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Short Communication

Computer-controlled generation of pH gradients in capillary zone electrophoresis

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ABSTRACT

A new capillary zone electrophoresis (CZE) apparatus was developed with the possibility of computer-controlled pH-gradient elution. The details of the apparatus are described together with an example showing the analysis of aspartame, benzoate and caffeine in diet Pepsi.

INTRODUCTION

Electrophoresis is a powerful technique for the separation of charged species in solution. Capillary zone electrophoresis (CZE) is a high-resolution separation method conducted in small-I.D. capillaries. Two effects are responsible for the migration of the charged solutes: electrophoretic and electroosmotic displacement. The strong electro-osmotic flow in CZE, which results from the substantial ζ-potential of the capillary surface, is sufficiently large under many conditions to result in the elution of ions with both positive and negative electrophoretic mobilities.

Typically CZE is performed with a uniform buffer [1]. Having complex samples, it is difficult to find the correct buffer to analyze different compounds in one run. To cope with this problem special conditions have to be generated. A temperature gradient [2] or pH gradient [3–6] are the most common approaches to get better separation. Here we report an approach which permits the generation of a smooth, computer-controlled pH gradient. Its effectiveness is illustrated with a simple sample (diet Pepsi).

EXPERIMENTAL

Apparatus

A schematic view of the apparatus used for the development of pH gradients is shown in Fig. 1. A high-voltage power supply (Brandenburg, Model alpha III type 3807) with reversible polarity was used. For acquisition of the UV–VIS spectra we used the Spectra-Physics Focus fast scanning detector with an IBM PS/2 computer. A laboratory-made box of Perspex with three buffer containers, a mixing system and a piece of capillary of 70 cm length (to the detector) are the main parts of the apparatus. Two of the containers (will be called

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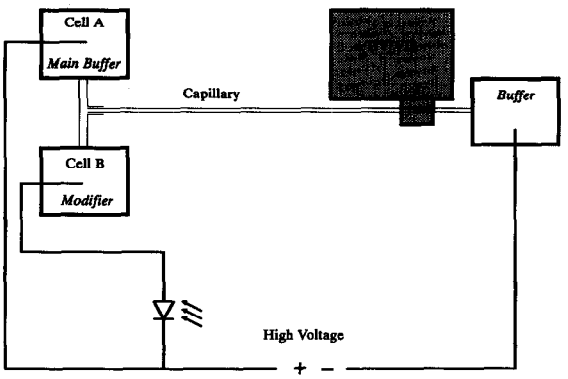

Fig. 1. Apparatus for the experiments with pH-gradient CZE.

cells A and B) are screwed into a Perspex block where the mixing chamber is situated. Cell A contains the main buffer (10 m*M* phosphate buffer of pH 2.5) the pH value of which is increased by the modifier out of cell B (100 m*M* phosphate buffer of pH 11.0). The mixing chamber (Fig. 2) is basically a T-piece channel of 350 μm diameter drilled in the Perspex block (2 mm length). To avoid a quick homogenization of the buffers due to the brownian migration, two short pieces of capillary (1 mm length and 10 μm I.D.) were fixed in a rubber seal to lower the flow of the buffer into the T-piece.

The mixing of the two buffers is controlled by a computer. The current is split continuously at a constant voltage over the two cells. A digital-to-analog converter output of the computer is interfaced by a "sweetspot" (light emitting transistor, RS 301-915) and a photodiode (RS 305-462) connected by a fibre optics cable to control the current splitting. The photodiode is installed in the direction of no conductivity. There is no current throught the photodiode unless light has been emitted from the "sweetspot". The current is (as far the photodiode allows) direct proportional to the emitted light intensity. Fig. 3 shows the programmed and measured currents during the separation. The programmed current is the calculated curve which the computer had to generate, the measured current was calculated from the measured voltage over a 100 kΩ resistor. The computer programme provided any linear, horizontal constant or exponential curve or combination of these, of up to ten different segments. The time per segment could be arbitrarily set to any time with a minimum dwell time of 1 s (gradients showing a non-linear, concave or convex current profile are not shown here).

