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NEW OPTION IN CAPILLARY ZONE ELECTROPHORESIS

USE OF A TRANSIENT IONIC MATRIX (DYNAMIC PULSE)

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SUMMARY

A method which allows the separation possibilities of capillary zone electrophoresis and zone electrophoresis to be improved and extended is described. The analyte, injected into the primary electolyte and migrating from the injection point to the detector, passes during this migration through the zone of a transient ionic matrix which separates selectively some of the components, and before detection (the analyte) returns to the primary electrolyte. The zone of the transient ionic matrix also migrates through the separation column (in the demonstrated cases in the direction from the detector to the sampling point) and both its composition and effective time can easily be changed in order to attain complete separation of all components as well as the minimum separation time. The transient ionic matrix may be advantageously formed by solvolytic (H^+, OH^-) and/or complex forming ions. This method extends the working pK_a of weak acids or bases separated in one analysis, *i.e.*, it enables single stage analyses which are not normally realizable. The advantages of the method are its speed, its suitability for automation and its perfect quantitative interpretation of the record (detection always proceeds in the original background electrolyte). It is in principle also possible to use more than one type of transient ionic matrix in a single analysis.

INTRODUCTION

In order to select an operational electrolyte in capillary electrophoresis which can effect the complete separation of substances, the effective mobilities of the substances must differ, *e.g.*, the relative differences must be greater than 2% and, simultaneously, they must all be sufficiently high, *e.g.*, greater than $3 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} to ensure reasonable migration velocities at the electric field strengths used in practice, *e.g.*, 50 kV m^{-1}.

In practice, it is often almost impossible to reach both complete separation of all substances and their reasonably rapid migration at a constant composition of the background electrolyte, even with the use of various selective effects such as acid–base equilibria, complex forming equilibria and formation of micelles.

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The case of acids and bases possessing similar ionic mobilities and a wide range of pK values is of interest here. Recently, a possibility of solving this problem has been suggested[1], based on the migration of substances in a dynamically generated pH gradient. As the generation of electrolyte systems in a multi-pole separation column[2] enables one to programme the composition of the ionic matrix in some part of the separation capillary, we can elaborate another method of solving difficult separation problems in capillary zone elctrophoresis (CZE). This method consists in the separation of substances by a dynamic limited change in the composition of the counter ion system (by a pulse), which may be generated by a time-controlled change in the working parameters of the equipment.

The advantage of this method lies first in its flexibility which makes it possible to operate with pulses of both pH and complexing ions, to control quantitatively the selective effects by controlling the composition of the pulse, its length (the time of starting and stopping its generation) and direction of migration. Secondly, the zones may be detected under constant and defined conditions of the primary electrolyte.

PRINCIPLE OF THE METHOD

In classical CZE the sample zones migrate in such a way that the ratios of the velocities of the sample components are constant along the whole separation path and composition of the background electrolyte is constant over the whole column. Moreover, if a stabilized electric power supply is used, the migration velocities of the components are constant. The background electrolyte forms an ionic surrounding, the so-called ionic matrix[2], which determines the electromigration properties of the ion considered.

The ions of the ionic matrix move under the influence of the electric field and the ionic matrix is, in fact, continuously restored by a continuous flow of ions of the same kind from the connecting electrode chambers. The volume of these chambers is selected in order to be great enough to ensure a constant composition of the ionic matrix during the whole time of migration of the sample through the column. Usually this volume is so large that one can perform a whole set of separations without any loss of constancy of the compositions of the electrolytes in the connecting chambers.

If, however, the sample components migrating from the injection point to the detector meet for a certain limited time a pulse of a matrix of different composition (with respect to the matrix formed by the original background electrolyte), then transient changes in their mobilities (and in the ratios of these mobilities) occur. This transient ionic matrix may thus selectively influence the migration and separation of sample substances.

