行政院國家科學委員會專題研究計畫　期中進度報告

微晶片電泳應用研究(1/2)

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微晶片電泳應用研究(1/2)
Application Study of Microchip Electrophoresis (1/2)

計畫編號：NSC 91-2113-M-009-025
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一、中文摘要

微晶片電泳(microchip electrophoresis)是在晶片上進行快速電泳分析，不但具有毛細管電泳高分離效率、高質量靈敏度、低溶劑使用量以及可分析微量樣品等特點，同時還具有極短分析時間及大量樣品平行分析等特點。本研究計畫將探討一些影響微晶片電泳分離效率的因素及相關對策，並利用不同的方法加強微晶片電泳分離的再現性，並應用於分析一些重要的生化反應，發展單石多孔性高分子進行晶片樣品前濃縮的研究，並以毛細管電泳輔助微晶片電泳研究。

關鍵詞：微晶片電泳、晶片樣品前濃縮、毛細管電泳

二、Abstract

The technique of microchip electrophoresis (μ-CE) is to perform electrophoresis in a microchip, which will be intensively investigated under this study. This technique possesses the advantages of the miniaturized separation systems, such as high separation efficiency, rapid analysis, high mass detection sensitivity, parallel analyses, and minimal sample consumption. The main objective of the present proposal is to optimize different experimental factors that influence the performance of microchip electrophoresis. In order to have a better understanding how different experimental setups will affect separation performance and signal intensity in the microchip electrophoresis, various experimental conditions will be manipulated. On-chip preconcentration by monolithic porous polymer will be examined by mixing various monomers. Capillary electrophoresis will also be used to assist microchip electrophoresis study.

Keywords: Microchip electrophoresis, On-chip preconcentration, Capillary electrophoresis

三、 Determination of chitin oligosaccharides by microchip electrophoresis on a

PMMA microfludic device

Introduction

A PMMA microfludic device was fabricated for use in the determination of chitin oligosaccharides is described. Chitin oligosaccharides were derivatized with 9-aminopyrene-1,4,6-trisulfonate (APTS) via reductive amination. The effects of chip dimension, applied voltage, sample matrix and concentration of Brij35 on the separation were discussed. The APTS-derivatized chitin oligosaccharides (1-6 DP) were successfully separated within 45 s by using a pH 3.6 citric acid-phosphate buffer containing 3.0% Brij35. By applying -600 V/cm electric field strength, the 960,000 theoretical plate number was achieved for the APTS-derivatized chitin monosaccharide. A double-T injection channel design in the PMMA microfluidic device was used to extend the sample-loading volume. The sample stacking by employing low concentration sample matrix for large sample loading was also accomplished in this design. The reproducibility of PMMA microfludic devices with 2-mm sample loading length was also examined. Moreover, the specificity of enzyme digestion on chitin polysaccharides using the optimized microchip electrophoresis was demonstrated.

Experimental

Channels were fabricated using PMMA Plexiglas pieces approximately 2.6 cm wide × 7.6 cm long × 2.0 mm thick. Steeliness wires with diameters of 50 μm were used to imprint the channels in the plastic substrate. The separation channel on PMMA was formed by heating the plastic at 103^{o}C for 6 min. The four holes were aligned with the ends of the channels in the imprinted substrate and adherence to a section tip bottom (about 250 μL) using hot glue to create the buffer, analyte, and two waste reservoirs as shown in Fig. 1.

Two identical power suppliers (CZE 2000, Spellman, Hauppauge, NY, USA) were utilized to furnish the loading and separation voltages, respectively, and the power switching was controlled by a program written in Lab-VIEW (National Instruments, Austin, TX, USA) running on a computer. The sample loading was performed by applying -600 V/cm between reservoir 1 and 2 for adequate time while keeping the separation channel floating. For the separation, -600 V/cm was applied to the separation channel while keeping the injection channel floating. Signals were detected on-chip with laser-induced fluorescence (LIF). The detection system was constructed through a commercial microscope (Model BH2-UMA; Olympus, Tokyo,

Japan). Briefly, an 8-mW, 488-nm argon-ion laser as the excitation source (JDS Uniphase; San Jose, CA) was focused at a position 4.5 cm downstream from the cross section within the channel using 20× working distance objective lens. Fluorescence was collected by the objective and passed through a dichroic cube with two band-pass filters, followed by photomultiplier detection operated at -800 V (Hamamatsu, Tokyo, Japan).

