Isotachophoresis superimposed on capillary zone electrophoresis

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ABSTRACT

Applying isotachophoresis (ITP) superimposed on capillary zone electrophoresis (CZE), which will be denoted the ITP/CZE mode, components migrate in an ITP way on top of a background electrolyte. In such an ITP/CZE system the leading electrolyte consists of a mixture of an ionic species L_1 with a high mobility (the leading ion of the ITP system), an ionic species L_2 with low mobility (the co-ions of the ZE system) and buffering counter ions, whereas the terminating solution contains only the ionic species L_2 and the buffering counter ions. The zones of the components migrating in the ITP/CZE mode are very sharp owing to the self-correcting effect and the concentrations of the components are adapted to the concentration of the L_1 ions of the system. Calculated mobility windows are given, indicating which components can migrate in the ITP/CZE mode and features and possibilities of ITP/CZE are discussed on the basis of several electropherograms.

INTRODUCTION

Although capillary zone electrophoresis (CZE) [1,2] has been developed into a worthwhile analytical separation method with a remarkable separation power, useful in a variety of application fields, a severe drawback is the high detection limits. Especially for components present in a sample at low concentrations, large sample volumes have to be injected in order to introduce detectable amounts of components and therefore the sample components have to be concentrated to obtain high plate numbers and a good resolution. For this purpose, field amplification [3,4] or sample stacking [5,6] is often applied. These techniques can result, however, in a disastrous decrease in separation power [7], especially for large sample volumes.

Isotachophoresis (ITP) can also be used as preconcentration technique. A separate ITP system can be coupled to a separate CE system [8–10], through which compounds can be selected from the sample in the ITP system and introduced into the CZE system. Another way is to convert an ITP system into a CZE system by filling the capillary partially with different electrolytes through which all the sample components move first in an ITP system and after some time in a CZE system [11]. In the conversion of an ITP system into a CZE system the choice of the electrolytes is limited, whereas components present in excess in a sample and concentrated at high concentrations in the ITP system can disturb the CZE system. Further, the narrow bands formed in the ITP mode at a high concentration broaden very quickly in the CZE mode owing to diffusion and electrodispersion and extra peak broadening [3] can occur owing to a mismatch in the electroosmotic flow (EOF) if the capillary is filled with different electrolytes. For these reasons, on-line preconcentration in ITP often gives scarcely better results than a simple sample stacking whereby the sample is introduced at a lower ionic strength than that of the background electrolyte. For components present at a low concentration in a complex matrix a concentration procedure often fails owing to the presence of a large excess of other components.

The fundamental aspect in applying the foregoing injection techniques is always how a trace component can be separated and concentrated from a complex matrix at high ionic strength. In some instances this problem can probably be solved by combining ITP and CZE in another way, which has not yet received attention, *viz.*, the principle of ITP superimposed on CZE, which will be denoted ITP/CZE to distinguish it from several other combinations of ITP and CZE, such as on-column transient and coupled-column ITP preconcentration in CZE [12] and the use of discontinuous buffer systems in CZE [13]. The basis of ITP/CZE can be found in ITP with two leading ions (a so-called 2L-ITP system) [14], whereby some components of a sample migrate in the ITP mode and others in the CZE mode, depending on the concentrations of the leading ions and the mobilities of the concerning ionic species.

In this work, the conditions under which ITP/CZE is possible were studied and some features and possibilities of ITP/CZE are discussed on the basis of several electropherograms.

THEORY

In CZE, the whole system is filled with a background electrolyte and sample components migrate in order of decreasing effective mobilities. In Fig. 1A, a schematic representation is given of the concentration profiles of the cationic species X, Y and Z migrating in a zone electrophoretic way in the separation compartment filled with the background electrolyte AB. Peaks will be diffuse owing to several peak-broadening effects such as electrodispersion and diffusion [15]. Especially when injecting long sampling zones, the sample components have to be concentrated in order to obtain a good separation and high plate numbers.

In a standard ITP electrolyte system [16], a leading ion L is chosen with an effective mobility higher than those of the sample components, whereas that of the terminating ion T must be lower than the latter. Components with an effective mobility higher than that of the leading ion or lower than that of the terminating ion will migrate in a zone electrophoretic way in the
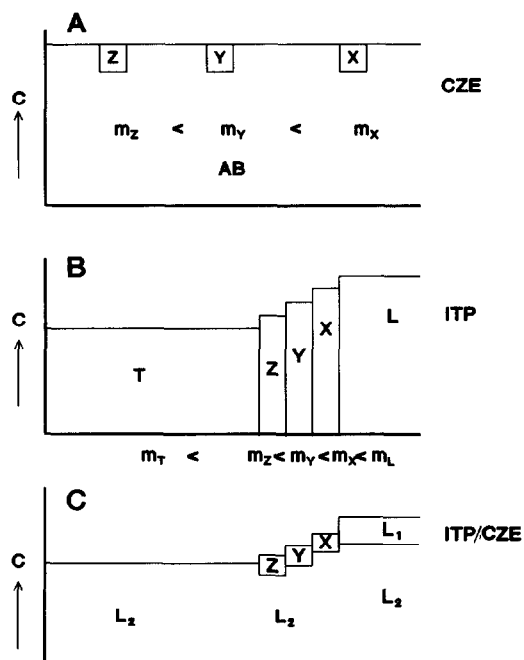