The zone of the transient ionic matrix apparently moves through the separation column, *i.e.*, it is not fixed at a given position in the column; therefore the whole process is a dynamic one. Hence, for each separation it is necessary to create the transient ionic matrix again. This can, however, easily be done by creating it outside of the separation capillary and then bringing it into the separation capillary by electromigration. The direction of migration of the transient ionic matrix can, in principle, be either the same or the opposite, with respect to the migration direction of the sample components. Although the transient ionic matrix may be formed by any arbitrary ion, matrices with solvolytic counter ions are of special interest, both owing

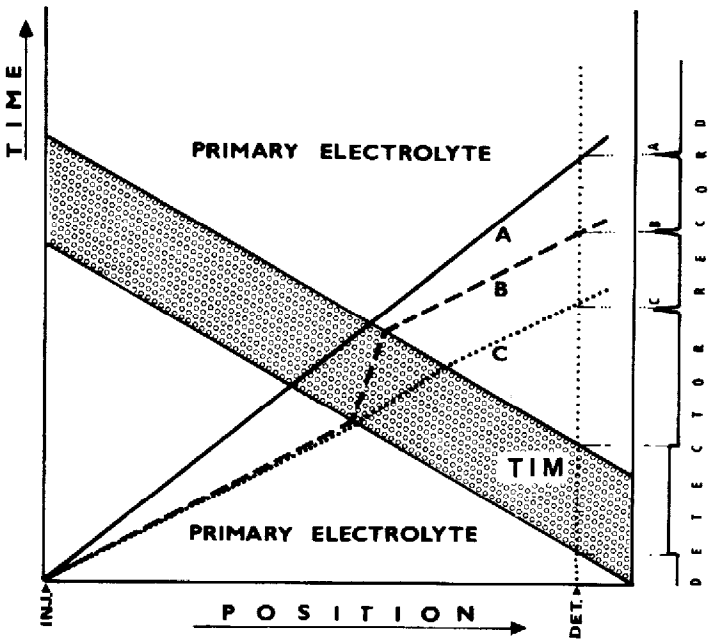

Fig. 1. Separation scheme in the transient ionic matrix. Substances A, B, C migrate in the column from the injection point INJ with constant velocity towards the transient ionic matrix (TIM). In the primary electrolyte, substance A with the lowest mobility is separated from B and C. In the transient ionic matrix, substances B and C differ in mobilities and are separated; A is not affected. After leaving the transient ionic matrix, the separated substances migrate with their own mobilities in the primary electrolyte and are detected in the detector (DET). The detection record is on the right.

to their simple principle and strong potential selectively to influence the effective mobilities of the sample components.

Fig. 1 shows the process where the transient ionic matrix moves through the column against the direction of migration of the sample components. The sample components, proceeding from the sampling point, change their velocities when entering the zone of the transient matrix (component B) and are separated; after leaving this zone the separated zones continue to migrate with their original velocities towards the detector and are recorded. This scheme is represented, *e.g.*, by the practical case where a pulse of a complexing counter ion (which has practically no effect on the conductivity of the background electrolyte) at constant pH is used for the separation of substances.

If a pulse of a solvolytic counter ion, *e.g.*, H^+, OH^- is used for the separation of substances, the situation is very similar but somewhat more complicated. One must take into consideration not only the effect of pH on the dissociation degree (and thus on mobility), but also the conductivity changes within the pulse (caused by a higher mobility of these ions) and thus the changes in the driving electric field strength.

The zone of the transient ionic matrix with a solvolytic ion broadens and changes by electromigration dispersion owing to its high mobility in a non-buffered electrolyte system. The rear boundary of this zone is sharp for a certain time and it

