capillary, three-electrode instrument is one of the smartest procedures to do that [2]. The principle of such a coupling is very simple. In the first, wider, capillary, the isotachophoretic step ensures stacking of the analytes into sharp zones that are then separated by CZE mode in the second narrower capillary. The basic features of cITP–CZE coupling are summarized in Table 1. The first isotachophoretic step enables injection of large sample volumes (up to several hundreds of μl) and concentration of analytes according to the Kohlrausch' regulating function. With respect to the minimal detectable zone an analyte can be up to 10^6 times concentrated during the cITP step. In a complex sample matrix constituents are separated into a stack of zones with trace analytes focused into narrow bands. The matrix constituents can be forced to migrate out of the separation path by proper column switching and the sample is thus cleaned up before its injection into the CZE capillary. The removal of the matrix is well defined and very reproducible because it is based on the signal from the detector of the cITP step. The sample clean up

*Corresponding author. Tel.: +420-2-2435-5117; fax: +420-2-3116-284.

E-mail address: frantisek.kvasnicka@vscht.cz (F. Kvasnička).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00616-1

Table 1
Basic features of cITP–CZE coupling

	cITP step	CZE step
Feature	High sample load (up to hundreds of μl)	High resolving power (~10^5 theoretical plates/m)
	Preconcentration and/or sample clean up (up to 10^6 fold in complex matrix)	Speed of analysis (~15 min)
	Well defined fraction for CZE (personal computer control of column switching)	Enhanced selectivity (second dimension of separation)
	Ideal sample injection for CZE (analyte is focused into narrow band)	

can be enhanced by the use of suitable discrete spacers [3]. The analytes in question present in the injected sample, concentrated and/or cleaned up from the matrix are completely transferred into a second capillary as a narrow sample pulse. The CZE step of high resolving power enables quick separation of the analytes. The selectivity of the analysis can be enhanced when the separation in the cITP and CZE steps are based on different mechanisms.

Generally, the column-coupling instrument enables different separation modes, i.e., cITP–cITP or cITP–CZE. In the cITP–cITP mode both preseparation (wider) and analytical (narrower) capillaries are filled with the same leading electrolyte (one-dimensional cITP) or different electrolytes (two-dimensional cITP). In the cITP–CZE mode the preseparation capillary is filled with leading electrolyte and the analytical capillary with terminating or some other background electrolyte (BGE).

From the point of view of electrolyte combinations in cITP–CZE coupling three types of electrolyte systems are described in the literature [4,5]. We will show that there is another electrolyte system as a logical consequence of existing electrolyte schemes:

(1) BGE–S–BGE

$$S = A + B;\ (m_L > m_A > m_B > m_T;$$
$$m_{BGE} > m_L \vee m_L \geq m_{BGE} \geq m_T \vee m_T > m_{BGE})$$

(2) L–S–L

$$S = A + B;\ (m_L = m_{BGE} > m_A > m_B > m_T)$$

(3) T–S–T

$$S = A + B;\ (m_L > m_A > m_B > m_{BGE} = m_T)$$

(m_i is the mobility of the corresponding ith constituent).

The first system so-called ''BGE–S–BGE'' is that where the leading (L) and terminating (T) electrolytes from the cITP step, and the BGE from the CZE step are quite different. The ''L–S–L'' system is that where the leading electrolyte from the cITP step serves as the BGE for the CZE separation of sample S composed of analytes A and B (A has a higher effective mobility than that of analyte B). In the ''T–S–T'' system the terminating electrolyte from the cITP step serves as the BGE in the CZE step. The electrolyte systems for anionic analysis are shown in Fig. 1. The BGE–S–BGE electrolyte system is the most general. According to the mobility of background co-ion there are three another subsystems. The mostly used system with background anion mobility lower than that of the leading anion and higher than that of the terminating anion is shown in Fig. 1A. There are two more systems with background ion mobility higher than that of the leading anion and/or lower than that of the terminating anion. At the beginning of analysis the sample S containing components A and B is injected between the terminating (T) and leading (L) electrolytes. The preseparation capillary is filled with leading electrolyte and the analytical capillary with the background electrolyte. Driving current is switched on (an arrow marked with the letter I shows its direction). At the end of the cITP step the sample components A and B are arranged into consecutive zones ordered according to their effective mobilities between the leading and terminating anions. When the rear boundary of leading anion zone reaches the bifurcation block, the driving current is switched to

