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Capillary zone electrophoresis of complex ionic mixtures with on-line isotachophoretic sample pretreatment

D. Kaniansky*, J. Marák, V. Madajová and E. Šimuničová

Department of Analytical Chemistry, Faculty of Natural Sciences, Komenský University, Mlynská Dolina CH-2, 842 15 Bratislava (Slovak Republic)

ABSTRACT

Separation modes provided by the column-coupling configuration of the separation unit in an on-line combination of capillary isotachophoresis (ITP) with capillary zone electrophoresis (CZE) were studied from the point of view of their potential in the (trace) determination of ions present in complex ionic matrices. Urine was arbitrarily chosen as such a matrix while sulphanilate and 3,5-dinitrosalicylate (currently not present in urine) served as model analytes. In one of these modes, ITP was employed to remove only the most abundant sample constituent (chloride) and concentrate the rest of those migrating between the leading and terminating zones for injection into the ZE stage. In the other mode, ITP was employed for maximum sample clean-up. Here, only the analyte(s) with a minimum of the matrix constituents was transferred for a final separation in the ZE stage. The fraction to be transferred was defined via a pair of discrete spacers added to the sample. Although a highly efficient sample clean-up was typical in this instance, the use of identical migration regimes in both stages (the separations according to ionic mobilities) did not prove the resolution of one of the analytes (sulphanilate) from the matrix constituent(s) in the ZE stage. A considerable improvement in this respect was achieved easily when the ITP clean-up was based on the separation according to pK values while the constituents present in the transferred fraction were finally separated via differences in their ionic mobilities. This two-dimensional approach provided a way to achieve a 150 ppb (10^{-6} mol l^{-1}) concentration detection limit for sulphanilate in a 1-μl volume of urine taken for the electrophoretic run.

INTRODUCTION

High separation efficiencies as typically achieved in capillary zone electrophoresis (CZE) favour the use of this technique for the determination of ionogenic compounds present in multi-component mixtures. This is a feature favouring its applicability in trace analysis, especially, when the samples are characterized by complex, multi-component ionic matrices (*e.g.*, determination of trace constituents present in samples of biological and environmental origins). However, the low load capacities of currently used columns and/or high concentration detection limits attainable by current detectors limit the wider use of CZE for such purposes. In this context, it should be noted that improvements in the detection systems (see *e.g.*, refs. 1–5) can provide only a partial solution to these problems as from a general point of view [6] a high frequency of peak overlaps (mixed zones) may be a key task to be solved.

Recently, considerable attention has been paid to various approaches enabling the concentration detection limits in CZE to be improved via higher sample loads. Many of them employ the inherent concentrating capability of zone electrophoretic (ZE) separation [7–10]. The sample preconcentration based on this capability is useful, *e.g.*, in the determination of ions present in water for steam generation in power plants [11]

* Corresponding author.

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or in the analysis of liquid chromatographic fractions when in-column neutralization of matrix ions is possible [12]. However, it cannot improve the concentration detection limits when the analytes are present in complex ionic matrices (see, *e.g.*, ref. 13).

Disc electrophoresis [14,15], *i.e.*, the sequential use of ITP concentration with ZE separation, was shown to be a convenient approach to the separations of dilute peptide solutions [16] and proteins [17]. A similar sequence of ITP and ZE is applied in the CZE analysis of samples containing a large excess of very mobile ions when the composition of the carrier electrolyte meets certain requirements [18–21]. The practical utility of these alternatives is limited when the number of matrix constituents is very high (peak overlaps) and/or the matrix is highly variable (*e.g.*, variabilities of the migration times of the separands from sample to sample). In this respect, a tandem arrangement of a pair of columns with the possibility of washing the ITP column after the analytes and the constituents migrating in front of them are transferred into the CZE column [22] will probably also be less effective in such situations.

It was shown by Mikkers [23] that the column-coupling configuration of the separation unit for ITP is convenient for electrophoretic separations in discontinuous buffer systems, *i.e.*, for a tandem arrangement of ITP with CZE. However, it is apparent that this concept of capillary electrophoresis instrumentation [23–25] provides wider possibilities, especially from the point of view of on-line ITP sample pretreatment before the CZE separation. We have already demonstrated some of them in previous work [26]. The determination of halofuginone in feedstuffs [27] showed the practical utility of combined ITP–CZE when performed with the column-coupling instrument. The analytical potential of this approach can be further improved from the point of view of concentration detection limits by using very sensitive detectors in the CZE stage [28,29].