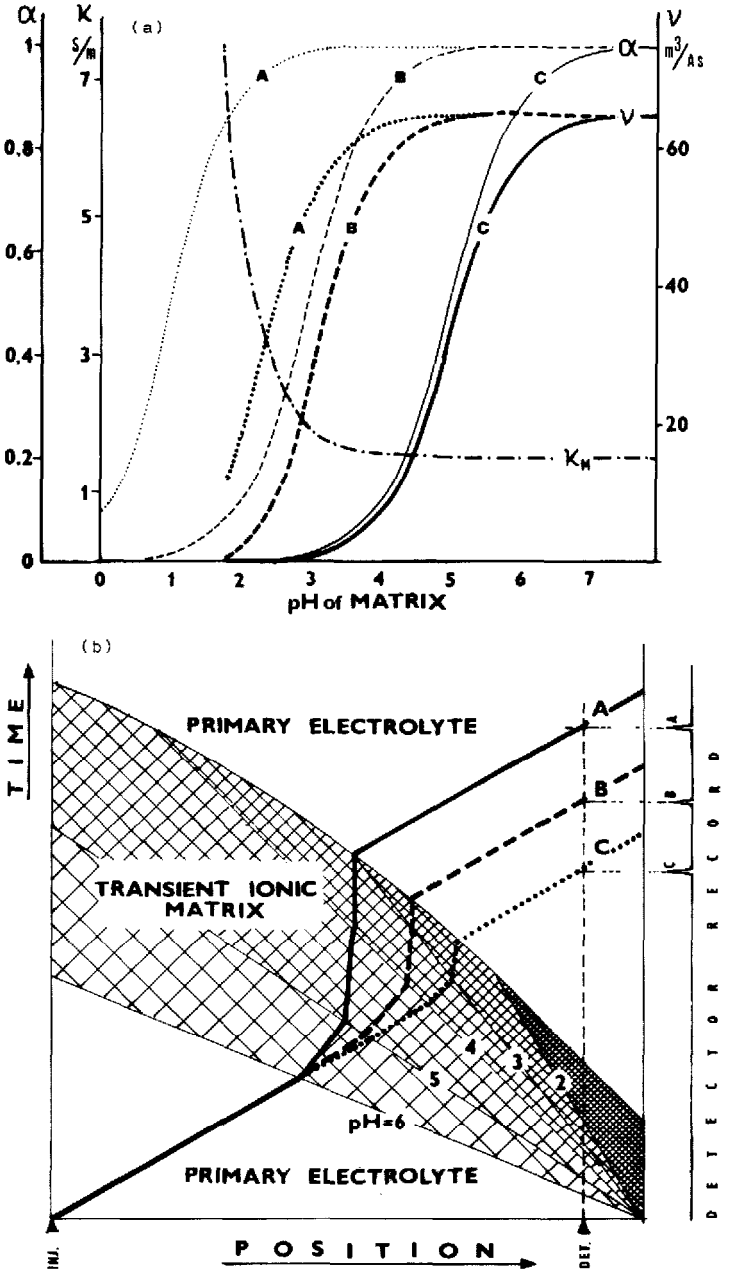

Fig. 2. (a) Dissociation degree, α, of model substances A, B and C, as a function of the pH of their matrix. The dependence of the conductivity, κ, of the matrix adjusted to the primary electrolyte (0.01 *M* KCl) on the pH of this matrix is also shown as are the dependences of the migration speed, *v*, of the first three substances (which show the same ionic mobility) on the pH of the matrix adjusted to 0.01 *M* KCl (primary electrolyte) per unit of electric current density. (b) Simplified separation scheme in the transient ionic matrix. Substances A, B, C migrate in the primary electrolyte from the injection point INJ with the same velocities towards the zone of the transient ionic matrix. In this matrix, the substances differ in mobilities and are separated into their own zones. After leaving the transient ionic matrix, the zones migrate again in the primary electrolyte with equal mobilities to the detector (DET). The detection record is on the right.

moves with constant velocity, whereas the leading edge becomes less steep with time[3,4]. The conductivity of the electrolyte rises in proportion to the increasing concentration of the solvolytic ions. At constant current density, this means decreasing electric field strength and thus decreasing migration velocity of the ions. For illustration, Fig. 2a shows the dissociation and velocity curves of model substances together with the conductivity of the model transient ionic matrix (system HCl–KCl), as a function of the pH of the transient ionic matrix.

In Fig. 2b are shown the trajectories of the sample components which migrate from the sampling point as a function of the pH at the given point in the column at a given time. The trajectories in the transient ionic matrix show increasing distances and thus separation. The scheme of the corresponding detection record is shown at the right-hand side where also the time of generation of the transient ionic matrix can be found.

EXPERIMENTAL

Equipment

All experiments were performed in a closed capillary system using the laboratory-made apparatus described earlier[2] equipped with a separation capillary of fused silica (95 mm x 0.3 mm I.D. between sampling point and detector). For detection, an UV-absorption detector (UVD-3A; Shimadzu, Kyoto, Japan) was used at 254 nm. The electric power supply was a laboratory-made one[5].