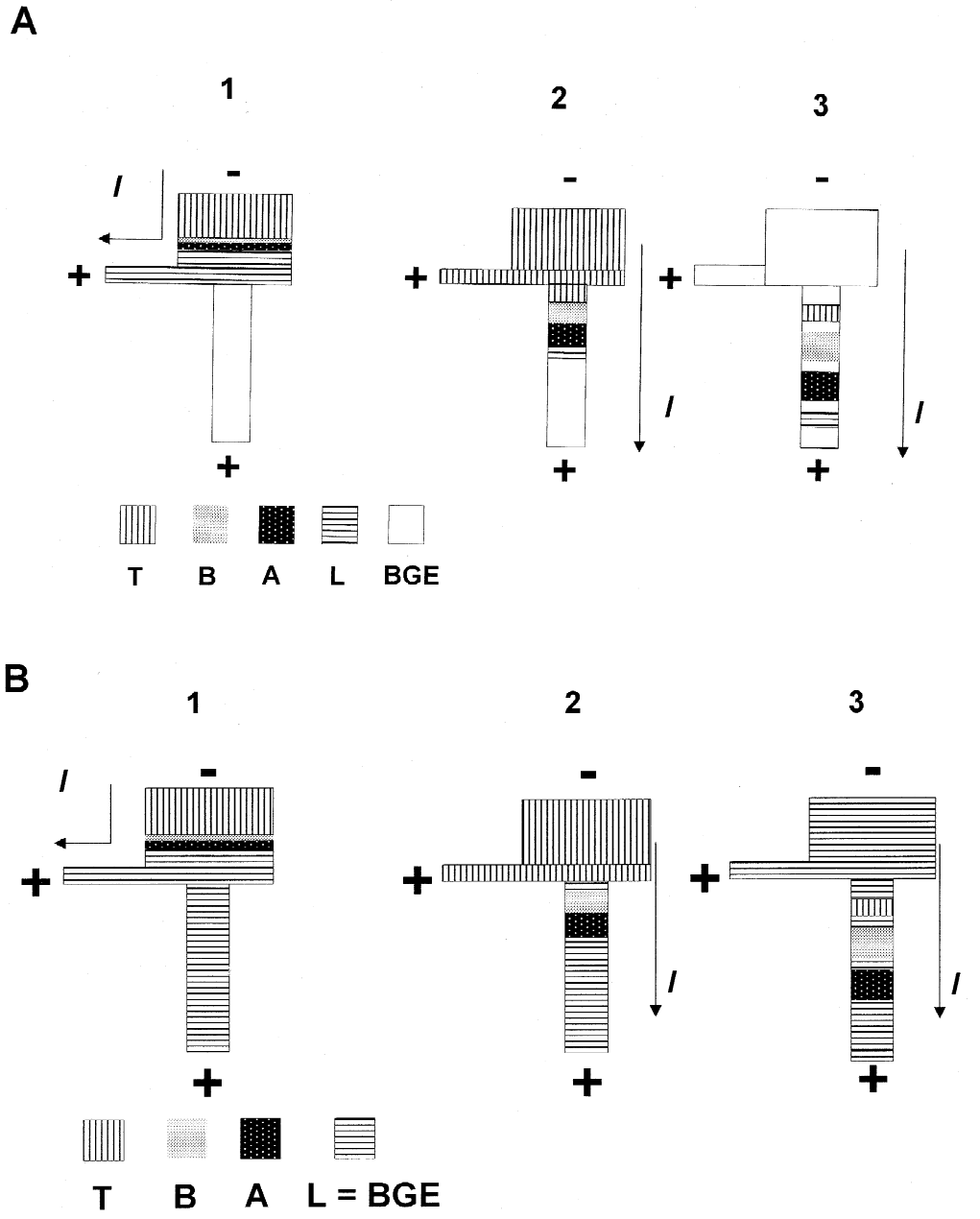

Fig. 1. Electrolyte schemes of on-line coupled cITP with CZE (anionic mode); (A) BGE–S–BGE; (B) L–S–L; (C) T–S–T; (D) T–S–BGE; 1 – situation after the cITP separation; 2 – the situation at the beginning of the CZE separation; 3 – the situation at the end of the CZE separation; I – direction of the driving current.

the analytical capillary. When the front boundary of the terminating anion enters the analytical capillary the driving current is switched off. The terminating and leading electrolytes are replaced with the BGE and the driving current is again switched on. The cITP stack of L, A, B and T anions becomes the

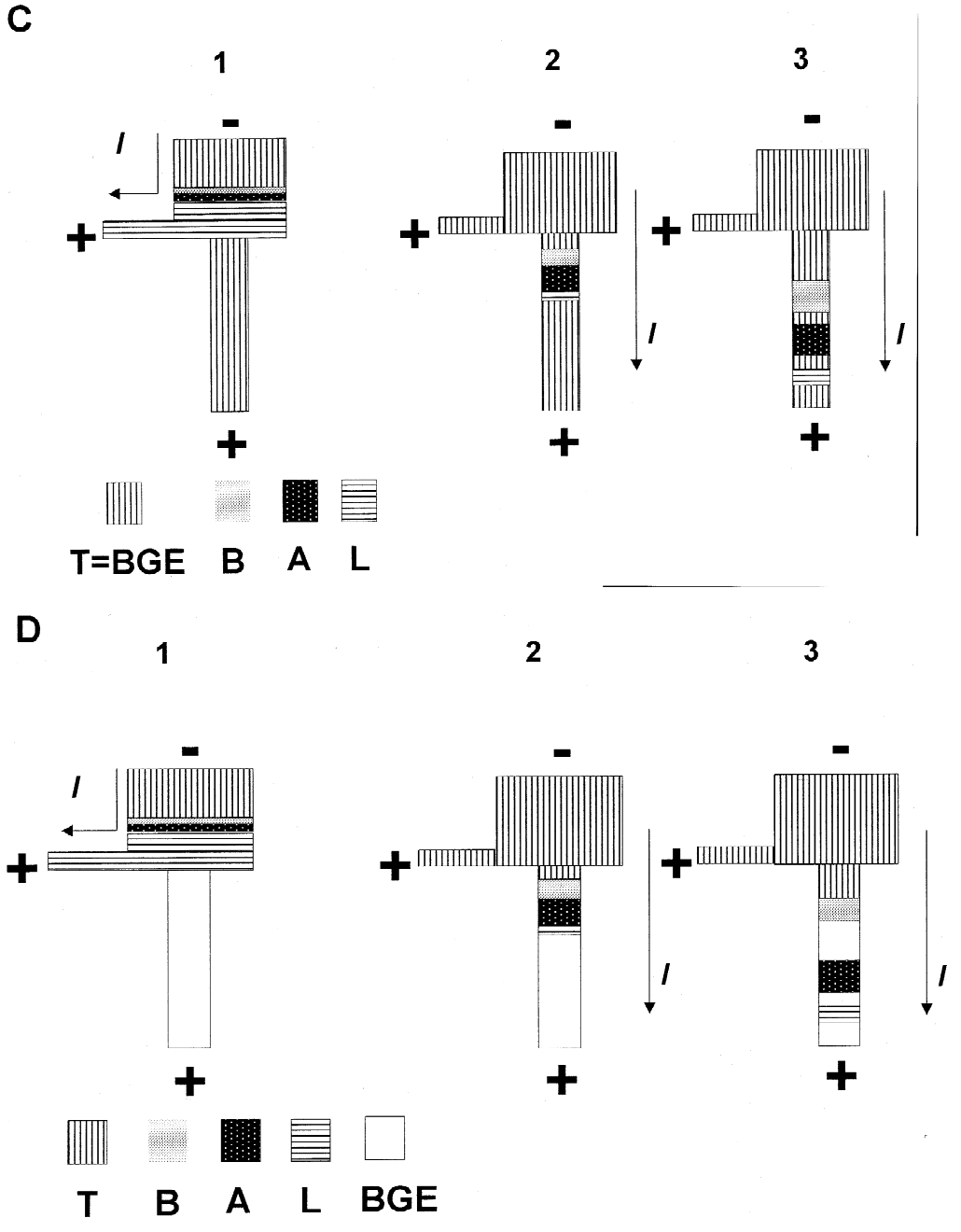

Fig. 1. (*continued*)

