Journal of Chromatography, 470 (1989) 309–312
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 381

Note

Dynamic programming of pH — a new option in analytical capillary electrophoresis

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(First received January 9th, 1989; revised manuscript received January 16th, 1989)

It is common in dynamic separation methods, *e.g.*, chromatography, that the analytical and/or separation possibilities are improved by the application of a suitable gradient. In gas chromatography, temperature gradients have been successfully used for a long time[1]; in liquid chromatography, gradient elution[2] has been improved progressively in the last decade. In both instances, the gradients used are dynamically programmed during the analysis itself by instrumental means according to the requirements of the user.

The use of gradients is also of great interest in electrophoretic techniques. In electrophoretic ion focusing[3] and isoelectric focusing[4], a pH gradient is used to focus the sample substances into narrow bands at defined positions in the separation column. In these techniques, it is essential to create a sufficiently stable (constant steepness) and stationary (not moving along the migration path) pH gradient along the separation column, serving subsequently for the separation and focusing of the sample substances. These stationary gradients may be mobilized in order to move through a fixed-point detection site by either hydrodynamic flow or substitution of the counter-ionic system (so that the stability of the gradient is lost)[5,6].

Recently, a paper[7] was published describing the gradient elution method in capillary zone electrophoresis (micellar electrokinetic capillary chromatography), in which a stepwise solvent programme with increasing 2-propanol content in the background electrolyte was used.

To our knowledge, so far no attempt has been made to perform capillary electrophoresis in a mobile pH gradient which is dynamically programmed. The aim of this paper is to illustrate this possibility by preliminary experiments. It involves a description of simple instrumentation that enables one to generate dynamic changes of pH in the separation capillary, thus forming a moving pH profile along the separation path. An example of the effect of a dynamic pH gradient on the migration behaviour of substances is given.

EXPERIMENTAL

The experiments were performed using equipment similar to that described elsewhere[8]; and shown schematically in Fig. 1. The separation capillary was placed

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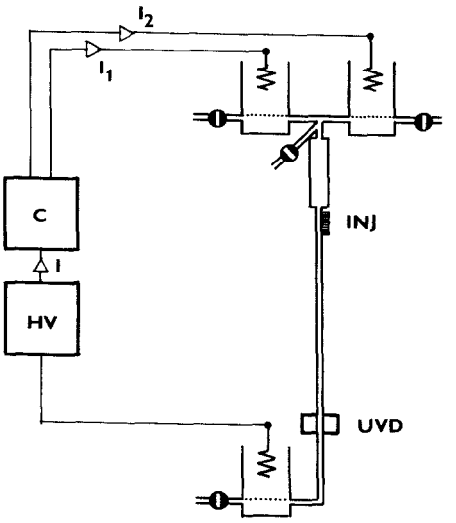

Fig. 1. Schematic diagram of the apparatus used. C = Electric current ratio controller; HV = high-voltage power supply; INJ = injection site; UVD = UV absorption detector.

between two electrode blocks. The starting electrode block consisted of two electrode chambers, one of them containing the primary (or background) electrolyte (0.01 *M* KCl) and the other one the modifying electrolyte (0.01 *M* HCl). The other electrode block contained 0.01 *M* KCl. The two electrode chambers in the starting electrode block were connected to the high-voltage power supply via an electric current ratio controller.

For the construction of the instrument, a CS Isotachophoretic Analyzer (Institute of Radioecology and Applied Nuclear Techniques, Spišská Nová Ves, Czechoslovakia) was modified in such a way that only one separation capillary was placed between the sampling valve and the UV absorption detector (254 nm). The modified electrode chamber was connected to the starting electrode block by a narrow-bore PTFE capillary; the mixing point was at the upper end of the sampling valve.

In all experiments, 0.01 *M* KCl served as the primary electrolyte. For its preparation, freshly boiled distilled water was used; the pH of the solution was adjusted to 4.25 by addition of HCl. The modifying electrolyte was 0.01 *M* HCl.

To prevent disturbances caused by the penetration of the electrode reaction products from the electrode chambers into the separation capillary, two precautions were taken. To suppress the penetration of OH^- ions into the capillary from the cathodic electrode chamber, this chamber was filled with 0.01 *M* HCl. In the anodic electrode chamber filled with 0.01 *M* KCl (see Fig. 1), an electrode made of silver was used to prevent H^+ production.