As already mentioned, peak overlaps (mixed zones) occur with a high frequency in the separation of complex mixtures also when very high separation efficiencies are achieved [6]. Coupled-column separation systems provide a way to solve these problems in high-efficiency chromatographic techniques [6,30]. Undoubtedly, it is reasonable to expect that such an approach will also be effective in capillary electrophoresis and the column-coupling configuration of the separation unit offers analytical benefits analogous to those of its chromatographic counterparts. In addition, when used with an ITP–CZE combination, some specific features of the ITP step (preconcentration of the analytes; a high sample load; transfer of a well defined fraction to CZE; ideal sample injection for CZE) can further increase these benefits. Previously, however, no attention has been paid to the use of this tandem arrangement to the determination of ions present at low concentrations in complex ionic matrices. The aim of this work was to carry out a feasibility study along these lines and various alternatives of tandem ITP–CZE in the column-coupling configuration of the separation unit were investigated from the point of view of their applicability to such analytical problems. Urine, containing hundreds of acids at very differing concentrations [31], represented such a matrix in this study. Sulphanilate and 3,5-dinitrosalicylate (not reported to be currently present in urine) added at appropriate concentrations to urine samples served as trace analytes.

EXPERIMENTAL

Instrumentation

A prototype of a micro-trace capillary electrophoresis system (Unitec, Vienna, Austria), assembled in the column-coupling mode, was employed. The samples were injected with the aid of a valve (25-μl sample loop). The columns provided with 0.30 mm I.D. capillary tubes (O.D. *ca.* 0.65 mm) made of fluorinated ethylene–propylene (FEP) copolymer were employed in both the preseparation (ITP) and analytical (ZE) stages. The analytical column was provided with an on-column UV detector. A rectangular slit (0.25 mm in height) defined the detection cell. The same type of detector was used in some experiments in the ITP stage to record a UV profile of the sample after the ITP separation. Detection was performed at 254 nm.

ZE experiments were carried out in the

column employed in the analytical stage of the separation unit. In this instance the column was directly connected to a CZE valve. The valve, suitable for various capillary electrophoresis techniques, was provided with a 200-nl internal sample loop in this work.

Chemicals

Chemicals used for the preparation of the electrolyte solutions were bought from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA) and Lachema (Brno, Czech Republic). Methylhydroxyethylcellulose 30 000 (MHEC), obtained from Serva, was used as an anticonvective additive in the electrolyte solutions. A 1% (w/v) aqueous solution of the cellulose derivative was demineralized on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, UK).

Water from a Rodem-1 demineralization unit (OOP, Tišnov, Czech Republic) was further purified by circulation through laboratory-made polytetrafluoroethylene cartridges packed with Amberlite MB-1 mixed-bed exchanger. The solutions used throughout were prepared from freshly recirculated water.

Samples

Urine samples were obtained from healthy individuals (mid-stream fractions). After receipt they were diluted fivefold with demineralized water to avoid gradual precipitation of the anionic constituents. When required, the samples or their aliquots were spiked at appropriate concentrations with sulphanilic and/or 3,5-dinitrosalicylic acids. No preservatives were added to the samples.

RESULTS AND DISCUSSION

Analysis of constituents present in complex ionic matrices by CZE and ITP

When CZE is applied to the determination of constituents present in biological and environmental matrices without an appropriate sample pretreatment, usually very small amounts of sample are injected on to the column to avoid its overloading. Consequently, higher concentration detection limits are typical for the analytes present in such matrices in comparison with samples in which they are accompanied by ionic constituents at lower concentrations. In some instances, however, it is advisable to overload the column with the sample as also under such circumstances reliable data can be obtained for some analytes (see, *e.g.*, refs. 20 and 21). This approach, inherently combining ITP and ZE separation principles, has certain analytically relevant implications [18,19]. The electropherograms in Fig. 1 illustrate some of them. For example, it can be seen that sulphanilate (migrating at a considerably longer migration time in comparison with the runs in which only aqueous solutions of this constituent were injected) was unresolved from matrix constituents and, in addition, its (very sharp) peak was fronting. This fronting, resembling the situation in the starting phase of the separation in ZE (see Fig. 3 in ref. 19), can be probably ascribed to the existence of a mixed zone of sulphanilate with more mobile macroconstituents (undetected at 254 nm). In

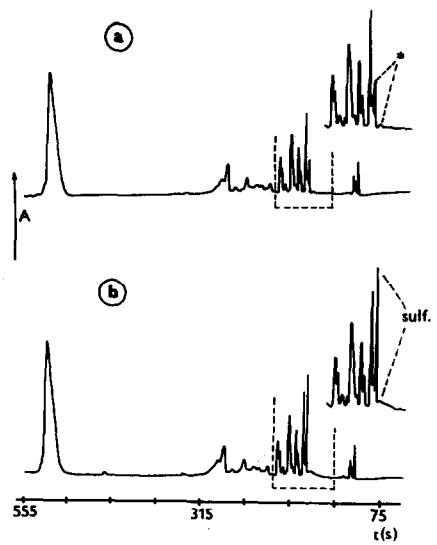

Fig. 1. Electropherograms from the separations of (a) urine and (b) urine spiked with sulphanilate at $2 \cdot 10^{-4}$ mol/l. Volumes of 200 nl of the samples diluted with water (1:5, v/v) were injected by a valve. The separations were carried out in the carrier electrolyte ZE-1 (Table I). The driving current was 150 μA. The parts of the electropherograms marked with dashed lines indicate the positions of the magnified details shown above the corresponding electropherograms. The asterisk marks the migration positions of sulphanilate in the unspiked urine. A = increasing light absorption; sulf. = sulphanilate.