sample for CZE analysis. After some time the background anion penetrates through the whole system and all zones are destacked and migrate in the CZE mode. In the second type of electrolyte combination (see Fig. 1B) called L–S–L, the cITP step of the separation proceeds in the same way as in the BGE–S–BGE system. The difference is that the analytical capillary is filled with the leading elec-

trolyte and serves as background for the CZE separation. In the CZE step, when the front boundary of the terminating anion enters the analytical capillary the driving current is switched off. The terminating electrolyte within the preseparation capillary and reservoir is replaced with the leading electrolyte and the driving current is again switched on. The cITP stack of L, A, B and T anions becomes the sample for CZE analysis. After some time the leading anion penetrates through the whole system and all of zones are destacked and migrate in the CZE mode.

The T–S–T electrolyte system uses the terminating anion as the BGE for CZE step. Here (see Fig. 1C) the preseparation capillary is filled with the leading and terminating electrolytes and the analytical capillary is filled with the terminating electrolyte. The cITP step of the separation is the same as in previous systems. Before the stack of analytes reaches the bifurcation block, the driving current is switched to the analytical capillary and this stack of analytes now becomes the sample for the CZE step. Also, after some time the terminating anion penetrates through the whole system and all of zones are destacked and migrate in the CZE mode.

The present paper describes a new combination of BGE–S–BGE and T–S–T systems, which will be called the T–S–BGE electrolyte system (see Fig. 1D).

(4) T–S–BGE

$$S = A + B; (m_L > m_A > m_{BGE} > m_B > m_T)$$

The main feature of this system is that the mobility of the BGE lies between L and T and furthermore this mobility is set up between mobilities of analytes of question. As regards comfort of handling, this system is similar to the T–S–T system. It means that there is no need to replace electrolytes after the cITP step. The new T–S–BGE electrolyte system uses for both the cITP and CZE steps different electrolytes. At the beginning of analysis the preseparation capillary is filled with the leading and the terminating electrolytes and the analytical capillary is filled with the BGE. This arrangement is the same as in the BGE–S–BGE system. The cITP step of the separation is the same as in previous systems. Before the stack of analytes reaches the bifurcation block, the driving current is switched to the analytical capillary and this stack of analytes now becomes the sample for the CZE step. As we mentioned above there is no need to replace the electrolytes as in case of the BGE–S–BGE or L–S–L electrolyte systems. As the mobility of the BGE lies between the mobilities of analytes A and B, the background anion is a leader for analyte B, while analyte A migrates in the CZE mode. It means there are two different modes in one run – the cITP mode for analyte B and the CZE mode for analyte A. The T–S–BGE system is especially suitable for the analysis of samples containing minor constituents of higher mobility than that of the major constituent. Due to the fact that both the CZE and cITP modes proceed in the analytical capillary we entitled this cITP–CZE combination[1] as the cITP–CZE–cITP mode. In this paper we further summarize results of comparison of computer simulation and real analyses. We compared several separation modes for both anionic and cationic analyses.

2. Experimental

2.1. Computer simulation

Computer simulations were performed on a personal computer using the SIMUL software that is freely available on the internet [6]. The SIMUL program is able to predict theoretically the movement of ions in the electric field. It solves the sets of non-linear partial differential equations and non-linear algebraic equations describing the continuity of ionic movement and acid–basic equilibria.

2.2. Chemicals

Standards of 3-methylhistidine (3-MeHis), hydroxypropylmethylcellulose (HPMC), morpholinethanesulfonic acid (MES), β-alanine, and ϵ-aminocaproic acid (EACA) were purchased from Sigma–Aldrich (Czech Republic); L-histidine (His) from Reanal (Hungary), creatinine (Creat) from Jenssen (Belgium); analytical-reagent grade ammonium hy-

[1] Using the T–S–BGE electrolyte system.

Table 2
Conditions of analyses

Mode/conditions of analysis	Conditions of anionic analysis	Conditions of cationic analysis
cITP–cITP	LE: 10 m*M* HCl + 12 m*M* β-alanine + 0.05% HPMC TE: 5 m*M* HAc BGE: – Driving current: 250 μA (presep.), 25 μA (anal.) Detection: conductivity and UV at 254 nm	LE: 10 m*M* NH_4OH + 20 m*M* MES TE: 10 m*M* EACA + 5 m*M* HAc BGE: – Driving current: 200 μA (presep.), 20 μA (anal.) Detection: conductivity and UV at 254 nm
cITP–CZE	LE: 10 m*M* HCl + 12 m*M* β-alanine + 0.05% HPMC TE: 5 m*M* HAc BGE: 20 m*M* HAc + 10 m*M* β-alanine + 0.05% HPMC Driving current: 250 μA (presep.), 50 μA (anal.) Detection: conductivity and UV at 254 nm	LE: 10 m*M* NH_4OH + 20 m*M* MES TE: 10 m*M* EACA + 5 m*M* HAc BGE: 10 m*M* EACA + 5 m*M* HAc Driving current: 200 μA (presep.), 20 μA (anal.) Detection: conductivity and UV at 254 nm
cITP–CZE–cITP	LE: 10 m*M* HCl + 12 m*M* β-alanine + 0.05% HPMC TE: 5 m*M* HAc BGE: 20 m*M* citric acid + 10 m*M* β-alanine + 0.05% HPMC Driving current: 250 μA (presep.), 50 μA (anal.) Detection: conductivity and UV at 254 nm	LE: 10 m*M* NH_4OH + 20 m*M* MES TE: 10 m*M* EACA + 5 m*M* HAc BGE: 10 m*M* pyridine + 10 m*M* MES Driving current: 200 μA (presep.), 20 μA (anal.) Detection: conductivity and UV at 254 nm

