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Short Communication

Adaptation of a microdrop injector to sampling in capillary electrophoresis

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Abstract

A micro injection system is presented that permits the injection of small sample droplets into capillaries suitable for capillary electrophoresis (CE). Drops of 60-μm diameter, *i.e.*, 113 pl, were used. The average size of the drops produced had a relative standard deviation (R.S.D.) of less than 1%. Good results, *i.e.*, R.S.D. values of less than 3%, were obtained with single-drop injection, whereas the injection of several drops in a row entailed problems with air bubbles during the measurements.

1. Introduction

Since its introduction in the 1980s, has the analytical potential inherent to modern capillary electrophoresis (CE) has attracted much attention [1–11]. A wide variety of charged and uncharged substances can be efficiently separated according to size, mass to charge ratio, isoelectric point, hydrophobicity or bioaffinity and determined by the various modes of CE. The introduction of the sample into the capillary is a crucial point in most of these methods. Commercially available CE systems commonly use electrokinetic or hydrodynamic injection [11–15]. In the former mode, charged sample components are forced to move from the bulk sample into the capillary under the influence of an electric field. In the second mode, pressure is applied to the sample vial and used to force a small sample portion, *i.e.*, a few picolitres, into the capillary.

The extreme smallness of the actual sample volume needed for a reliable analysis constitutes a major advantage of CE. In principle this would even allow the determination of, *e.g.*, the amino acid or peptide/protein content of a single cell. Amino acids and peptides can be determined without derivatization at levels down to 200 fmol [16]. The use of the highly sensitive laser fluorescence detection allows the determination of less than attomoles of amino acids [17], but requires pre- or postcolumn sample derivatization. Electrochemical detection allows the detection and determination of biogenic amines at levels down to 34 amol, as demonstrated by Wallingford and Ewing [18] for 440-pl samples from single nerve cells of *Planorbis corneus*. A combination of micro injection and fluorescence or electrochemical detection should thus further the development of CE microanalysis considerably. How-

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ever, none of the above-mentioned standard injection systems can handle such small sample volumes. The micro injector recently introduced by Wallingford and Ewing [18,19] is still under development and not commercially available.

In this paper, we introduce a reliable and elegant solution to the problem of micro injection in CE. A commercially available ink-jet system, originally developed for use in an ink-jet printer, has been adapted for the contact-free shooting of sub-nanolitre sample droplets into the capillary of a standard CE system.

2. Experimental

All chemicals used were obtained from Sigma and were of the highest purity available. A 0.05 *M* phosphate buffer of pH 2.5 served as the electrophoresis buffer. Doubly distilled water was used for sample preparation. The amino acid standard solution contained the indicated amino acid at a concentration of 10 mmol/l. Unless indicated otherwise, arginine was used as a standard.

A Beckman P/ACE 2000 system with P/ACE system software controlled by an IBM computer was employed for the capillary electrophoresis experiments. Data were collected and analysed by Beckman System Gold software. An applied voltage of 20 kV and a detection wavelength of 214 nm were used throughout. Untreated fused-silica capillaries were obtained from CS-Chromatographie Service. Unless indicated otherwise, capillaries of 27 cm × 50 μm I.D. were used. The detection window was created by mechanically removing the outer polyimide coating of the capillary 6.5 cm from one end. Prior to the first use, the inner surface of each capillary was etched with 0.1 *M* NaOH for 15 min followed by thorough rinsing with distilled water. After each measurement the capillary was rinsed for 60 s with 0.1 *M* NaOH, followed by air (60 s) and electrophoresis buffer (60 s).

A microdrop ink-jet system (Fig. 1) was used for the micro injections. The ejector was supplied with a storage bottle containing several millilitres of the amino acid standard solution.

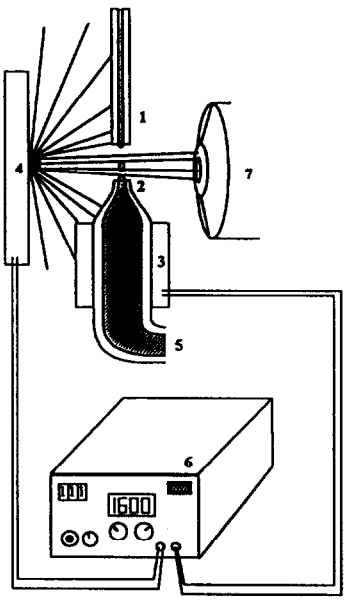

Fig. 1. Schematic diagram of the Microdrop system. 1 = Capillary; 2 = microdrop ejector; 3 = piezo element; 4 = LED; 5 = sample storage; 6 = control unit; 7 = microscope.