Fig. 1. Schematic representation of different modes in capillary electrophoresis. (A) In the CZE mode the whole system is filled with a background electrolyte AB and the cations X, Y and Z migrate in a zone electrophoretic way (from the left-to the right-hand side) in order of decreasing mobilities. (B) In an ITP system the cations X, Y and Z migrate in consecutive zones between the leading ions L and terminating ions T. (C) In a 2L-ITP system the leading electrolyte contains two leading ions L_1 and L_2. The ion with the lowest ionic mobility, L_2, forms the terminating zone. The cations X, Y and Z migrate in consecutive zones in the ITP mode superimposed on a background electrolyte of L_2 and the counter ions in the so-called ITP/CZE mode.

leading or terminating electrolyte, respectively. All other components migrate in an ITP way between leading and terminating ions. The zone boundaries are sharp owing to the self-correction of the zones. The concentrations of all components migrating in the ITP more are adapted to that of the leading electrolyte. In Fig. 1B the concentration profiles of the different zones are schematically represented.

In an ITP system with two leading ions (a so-called 2L-ITP system), components behave in a similar way, on the understanding that not only the effective mobilities of the leading and terminating ions, but also the concentrations of the

two leading ions regulate the migration behaviour. In the model of a 2L-ITP system a leading electrolyte is used with two leading ions whereas the terminating electrolyte contains only that with the lowest effective mobility. The leading ion with the lowest effective mobility, L_2, remains partially behind the leading ion with the highest mobility, L_1, and creates a terminating L_2 zone with a fixed E gradient and specific zone resistance (SZR) [17], characterized by a specific R_E value [18]. Beckers and Everaerts [14] have already described a mathematical model for 2L-ITP systems and with this model it can be calculated whether components migrate in the ITP or the ZE mode.

Sample ions migrate in the ITP mode if the calculated value of the SZR or R_E values of the S/L_2 zone (the sample ions S always migrate in a mixed zone with L_2 ions) are smaller than those of the terminating zone L_2 (see Fig. 1C for the concentration profiles). In fact, an ITP system is created superimposed on a zone electrophoretic system of the L_2 ions and the counter ions, the so-called ITP/CZE mode.

Mobility windows in 2L-ITP systems

With the model of 2L-ITP [14], the R_E values of the terminating L_2 zone and sample zones can be calculated and the sample components migrate in the ITP/CZE mode if their R_E values are smaller than that of the L_2 zone. To demonstrate the features of a 2L-ITP system for negative ions, the calculated R_E values for the terminating L_2 zones are given (dashed lines) in Fig. 2 (right-hand scale) of (A) a leading electrolyte consisting of 0.01 M chloride as L_1 ions and varying concentrations of 2-(N-morpholino)ethanesulphonate (MES) as L_2 ions and (B) a leading electrolyte consisting of 0.01 M MES as L_2 ions and varying concentrations of chloride as L_1 ions. As terminating electrolyte 0.02 M MES was used. All electrolytes were adjusted to pH 6 by adding histidine. In Table I the pK values and mobilities at infinite dilution are given for the ionic species used in the calculations and experiments.

Further, we calculated with the mathematical model for 2L-ITP systems the mobilities (at infinite dilution) of components, with assumed

pK values of 3, which migrate in the ITP mode in these 2L-ITP systems. In Fig. 2A and B, the mobility windows for the components migrating in the ITP mode are indicated by the hatched areas (left-hand scale). The upper limit of the mobility window is always the mobility of the leading ion L_1 with the highest mobility (in this instance chloride), whereas the lower limit is determined by both the mobilities and the concentrations of L_1 and L_2 in the leading zone. This in contrast with standard ITP, where only the mobilities of the terminating ions are of importance. Beyond the lower and upper limits of the mobility window, ITP changes into CZE. The dashed lines labelled B give the R_E values for benzoic acid in the different systems. Because the R_E values of benzoic acid in Fig. 2A are always smaller than the R_E values of the terminating L_2 zone, it migrates in the ITP/CZE mode. This can also be concluded from its mobility of $-33.6 \cdot 10^{-5}$ cm^2/V·s that is covered by the mobility window. In all systems in Fig. 2B the R_E values of benzoic acid are larger than that of the terminating L_2 zone and it always migrates in the CZE mode.

The mobility window is reduced with decreasing values of the concentration of L_1 and increasing values of the concentration of L_2 and has a maximum width in the pure ITP mode (left-hand side of Fig. 2A with $[L_2] = 0$ M and $[L_1] = 0.01$ M) and a minimum width in the pure CZE mode (right-hand side of Fig. 2B with $[L_2] = 0.01$ M and $[L_1] = 0$ M). In fact, this mobility window indicates which components can migrate in an ITP system with leading ions L_1 superimposed on a background electrolyte consisting of L_2 and the counter ions. In the combined plot of calculated mobility windows (left-hand scale) and calculated R_E values (right-hand scale) of 2L-ITP systems the mobilities and R_E values are not linearly related.