droxide, hydrochloric acid, pyridine, malic acid, fumaric acid, citric acid and acetic acid (HAc) were purchased from Lachema (Czech Republic). Ammonium hydroxide, hydrochloric acid and acetic acid were purified by isothermal distillation.

2.3. On-line capillary isotachophoresis–capillary zone electrophoresis

The electrophoretic analyser used for our experiments was the EA 100 (Villa-Labeco, Slovak Republic) with column coupling. The separation was performed in a PTFE pre-separation capillary (90 mm × 0.8 mm I.D.), which was coupled to a PTFE analytical capillary (90 mm × 0.3 mm I.D.). Zones were detected by conductivity and UV detection, respectively. The isotachopherograms were evaluated by a software package supplied with the analyser. Electrolyte systems and analysis conditions are described in Table 2.

3. Results and discussion

Within our experiments we compared three separation modes, namely, cITP–cITP (one dimensional), cITP–CZE (T–S–T electrolyte system) and cITP–CZE–cITP (new T–S–BGE electrolyte system) on both cationic and anionic analyses that are not only of theoretical value but also have a practical importance. We performed a computer simulation of a model mixture and a real analysis of the same model mixture. Additionally we analyzed real samples.

It is generally accepted that the lean meat content is the best quality parameter of meat products [7]. There are some typical components of meat proteins called lean meat markers. For cationic analyses we therefore chose the separation of such markers, namely, 3-MeHis and creatinine [8]. For computer simulation we used L-histidine instead of 3-MeHis due to known physicochemical properties.

As an application of anionic separation we chose the determination of fumaric and malic acid in apple juice [9]. Malic acid as a major component is an important analyte because it is the natural component of apple juice and its content varies within a specific range. Apple juice could be potentially adulterated with the addition of synthetic malic acid. Malic acid contains a trace of fumaric acid from its synthesis. The natural content of fumarate in apple juice is very low (lower than 5 mg/l). Apple juice with levels of

fumarate higher than 8 mg/l is to be suspected of adulteration.

3.1. Cationic cITP–cITP mode

A mixture of 5 m*M* L-histidine and 5 m*M* creatinine was used for computer simulation of cationic analyses. Contrary to real analyses, we used for computer simulation the same concentrations of L-histidine and creatinine because very short zones cannot be well resolved by simulations. The composition of the electrolytes used for all computer simulations was the same as for real analyses. Fig. 2A and B show the situation at the beginning and at the end of computer simulation, respectively. The calculated steady-state concentrations of L-histidine and creatinine are 7 m*M* and 7.7 m*M*, respectively. As it is obvious from the conductivity trace (Fig. 2B)

