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Theoretical and experimental studies on sequential two-diffusional sample injection for capillary electrophoresis

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ABSTRACT

We report here theoretical and experimental studies on the sequential diffusion injection (SDI) for CE analysis. Based on the Fick's second law, a theoretical model for two-dimensional (2-D) diffusion has been developed for our SDI system. The 2-D diffusion model has been demonstrated via systematic experimental studies using standard nicotinamide adenine dinucleotide (NADH) as the model analyte. The results show that the dependence of the NADH peak area (corresponding to the injection amount) on the initial sample concentration, the injection time or the capillary-gap distance is consistent with the deduction of the 2-D diffusion model. It is indicated that the 2-D diffusion, both in longitudinal and transverse directions of the capillary, enhances the injection efficiency in comparison to classical concentration diffusion on the plane interface, and improves the accuracy of the sequential injection without any physical disturbance of the capillary inlet. With the insight understanding of the injection mechanism, we have successfully applied the SDI method for sequential CE analysis of amino acids mixture and online assay of the glucose-6-phosphate dehydrogenase-catalyzed reaction. The present study showed that the SDI is a versatile tool for efficient and accurate sequential CE analysis, not only for online monitoring various bioprocesses but also for continuous analyzing complex samples based on capillary electrophoresis.

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1. Introduction

Sequential analysis based on capillary electrophoresis (CE) is of vital importance especially for online monitoring application in various research fields, such as drug dissolution, bioreactor, process stream, and organic synthesis monitoring, etc. [1–3]. In order to achieve accurate sequential CE analysis, a great challenge is to develop an automatic and sequential sample injection method to perform repeatable small-amount sampling without any physical disturbance of the capillary inlet [4], which is quite a difficult task for the two most commonly used CE injection modes, i.e., hydrodynamic and electrokinetic injection. During the past several decades, some exciting approaches and devices have been reported regarding sequential capillary injection, including on-line flow injection (FI) [5–8] and sequential injection (SI) [2,9–12], flow-gated injection [13–16], optical-gated injection [17–20], split flow injection [21–23], as well as automated sampling devices coupled to a microchip CE [24–30]. These approaches could carry out automatic online analysis and allow one to sequentially inject the sample without interrupting the CE separation. Sample throughput and sampling accuracy in CE were improved and various on-line treatment procedures for samples were implemented.

Recently, our group developed a simple and easy-to-operate sequential CE analysis method [31,32]. The system was constructed by coaxially aligning two capillaries through a sample vial with a distance of 5 μm between the capillary ends. Direct online sample injection and sequential CE analysis were easily achieved by periodically switching the high-voltage power supply off (for sample injection) and on (for CE separation). It is indicated that the sample in the vial is injected into the capillary via diffusion as the high-voltage power supply is at off position. Without any physical disturbance of the capillary inlet during analysis, this method has been successfully applied for the sequential online CE enzyme assay. Comparing to other sequential injection methods, our approach does not require sophisticated experimental set-up and has great repeatability of the sequential online sample injection (1.01% RSD (peak height, $n = 20$)).

However, the detail injection mechanism of our sequential CE analysis method has not been addressed until now. Two questions about the sequential diffusion injection (SDI) in our method have to be answered. First, how is rapid and accurate online sample injection achieved, considering that it generally requires long time for

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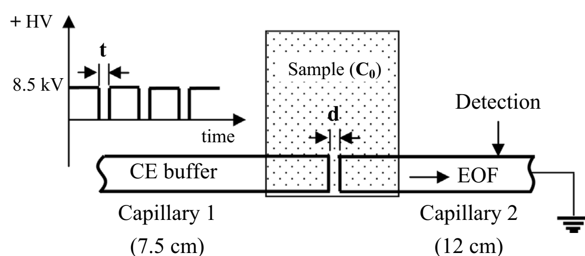

Fig. 1. Schematic diagram of the SDI CE system. The three parameters, gap distance (d) of the two capillaries, initial sample concentration (C_0) in the sample vial, and injection time (t) as the high-voltage is at the off-position, are indicated in the diagram. For detail description of the method, see Refs. [31,32].

the sample to diffuse into the capillary? Second, is it a versatile method for sequential CE analysis, or only applicable in the case that rapid derivatization reaction at the capillary interface occurs (as in our previous papers [31,32])?

Therefore, the purpose of this paper is to carry out theoretical and experimental studies to reveal the mechanism of the SDI in our method. Based on the Fick's second law, we have developed a theoretical model of two-dimensional (2-D) SDI for CE, which is demonstrated by a systemic CE experiments using nicotinamide adenine dinucleotide (NADH) as the analyte. The results show that the 2-D diffusion, both in longitudinal and transverse directions of the capillary, enhances the diffusion efficiency in comparison to classical concentration diffusion on the plane interface. The method was successfully applied for sequential CE separation of a mixture of dinitrophenyl (DNP)-labeled amino acids (AAs) and on-line analysis of glucose-6-phosphate dehydrogenase (G6PDH)-catalyzed reaction, both of which do not need any derivatization for analysis. Our study proves that the method presents versatile applications for accurate, repeatable and efficient sequential CE analysis in a variety of fields of analytical chemistry.

