行政院國家科學委員會補助專題研究計畫成果報告

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※　電滲透流動注射分析系統：開發、整合與應用　※

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC89－2313－B－002－247

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：陳力騏

共同主持人：張炳華

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：台灣大學生物產業機電工程系

中　華　民　國　90 年　9 月　1 日

行政院國家科學委員會專題研究計畫成果報告

電滲透流動注射分析系統：開發、整合與應用

Electroosmotic Flow Injection System : Development, Integration and Applications

計畫編號：NSC 89-2313-B-002-247
執行期限：89 年 8 月 1 日至 90 年 7 月 31 日

主持人：陳力騏　台灣大學生物產業機電工程系
rlcchen@ccms.ntu.edu.tw
共同主持人：張炳華　弘光技術學院醫務管理系
計畫參與人員：黃怡仁博士　台糖公司研究所產程開發系
林俊勳、張文彥、陳柏中、謝博全、楊凱元
台灣大學生物產業機電工程系學生

一、中文摘要

自行組裝一以電滲透流為送液基礎的流動注射分析系統。相對於一般流動注射分析系統(管徑約 1mm)，由於管徑小(75μm)質傳效率高，容易進行液相萃取，故利用此系統開發出第一套毛細管內微萃取式分離/分析系統，並應用於昇壓素轉換胺活性的檢測，對於本態性高血壓的診斷與抗高血壓藥物的快速篩選助益很大，詳見 SCI 線上期刊 Anal. Biochem. (2001) 291, 84-88 (附件)。

關鍵詞：電滲透流、流動注射分析、毛細管內微萃取、昇壓素轉換胺

Abstract

We constructed a flow-injection analytical system based on electroosmotic flow. Compared with an ordinary flow-injection system (i.d. ca. 1mm), the narrow bore (75μm) system has high mass transfer efficiency and an in-capillary microextraction procedure was easily performed. The system was used to quantify the angiotensin-converting-enzyme-catalyzed reaction and was proved to be valuable in diagnosing systematic hypertension and screening effective anti-hypertensive agents.

Keywords: Flow-Injection Analysis, Electroosmotic Flow, In-Capillary Microextraction, Angiotensin Converting Enzyme

二、緣由與目的

Flow-injection analysis (FIA) is a rapid and versatile analytical technique, but the selectivity is generally not sufficient for biological application [1]. Combining with biosensing strategies, flow-injection biosensing techniques did meet some bioanalytical demands [2], but the running costs were still high and separation units such as membranes (e.g. Teflon, Nafion, cellulophane, cellulose acetate membranes) and cartridges (ion-exchanger, solid-phase extraction columns) were frequently required. The objective of the project is to construct a micro-scaled FIA system to improve the mass transfer for an on-line extraction-based separation process and to reserve the sample dispersion in the same time.

Limited sample dispersion is the basic principle of FIA, and therefore any agitation of injected sample plug should be avoided. To enhance extraction efficiency of an FIA manifold, diaphragm membrane, hollow fiber or solid-phase extraction approaches were attempted, but the separation units of the manifolds should be replenished frequently

especially for complicated biological samples. We therefore incorporated a traditional liquid phase extraction process into a flow-injection system to eliminate the mentioned renewing requirement.

However, the back pressure of a micro-bore FIA system is too high for a peristaltic pumping system, and the resulting flow is pulsative. Electroosmotic pumping approaches are promising for a microfabricated system [3], and more importantly, extraction and separation process can be performed simultaneously. We thus constructed here an electroosmotic flow-injection system and conducted a extraction/separation process to quantify benzoate derivatives. The system was proved to be valuable for biomedical applications.

三、結果與討論

We initially planed to micro-fabricate the FIA system by MEMS technique, but the training of students was not sufficient to complete the project in a single year. The system (Fig. 1) was thus assembled according to capillary electrophoresis technique.