experiments carried out with the same sample in the column-coupling configuration of the separation unit (see, *e.g.*, Fig. 5), such a fronting was not observed while sulphanilate remained unresolved from matrix constituents. As in these experiments removal of chloride from the separation compartment after the ITP stage was, in fact, the only difference in comparison with the CZE runs shown in Fig. 1, the above explanation of the fronting (a transient mixed zone) seems reasonable. Analytical consequences of the described behaviour of the separands in CZE are obvious (uncertainty in the identification; systematic error in the quantification). We can expect that in the analysis of complex ionic mixtures such situations will arise and there may not be simple means for their detection, especially for trace analytes.

When ITP is considered for use in the above analytical application, it is apparent that the load capacities [23,32] of current single-column separation compartments are not sufficient when the analytical evaluation is to be derived from the response of a high-resolution universal detector (a.c. or d.c. conductivity). ITP used in the spike mode (see, *e.g.*, refs. 33 and 34) is in this respect more convenient. The isotachopherograms given in Fig. 2 (obtained for the same samples as in CZE in Fig. 1) illustrate the advantages and drawbacks of the spike mode for this type of application, especially, when less selective spectrophotometric detection (254 nm) is employed for evaluation. The high concentrating power of ITP is clearly visible from these isotachopherograms. At the same time, however, it can be seen that the sample constituents focused in the migration position of sulphanilate (defined by the zones of spacing constituents, S_1, and, S_2) may introduce a serious systematic error into its determination and/or make its identification problematic. The use of more appropriate spacing constituents could decrease this bias, but a search for ideal spacers may be a tedious task for the matrix of interest [35].

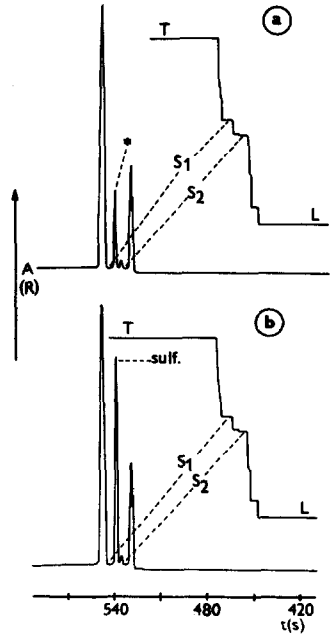

Fig. 2. Isotachopherograms from the separations of (a) urine and (b) urine spiked with sulphanilate at $2 \cdot 10^{-4}$ mol/l. Volumes of 25 μl of the samples diluted with water (1:250, v/v) were taken for the analyses. The electrolyte system ITP-1 (Table I) was used. The driving current was 150 μA. The injected samples contained iminodiacetate (S_1) and β-bromopropionate (S_2), each at a $3 \cdot 10^{-4}$ mol/l concentration, as discrete spacers. L, T = leading and terminating zones, respectively; A = increasing light absorption; R = increasing resistance.

ITP in the sample injection mode for ZE

Experiments carried out with model mixtures in our previous work [26] and the results presented by other workers [16,17,22,27–29] clearly show that ITP can be used as a very convenient sample injection technique for CZE. When the column-coupling configuration of the separation unit is used in this mode of operation (see Fig. 3), it is apparent that the sample need not be injected completely into the ZE stage. Its injection can be combined with removal of constituents having effective mobilities higher than or equal to that of the leading ions. Such a possibility may be of interest, *e.g.*, in the analysis of various biological fluids (containing Cl^- and Na^+ at high concentrations) to avoid some of the problems discussed above. From the scheme in Fig. 3 we can deduce that this mode represents a tandem arrangement of the columns in the separation system with limitations inherent to this arrangement from the point of view of multidimensional separations [30].

In the ITP phase not only are the sample

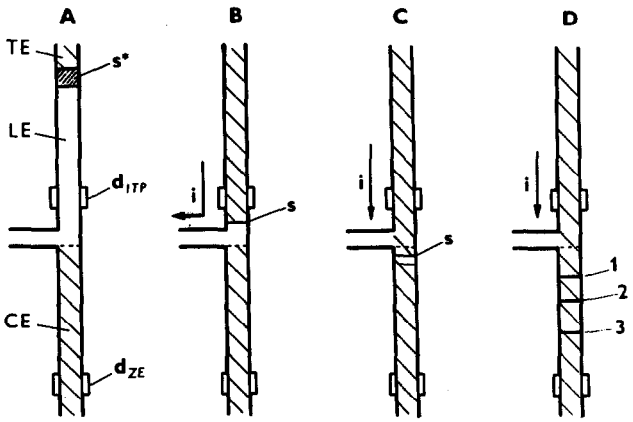

Fig. 3. Schematic illustration of the use of ITP for sample injection into ZE in the column-coupling configuration of the separation unit. (A) Starting phase; (B) end of the ITP separation; (C) transfer of the sample into the ZE stage; (D) ZE separation. s* = Injected sample; s = sample after the ITP stage; 1–3 = constituents separated by ZE; i = direction of the driving current; LE, TE, CE = leading, terminating and carrier electrolytes, respectively; d_{ITP}, d_{ZE} = detectors in the ITP and ZE columns, respectively.

