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Short communication

Fast analysis of antibacterial isothiazolones by capillary electrophoresis[†]

Petr Barták[a,b,*], Petr Bednář[b], David Friedecký[b], Antonín Haviger[b], Juraj Ševčík[a,b]

[a]*Centre of Bioanalytical Research, Palacký University, Třída Svobody 8, 771 46, Olomouc, Czech Republic*
[b]*Department of Analytical Chemistry, Palacký University, Třída Svobody 8, 771 46, Olomouc, Czech Republic*

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Abstract

Some technical aspects influencing the total time of CE analysis are discussed. A high throughput electrophoretic system based on micellar electrokinetic chromatography (MEKC) is demonstrated as an example. A short capillary, strong electric field, alkaline buffer (pH 9.5) generating strong electroosmotic flow, and parallel hydrodynamic pressure allow the separation of two uncharged isothiazolone derivatives within 45 s.

Keywords: Isothiazolones

1. Introduction

Modern separation methods must be as quick as possible in order to keep up with other high-throughput techniques used in the biochemical and pharmaceutical sciences. The family of capillary electrophoretic techniques covers fast analytical separation techniques based on the different mobilities of separated compounds in an electric field. Because of its high separation efficiency, flexible changes of separation conditions, low operating expense and time consumption, capillary electrophoresis (CE) has replaced common liquid and gas chromatographic methods [1,2]. CE has expanded into clinical [3–7], industrial, pharmaceutical and research [7–10] laboratories.

Most CE assays are performed in uncoated fused-silica capillaries in the presence of electroosmotic flow (EOF). In some cases, EOF can be used to reduce the total analysis time. The EOF velocity (v_{EOF}) can be expressed in terms of the dielectric constant (ε), the zeta potential (ξ), the viscosity (η) and the intensity of the electric field (E) given as a quotient of the applied voltage (U) and the total length of the capillary (L) [8]:

$$v_{EOF} = \left(\frac{\varepsilon \times \xi}{\eta}\right) \times E = \left(\frac{\varepsilon \times \xi}{\eta}\right) \times \frac{U}{L} \quad (1)$$

The important role of the ionic strength should be emphasized. An increase in the ionic strength results in double layer compressing, a decrease of the zeta potential and reduction of the EOF. Low ionic strength encourages EOF and reduces the analysis time by enhancement of the zeta potential and by decreasing the electric conductivity, allowing a higher voltage to be applied. Utilization of short capillaries

[†]Dedicated to Antonín Haviger in memoriam.

*Corresponding author. Tel.: +420-68-563-4441; fax: +420-68-523-0356.

E-mail address: bartak@risc.upol.cz (P. Barták).

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and ''short end'' injection are techniques for speeding up the analysis when the resolution is sufficient [11–13]. Electrophoresis in chip format and multicapillary systems [7,14,15] are other possibilities to achieve high throughput of samples.

The requirements for fast CE analysis can be concluded, although all of these requirements cannot generally be fulfilled:

1. short and narrow capillaries;
2. high intensity electric field;
3. low ionic strength of buffers;
4. strong electroosmotic flow (high pH of the BGE);
5. parallel hydrodynamic flow.

This report describes the application of a high-throughput separation system based on micellar electrokinetic chromatography (MEKC). Two antibacterial isothiazolones served as a model for the demonstration of the proposed system.

2. Experimental

All experiments were performed on a P/ACE 5510 with a diode array detector (Beckman Instruments, Fullerton, CA, USA) in an uncoated fused-silica capillary (75 μm I.D.×375 μm O.D., Polymicro Technologies, Phoenix, AR, USA) (effective length 20 cm, total length 27 cm, 15 kV, ~120 μA, 25°C). The sample was loaded by low-pressure injection (0.5 p.s.i.) for 4 s (3% of the total capillary length). Phosphate buffer (0.05 *M*, pH 7.0) with 0.025 *M* SDS and 0.05 *M* borate buffer pH 9.5 with 0.1 *M* SDS were used for MEKC. A Finnigan MAT (San Jose, CA, USA) LCQ mass spectrometer was used off-line for the confirmation of the presence of both isothiazolones in an unknown sample.

A series of ProClin 300 solutions and unknown samples were injected directly into the capillary. Samples with a low concentration of active substance were adjusted to pH 12 and extracted by diethyl-ether. The extract was evaporated to dryness and reconstituted in running buffer diluted 1:2 with deionized water. The enrichment factor was adjusted in the range 2–100 according to the sample concentration. Recovery was in the range 80–90%.