The system is capable of shooting micro drops in any desired direction, even upwards. The drops can be precisely aimed over a distance of several centimetres. The glass capillaries of the ejector (I.D. 1 mm) were drawn out at the tip to give a final I.D. of 60 μm at the aperture. As a consequence, the droplets produced had a diameter of 60 μm with a concomitant volume of 113 pl.

Prior to an injection, the capillary of the CE was filled with electrophoresis buffer by applying pressure to the buffer reservoir. The tip of the microdrop system was rinsed by ejecting 100 drops to waste. Subsequently, the capillary holder was removed from the CE apparatus and fixed together with the capillary under the micro drop ejector. By use of a micro manipulator working in three dimensions, the ejector was positioned over the capillary. The drops were injected directly on the end of the column (Fig. 2). The droplets could be observed by means of a stroboscope, which had been coupled to the micro ejector control unit for this purpose. A simple LED was a sufficient for illumination. After correct injection, the capillary holder was

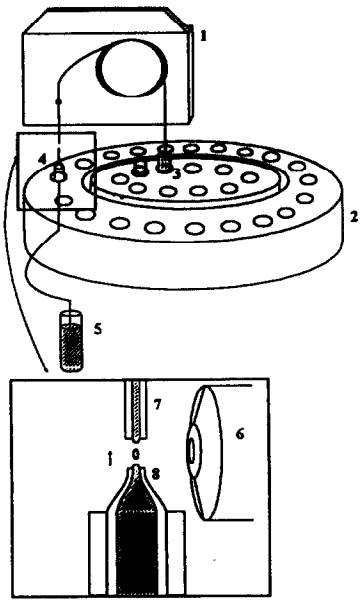

Fig. 2. Application of the Microdrop system as a CE injector. 1 = Capillary holder; 2 = autosampler; 3 = buffer storage; 4 = microinjection system; 5 = sample storage; 6 = microscope; 7 = capillary; 8 = microdrop ejector.

positioned back in the CE apparatus as quickly as possible and the measurement was made as described above. For reference measurements samples were introduced into the capillary by pressure injection for 3 s.

3. Results and discussion

A commercially available ink-jet system, originally developed for use in an ink-jet printer, was studied for its potential as a micro injector for capillary electrophoresis. The formation of a micro drop and its pathway into the capillary were checked by use of a stroboscope. Several hundred droplets were taken into account. Preliminary experiments had shown that the droplets could be precisely aimed over a distance of several centimetres. The reproducible injection of droplets into a CE capillary after careful alignment of the ejector and the CE capillary by use of the micro manipulator thus presented no problem. The injection system had to be adapted, however, to produce droplets of uniform size, otherwise smaller secondary drops were formed and the ejection was not reproducible. Once adjusted correctly, the system remained stable for several days of constant use. The average diameter of the micro drops produced by the micro ejector was 60 μm according to the stroboscope measurements. This corresponds to an average volume of 113 pl. A relative standard deviation of 1% was calculated for the droplet size.

Amino acids are directly assessible by UV detection at 214 nm. No derivatization is necessary. By adapting the free zone capillary electrophoretic separation described in ref. 16, we were able to detect as little as 250 fmol of methionine with a signal-to-noise ratio of 10:1. The best results were obtained when the shortest possible capillary length of 27 cm, a capillary I.D. of 50 μm and a voltage of 20 kV were used [20]. In general, the highest sensitivities were observed for aromatic amino acids and methionine. However, because of its short retention time, arginine rather than methionine or an aromatic acid was chosen as a standard for the micro drop injection experiments.