Results and Discussion

Fig. 2 displays the influence of the four different dimensions of sample loading length on the electrophoretic analysis. It is obviously that the fluorescence intensity was increased as extending sample loading length. However, considering the separation efficiency and peak intensity, 2 mm sample loading length was the preferred choice to separate the APTS-derivatized chitin oligomers.

As shown in Fig. 3, both the enhanced electrophoretic velocity and separation efficiency were observed with an increasing applied voltage ranging from -450 V/cm to -600 V/cm. For the APTS-derivatized chitin monomer, the theoretical plate number can reach to 960,000 at –600 V/cm. Although applied higher electric field strengths than -600 V/cm can be achieved, the increased Joule heat that inevitably causing bubble produced resulted in breaking current. The six APTS-derivatized chitin oligomers were well separated within 45 seconds by applying electric field strength at -600 V/cm.

Fig. 4 depicts the effect of Brij35 concentration on the chip electrophoresis separation of the six analytes. Nonionic surfactant Brij35 was used for coating inner PMMA surface that made it compatible with aqueous separation medium. Considering the stability of electrophoretic process and adequate fluorescence intensity, 3.0% Brij35 was added into the separation buffer solution in subsequent analysis.

As shown in Fig. 5, the sample matrix plays an important factor on sensitivity while injecting large volume of sample solution. The results indicated that the largest signal was obtained by diluting 10× analytes solution with H_2O as shown in Fig. 5(a). Diluting analytes with 10% boric acid/borax buffer solution was the preferred choice under electric field stabilization.

Fig. 6 displays the analysis of six APTS-derivatized chitin oligomers on a 2 mm sample loading length PMMA chips. Consecutive injections and separations could be completed by voltage switching as shown in Fig. 6(a). The RSDs for the migration time were less than 1.68%. The electropherograms of six analytes in 5 chips were depicted in Fig. 6(b)-6(f). For each separation, all the six derivates could

be well resolved within 45 s.

Table 1 lists the chip-to-chip variation of average migration times, reproducibilities and the detection limits of the six APTS-chitin oligosaccharide derivatives. The RSDs for the migration times in 5 different chips were less than 4.72%. The detection limits of the six chitin oligosaccharides ranged from 9.5 to 22.5 amol.

The six APTS-derivatized chitin oligomers could be identified by compare with individual derivate as shown in Fig. 7. Fig. 8 shows an electropherogram of APTS-derivatized chitin oligosaccharides from an enzymatic digestion under the optimized microchip electrophoresis conditions. According to this Fig., the major chitin oligomer products of enzyme A digestion was dimmer. Chitin trimer and tetramer were produced when enzyme B was used.

In this study, a simple design of wire-imprinting double T injection channel on PMMA chip for enhancing detection sensitivity in microchip electrophoresis was developed for the analysis of chitin-oligosaccharides. The six APTS-derivatized chitin-oligosaccharides were well separated within 45 s using a pH 3.6 citric acid/phosphate buffer containing 3.0% Brij35 under -600 V/cm electric field strength. By applying -600 V/cm electric field strength, the theoretical plate number of 960,000 was achieved for APTS-derivatized chitin monomer.

The detection limits of the APTS-derivatized chitin-oligosaccharides (1-6 DP) ranged from 9.5 to 22.5 amol. The RSDs for the migration time were less than 1.68%. The reproducibility of fabrication of 2 mm sample loading PMMA chip was also examined. The RSDs of migration times among 5 chips were below 4.72%. Chitin-polysaccharides, depolymerized by chitinase digestion, were adequately analyzed using the optimized microchip electrophoresis method. Enzyme specificity in the digestion of chitin-polysaccharides was also demonstrated.