

附件：封面格式

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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計畫主持人：蕭明文

共同主持人：

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- 國際合作研究計畫國外研究報告書一份

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行政院國家科學委員會專題研究計畫成果報告

以毛細管電泳分析大蒜中的 Allicin

Separation of Allicin in the garlic by Capillary Electrophoresis

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執行機構及單位名稱：中山醫學大學醫研所

一、中文摘要

本研究利用毛細管電泳來分離大蒜精中的主要活性成分 DAS, DADS, DATS. 對於施加電壓, 溫度, 有機修試劑, 等參數也予以探討。

關鍵詞：大蒜精、毛細管電泳

Abstract

This study used capillary electrophoresis to separate the main active components, DAS, DADS, and DATS in the garlic. The parameters as applied voltage, temperature and organic modifiers were also examined.

Keywords: garlic, capillary electrophoresis

二、Introduction

Garlic is common food. Allicin is the main component of garlic. It can increase the function of antiplatelet activity and prevent tumors from forming, and so on. (1)

Gas chromatography and high performance of liquid chromatography are the methods to determine their concentrations in the literature. Capillary electrophoresis can be utilized to analyze a wide variety of charged and uncharged species. Sizes range from those of small analytes such as amino acids, proteins, DNA and RNA. The results have confirmed its high proficiency and

accuracy. Capillary zone electrophoresis (CZE) separate ionic compounds of different charge to mass ratios. Nonionic analytes are not resolved by CZE. Micellar electrokinetic capillary chromatography (MECC) was introduced by Terabe in 1984. (2) In this method, the separations are based on a differential partitioning of analytes between the solvent phase and the micellar phase. MECC has proven very useful separation of water-soluble analytes and nonionic molecules. Sodium dodecyl sulfate and sodium cholate are the common surfactants used in these methods. (3-5)

Varying organic solvents, surfactant, and buffer systems in the experimental analysis were necessary to obtain the best analytical conditions. The three major elements, DAS, DADS and DATS of the most appropriate analytic conditions of separating allicin are studied.

三、Experimental

A Beckman 5500 P/ACE electrophoresis system equipped with gold software for data collection (Beckman, Fullerton, CA) was employed for CE. The P/ACE system included a temperature-controlled cartridge enclosing a capillary column, an autosampler, a wavelength-selectable detector, and an electric interface. Spectra were collected with the use of the 168 diode-array detector using a scan graphic option. Fused silica capillary was used. The P/ACE instrument was

controlled automatically via an IBM-compatible personal computer with system Gold software. Data were collected with the P/ACE software system.

Reagents were obtained from Sigma (St. Louis, MO) and the water was obtained from Millipore (Bedford, MA) water purification system with at least 18.2 M resistance. Stock solutions were prepared from the reagents and pure water. The desired concentration was obtained from the stock solution before running the experiment. 1 N HCl was used to clean the capillary for each new capillary. 0.1N NaOH was used to generate the capillary before and after each run of the experiment. The solutions were filtered through the 0.45µm membrane filters.

四、Results and Discussion

Fig. 1 shows the chromatogram of DAS, DADS and DATS in 0.6M phosphate buffer system (pH=4.7) and acetonitrile (1:1) was added. Three major components were appeared in the three different elution time. While the buffer was adjusted at its pH=6.5 and 7.4, the results show the elution pattern of two out of three major components was overlapped in the chromatogram. Another organic modifier as methanol was adopted to increase the separation but the elution time is longer than the acetonitrile. Sodium cholate, Sodium dodecyl sulfate and cyclodextrin were used as surfactant to increase the resolution pattern. However, our results indicated that figure 1 is the best way to separate those three analytes.

