Wide-bore Electrophoresis System with Single Channel Inner-cooling and UV Detection

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(Received July 28, 2006; CL-060862)

A novel, high performance wide-bore electrophoresis (WBE) system with inner cooling has been developed. By introducing the mode of shell and tube heat exchanger into this system to remove Joule heat generated during electrophoresis, it is feasible to extend the conventional capillary electrophoresis tube into wide bore (>1.0 mm ID) diameter. The wider tube allows loading larger volume of sample with improved detection limit. Several separations of model compounds were achieved with relatively satisfactory performance on this device preliminarily. Further work is undergoing.

Capillary electrophoresis (CE) has been widely recognized as a powerful separation tool for chemical and biochemical analyses.[1–3] CE has the advantages of short analysis time, high-resolution, low sample consumption, high-speed separation with automation, and the potential of "lab-on-a-chip" integration. The fundamental reason behind all of these is capability to effectively disperse Joule heat because of its extremely small capillary (<100 µm ID). However, from a practical viewpoint, the small dimension of the CE brings challenge on detection sensitivity. Further, CE can only handle a small volume (ca. 1.0–10.0 nL) of sample, which also makes it extremely difficult to integrate it into multidimensional separation processes and miniaturized total analytical system (μ-TAS). More importantly, the rapid development of proteomics and genomics demands new technologies, which can handle the amount of samples beyond the reach of the current CE technique. Thus, various attempts have been made to enhance sample-loading capability of CE.[4–7] So far, none of them can be used as a general approach but works only in a specific condition. For example, it is often necessary to add organic solvents to reduce the current and thus the Joule heat. If the sample load is further increased, none of these approaches is adequate for removing the Joule heat.

Therefore, a more efficient and universal method, which can greatly increase the sample-loading capability of CE and effectively remove Joule heat, is desirable. The wide-bore electrophoresis (WBE) reported here was developed under this background.

The proposed technique applied the principle of shell and tube heat exchanger to the electrophoresis system subtly. A coolant liquid was pumped passing through the inner tube while the electrophoretic buffer flowed through between the shell and the inner cooling tube. It brings all advantages of shell and tube heat exchanger into play to disperse the Joule heat in situ in real time, which is helpful to eliminte the formation of temperature gradients inside the separation channel. It is well known that this internal temperature gradient is the primary cause for preventing the expension of the capillary diameter in CE. By eliminating this temperature gradient, it is possible to achieve reasonable separation in WBE system.

In this study, all chemicals used were of analytical grade or higher unless otherwise stated. The samples were dissolved in deionized water at proper concentrations. All solutions were filtered through 0.22 µm membrane before use.

The WBE system was designed and constructed in-house. The separation channel is the opening space between a 1.0 mm ID quartz tube and a coaxially inserted capillary with an OD smaller than 1.0 mm. The exterior surface coating of the capillary was removed to create a uniform surface. This cooling capillary (530 µm ID, 690 µm OD) is connected to a high-pressure pump, which drives the coolant liquid pass through. The rest components of the system include a UV detector; high voltage power supply, etc. provide all necessary function to the system. A schematic drawing is shown in Figure 1.

Notice that the cooling water should be introduced into the WBE before experiment and shut off after all experimental procedures completed lest Joule heat generated by electrophoresis destroyed the system.

The sample-loading capacity and Joule heat in the WBE system can be evaluated based on the following:

$$A = \pi(R^2 - r^2), \quad (1)$$

$$I = A\lambda CE, \quad (2)$$

$$Q_J = A\lambda CE^2, \quad (3)$$

where A is the effective cross section area of the separation channel, R is the inner radius of the separation tube, r is the outer radius of the cooling capillary, I is the electric current under field strength E, λ is the molar conductivity of the buffer solution, C is the buffer concentration, Q_J is the amount of Joule heat per unit length. In the current WBE system, the cross section area is larger than that of a typical capillary with 720 µm ID without inner cooling. Its potential loading capacity of WBE is much larger than that of conventional capillary (<100 µm ID) in CE. It is expected that the Joule heat generated in the WBE system is much higher than that in CE. Therefore, the configuration of shell-and-tube heat exchanger was introduced into the WBE system in order to remove the Joule heat in time.

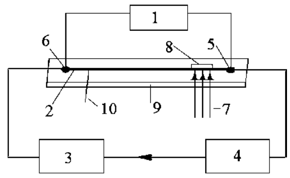

Figure 1. Schematic drawing of the WBE system. (1) high voltage power supply; (2) separation tube; (3) high-pressure pump; (4) circulating cooler; (5) buffer reservoir (6) buffer reservoir; (7) UV light from detector; (8) photodiode to signal preamplifier and data acquisition system; (9) a mainframe supporting the WBE system; (10) sampling capillary (100 µm ID, 375 µm OD) connecting to 1.0 µL syringe.

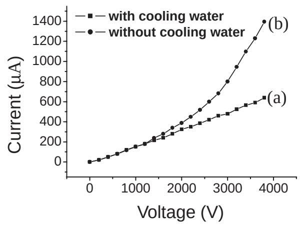

Figure 2. Effect of voltage on current for WBE system. Experimental conditions: 10 mmol/L tris-acetic acid, hydroxypropyl methyl cellulose % = 1% (w/w), pH 4.5, total separation length 15 cm, the effective separation length 8 cm, detection at 254 nm, the flow rate of coolant in cooling capillary 8.0 mL/min at room temperature.