Chemicals

All chemicals were of p.a. grade. 3,5-Dinitrobenzoic acid was from Reanal (Budapest, Hungary), 2,4-dinitrophenol from Reachim (Moscow, U.S.S.R.); all other chemicals were from Lachema (Brno, Czechoslovakia).

RESULTS AND DISCUSSION

The principle of application of the complexing matrix was verified experimentally by the separation of 3,5-dinitrobenzoic and 3,5-dinitrosalicylic acids for which one may expect almost the same mobilities but a great difference in stability constants of complexes with heavy metal cations. As the primary electrolyte, 0.01 *M* $NaNO_3$ (pH 5.75) was used and 0.01 *M* $Cu(NO_3)_2$ (pH 4.25) served as the modification electrolyte. The ion Na^+ was selected to be the cation of the primary electrolyte as its mobility is similar to that of Cu^{2+} ($u_{Na} = 51.9 \cdot 10^{-9}$, $u_{Cu} = 55.5 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$). Owing to this, the pulse of the transient ionic matrix of Cu^{2+} should maintain both its boundaries sharp during the whole migration time, being broadened only by diffusion and not by electromigration dispersion.

Fig. 3a shows the UV-absorption record of an electrophoresis of a mixture of both acids (sample: 1 μl of $5 \cdot 10^{-5}$ *M* 3,5-dinitrobenzoic acid and $5 \cdot 10^{-4}$ *M* 3,5-dinitrosalicylic acid) in the primary electrolyte. The mobilities of the two acids are almost the same, and there is no separation (I = constant = 200 μA).

In Fig. 3b the separation of the same mixture is shown where the transient ionic matrix was used. The latter was generated by a 30-s pulse of Cu^{2+} sampled into the capillary immediately after sample introduction and switching on the current. The

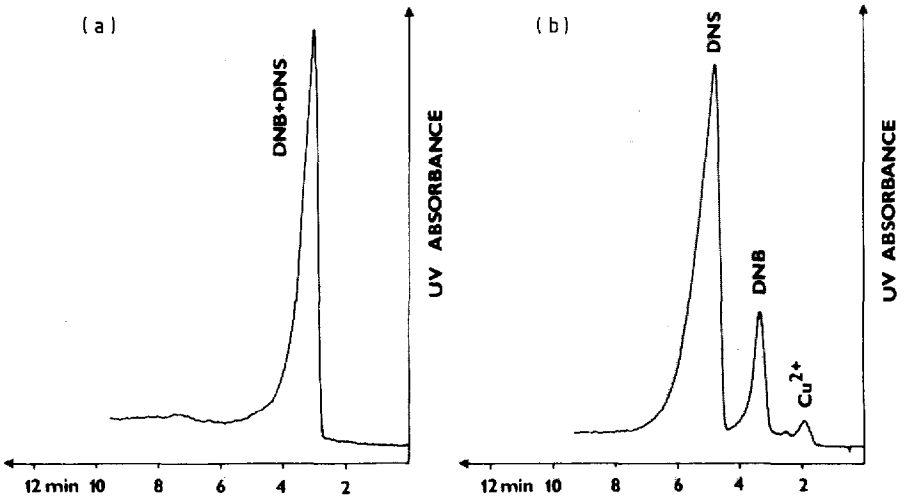

Fig. 3. (a) Record of the separation of 3,5-dinitrobenzoic and 3,5-dinitrosalicylic acids in the primary electrolyte (without the use of the transient ionic matrix). The primary electrolyte was 0.01 *M* $NaNO_3$ (pH 5.75), $I = 200$ μA. (b) The same separation but with the use of the transient ionic matrix. A 30-s pulse of Cu^{2+} was applied.

transient ionic matrix caused a selective retardation of 3,5-dinitrosalicylic acid and the two substances were separated. A comparison of Fig. 3a and b shows that the transient ionic matrix had no influence on the retention time of 3,5-dinitrobenzoic acid.