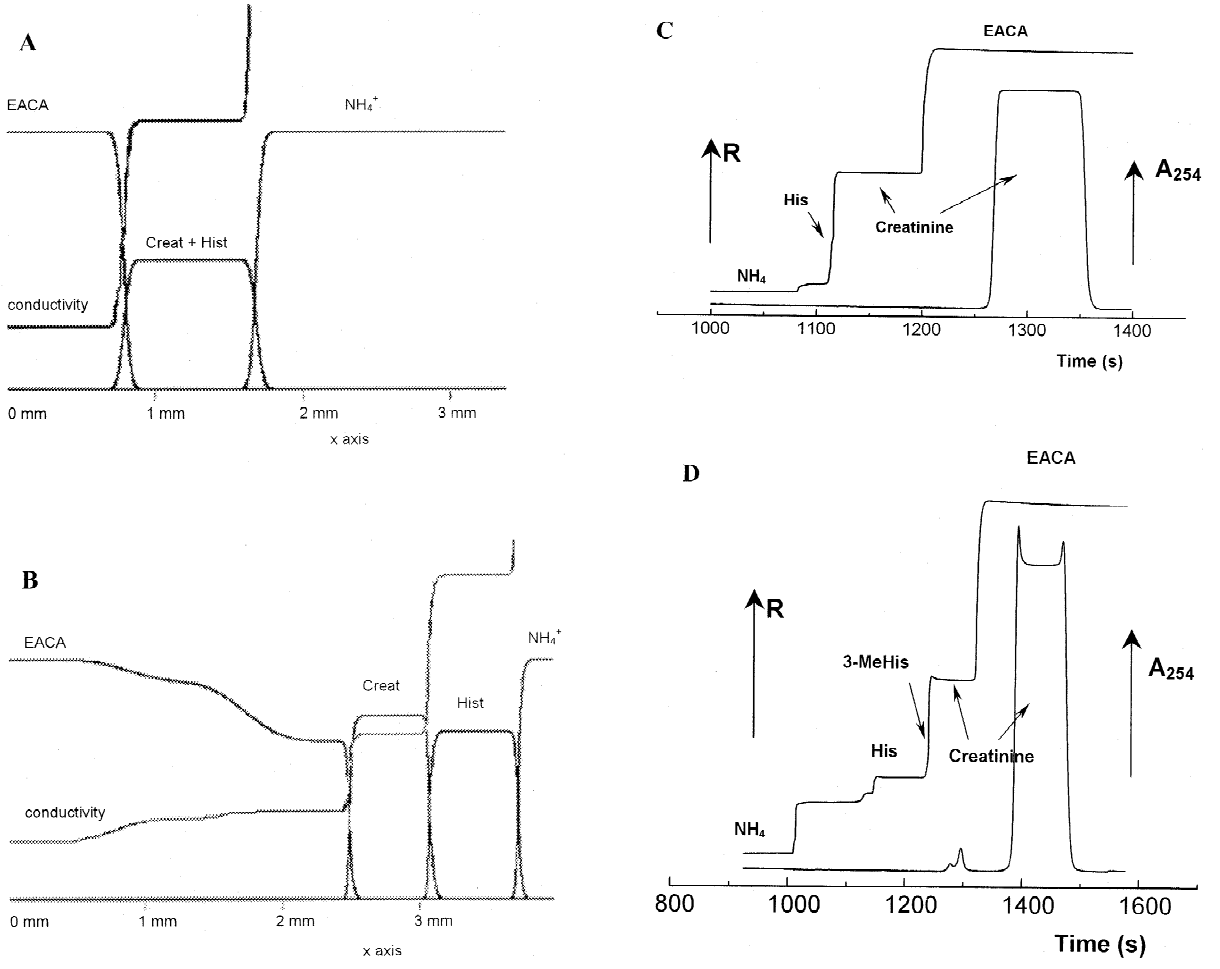

Fig. 2. Results of computer simulation of separation of 5 m*M* L-histidine and 5 m*M* creatinine and analyses of real sample by the cationic cITP–cITP mode. (A) Situation at the beginning of simulation of cITP step; (B) situation at the end of simulation of cITP step. Gray line: conductivity (upwards increasing) trace. Black lines: concentration (upwards increasing) profile of compounds. Creat: creatinine; His: L-histidine; EACA: ϵ-aminocaproic acid; NH_4^+: ammonium. (C) Conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 m*M* creatinine and 4 μ*M* His by the cITP–cITP mode; (D) conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of hydrolysate of duck leg by the cITP–cITP mode.

the mixture of L-histidine and creatinine is well separated. This result was confirmed by a real analysis of a model mixture of 0.2 m*M* creatinine and 4 μ*M* L-histidine (Fig. 2C). The record from conductivity detection at the analytical capillary in the analysis of hydrolysate [10] of duck leg [5 g of duck leg was hydrolysed by 30% sulfuric acid at 110°C for 24 h, 10-times diluted and neutralized by $Ba(OH)_2$] is shown in Fig. 2D. It is clear, that all the analytes (L-histidine, 3-MeHis and creatinine) are well separated as was predicted by the computer simulation. It is also clear that it is very difficult to quantify very short zones of minor sample components (3-MeHis) by the cITP–cITP mode. On the other hand the creatinine as a major sample constituent is easily determined by the cITP–cITP mode.

3.2. Cationic cITP–CZE mode

The calculated steady-state concentrations of L-histidine (7 m*M*) and creatinine (7.7 m*M*) from the cITP step were used as a sample for simulation of the CZE step (see Fig. 3A and B). The conductivity record of the CZE step (Fig. 3B) implies that the