For a further illustration of the features of ITP/CZE systems, the same parameters as in Fig. 2A and B are given in Fig. 2C and D for 2L-ITP systems consisting of various concentrations of an ionic species L_1 with a mobility of $-40 \cdot 10^{-5}$ cm^2/V·s and of L_2 with a mobility of $-20 \cdot 10^{-5}$ cm^2/V·s at a pH of 6 by adding histidine (assumed pK values of the sample
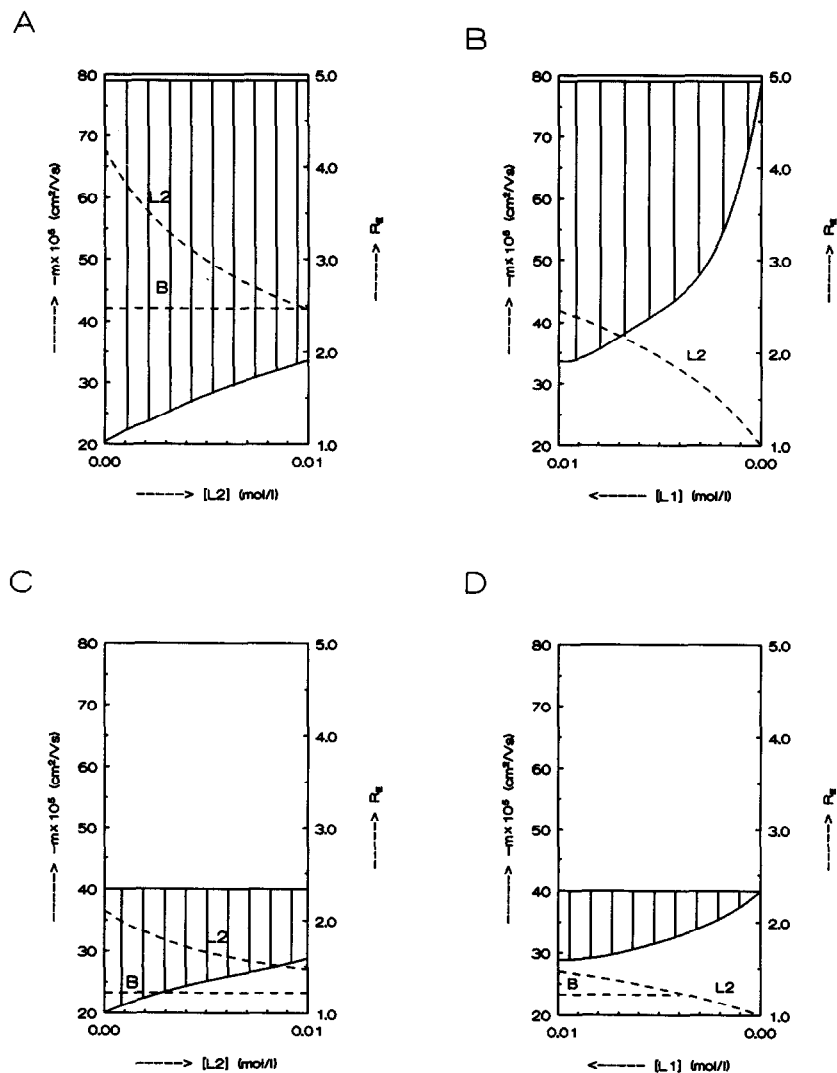
A

B

C

D

Fig. 2. Combined plot of mobility windows (left-hand scale) and R_E values (right-hand scale) for several 2L-ITP systems. The calculated mobility windows (hatched areas) indicate which negative components migrate in the ITP mode in 2L-ITP systems for varying concentrations of the leading ions (A, B) chloride and MES and (C, D) leading ions with mobilities of $-40 \cdot 10^{-5}$ and $-20 \cdot 10^{-5}$ cm^2/V·s, respectively. The dashed lines labelled L2 show the R_E values of the terminating L_2 zone and those labelled B show those of the component benzoic acid. From (C) and (D) it can be concluded that the mobility window width is reduced if the mobilities of L_1 and L_2 approach each other. The mobility window width is maximum for a pure ITP system [left-hand side of (A) and (C)] and minimum for a pure CZE system [right-hand side of (B) and (D)]. For further details, see text.

components are 3). It is clear from Fig. 2 that the mobility window can be reduced if the mobilities of L_1 and L_2 approach each other and with decreasing concentrations of the L_1 ions. In Fig. 2C benzoic acid always migrates in the ITP/ CZE mode because the R_E values are always smaller than that of the terminating L_2 zone and from Fig. 2D it can be concluded that benzoic acid migrates in the CZE mode in a background

electrolyte consisting of a mixture of 0.01 M L_1 and a concentration of L_2 lower than about 0.004 M.