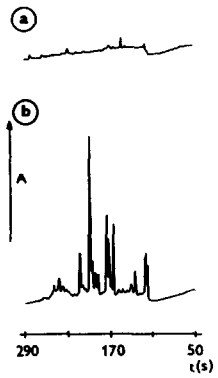

Fig. 4. Influence of impurities preconcentrated from the electrolyte solutions in the ITP stage on the ZE profile of urine. (a) ZE profile of ionogenic constituents accumulated between the leading and terminating zones in a blank ITP run preceding the ZE separation (the solution of the terminating electrolyte served as a sample); (b) ZE profile of urine (diluted 1:250 with water) with ITP injection, 25-μl volumes of the samples being taken for the analyses. ITP-1 and ZE-1 electrolyte systems (Table I) were used in the ITP and ZE stages, respectively. The driving current in both stages was 150 μA. A = increasing light absorption.

constituents separated and concentrated (diluted) in a well known way [23,32,36], but also the corresponding impurities present in the electrolyte solutions are focused between the leading and terminating zones in accordance with principles of moving boundary electrophoresis [36–38]. Hence the ITP stage can introduce some disturbances into the CZE analysis. The electropherograms in Fig. 4 show the effect of impurities accumulated between the leading and terminating anions in the ITP stage on the CZE profile of a urine sample. Here, a blank ITP run which gave a maximum background in the ZE stage in terms of the number of peaks and their total area was chosen amount those obtained with the electrolyte solutions employed in this work.

The electropherograms in Fig. 5 were obtained from the separations of urine and urine spiked with 3,5-dinitrosalicylate and sulphanilate. For the reasons mentioned above, chloride present in the sample was removed from the separation compartment after the ITP stage and the sample amount (in terms of total ionics) loaded on to the CZE column was thus considerably reduced. The electropherograms show that whereas 3,5-dinitrosalicylate was resolved from the matrix constituents, sulphanilate migrated unresolved from them. Optimization of the separation conditions could probably solve this particular resolution problem but a detailed study along these lines was beyond the scope of this work.

ITP sample clean-up with the injection of the analyte-containing fraction into the ZE stage

The above results suggest that the injection mode combined with removal of very mobile sample macroconstituents can be convenient, *e.g.*, in a CZE profiling of various samples or in a multi-component analysis of ions when the qualitative complexity of the sample is not high. In the analysis of constituents present in complex mixtures of ionogenic compounds, however, a sample clean-up step may become essential. The separation unit as used in this work enables such a clean-up to be carried out on-line when used in the mode shown in Fig. 6. Here, a pair of appropriately chosen spacing constituents added to the sample defines in a straightforward way the fraction to be transferred from the ITP stage for a final ZE separation in the second column. Iminodiacetate (S_1 in Fig. 7) and β-bromo-

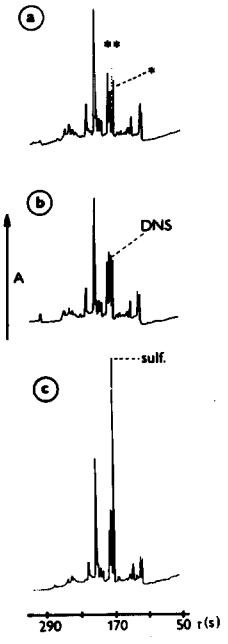

Fig. 5. ZE profiles of urine and urine spiked with the studied analytes in the column-coupling instrument with ITP used for the sample injection. (a) Urine diluted with water (1:250, v/v; *, * * = migration positions of sulphanilate and 3,5-dinitrosalicylate, respectively); (b) the same sample as in (a) except that it was spiked with 3,5-dinitrosalicylate (DNS) at a 10^{-4} mol/l concentration before dilution; (c) the same as in (b) except sulphanilate (sulf.) was the analyte. Other conditions as in Fig. 4.

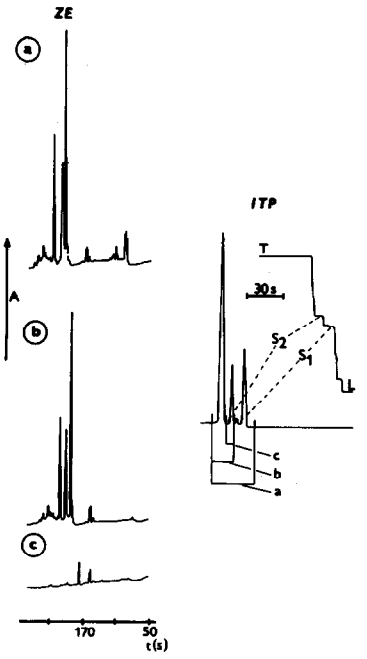

Fig. 7. Alternatives of the ITP clean-up of a urine sample with the use of a pair of discrete spacers. (a) Fraction a [marked on the isotachopherogram (ITP) from the UV detector] was transferred for a final ZE separation; (b) ZE profile of fraction b; (c) ZE profile of fraction c. Volumes of 25 μl of diluted urine (1:250, v/v) containing iminodiacetate (S_1) and β-bromopropionate (S_2) as discrete spacers (each at a $3 \cdot 10^{-4}$ mol/l concentration) were taken for the separations. L, T = leading and terminating zones, respectively. Other conditions as in Figs. 2 and 4.