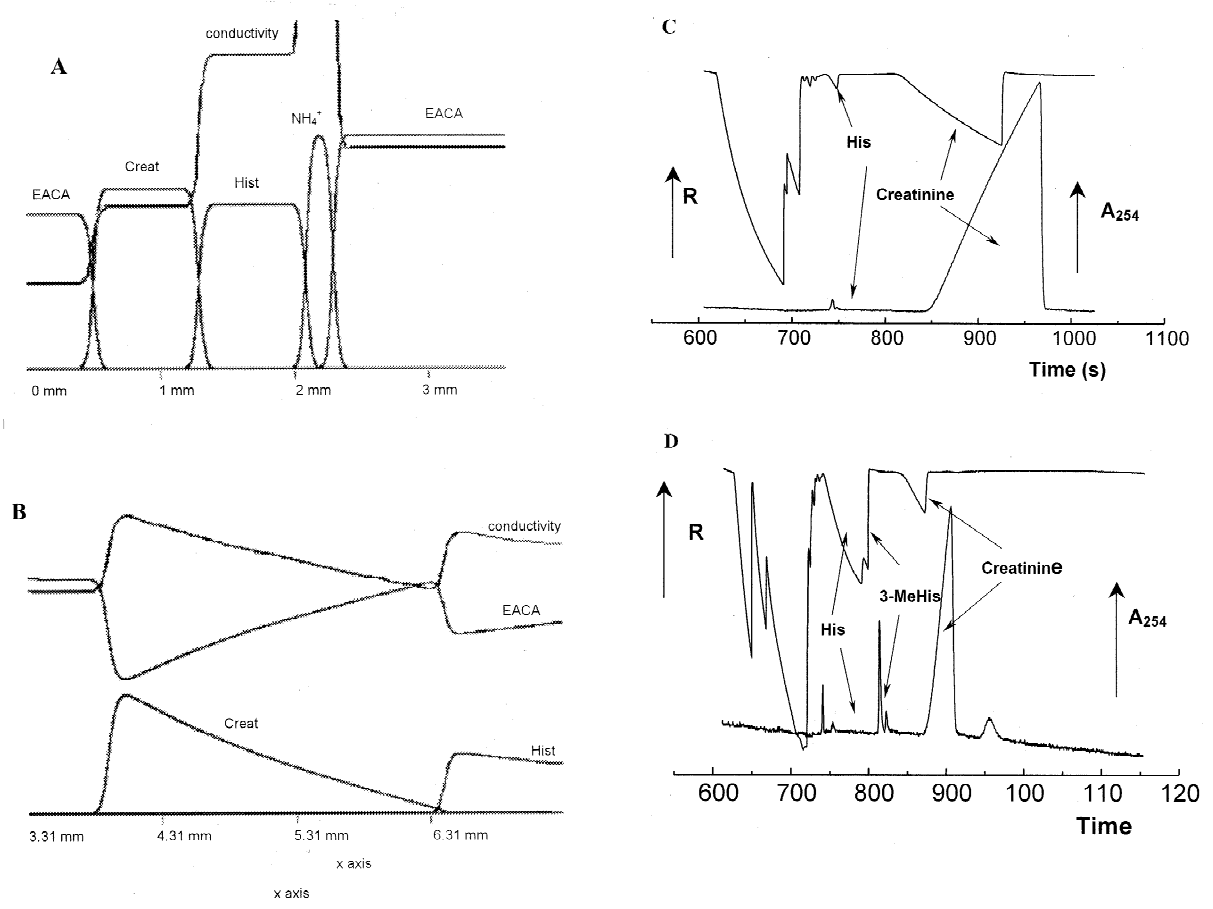

Fig. 3. Results of computer simulation of separation of 5 m*M* L-histidine and 5 m*M* creatinine and analyses of real sample by the cationic cITP–CZE mode. (A) Situation at the beginning of simulation of the cITP step; (B) situation at the end of simulation of the cITP step. Gray line: conductivity (upwards increasing) trace. Black lines: concentration (upwards increasing) profile of compounds. Creat: creatinine; His: L-histidine; EACA: ϵ-aminocaproic acid; NH_4^+: ammonium. (C) Conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 m*M* creatinine and 4 μ*M* His by the cITP–CZE mode; (D) conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of 10-times diluted hydrolysate of duck leg by the cITP–CZE mode.

mixture of L-histidine and creatinine is well separated. This expectation was again confirmed by the real analysis of the model mixture (Fig. 3C) and by the analysis of the real sample of duck leg hydrolysate (Fig. 3D). Here quantification of the minor component (L-histidine or 3-MeHis) is easier than in the case of the cITP–cITP mode. Since L-histidine and 3-MeHis do not absorb at 254 nm, the quantification of these analytes have to be performed from the conductivity trace, while creatinine due to its absorption at 254 nm could by quantified from both the conductivity and UV traces. Fig. 3D indicates that the quantification of 3-MeHis is still difficult because 3-MeHis is not fully separated from L-histidine.

3.3. Cationic cITP–CZE–cITP mode

The same model mixture and sample were analysed by the cITP–CZE–cITP mode using the T–S–BGE electrolyte system. Firstly the cITP step was simulated (Fig. 4A) and the theoretically calculated concentration of analytes was used for the simulation of the CZE–cITP step. Pyridine was chosen as a background cation. Its mobility is higher than that of creatinine and lower than that of L-histidine and

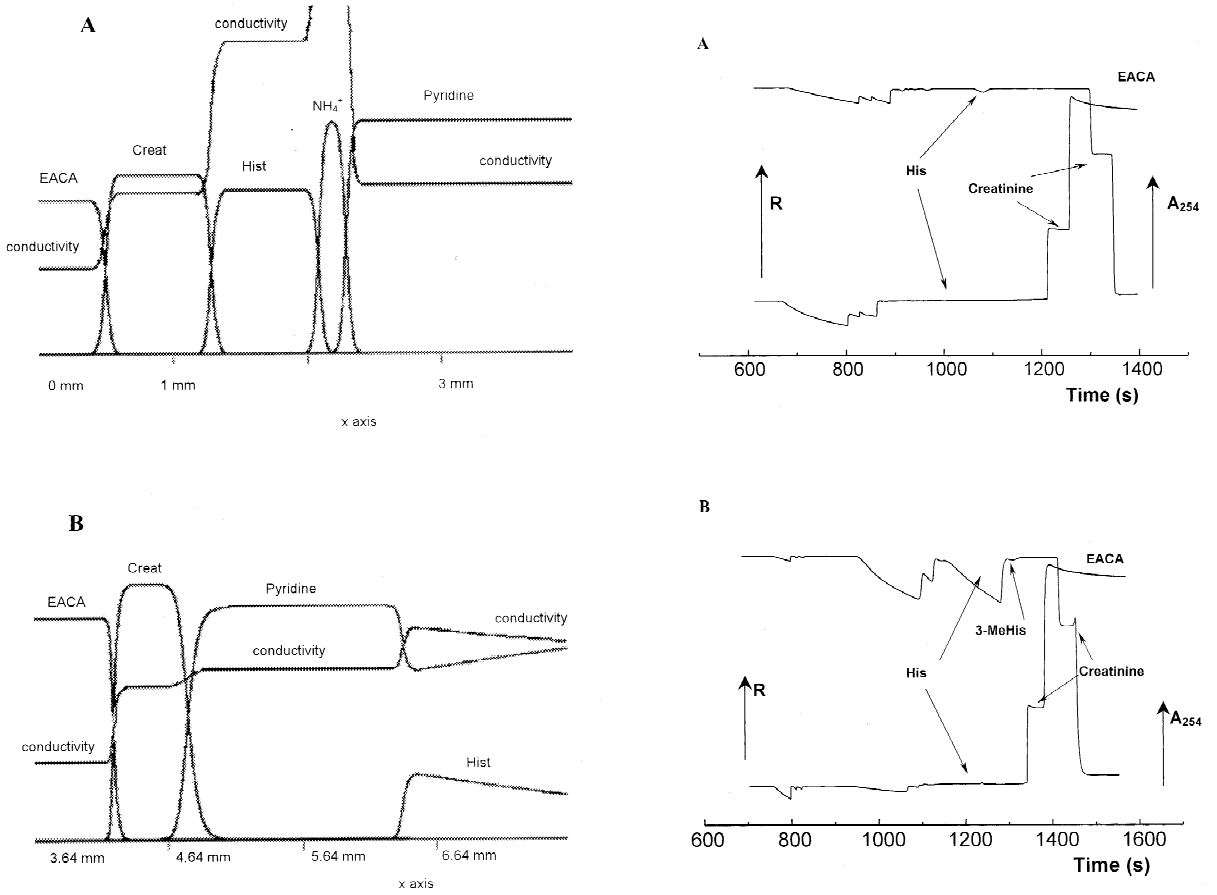

Fig. 4. Results of computer simulation of separation of 5 m*M* L-histidine and 5 m*M* creatinine and analyses of real sample by the cationic cITP–CZE–cITP mode. (A) Situation at beginning of simulation of the cITP step; (B) situation at the end of simulation of the CZE step. Gray line: conductivity (upwards increasing) trace. Black lines: concentration (upwards increasing) profile of compounds. Creat: creatinine; His: L-histidine; EACA: ϵ-aminocaproic acid; NH_4^+: ammonium. (C) Conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 m*M* creatinine and 4 μ*M* His by the cITP–CZE–cITP mode; (D) conductivity (*R*) and UV trace (A_{254}) from the analytical capillary of the real analysis of hydrolysate of duck leg by the cITP–CZE–cITP mode.