Concentrations in ITP/CZE systems

As already indicated, components can migrate in an ITP system superimposed on a background electrolyte. In such an ITP/CZE system the concentrations of the sample components, mi-

TABLE I

IONIC MOBILITIES AT INFINITE DILUTION, m $(10^{-5}$ $cm^2/V \cdot s)$, AND pK VALUES FOR IONIC SPECIES USED IN THE CALCULATIONS AND EXPERIMENTS

Ionic species[a]	m	pK
Acetic acid	−42.4	4.76
BALA	36.7	3.552
Benzoic acid	−33.6	4.203
Histidine	29.7	6.03
Hydrochloric acid	−79.1	−2.0
Imidazole	48.7	6.953
MES	−28.0	6.095
Potassium	76.2	−
Salicylic acid	−35.4	3.107
Sodium	51.9	−
Sulphosalicylic acid	−54.5	−
Tris	29.5	8.1

[a] BALA = β-Alanine; MES = 2-(N-morpholino)ethanesulphonic acid; Tris = tris(hydroxymethyl)aminomethane.

grating in the ITP mode, are adapted to that of the leading ion with the highest mobility, L_1. In Fig. 3, the calculated total concentrations of components are given as a function of their mobility at infinite dilution, assuming pK values of 3, in a pure ITP system (dashed line) for a leading electrolyte of 0.01 M HCl adjusted to pH 6 by adding histidine and for an ITP/CZE system

with a leading electrolyte consisting of a mixture of 0.01 M HCl (L_1) and 0.01 M MES (L_2) at pH 6 adjusted by adding histidine. From Fig. 3 it can be concluded that the sample concentrations in ITP/CZE system decrease strongly at mobilities where ITP changes into CZE. The mobility window for the corresponding 2L-ITP system (see Fig. 2A) covers anionic mobilities of the sample components from $79.1 \cdot 10^5$ to $ca.$ $34 \cdot 10^{-5}$ $cm^2/V \cdot s$. In Fig. 4 the calculated total concentrations of components with anionic mobilities of $70 \cdot 10^5$, $60 \cdot 10^5$ and $50 \cdot 10^{-5}$ $cm^2/V \cdot s$ are given for 2L-ITP systems consisting of a mixture of 0.01 M MES (L_2) and various concentrations of chloride (L_1) at a pH of 6 adjusted by adding histidine. An interesting point is that the concentrations of the sample components migrating in the ITP mode in a 2L-ITP system are adapted to the concentration of the L_1 ion. Calculations for cations gave analogous results.

Optimization of mobility windows

It has been shown that ITP/CZE systems can be applied and by an appropriate choice of the electrolyte system it can be managed that a specific component of a complex sample with a specific mobility migrates in the ITP/CZE mode,
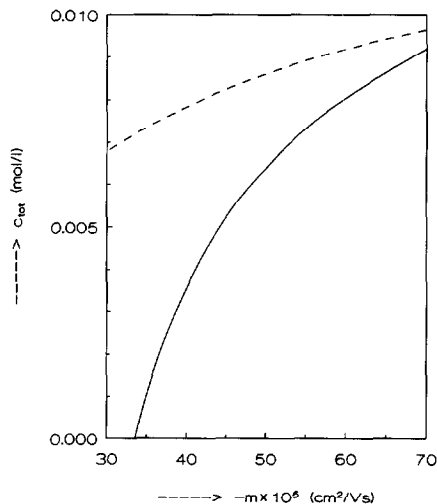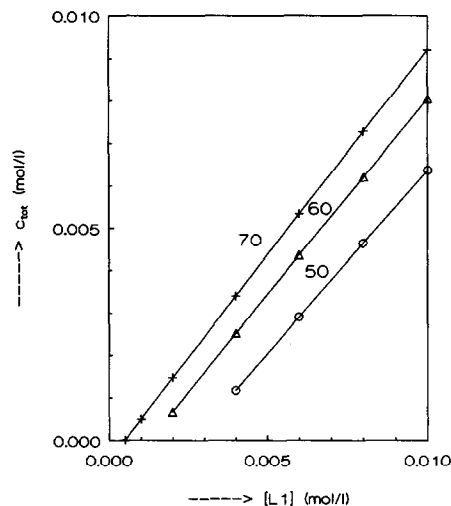

Fig. 3. Total concentrations of components as a function of their mobilities for an ITP system (dashed line) and for an ITP/CZE system. For components with low mobilities, where the ITP/CZE mode is converted into the CZE mode, the concentration decreases. For further details, see text.

Fig. 4. Relationship between total concentrations of components migrating in the ITP/CZE mode and the concentration of L_1. The concentrations of the components are adapted to that of L_1. The numbers refer to the anionic mobilities of the components in 10^{-5} $cm^2/V \cdot s$. For further details, see text.

with a concentration that can be regulated by the choice of the concentration of the leading L_1 ions and with the advantage of self-correcting zone boundaries. The difference from the normal ITP mode applying the same concentration of a leading ion L_1 is that in the ITP/CZE mode the loadibility of the system can be expected to be larger owing to the presence of the large concen-

tration of background electrolyte. The question now is how to choose an optimum electrolyte system.