propionate (S_2) were found to be convenient spacing constituents for sulphanilate and 3,5-dinitrosalicylate in the ITP phase when the separation was carried out in the electrolyte system ITP-1 (Table I).

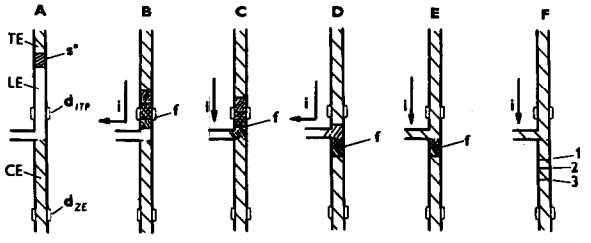

Fig. 6. Schematic illustration of the use of ITP as a sample clean-up technique for ZE. (A) Starting phase of the separation; (B) end of the ITP separation; (C) transfer of the fraction containing the analyte (f) into the ZE stage; (D) removal of the sample constituents migrating behind the transferred fraction; (E) starting phase in the ZE stage; (F) ZE separation. Other conditions as in Fig. 3.

The electropherograms in Fig. 7 illustrate alternatives of the clean-up attainable under these conditions for a urine sample. In the run shown in Fig. 7a, only the main part of the chloride and the sample constituents migrating zone electrophoretically in the terminating zone were removed from the separation compartment after the ITP stage. As in this instance the sample constituents migrating between the leading and terminating zones (including the spacers) were injected into the ZE stage it may serve as a reference to which the runs in Fig. 7b and c are related. In the run shown in Fig. 7b, the sample fraction transferred into the ZE stage was defined by the spacing constituent S_1 and by the terminating zone. The overall sample clean-up was not high in this instance as the main part of the UV-absorbing separands migrated in the ITP stage behind the rear spacer. On the other hand,

TABLE I

ELECTROLYTE SYSTEMS

Parameter	Electrolyte[a]				Carrier	
	ITP-1		ITP-2		ZE-1	ZE-2
	Leading	Terminating	Leading	Terminating		
Solvent	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O
Anion	Cl^-	MES^-	Cl^-	$Prop^-$	MES^-	$Prop^-$
Concentration (m*M*)	40	10	25	10	100	50
Counter ion	HIS	HIS	BALA	BALA	HIS	HIS
pH	6.0	6.0	3.3	4.0	5.2	4.8
Additive	MHEC	–	MHEC	–	MHEC	MHEC
Concentration (%, w/v)	0.2	–	0.2	–	0.2	0.2

[a] MHEC = Methylhydroxyethylcellulose; MES = morpholinoethanesulphonic acid; HIS = histidine; BALA = β-alanine; Prop = propionic acid.

95% removal of the matrix constituents (when compared via the peak areas with the run in Fig. 7a) was achieved when only the separands migrating between the spacers were transferred into the CZE stage (Fig. 7c). It should be noted that such results could be expected from a general model describing the clean-up efficiency in two-dimensional ITP analysis of ions present in complex matrices [35].

The electropherograms in Fig. 8 illustrate the impact of the ITP clean-up on the resolutions of the studied analytes from the urine matrix. It is apparent that 3,5-dinitrosalicylate was baseline resolved from the matrix constituents transferred with it into the ZE stage (compare Fig. 8a and b). A comparison with the run in which ITP was used for the same sample in the injection mode (Fig. 5b) clearly shows an overall improvement of the analytical conditions for this analyte owing to the clean-up step. On the other hand, sulphanilate was not resolved from the matrix constituent(s) under identical clean-up conditions (Fig. 8c). Although optimizations of the separation conditions could improve the resolution of this model analyte, no investigations along these lines were performed in this work.