3-MeHis. In such a BGE L-histidine and 3-MeHis migrate in the CZE mode, and creatinine in the cITP mode, see Fig. 4B. The results of computer simulation were again fully confirmed by a real analysis of the model mixture (Fig. 4C) and sample (Fig. 4D). As pyridine is a strongly UV-absorbing compound, the UV detection here acts actually in the indirect mode. Note that the peak of L-histidine or 3-MeHis on the conductivity trace (Fig. 4C and D) is hardly visible, because its mobility is very close to the background cation mobility. From Fig. 4C and D it also is clear, that both analytes (minor and major component) are well separated as was predicted by computer simulation and can be quantified in one run. L-Histidine or 3-MeHis as an almost symmetrical peak and creatinine as a step. This is the main advantage of the new configuration proposed. Another benefit is that the peak of L-histidine or 3-MeHis is almost symmetrical due to low electrodispersion. Note also that the separation of 3-MeHis from L-histidine is better than that of the previous cITP–CZE mode.

3.4. Anionic cITP–cITP mode

We did analogous experiments and simulations for the anionic separation of the model mixture of fumaric and malic acid and apple juice as a sample. We use the same leading and terminating electrolytes for all modes. Conditions of analysis are given in Table 2. Within anionic experiments we found very good agreement between the results of computer simulation (data for anionic separation are not shown) and real analyses alike as in case of cationic analysis. Fig. 5A shows the record of analysis of a model mixture, 0.2 m*M* malic acid + 1 μ*M* fumaric acid, by the anionic cITP–cITP mode. Again quantification of minor component (fumarate) is rather problematic in the cITP–cITP mode, similarly to the cationic cITP–cITP mode. Furthermore, when determining fumaric acid in apple juice (see Fig. 5B) some sample components were found migrating close to fumarate, which was proved by a standard addition method. For these reasons the simultaneous determination of fumarate and malate in apple juice by the cITP–cITP mode is not possible.

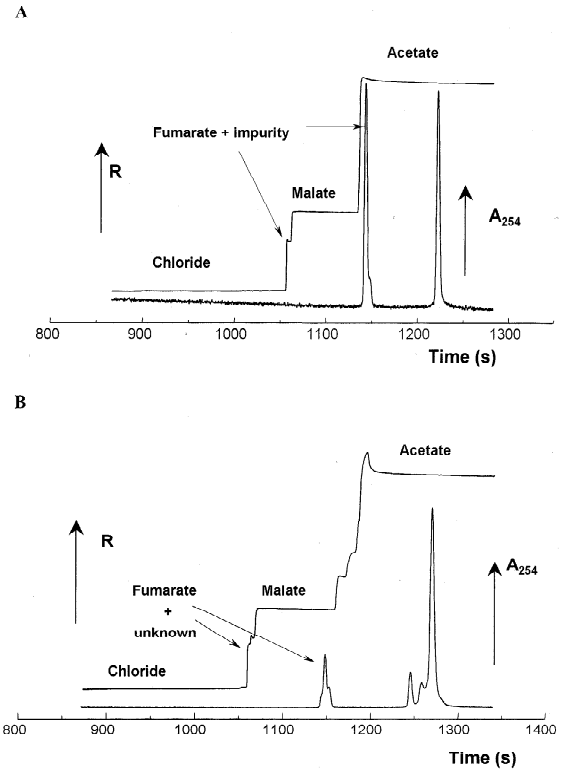

Fig. 5. Results of analyses of model mixture and real sample by the anionic cITP–cITP mode. (A) Conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 m*M* malic acid and 1 μ*M* fumaric acid by the cITP–cITP mode; (B) conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of 200-times diluted apple juice by the cITP–cITP mode.

3.5. Anionic cITP–CZE mode

The record of the model mixture analysis is shown in Fig. 6A. Fumarate is very well resolved from malate and can be easily quantified from the UV trace while malate from the conductivity trace. Fig. 6B shows the electropherogram of analysis of 200-times diluted apple juice. From this picture it is clear that fumarate can by quantified while for malate determination it should be recommended another analysis of a more diluted sample. Due to a high amount of malate, surviving transient cITP in the CZE step causes poor separation of malate and slower sample components (see Fig. 6B).