2. Materials and methods

2.1. Chemicals

G6PDH (EC1.1.1.49), D-glucose-6-phosphate (G6-P), NADH, β-nicotinamide adenine dinucleotide (β-NAD^+), L-arginine (Arg), L-tryptophan (Trp), L-glycine (Gly) and L-glutamic acid (Glu) were purchased from Sigma Chemical Co. 2,4-Dinitrochlorobenzen was purchased from Tianjin Fine Chemicals Co., Ltd. (Tianjin, China). All other reagents were of analytical grade and were used without further purification. All solvents and solutions were filtered using 0.2 μm membrane filters prior to use.

2.2. Sequential CE analysis

Fig. 1 presents a schematic diagram of the SDI CE system. The detail description about the system has been presented in our previous study [31]. Briefly, the system was simply constructed by coaxially aligning two capillaries and passing them through a sample vial. The alignment of the two capillaries and the distance between the smooth ends were ensured under a microscope. A high voltage power supply, which can be automatically turned on and off with controllable periodic time, was applied across the two capillaries. By periodically switching the high-voltage power supply off (for sample injection) and on (for CE separation and detection), sequential CE analysis was achieved without any physical disturbance of the capillary inlet. The injection time refers to the time during which the power supply is at off position.

All experiments were carried out in a home-built CE apparatus with a 6000PVW UV–visible detector (Cometro Technology Ltd., USA). Two fused silica capillaries (365 μm o.d., 50 μm i.d. Hebei Yongnian Optical Fiber Factory, China) were used for the sequential CE analysis, with the total length of 12 and 7.5 cm, respectively. The 12-cm capillary was used as the separation and detection channel with an effective length of 6 cm. The capillaries were pressure-rinsed successively with 0.1 M NaOH for 2 min, distilled water for 3 min, the running buffer for 5 min, and the sample vial was rinsed with distilled water and the running buffer using a syringe. The high voltage for CE was 8.5 kV.

For sequential CE analysis of standard NADH sample and the DNP-AAs mixture, the sample vial contained the corresponding analyte with a given concentration. Phosphate buffer (10 mM, pH 7.5) and sodium tetraborate solution (10 mM, pH 9.2) were used as the running buffer for analysis of NADH and DNP-AAs, respectively. NADH and DNP-AAs were detected by UV absorption at wavelength of 340 nm and 360 nm respectively. For sequential online enzyme assay, the reaction mixture (50 μL) contained 10 mM phosphate buffer (pH 7.5), the substrate NAD^+ and G6-P was initially put into the sample vial. Reaction was initiated by the addition of G6PDH into the mixture. The product NADH was sequentially injected and detected as a function of reaction time to achieve the online CE enzyme assay.

2.3. Off-line CE enzyme assay

For off-line analysis of the G6PDH-catalyzed reaction, the reaction mixture (120 μL) contained G6-P and NAD^+ of different concentrations in 10 mM phosphate buffer (pH 7.5). Reaction was initiated by the addition of 6 μL of 20 U/mL G6PDH enzyme into the mixture. Aliquots of 10 μL were periodically removed from the reaction mixture, and the G6PDH enzyme was inactivated by the addition of 2 μL of 0.1 M HCl to each aliquot. The CE running buffer was 10 mM phosphate buffer (pH 7.5) and the sample was injected at a height of 10 cm for 3 s. The product NADH was detected by UV absorption at the wavelength of 340 nm and measured at a separation electric potential of 400 V/cm. The total length of the separation capillary (50 μm i.d., 365 μm o.d.) was 50 cm with an effective length of 42 cm.

2.4. Derivatization of AAs

Derivatization of AAs was achieved following the procedure reported in the literature [33]. Stock solutions of the standard amino acids (50 mM) were prepared in 0.1 mM HCl, and diluted with 50 mM sodium tetraborate solution (pH 9.2) for derivatization with 2,4-dinitrofluorobenzene (molar ratio, 1:5) in the alkaline solution for 2 h at 90 °C water bath in the dark. The DNP-AAs were contained in the sample vial for sequential CE analysis.

3. Results and discussion

3.1. Principle of sequential diffusion injection (SDI)

In our previous studies [31,32], rapid derivatization reactions occurred at the interface of the capillaries which was argued to enhance the diffusion efficiency via changing the concentration gradient. In this study, however, we show that rapid derivatization reactions are not a prerequisite for the efficient SDI. Actually, diffusion of the sample from the vial to the capillary-gap in our system is two-dimensional (2-D), i.e., both in the longitudinal and transverse directions of the capillary. Such 2-D diffusion can be well described by Fick's second law predicting the concentration to change with time,

$$\frac{\partial C}{\partial t} = D_m \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) \quad (1)$$

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