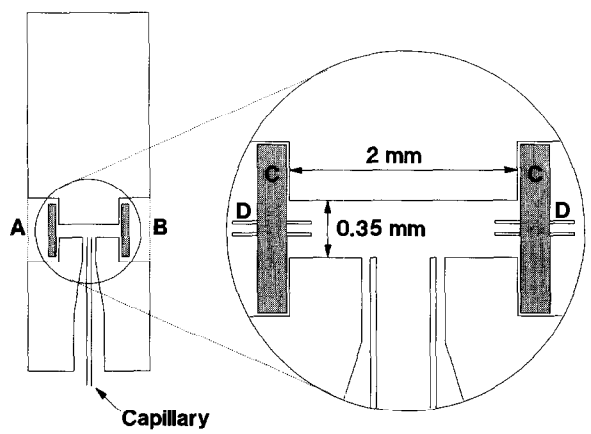

Fig. 2. Scheme of the mixing chamber. A = Connection to cell A; B = connection to cell B; C = rubber seak; D = capillary for flow reduction (1 mm length and 10 μm I.D.).

Procedure

In our example experiment, cell A contains 10 m*M* phosphate buffer of pH 2.5 which is modified by 100 m*M* phosphate buffer of pH 11.0 out of the cell B.

The T-piece was filled by pumping (by air pressure on the orifice where the buffer is filled into the cell) the buffer out of cell B into the T-piece while

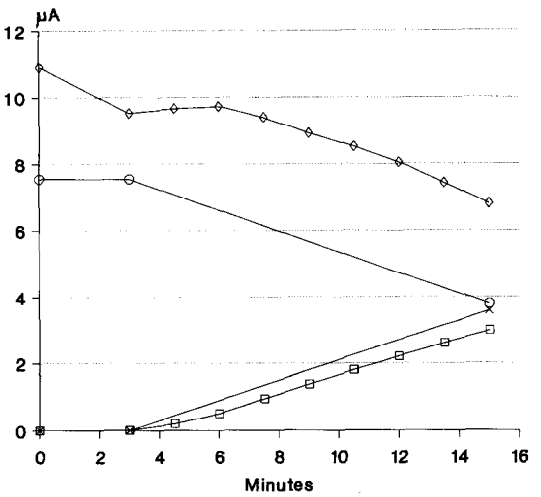

Fig. 3. Diagrammatic presentation of the currents through cell A and B during the pH-gradient CZE. ○ = Cell A calculated; ◇ = cell A measured; × = cell B calculated; □ = cell B measured.

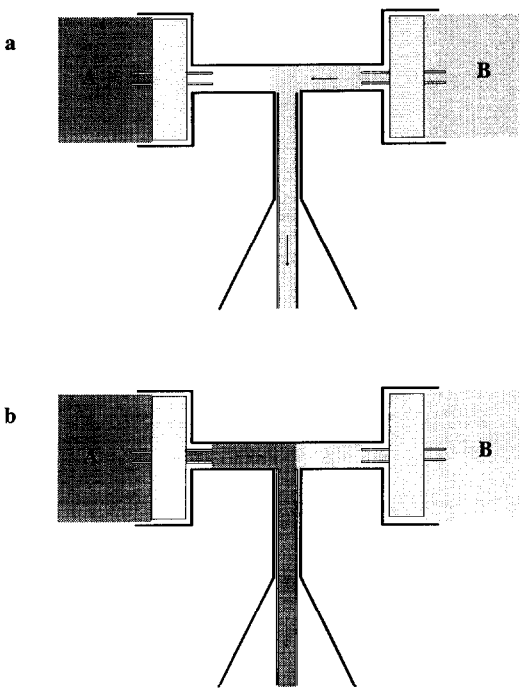

Fig. 4. Principle of filling the T-piece of the mixing chamber with (a) the modifier and (b) the main buffer.

cell A was plugged to avoid entrance of the modifier into cell A (Fig. 4a). Then cell B was plugged and the buffer of cell A was pumped to fill the T-piece to the junction (Fig. 4b).

In the beginning of the experiment the capillary was flushed (by vacuum using a small rotary pump which was connected by a plastic tubing with a reduction in the end to the capillary) with 1 *M* sodium hydroxide solution for 5 min and with the main buffer (10 m*M* phosphate buffer of pH 2.5) for an additional 10 min.