All chemicals used were obtained from Lachema (Brno, Czechoslovakia). The sample (0.1 μl of 0.005 *M* pyridine + 0.005 *M* *p*-bromoaniline, pH 4.9) was introduced with a 2-μl syringe (Hamilton, Bonaduz, Switzerland) via the septum up to the point of connection between the sampling valve and the detection capillary.

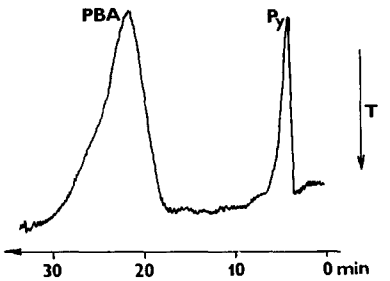

Fig. 2. UV detection record of an analysis in the zone electrophoresis mode. Background electrolyte, 0.01 *M* KCl (pH 4.25); I = 200 μA. Py = pyridine; PBA = *p*-bromoaniline. T = transmittance.

The rate of increase of the pH gradient was controlled electrically by setting up the ratio of the two electric currents, I_1/I_2 (*cf.*, Fig. 1) while keeping the total electric current ($I = I_1 + I_2$) constant (200 μA).

RESULTS

In order to show the possible influence of a dynamic pH gradient in zone electrophoretic analysis, experiments were performed with a model sample containing pyridine and *p*-bromoaniline. Fig. 2 shows the analysis of the sample by classical (isocratic) zone electrophoresis (background electrolyte 0.01 *M* KCl, pH 4.25). It can be seen that the separation of the two substances was effective, but the analysis time was fairly long owing to the relatively slow migration of the *p*-bromoaniline zone, accompanied by considerable broadening of its peak. The pyridine peak was asymmetric owing to electromigrational dispersion caused by the relatively large amount of sample that was necessary to keep the signal-to-noise ratio at a reasonable level.

Fig. 3 shows the result of an analysis of the same mixture at low pH. Electrophoresis was carried out in 0.0167 *M* HCl (pH 1.77), which is the most acidic medium obtainable with 0.01 *M* KCl as the primary electrolyte (when $I_1 = 0$, see Fig. 1). The analysis time for pyridine was three times longer than that in the previous experiment and the peak of *p*-bromoaniline was hardly detected owing to the decrease in its molar absorption coefficient with decrease in pH.

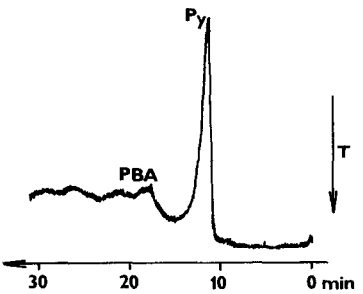

Fig. 3. UV detection record of an analysis in the zone electrophoresis mode. Background electrolyte, 0.0167 *M* HCl (pH 1.77); I = 200 μA. Abbreviations as in Fig. 2.

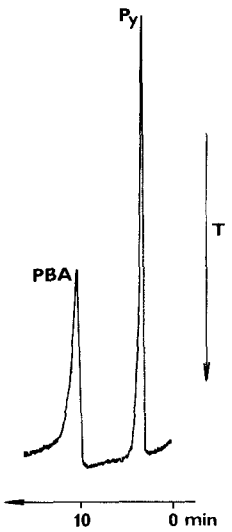

Fig. 4. UV detection record of an analysis with a pH gradient. Primary electrolyte, 0.01 *M* KCl (pH 4.25); modifying electrolyte, 0.01 *M* HCl; I = 200 μA; I_2 was increased and I_1 was decreased at 4 μA/min. Abbreviations as in Fig. 2.

Fig. 4 shows the result of an analysis using a pH gradient. The starting conditions were identical with those in the previous experiment [background (*i.e.*, primary) electrolyte 0.01 *M* KCl, pH 4.25]; the dynamic gradient was then programmed by a stepwise increase in the modifying current (4 μA/min). It can be seen that the analysis time for both substances has decreased and that their peaks have become sharper.

The use of migrating pH gradients in capillary zone electrophoresis seems to be promising and work on this topic is continuing.

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