Multi-dimensional approach in CZE coupled with ITP sample pretreatment

In the experiments described above, no special attention was paid to the choice of electrolyte systems. Considering the acid–base properties of sulphanilic acid ($pK_a = 3.23$), it is apparent that in both columns its effective mobility was determined mostly by its ionic mobility. An evaluation of the electrolyte systems for ITP from the point of view of

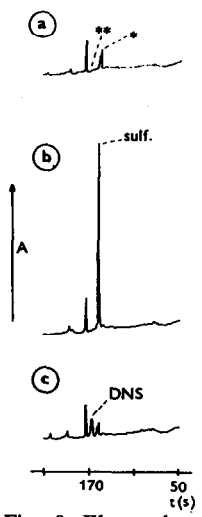

Fig. 8. Electropherograms from the separations of urine fractions obtained by the ITP clean-up. (a) Urine (diluted 1:250, v/v; *, * * = migration positions of sulphanilate and 3,5-dinitrosalicylate, respectively); (b) urine spiked with sulphanilate (sulf.) at a $1 \cdot 10^{-4}$ mol/l concentration [dilution as in (a)]; (c) urine spiked with 3,5-dinitrosalicylate (DNS) at a 10^{-4} mol/l concentration [dilution as in (a)]. Volumes of 25 μl of the samples were injected. The electrolyte systems and driving currents were the same as in Fig. 4. For further details, see the text.

information theory [39] suggests that in the coupled column systems our chance of achieving a desired resolution of the sample constituents will be significantly higher when using electrolyte solutions characterized by low similarities (*e.g.*, by combining the separations according to ionic mobilities and p*K* values). It was shown recently [35] that the choice of electrolyte systems following this general rule provides, for example, maximum benefit in two-dimensional separations by ITP. Undoubtedly, the ITP–CZE tandem arrangement in the separation unit used in this work can also follow this rule. Therefore, to resolve sulphanilate from a urine matrix the electrolyte system ITP-2 (Table I) was used in the ITP stage. Its role was to minimize the number of isotachophoretically migrating constituents and provide their separations according to p*K* values. The ZE separations were performed in the carrier electrolyte ZE-2 (Table I). Here, the resolution of separands was governed mostly by the differences in their ionic mobilities.

The separations were carried out in both the sample injection (Fig. 3) and clean-up (Fig. 6) modes to demonstrate their various capabilities under otherwise identical working conditions. To avoid disturbances which can occur in a "pure" sample injection mode (see Fig. 1 and the accompanying discussion), chloride present in the sample was removed from the separation compartment after the ITP stage.

From the electropherograms in Fig. 9 it can be seen that in comparison with the corresponding runs in Fig. 5 an improved resolution of sulphanilate from the matrix was obtained, probably because the electrolyte system used in the ITP stage was more restrictive for the ITP migrations of the anionic sample constituents (lower pH) than its counterpart used in the runs shown in Fig. 5.

A pair of spacing constituents (tartrate and citrate) were added to the sample when it was analysed by ITP in the sample clean-up mode (Fig. 10). These constituents were chosen in the way described elsewhere [35] and their capability to space sulphanilate from the other separands is clear from the isotachopherogram obtained with the UV detector (ITP, in Fig. 10). Electropherograms for the analyses of the transferred ITP fractions (ZE, in Fig. 10) of relevant samples show that the analyte was resolved from the comigrants accompanying it in the migration position in the ITP stage. When we consider differences in pH values of the electrolyte systems, the pH sequence of these systems in the coupled columns and the removal of the main part of the sample constituents from the separation compartment after the first separation stage, it seems reasonable to assume that a two-dimensional separation approach was also responsible for the resolution achieved.

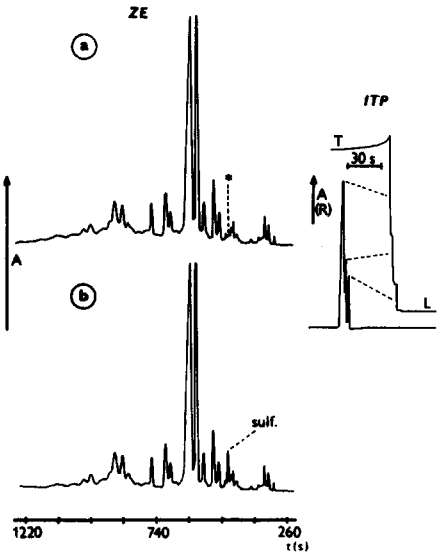

Fig. 9. Electropherograms from the separations of urine and urine spiked with sulphanilate ($1 \cdot 10^{-4}$ mol/l) at pH 4.8 with the ITP preseparation carried out at pH 3.3. Isotachopherograms (ITP) as obtained from the conductivity and UV detectors show ITP profiles of the sample constituents transferred into the ZE stage. (a) Urine (diluted 1:250, v/v); (b) the same as (a) except the injected sample contained sulphanilate (sulf.). ITP-2 and ZE-2 electrolyte systems were used for the separation in the ITP and ZE stages, respectively. The driving currents in both stages were 150 μA.