Fig. 3 shows an electropherogram obtained for a standard solution containing 10 mmol/l of arginine. The sample was injected by standard pressure injection, *i.e.*, by applying pressure to the sample vial for 3 s. The arginine peak appears after 1.33 min. Relative standard devia-

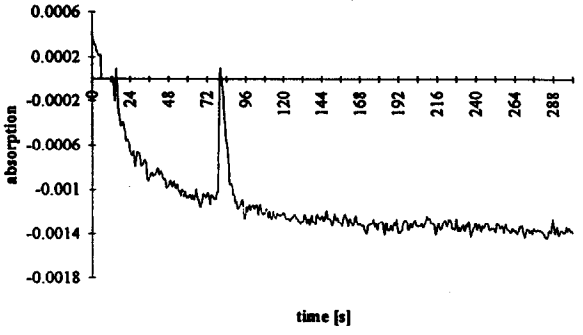

Fig. 3. Electropherogram obtained with standard pressure injection. Sample, 10 mmol/l arginine; electrophoresis buffer, 50 mmol/l phosphate buffer (pH 2.5); conditions, 25°C, 20 kV, 27 cm × 50 μm I.D. capillary, detection wavelength 214 nm, pressure injection for 3 s.

tions of less than 1% were calculated for the retention times and the peak areas. The results were compared with electropherograms recorded for a sample that had been introduced into the capillary by micro injection of one or several droplets of sample solution. At present, sample droplets produced by the micro injector have a diameter of 60 μm, *i.e.*, larger than the I.D. of the CE capillary. As a consequence, care had to be taken to fill the capillaries completely with buffer before an injection was attempted, otherwise the introduction of air bubbles became a problem. Especially the injection of more than one droplet in a row caused problems with air bubbles during the measurements and tended to give uninterpretable electropherograms. Microdrop also offer a different ejector capillary that produces 20-μm droplets. However, this system was not available to us. The use of CE capillaries with larger diameters, on the other hand, caused problems with heat dissipation.

The injection of one drop, on the other hand, gave excellent and reproducible results. In Fig. 4 an electropherogram obtained after the injection of one 113-pl micro drop of an arginine-containing sample is shown. The retention times and relative standard deviations thereof were similar to those found for the standard pressure injection mode. A higher relative standard deviation of 3% was calculated for the peak area. As the droplet size varied only by about 1%, this larger deviation is possibly due to the handling of the capillary holder that is at present still required before and after sample injection. Permanent incorporation of a micro injector in the CE system is expected to reduce the deviation to 1%, *i.e.*, within the range of the standard pressure injection.

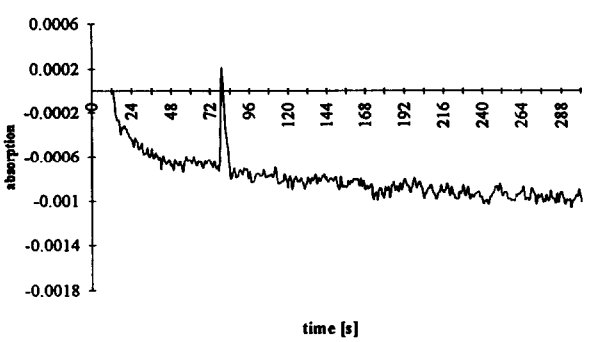

Fig. 4. Single-drop micro injection of a 113-pl sample. Sample, 10 mmol/l arginine; electrophoresis buffer, 50 mmol/l phosphate buffer (pH 2.5); conditions, 25°C, 20 kV, 27 cm × 50 μm (I.D.) capillary, detection wavelength 214 nm.

4. Conclusions

A commercially available micro injector for capillary electrophoresis has been introduced. The CE system itself including the capillary did not have to be modified. The micro injection system was tested with an arginine standard solution. It was shown that extremely small sample volumes can be handled with great precision and good reproducibility. The best results were obtained for single drop injection, whereas air bubbles caused problems if several droplets were injected in a row. This was most likely the case because the average droplet diameter was 60 μm compared with 50 μm for the I.D. of the CE capillaries. The use of a system with a smaller diameter of the ejector capillary should eliminate the problem. The fact that no actual contact between the injector outlet and the CE capillary takes place during injection constitutes another advantage of the micro injector. An automatic micro-positioning system for the alignment of the ejector and the CE capillary would be highly desirable, as this would allow the integration of an automatic micro injector into standard CE systems.

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