Other parameters as temperature and applied voltage were studied in this research. Variation of voltage to change the electrical field can have the several effects (e.g. variation in the migration time of samples, EOF, peak area and joule heating). The joule heating that results from an increase in voltage might lead to changes in EOF, ion mobility, analyte diffusion and band broadening. The result shows that the migration time increase with the increasing the applied voltage. This effect in the retention time is due to the change in the mobility of analyte and EOF. Viscosity is a

function of time. As the temperature increases, the viscosity decreases. The electrophoretic mobility and EOF increase too. Some analytes may not be stable at higher temperatures and the variation of temperature may lead to conformational change. The results indicate that the elution time is increased with increasing the temperature. An increase in EOF and electrophoretic mobility with increasing temperature leads to a shorter retention time for this case.

In conclusion, to determine DAS, DADS and DATS separately can be used a phosphate buffer (pH=4.7) with acetonitrile (1:1).

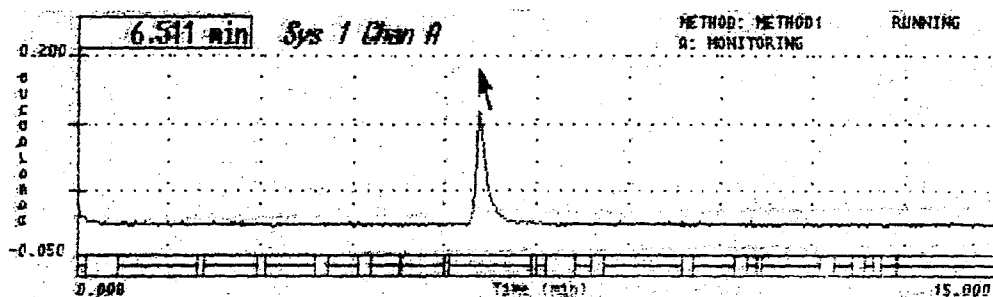
五、Acknowledgement

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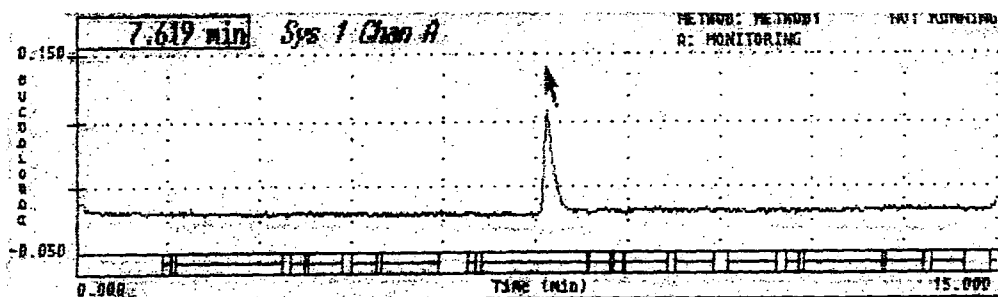
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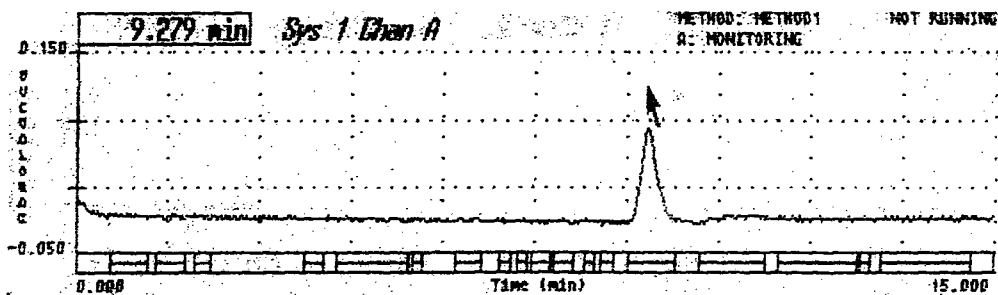
a.



b.



c.



圖一 磷酸緩衝溶液 (pH=4.7) 加入 acetonitrile (1:1, v/v)

(a) DAS (b) DADS (c) DATS