Considerable overloading of the CZE column was unavoidable when a 25-μl volume of urine diluted 1:25 (v/v) was taken for the analysis and in the ITP stage only chloride was removed from the separation compartment (Fig. 11). Obviously, in this instance hardly any useful information can be obtained from the response of the detector in the ZE stage. On the other hand, the ITP sample clean-up improved the situation in the

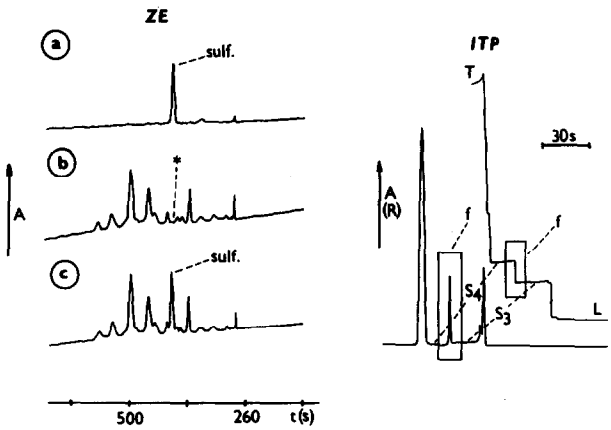

Fig. 10. Electropherograms from the separations of the constituents present in a urine fraction obtained by the ITP clean-up. (a) Control run with sulphanilate ($4 \cdot 10^{-7}$ mol/l concentration in an aqueous solution containing also Na_2SO_4 as an adsorption-eliminating additive at a $1 \cdot 10^{-3}$ mol/l concentration); (b) urine (diluted 1:250, v/v); (c) urine spiked with sulphanilate at $1 \cdot 10^{-4}$ mol/l (diluted 1:250, v/v). The samples contained tartrate (S_3) and citrate (S_4) as spacing constituents (each at a $3 \cdot 10^{-4}$ mol/l concentration). The fractions f [marked by boxes on the isotachopherograms (ITP)] from the conductivity and UV detectors were transferred into the ZE stage. Volumes of 25 μl of the samples were taken for these runs. Other conditions as in Fig. 9.

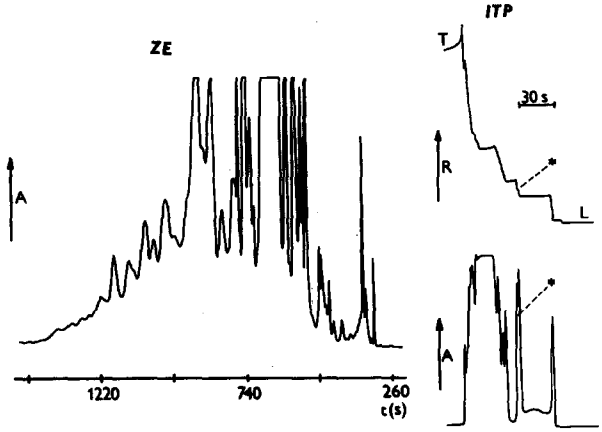

Fig. 11. Electropherograms from the separations of urine with a high sample load. A 25-μl volume of urine (diluted 1:25, v/v) was taken for the separation. All sample constituents after the ITP stage (see isotachopherograms on the right) were transferred for a final ZE separation. The asterisk marks the migration position of sulphanilate. Other conditions as in Fig. 9.

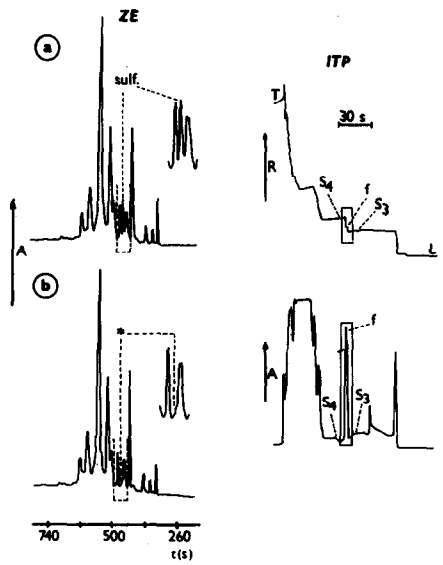

Fig. 12. Electropherograms from the separations of urine fractions obtained by the ITP clean-up for a high sample load. (a) Urine spiked with sulphanilate at $1 \cdot 10^{-5}$ mol/l (diluted 1:25, v/v); (b) urine (diluted 1:25, v/v). The parts of the electropherograms marked with dashed lines indicate the positions of the magnified details shown above the corresponding electropherograms. The injected samples (25 μl) contained the same spacers as in Fig. 10 to define the fractions to be transferred for the ZE separations. The fractions (f) are marked with boxes on the isotachopherograms (ITP) as obtained from the conductivity and UV detectors. The asterisk indicates the migration position of sulphanilate. Other conditions as in Figs. 9 and 10.

ZE stage dramatically (Fig. 12). In this way almost baseline resolution of sulphanilate from the matrix constituents was achieved. In addition, a high sample load provided the possibility of decreasing the concentration detection limit for this analyte. We found that its value under our working conditions was *ca.* 150 ppb (10^{-6} mol l^{-1}), *i.e.*, a tenfold improvement in comparison with the run (Fig. 10) in which the same sample was diluted 1:250 (v/v).

CONCLUSIONS

This work has shown that various modes of the on-line combination of ITP with CZE in the column-coupling configuration of the separation unit provide very promising possibilities for the CZE analysis of complex ionic mixtures. The use of ITP for sample clean-up seems very conveni-

ent for trace analysis problems in which matrix constituents with physico-chemical properties close to those of the analyte(s) are key interferents. The two-dimensional features of this mode can considerably increase the means available for the optimization of the working conditions.