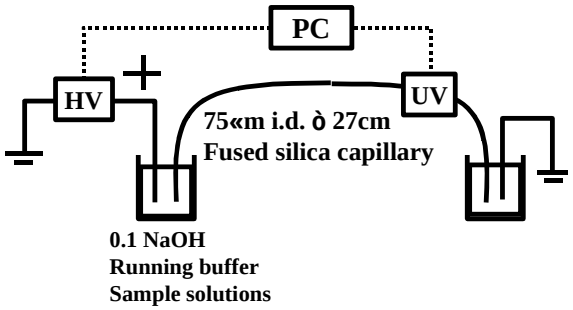

Fig.1Schematic representation of the system. HV: high voltage D.C. source; UV: UV detector; PC: personal computer.

A high voltage (up to 30kV; with a 5V D.C. on-off relay) D.C. power supply (EH30R3, Glassman High Voltage Inc.) was used to drive the electroosmotic flow in an untreated fused silica capillary (75μm × 27cm). The signal (A214nm) was monitored with an UV detector (UV1500, Jasco). Rinse of capallary (0.1N NaOH), sample injection (ca. 10nl) and separation were performed by applying 10kV (inlet to outlet) for 1.5min, 1s, 4min, respectively. The operation sequence and data acquisition were software-controlled *via* a multi-IO board (6030E, NI) under LabView™ environment.

Prior to the experiment, samples (typically, 1 ml) containing benzoate derivatives were acidified by adding sufficient amount of aqueous hydrochloric acid (0.1 M × 1 ml) and then extracted with 2 ml of ethyl acetate. Omitting a laborious phase separation process, about 10nl of the benzoate containing organic phase solution (the upper layer) was introduced directly into the analytical capillary by electroosmotic injection. Benzoates were extracted back into the aqueous phase, the alkaline running buffer (20 mM sodium borate, pH 9.2), within the capillary, and electrophoresed simultaneously against the high electric field (10 kV, 370 V/cm). The electrophoretic separation process greatly enhanced the efficiency of the microextraction procedure (Fig. 2).

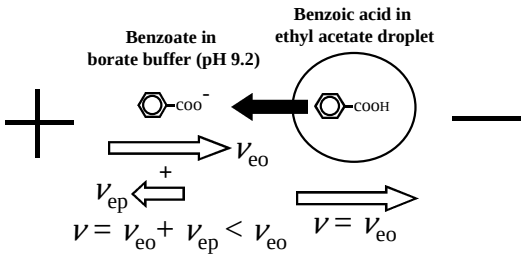

Fig. 2 Electric-field enhanced in-line micro-extraction of benzoic acid into a alkaline running buffer. v_{eo}= electroosmotic velocity; v_{ep}= electrophoretic velocity;

For samples with a high ionic strength such as sauce and serum, capillary electrophoresis can be performed without a lengthy desalting pretreatment (such as those using electrodialyzer, solid-phase extraction and chromatography). Unwanted signals of probably peptides and amino acids were excluded from the electrophoregram (Fig. 3).

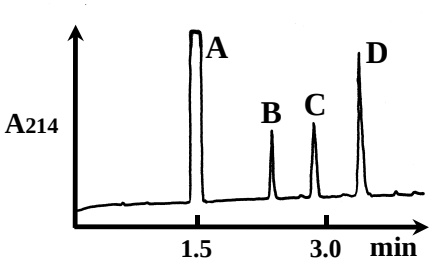

Fig. 3 Extraction/separation of an ethyl acetate (A) solution containing 100 mg/ml of hippurate (B), 50 mg/ml of benzoate (C) and 100 mg/ml of *p*-hydroxy benzoate (D).

Recently, the method has being widely used by our colleagues to determine the preservative content of soy sauce [5], to screen naturally occurring angiotensin converting enzyme inhibitors [4], to diagnose systematic hypertension [4], and to evaluate the hepatic activity of bioconversion of benzoate to hippurate, etc.

四、計畫成果自評

Our work was published recently in an SCI-on-line journal [4]. We also orally presented the microextraction technique in the 5th Chemical Sensor Technology Conference [5]. The liquid phase microextraction technique is a practical and promising analytical approach; its simple operation process will benefit future high-throughput automation procedure for bioanalytical purposes.

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