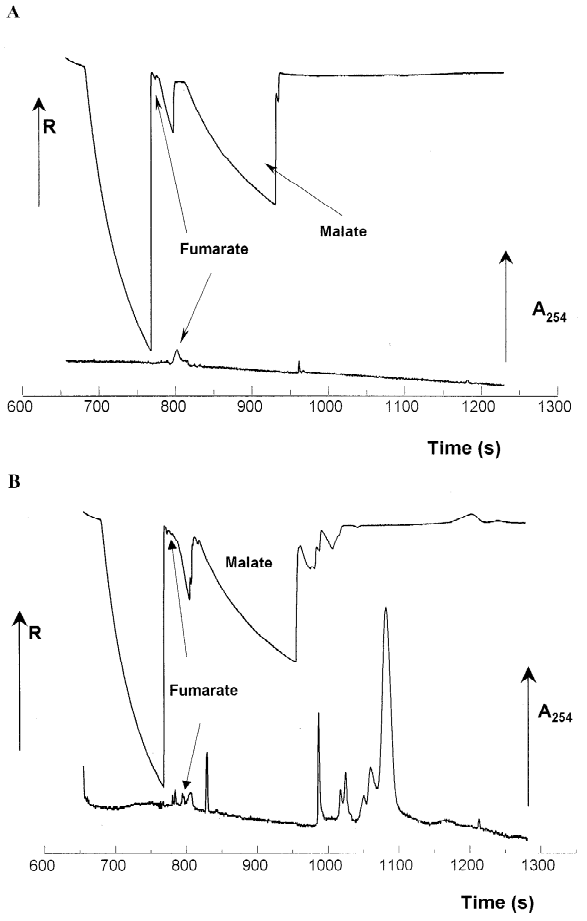

Fig. 6. Results of analyses of model mixture and real sample by the anionic cITP–CZE mode. (A) Conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 mM malic acid and 1 μM fumaric acid by the cITP–CZE mode; (B) conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of 200-times diluted apple juice by the cITP–CZE mode.

3.6. Anionic cITP–CZE–cITP mode

The same model mixture and sample was analysed by the cITP–CZE–cITP mode using the new T–S–BGE electrolyte system. Citric acid was chosen as the background anion the mobility of which is higher than that of malate and lower than fumarate. Analogically to cationic analyses discussed above, in such electrolyte fumarate migrates in the CZE mode and malate in the cITP mode. Analysis of model mixture is shown in Fig. 7A. The peak of fumarate on the conductivity trace is hardly visible as its mobility is very close to the mobility of citrate and must be quantified from the UV trace (note the symmetry of the peak due to low electrodispersion). It is clearly seen from Fig. 7A and B that both analytes are well separated as was predicted by computer simulation. It is also clear from this picture that minor and major components can be quantified by this mode in one run – fumarate as the almost symmetrical and good resolved peak and malate as the isotachophoretic step. The UV trace is more readable and separation

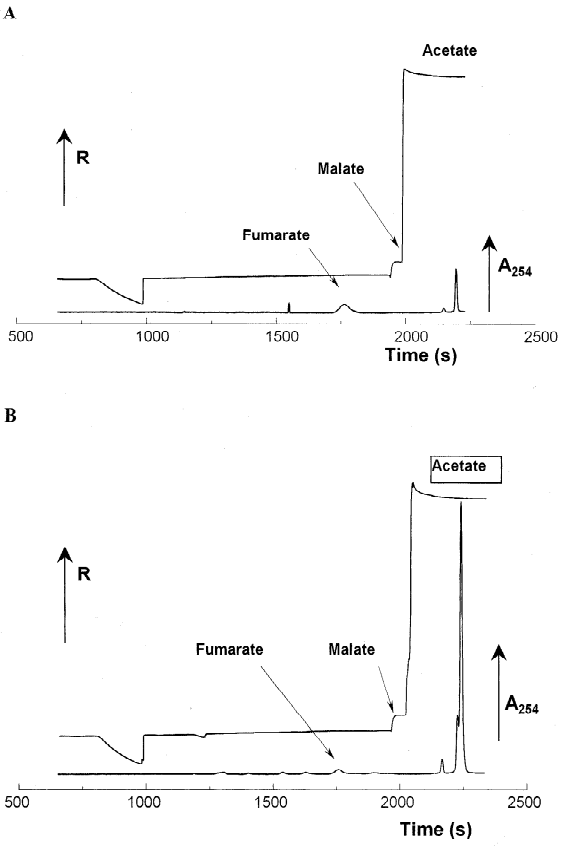

Fig. 7. Results of analyses of model mixture and real sample by the anionic cITP–CZE–cITP mode. (A) Conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of a model mixture of 0.2 mM malic acid and 1 μM fumaric acid by the cITP–CZE–cITP mode; (B) conductivity (R) and UV trace (A_{254}) from the analytical capillary of the real analysis of 200-times diluted apple juice by the cITP–CZE–cITP mode.

of fumarate from unknown is better than that of the cITP–CZE mode.

4. Conclusions

We found that the results of computer simulation are in a very good agreement with real analyses. The program SIMUL serves as a useful tool in this field. From the practical point of view the new configuration, so-called cITP–CZE–cITP, using the new electrolyte system T–S–BGE brings:

(1) Extending of cITP–CZE applications.

(2) Both minor and major components could be separated and determined in one run.

(3) Nearly symmetrical peaks of analytes (lower electrodispersion)

(4) Easy to operate – no need to change electrolytes after the cITP step.

Acknowledgements

The authors are grateful for financial support provided by the Grant Agency of the Czech Republic (project No. 525/99/1279) and the Ministry of Education of the Czech Republic (project No. J13/98/11310001).

References

[1] D.M. Osbourn, D.J. Weiss, C.E. Lunte, Electrophoresis 21 (2000) 2768.
[2] D. Kaniansky, J. Marák, J. Chromatogr. 498 (1990) 191.
[3] D. Kaniansky, J. Marák, V. Madajová, E. Šimuničová, J. Chromatogr. 638 (1993) 137.
[4] L. Křivánková, P. Gebauer, W. Thormann, R.A. Mosher, P. Boček, J. Chromatogr. 638 (1993) 119.
[5] J.L. Beckers, P. Boček, Electrophoresis 21 (2000) 2747.
[6] http://www.natur.cuni.cz/~gas/, accessed September 2000.
[7] H.F. De Brabander, K. De Wasch, P. Batjoens, G. Temmermen, in: Proceedings of Euro Food Chem IX, Interlaken, 24–26 September 1997, pp. 17–22.
[8] F. Kvasnička, M. Voldřich, Electrophoresis 21 (2000) 2848.
[9] F. Kvasnička, M. Voldřich, J. Chromatogr. A 891 (2000) 175.
[10] F. Kvasnička, J. Chromatogr. A 838 (1999) 191.