The 150 ppb concentration detection limit achieved for one of the model analytes (sulphanilate) in urine is obviously not an ultimate minimum. For example, in the analysis of model mixtures containing various ionic constituents we were able to detect it with confidence at the 5 ppb level for a 25-μl injection volume. A further improvement in the concentration detection limit of the ITP–CZE tandem system by increasing the injection volume is closely related to the load capacity of the ITP stage. However, a very high ratio of the load capacity of the ITP column to that of the CZE column may require sample splitting [29] and thus an inefficient use of the ITP pretreatment. A detailed investigation along these lines can define limits of the combination of ITP and CZE in trace analysis applications. Obviously, the potential associated with the use of more sensitive and/or more selective detectors should also be taken into consideration in such an investigation.

REFERENCES

1 W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, 64 (1992) 389R.
2 K.C. Waldron and N.J. Dovichi, *Anal. Chem.*, 64 (1992) 1396.
3 T.T. Lee and E.S. Yeung, *J. Chromatogr.*, 595 (1992) 319.
4 T. Wang, J.H. Aiken, C.W. Huie and R.A. Hartwick, *Anal. Chem.*, 63 (1991) 1372.
5 J.P. Chervet, R.E.J. van Soest and M. Ursen, *J. Chromatogr.*, 543 (1991) 439.
6 J.C. Giddings, *Unified Separation Science*, Wiley, New York, 1991.
7 F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
8 F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
9 J.L. Beckers, Th.P.E.M. Verheggen and F.M. Everaerts, *J. Chromatogr.*, 452 (1988) 591.
10 R.-L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 489 A.
11 G. Bondoux, P. Jandik and W.R. Jones, *J. Chromatogr.*, 602 (1992) 79.
12 R. Aebersold and H.D. Morrison, *J. Chromatogr.*, 516 (1990) 79.
13 B.J. Wildman, P.E. Jackson, W.R. Jones and P.G. Alden, *J. Chromatogr.*, 546 (1991) 459.
14 L. Ornstein, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
15 B.J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
16 V. Dolník, K.A. Cobb and M. Novotný, *J. Microcol. Sep.*, 2 (1990) 127.
17 S. Hjertén, K. Elenbring, F. Kilár, J.-L. Liao, A.J.C. Chen, C.J. Siebert and M.-D. Zhu, *J. Chromatogr.*, 403 (1987) 47.
18 J.L. Beckers and F.M. Everaerts, *J. Chromatogr.*, 508 (1990) 3.
19 J.L. Beckers and F.M. Everaerts, *J. Chromatogr.*, 508 (1990) 19.
20 Th.P.E.M. Verheggen, A.C. Schoots and F.M. Everaerts, *J. Chromatogr.*, 503 (1990) 245.
21 A.C. Schoots, Th.P.E.M. Verheggen, P.M.J.M. De Vries and F.M. Everaerts, *Clin. Chem.*, 36 (1990) 435.
22 F. Foret, V. Šustáček and P. Boček, *J. Microcol. Sep.*, 2 (1990) 229.
23 F.E.P. Mikkers, *Thesis*, University of Technology, Eindhoven, 1980.
24 F.M. Everaerts, Th.P.M. Verheggen and F.E.P. Mikkers, *J. Chromatogr.*, 169 (1979) 21.
25 D. Kaniansky, *Thesis*, Komenský University, Bratislava, 1981.
26 D. Kaniansky and J. Marák, *J. Chromatogr.*, 498 (1990) 191.
27 L. Křivánková, F. Foret and P. Boček, *J. Chromatogr.*, 545 (1991) 307.
28 D.S. Stegehuis, H. Irth, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 538 (1991) 393.
29 D.S. Stegehuis, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 591 (1992) 341.
30 C.J. Giddings, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 319.
31 H.M. Liebich and C. Foerst, *J. Chromatogr.*, 525 (1990) 1; and references cited therein.
32 F.E.P. Mikkers, F.M. Everaerts and J.A.F. Peek, *J. Chromatogr.*, 168 (1979) 293.
33 M. Svoboda and J. Vacík, *J. Chromatogr.*, 119 (1976) 539.
34 D. Kaniansky, V. Madajová, J. Marák, E. Šimuničová, I. Zelenský and V. Zelenská, *J. Chromatogr.*, 390 (1987) 51.
35 J. Marák, J. Laštinec, D. Kaniansky and V. Madajová, *J. Chromatogr.*, 509 (1990) 287.
36 F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, *Isotachophoresis: Theory, Instrumentation and Applications*, Elsevier, Amsterdam, 1976.
37 J. Vacík, *D. Sc. Thesis*, Charles University, Prague, 1980.
38 V. Dolník, *Thesis*, Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, 1987.
39 E. Kenndler, *Anal. Chim. Acta*, 173 (1985) 139.