To be effective, cooling water was pumped through the cooling capillary, which not only can reduce system temperature but also minimize radical temperature gradients inside the buffer by adjusting the coolant temperature and flow rate. It is worth noting that, in reference to the flow direction of buffer, a counter flow was selected as it is a more effective mode for heat transfer[8] according to the shell and tube heat exchanger principles.

To evaluate the efficiency of cooling, the current change as a function of the applied potential in the WBE system was monitored with or without a stream of cooling water passing through the inner capillary. Figure 2 shows the Ohm plots of the electrophoresis system with and without the coolant flowing through.

Figure 2 clearly indicates that the current is reasonably stable following Ohm's law (a) when the coolant passes through the heat sink systems. If coolant does not exist, the current will deviate from Ohm's law quickly (b). Therefore, the WBE system allows the use of much higher voltage during the electrophoresis process only when proper cooling exists. Figure 2 also indicates that the interior cooling could significantly improve the heat dispassion and extend the linear range of the Ohm plot. There should be no Joule heat accumulation in WBE system if the heat sink system works well. It is this effective cooling that makes it possible to run electrophoresis in the WBE system like a traditional CE without the concern of Joule heat.

To evaluate the separation properties of the WBE system, some model compounds were analyzed on this system. The separation results were shown in Figures 3 and 4.

These figures demonstrate that the WBE system is capable of performing electrophoretic separations with relatively high efficiency. Taking Figure 3 as example, the separation efficiency achieved for FD&C Yellow No. 5, salicylic acid and FD&C Blue No. 1 was 29,498, 67,121 and 37,304 theoretical plates per meter, respectively. However, the efficiency is lower than traditional CE but better than a typical HPLC. At the same time, in order to evaluate the detection ability of WBE, five measurements were processed at 214 nm on p-toluenesulfonic acid. The limit of detection (LOD) for p-toluenesulfonic acid was 3.0×10^{-4} mg/mL (signal-to-noise ratio 3:1).

In WBE system, a 1.0-mm separation tube containing a 690 μm OD cooling capillary coaxially inserted can be used as an effective separation technique. The novel system presents an efficient approach to removing the Joule heat associated with the electrophoresis process. Several limitations of CE, such as low sample loading capacity and sensitivity, can be improved

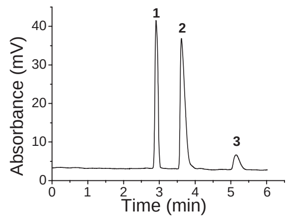

Figure 3. Electrophoregram of salicylic acid and food colors. Experimental conditions: the same as Figure 2, plus a 2000 V electrical potential, sample injection volume 200 nL, sample concentration 0.1 mg/mL. Samples: 1. FD&C Yellow No. 5; 2. salicylic acid; 3. FD&C Blue No. 1.

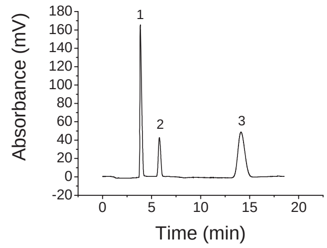

Figure 4. Electrophoregram of mixture of aromatic acid. Experimental conditions: the same as Figure 3, sample injection volume 400 nL, sample concentration 0.2 mg/mL. Samples: 1. toluene-p-sulfonic acid; 2. benzoic acid; 3. p-aminobenzene sulfonic acid.

by using the proposed method significantly. Although not optimized, the separation efficiency is higher than any known wide-bore electrophoresis systems. The bigger advantage of this novel system is that its potential can be developed as a preparative or semi-preparative electrophoretic separation technique. It is expected that much more efficient separation results can be achieved if the conditions are further optimized.

Financial support from Cheung Kong Scholar Programme is gratefully acknowledged. This work is also supported by the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of People's Republic of China (704013). Project supported by the Tianjin Foundation for Development of Science and Technology, China (No. 05YFJPGX07900).

References and Notes

1 A. R. Timerbaev, *Electrophoresis* **2002**, *23*, 3884.
2 A. Cifuentes, *Electrophoresis* **2006**, *27*, 283.
3 J. W. Cooper, Y. Wang, C. S. Lee, *Electrophoresis* **2004**, *25*, 3913.
4 H. Yin, C. Keely-Templin, D. McManigill, *J. Chromatogr., A* **1996**, *744*, 45.
5 S. P. Porras, M. Jussila, K. Sinervo, M.-L. Riekkola, *Electrophoresis* **1999**, *20*, 2510.
6 J.-R. Chen, R. N. Zare, E. C. Peters, F. Svec, J. J. Frechét, *Anal. Chem.* **2001**, *73*, 1987.
7 Q. Qu, Y. He, W. Gan, N. Deng, X. Lin, *J. Chromatogr., A* **2003**, *983*, 255.
8 J. P. Holman, *Heat Transfer*, 9th ed., McGraw-Hill Companies, Inc., New York, **2002**.

Published on the web (Advance View) November 11, 2006; doi:10.1246/cl.2006.1386