We shall answer this question on the basis of the determination of imidazole in a mixture of cations and try to find a system with a small mobility window at a low L_1 concentration. Imidazole is a cation with a pK value of 6.953
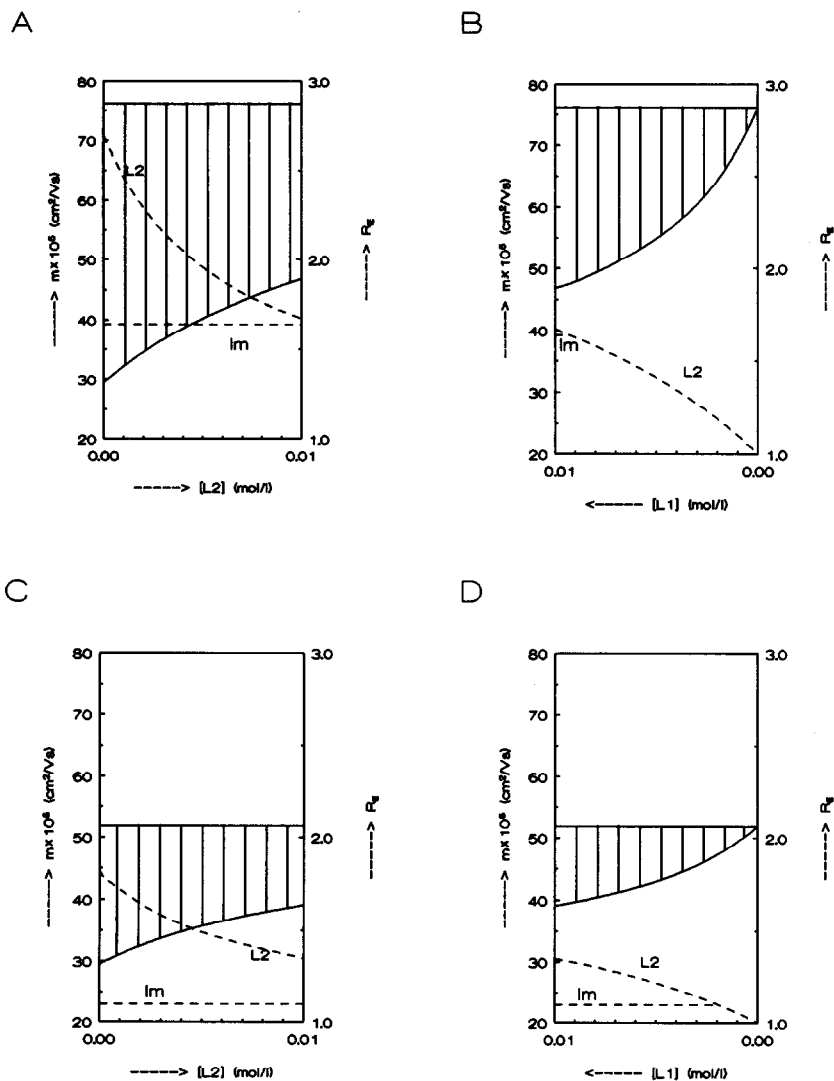

Fig. 5. Combined plot of mobility windows (left-hand scale) and R_E values (right-hand scale) for several 2L-ITP systems. The mobility windows (hatched areas) indicate which positive components migrate in the ITP mode in 2L-ITP systems for varying concentrations of the leading ions (A, B) potassium and Tris and (C, D) sodium and Tris. The dashed lines labelled L2 show the R_E values of the terminating L_2 zone and those labelled Im show those of the component imidazole. From (C) and (D) it can be concluded that the mobility window width is reduced if the mobilities of L_1 and L_2 approach each other. The mobility window width is maximum for a pure ITP system [left-hand side of (A) and (C)] and minimum for a pure CZE system [right-hand side of (B) and (D)]. For further details, see text.

and a mobility of $48.7 \cdot 10^{-5}$ cm^2/V·s. This mobility is determined by ITP measurements according to a procedure described previously [17,19], applying a leading electrolyte of 0.01 M NaOH adjusted to pH 4.75 by adding acetic acid and a terminator of 0.01 M LiCl. For a first impression, the mobility windows and the R_E values of the terminating L_2 zone and imidazole zone were calculated for a system with various concentrations of potassium (L_1) and Tris (L_2). In Fig. 5 the mobility windows (hatched areas) and R_E values (dashed lines) of the terminating L_2 zone and of imidazole are given for (A) varying L_2 concentrations at a concentration of L_1 of 0.01 M and (B) varying L_1 concentrations at a concentration of L_2 of 0.01 M, and it can be seen that in (A) imidazole always migrates in the ITP mode and in (B) it migrates in the ITP mode above a concentration of *ca.* 0.009 M of L_1 ($R_{E,\text{Im}} < R_{E,L_2}$). In these systems the widths of the mobility windows are too large and the necessary concentration of the L_1 ions is high, in order that imidazole migrates in the ITP/CZE mode. In Fig. 5C and D the same parameters are given for electrolytes with various concentrations of sodium (L_1) and Tris (L_2). The effective mobility of sodium is slightly higher than that of imidazole. The width of the mobility window is much smaller and the lowest concentration of L_1, whereby imidazole migrates in the ITP mode, is now *ca.* 0.002 M. For a further minimization of the mobility window, the mobility windows were calculated for several systems with L_2 ions with a mobility close to that of sodium. Although the mobility windows were narrower, the L_1 concentration could not be lowered further.

From the above calculations, it can be concluded that an optimum ITP/CZE system can be obtained by choosing an L_1 ion with a mobility just slightly higher than that of the sample component and the width of the mobility window can be diminished by choosing an L_2 ion with mobility close to that of L_1.