The principle of application of the solvolytic transient ionic matrix was verified by the separation of a model mixture of acids, *viz.*, picric (PIC), 3,5-dinitrobenzoic (DNB), sorbic (SOR) and cinnamic (CIN) acids and 2,4-dinitrophenol (DNP). Table I shows their limiting ionic mobilities and dissociation constants. The primary electrolyte used was 0.01 *M* KCl (pH 5.6) and the modification electrolyte was 0.01 *M* HCl.

Fig. 4a shows the electrophoretic record of this mixture in the primary electrolyte. The substances are not separated (sample: 1 μl of a solution, 10^{-4} *M* in each of the sample components, current 300 μA).

Fig. 4b presents the electrophoretic separation of the same model mixture of substances with the use of the transient ionic matrix. The latter was formed by a 60-s

TABLE I

LIMITING IONIC MOBILITIES AND pK_a VALUES OF THE MODEL SUBSTANCES[6–8]

Substance	$u_o \cdot 10^9$ ($m^2\ V^{-1}\ s^{-1}$)	pK_a
Picrate	31.5	0.708
3,5-Dinitrobenzoate	29.3	2.82
2,4-Dinitrophenol	31.3	4.111
Cinnamate	28.3	4.438
Sorbate	33.4	4.77

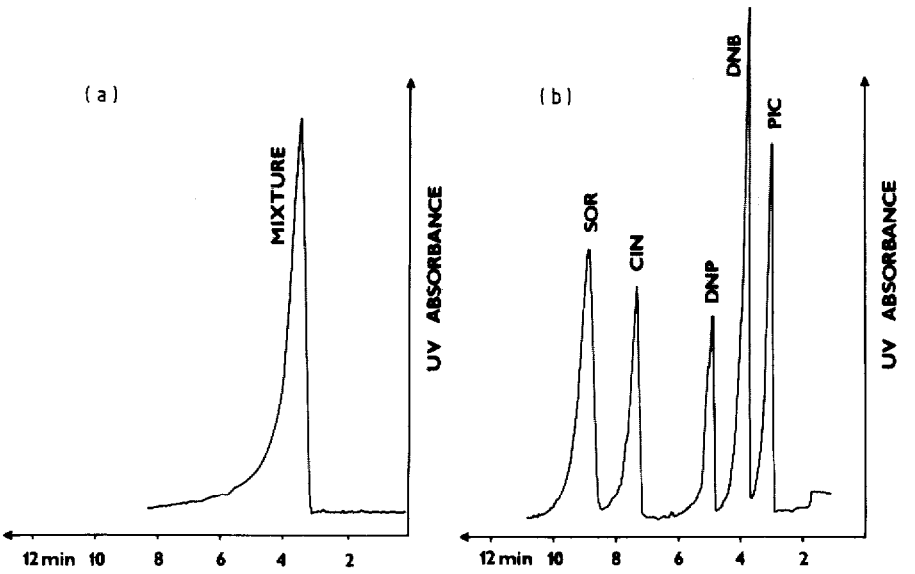

Fig. 4. (a) Record of the separation of a mixture of acids (PIC, DNB, DNP, CIN, SOR) in the primary electrolyte (without the use of the transient ionic matrix). The sample was 1 μl of a solution 10^{-4} M in each of the components. The primary electrolyte was 0.01 M KCl (pH 5.6), I = 300 μA. (b) The same separation but with a 1-min pulse of H^+.

pulse of H^+ into the column just at the beginning of the analysis. Under the influence of the transient ionic matrix, the sample was separated into the individual components which migrate in the order of increasing values of pK_a. The difference in the pK_a values for this mixture is 4 and this mixture cannot, in principle, be separated in a single electrolyte.

CONCLUSIONS

The utilization of the transient ionic matrix extends greatly the separation potential of CZE and enables one to separate in one analysis mixtures which cannot be separated in one constant ionic matrix formed by the background electrolyte. The transient ionic matrix is readily formed from complexing or solvolytic counter ions by generation of a pulse of modifying counter ions which electromigrate.

The transient ionic matrix may result in a shortening of the analysis time even in cases where the separation is possible in a constant electrolyte.

An outstanding advantage consists in the fact that the components pass through the detector in an electrolyte of known and constant composition which is independent of the transient ionic matrix. This fact the analyst may appreciate when performing quantitative analysis.

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