3. Results and discussion

ProClin 300 is a registered trademark of Rohm and Haas (Philadelphia, USA) for a new antimicrobial reagent based on two isothiazolones (2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one) (for structures, see Fig. 1). These compounds are not charged above pH 1.8, thus a micellar system was suggested for separation. Total separation was achieved in 2 min using 0.05 *M* phosphate buffer as BGE, pH 7.0, 0.025 *M* SDS. Electroosmotic flow generated in 0.05 *M* borate, pH 9.5, with 0.1 *M* SDS allowed shortening of the separation time. Application of parallel hydrodynamic flow developed by an additional pressure of 0.5 p.s.i. was used to further accelerate the process. An analytical time of about 45 s was achieved under these conditions (Fig. 2).

In fact, parallel hydrodynamic flow has the same effect as shortening the capillary. Unfortunately, the current instrumentation (see Experimental) disables the operation of capillaries shorter than 27 cm and short end injection.

In addition to its role of speeding up the analysis, strong hydrodynamic flow, induced by electroosmosis and parallel pressure, replaces the background electrolyte in the capillary during analysis. Therefore, the capillary is washed throughout the analysis and particular washing between runs may be reduced or even completely omitted. In such a case the capillary is prepared for the next injection at the moment when the previous analysis is completed. Furthermore, in principle, self-conditioning of the capillary allows to increase the throughput by sequential injection (the next sample can be injected while the previous sample is still running). However, the limited speed of the carousel and the instrument–PC communication precludes the utilization of the sequential technique for such fast analyses with the current instrumentation.

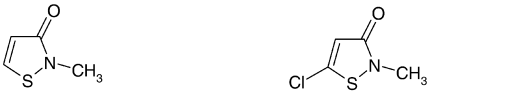

Fig. 1. Structures of the isothiazolones.

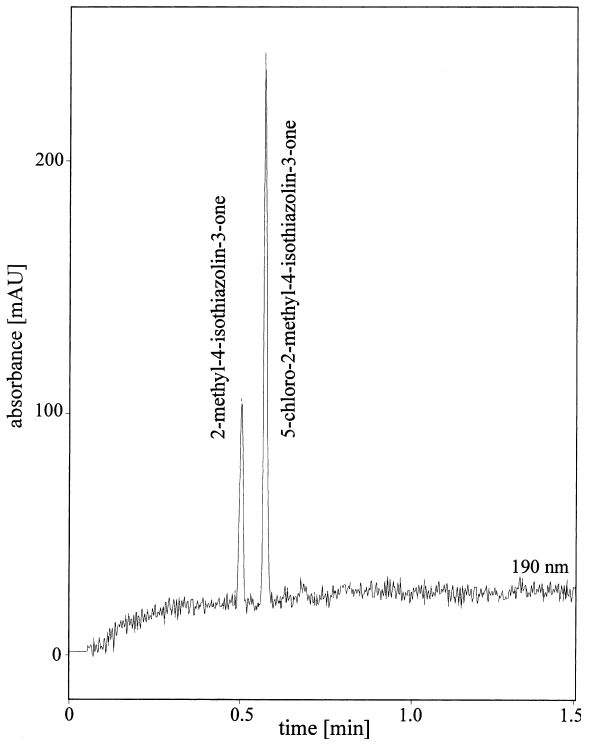

Fig. 2. Electropherogram of extract containing both isothiazolones (0.05 *M* borate buffer, pH 9.5, 0.1 *M* SDS, 15 kV, additional pressure 0.5 p.s.i.).

The migration behavior and UV spectra were used for the identification of the two major components in the unknown solution of the commercial preparation used for the conservation of blood. The final identity of the main components of ProClin 300 and the unknown sample was confirmed by the detection of the molecular ions in the mass spectrum of a directly injected extract.

The proposed method was used for the determination of both active compounds in a solution routinely used for the conservation of blood. Direct injection into the capillary is acceptable for samples containing >100 μmol/l. Extraction by diethylether was used for analysis of samples containing <100 μmol/l of each isothiazolone. Samples containing 5–100 μmol/l of each derivative were analyzed successfully by using an extraction procedure. The relative standard deviation (RSD) of the migration times was about 1.3% ($n = 3$), the RSD of peak areas was about 1.5% ($n = 3$) and the RSD of the extraction procedure was from 5 to 10% ($n = 3$).

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