EXPERIMENTAL

Instrumentation

For all CZE experiments a P/ACE System 2000 HPCE (Beckman, Palo Alto, CA, USA) was used. All experiments were carried out with Beckman eCAP capillary tubing (75 μm I.D.) with a total length 46.70 cm and a distance between injection and detection of 40.00 cm. The wavelength of the UV detector was set at 214 nm. All experiments were carried out in the cationic mode applying a constant voltage of 10 kV, unless stated otherwise, and the operating temperature was 25°C. Sample introduction was performed by applying pressure injection, where a 1-s pressure injection represents an injected amount of *ca.* 6 nl and an injected length of 0.136 cm. Data analysis was performed using the laboratory-written data analysis program CAESAR.

Chemicals

All chemicals were of analytical-reagent grade. Deionized water was used for the preparation of all buffer and sample solutions. The cationic surfactant FC 135 [20] was donated by 3M (Zoeterwoude, Netherlands).

RESULTS AND DISCUSSION

In 2L-ITP systems, components with mobilities covered by the previously described mobility windows migrate in the ITP/CZE mode whereas the other components migrate in the CZE mode. Components migrating in the ITP/CZE mode are concentrated to a concentration determined by that of the L_1 ions. In order to study this concentration effect, other concentration effects must be eliminated. This means that (1) the sample components may not be dissolved in water or dilute buffer to avoid sample stacking, (2) the sample components may not be injected after a plug of water or of diluted background electrolyte to avoid field amplification, (3) the sample may not contain an excess of ionic species with high mobility to avoid the concentration effect of a 2L-ITP system and (4) the sample solution may not contain a large excess of sample ions with low mobility in order to avoid the concentration effect due to the presence of a terminator. For all experiments the sample components were dissolved in background electrolyte.

To visualize the concentration effect of ITP/

CZE systems, in Fig. 6 the electropherograms are given for a sample solution of 0.0001 M imidazole and 0.0001 M histidine dissolved in 0.01 M Tris acetate at pH 5.5 for leading electrolytes of (A) 0.01 M Tris, (B) a mixture of 0.01 M Tris and 0.003 M sodium hydroxide, (C) a mixture of 0.01 M Tris and 0.004 M NaOH and (D) a mixture of 0.01 M Tris and 0.01 M NaOH. All electrolytes were adjusted to pH 5.5 by adding acetic acid. In all experiments (cationic mode) the anode compartment was filled with the terminating electrolyte, 0.01 M Tris acetate at pH 5.5. The sample solutions were introduced by pressure injection for 10 s. The very sharp concentration effect in the ITP/CZE systems with 0.004 M and 0.01 M sodium (L_1) ions compared with the CZE system can be clearly seen (Fig. 6A). In fact, the sharpening effect was expected at a lower concentration of sodium (see Fig. 5D). It must be remembered,
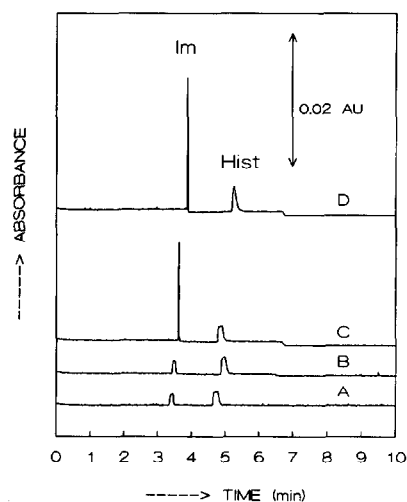
however, that at low concentrations of the L_1 ions the differences between the R_E values of leading, terminating and sample zones are very small, hence the separation force is small.

In order to determine the imidazole concentration in ITP/CZE systems with various sodium concentrations the following experiments were carried out. First, the UV absorbances were measured of known concentrations of imidazole dissolved in 0.01 M Tris adjusted to pH 5.5 by adding acetic acid. The UV detector was set to zero by applying a solution of 0.01 M Tris acetate (pH 5.5). In Fig. 7 the relationship between UV absorbance and imidazole concentration is given (solid line). Then the maximum UV absorbances, with injection of increasing amounts, of the imidazole peaks were determined in ITP/CZE systems with several sodium concentrations. Using the relationship between UV absorbance and the imidazole concentration in Fig. 7 (solid line), the concentration of imidazole in the ITP/CZE systems can be determined. In an ITP/CZE system with, e.g., a concentration of Na^+ of 0.01 M, the concentration of IM^+ seems to be ca. 0.008 M. In Table II, the concentrations of imidazole calculated with the 2L-ITP model and measured concentrations in ITP/CZE systems are given, showing good agreement.

Applying ITP/CZE systems, a gain in detection limits can be expected. In Fig. 8 the electropherograms are given for CZE separations with a background electrolyte of 0.01 M NaOH and 0.01 M Tris adjusted to pH 5.5 by adding acetic acid for sample solutions of imidazole and histidine dissolved in 0.01 M Tris acetate (pH 5.5) at concentrations of (A) $1 \cdot 10^{-5}$ M and (B) $1 \cdot 10^{-4}$ M (5-s pressure injection) and separations with an ITP/CZE system with a leading electrolyte of 0.01 M NaOH and 0.01 M Tris (pH 5.5) and a terminator solution of 0.01 M Tris acetate (pH 5.5) for sample solutions of imidazole and histidine at concentrations of (C) $1 \cdot 10^{-5}$ M and (D) $1 \cdot 10^{-4}$ M (5-s pressure injection). From Fig. 8 a gain in detection limit of about a factor of ten can be concluded.