The sample was injected without further treatment after degassing for 5 min in a ultrasonic bath. It was injected by syphoning (lifting the sample tube with the capillary entrance to 30 cm above the other capillary end). The determined sample volume was approximately 60 nl. The capillary was introduced into the T-piece of the mixing chamber, fastened by a ferrule and a nut and the door of the perspec box was closed to switch on the power supply by a micro switch situated next to the edge of the box. The acquisition and the gradient programme were started.

RESULTS AND DISCUSSION

A simple sample, diet Pepsi which contains large quantities of aspartame, benzoate and caffeine, was chosen.

Fig. 5 shows three electropherograms of diet Pepsi at pH 2.5, 4.9 and 8.3 respectively. Aspartame (1) is the fastest compound at pH 2.5. If the pH value is increased, its direction of migration changes and the corresponding peak can be observed at reversed polarity. Peak 2 has not yet been identified. Compounds 2 and 2′ show identical UV spectra. Peaks 3 and 4 were found to be caffeine and benzoate respectively, by comparison of the retention time and UV spectra of the pure compounds.

The pH-gradient electropherogram is shown in Fig. 6. Compared to the data shown in Fig. 5 aspartame appears not to be affected, but the retention times of the other compounds change. The applied gradient considerably speeds up elution of caffeine. Furthermore, peak 2 (unknown compound) is retarded. This experiment proves the functionality of

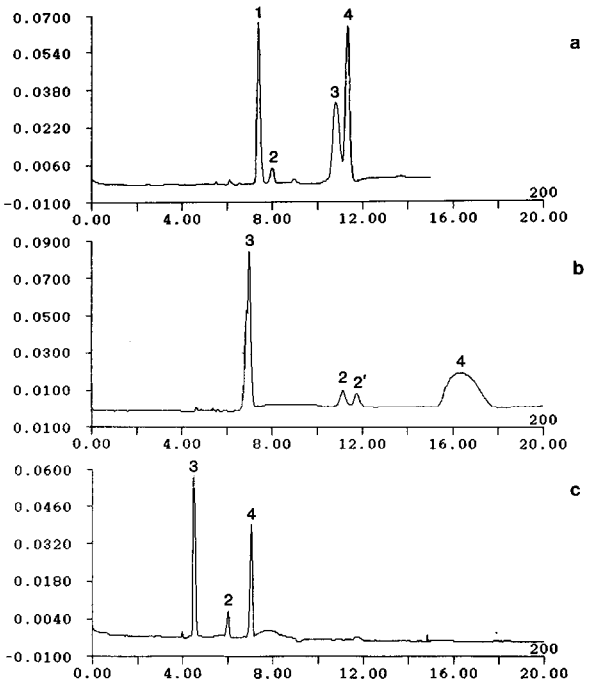

Fig. 5. Electropherograms of diet Pepsi at three different pH values with 100 m*M* phosphate buffer: (a) pH 2.5; (b) pH 4.9; (c) 8.3. Time scale in min. *y*-axes represent absorbance units. Peaks: 1 = aspartame; 2 and 2′ = unknown; 3 = caffeine; 4 = benzoate.

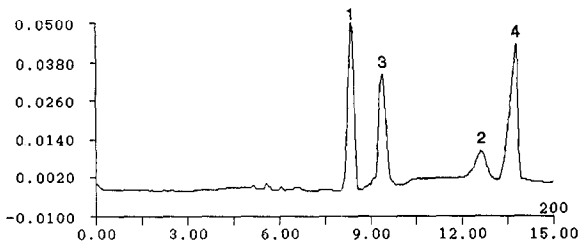

Fig. 6. Electropherogram of diet Pepsi with the use of pH-gradient CZE. Scales and peaks as in Fig. 5.

the new technique for pH-gradient generation. It is important to introduce the capillary carefully into the mixing chamber to get reasonable reproducibility. The retention time of the last peak may vary up to ± 1 min. We expect that changes in temperature and chemistry on the capillary surface (activation–desactivation) are rather responsible for that variation in retention time than the generation of the gradients, because the relative retention time between the peaks do not vary a lot.

CONCLUSION

In a simple experiment we have proved that the new technique of generating pH gradients is a powerful method. There is no doubt that it can be used in many separation problems. More work is required to produce a better reproducibility.

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