Calibration graphs were also constructed for several systems. Although the experiments were carried out in the fast rise mode of the UV
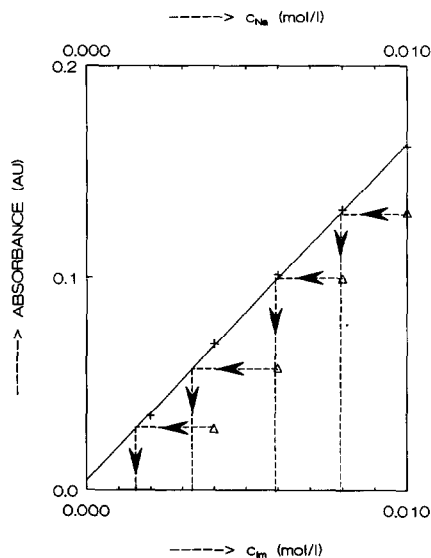

Fig. 6. Electropherograms for the separation of 0.0001 M imidazole (Im) and 0.0001 M histidine (Hist) dissolved in Tris acetate (pH 5.5), applying 2L-ITP systems consisting of 0.01 M Tris (L_2 ions) and (A) 0, (B) 0.003, (C) 0.004 and (D) 0.01 M sodium ions as L_1 ions. All electrolytes were adjusted to pH 5.5 by adding acetic acid. All experiments were carried out in the cationic mode, applying 10 kV with pressure injection for 10 s. The anode compartment (at the inlet) was always filled with 0.01 M Tris acetate (pH 5.5) as terminating electrolyte. It can be clearly seen that from a concentration of 0.004 M sodium ions, imidazole migrates in the ITP/CZE mode resulting in very sharp peaks.

Fig. 7. Relationship between measured UV absorbances and concentrations of standard solutions of imidazole (C_{Im}) dissolved in 0.01 M Tris acetate at pH 5.5 (solid line). From ($\triangle$) measured UV absorbances of the imidazole peaks in ITP/CZE systems with leading electrolytes consisting of 0.01 M Tris (L_2) and several concentrations of sodium (L_1), the concentration of the imidazole zone migrating in the ITP/CZE mode can be deduced.
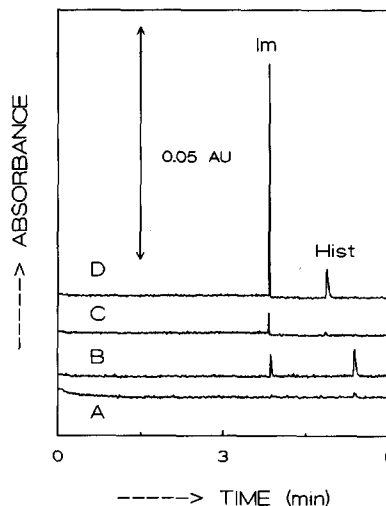

Fig. 8. Electropherograms for the separation of imidazole (Im) and histidine (Hist) dissolved in 0.01 M Tris acetate (pH 5.5) at concentrations of (B, D) $1 \cdot 10^{-4}$ M and (A, C) $1 \cdot 10^{-5}$ M in (A, B) CZE systems with a background electrolyte of 0.01 M NaOH and 0.01 M Tris adjusted to pH 5.5 by adding acetic acid and (C, D) applying ITP/CZE with a leading electrolyte of 0.01 M NAOH and 0.01 M Tris (pH 5.5) and a terminating electrolyte of 0.01 M Tris acetate (pH 5.5). Pressure injection time, 5 s. In the ITP/CZE systems a gain in detection limit of a factor of $ca.$ ten can be obtained.

detector and a data rate of 10 Hz (highest value of the apparatus), bad calibration graphs were obtained on injecting small sample amounts because often peak widths of 0.1–0.2 s were obtained. Applying ITP/CZE systems high demands are made on the detector electronics and the data system concerning handling of extremely sharp peaks.

To visualize the concentration effects in ITP/

TABLE II

CALCULATED, c_{calc}, AND MEASURED, c_{meas}, VALUES OF THE CONCENTRATION OF IMIDAZOLE IN ITP/CZE SYSTEMS WITH LEADING ELECTROLYTES CONSISTING OF 0.01 M TRIS (L_2) AND VARIOUS CONCENTRATIONS OF SODIUM (L_1)

c_{Na} in 2L-ITP system (M)	c_{calc} (M)	c_{meas} (M)
0.010	0.0079	0.0079
0.008	0.0060	0.0059
0.006	0.0039	0.0033
0.004	0.0021	0.0015

CZE for negative ionic species, experiments were carried out in the anionic mode with reversed EOF by adding the cationic surfactant FC 135 (5 μg/ml) to the background solution. In Fig. 9 the electropherograms are given for a mixture of 0.0001 M sulphosalicylic acid and 0.0001 M salicylic acid dissolved in 0.01 M MES–histidine (pH 6) for leading electrolytes consisting of 0.01 M MES (as L_2 ions) with (A) 0, (B) 0.003, (C) 0.005 and (D) 0.009 M HCl as L_1 ions. All electrolytes were adjusted to pH 6 by adding histidine. The experiments were carried out in the anionic mode (anode placed at the outlet), the cathode compartment being filled with 0.01 M MES adjusted to pH 6 by adding histidine. In Fig. 9E the isotachopherogram obtained by applying a leading electrolyte of 0.01 M HCl and a terminating electrolyte of 0.01 M MES, both adjusted to pH 6 by adding histidine, is shown. All sample solutions were introduced by pressure injection for 10 s. From Fig. 9 it can be concluded that the concentration effect due to the ITP/CZE mode for sulphosalicylic acid acts
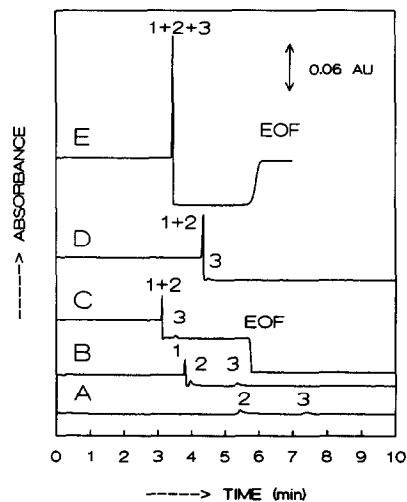
Fig. 9. Electropherograms for the separation of (2) 0.0001 *M* sulphosalicylic acid and (3) 0.0001 *M* salicylic acid in 0.01 *M* MES adjusted to pH 6 by adding histidine for 2L-ITP systems consisting of 0.01 *M* MES as L_2 ions and (A) 0, (B) 0.003, (C) 0.005 and (D) 0.009 *M* chloride as L_1 ions. All electrolytes were adjusted to pH 6 by adding histidine. Pressure injection time, 10 s. The experiments were carried out in the anionic mode whereby the EOF was reversed by addition of FC 135 (5 μg/ml) and the cathode compartment (at the inlet site) was filled with a solution of 0.01 *M* MES adjusted to pH 6 by adding histidine. In (E) the separation was carried out in a pure ITP system with leading electrolyte 0.01 *M* HCl and terminating electrolyte 0.01 *M* MES, both adjusted to pH 6 by adding histidine. In (C) and (D) sulphosalicylic acid migrates in the ITP/CZE mode. (1) System peak, possibly due to the presence of iodide ions in FC 135, always migrating in the ITP/CZE mode.
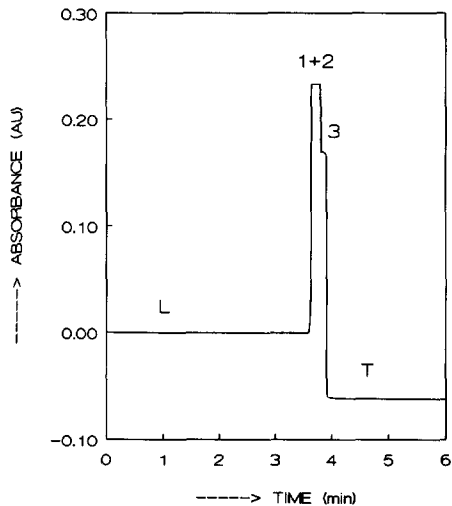

Fig. 10. Isotachopherogram for the separation of (1) system peak, (2) 0.005 *M* sulphosalicylic acid and (3) 0.005 *M* salicylic acid with (L) leading electrolyte 0.01 *M* histidine chloride at pH 6 and (T) terminator 0.01 *M* MES–histidine at pH 6. Pressure injection time, 5 s.

water is given. The separate steps can be clearly seen.

CONCLUSIONS

By applying a leading electrolyte consisting of two ionic species, components can migrate in an ITP system superimposed on CZE, the so-called ITP/CZE mode, whereby the leading ions L_1 with the highest mobility act as the leading ions in the ITP system and the leading ions L_2 with the lowest mobility create the terminating zone and act as co-ions of the CZE system. With the mathematical model of 2L-ITP, mobility windows can be calculated, indicating which components migrate in the ITP/CZE mode, and these components migrate at a concentration determined by the concentration of the L_1 ions. By choosing a suitable leading electrolyte in ITP/ CZE systems, one can select which components migrate in the ITP mode and at what concentration. Components migrating in the ITP mode in such an ITP/CZE system show sharp peaks owing to the self-correcting property of the zones. By this means a gain in detection limit of a factor of *ca.* ten could be established. Great

at a concentration of about 0.004 *M* chloride in the background electrolyte, whereas salicylic acid migrates in the CZE mode (see Fig. 9A–D). In a pure ITP system it migrates in the ITP mode. In the systems in Fig. 9B–E an extra system peak (1) was always present, possibly owing to the presence of iodide ions in FC 135 [20]. In all systems, however, the increase in this peak due to the presence of peak 2 (Fig. 9C and D) or peaks 2 and 3 (Fig. 9E) was carefully checked. To demonstrate that in an ITP system sulphosalicylic acid and salicylic acid migrate in the ITP mode, in Fig. 10 the isotachopherogram obtained under the same conditions as in Fig. 9E for a sample composition of 0.005 *M* sulphosalicylic acid and 0.005 *M* salicylic acid in

demands, however, are made on the detection system and data acquisition in order to handle the very sharp peaks in a proper quantitative way. Experimental work is in progress to investigate the applicability and ruggedness of ITP/CZE systems.

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