รายงานฉบับสมบูรณ์

โครงการ

การหาสภาวะที่เหมาะสมของการเตรียมแพคแคปปิลารีสำหรับใช้ในแคปปิลารีอิเล็กโทรโครมาโทรกราฟีและการวิเคราะห์โพลีไซคลิกอะโรเมติกไฮโดรคาร์บอน

Determination of Optimal Conditions of Preparation of Packed Capillaries for Use in Capillary Electrochromatography and the Analysis of Polycyclic Aromatic Hydrocarbons

โดย

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31 มิถุนายน 2546

สัญญาเลขที่ **TRG4580008**

รายงานวิจัยฉบับสมบูรณ์

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ผู้วิจัย

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สังกัด

ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย ที่ให้การสนับสนุนในด้านการเงินของงานวิจัยครั้งนี้ นอกจากนี้ ขอขอบคุณ รองศาสตราจารย์ ดร.เพริศพิชญ์ คณาธารณา อาจารย์ประจำภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ที่ให้ความกรุณาเป็นนักวิจัยพี่เลี้ยง และคำแนะนำต่าง ๆ ตลอดโครงการวิจัย

บทคัดย่อ

รหัสโครงการ TRG4580008

ชื่อโครงการ การหาสภาวะที่เหมาะสมของการเตรียมแพคแคปปิลารีสำหรับใช้ในแคปปิลารีอิเล็กโทรโครมาโทรกราฟีและการวิเคราะห์โพลีไซคลิกอะโรเมติกไฮโดรคาร์บอน

ชื่อนักวิจัย นายสมศักดิ์ ศิริไชย

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ระยะเวลาโครงการ 1 ปี

งานวิจัยนี้เป็นการศึกษาวิธีการเตรียม frit และการแพคในฟิวส์ซิลิก้าแคปปิลารีที่ใช้ในแคปปิลารีอิเล็กโทรโครมาโทรกราฟี วัสดุที่แพคในแคปปลารีคือนิวคลีโอซิล C_{18} Frit และการแพคทำบน แคปปิลารีซึ่งมีขนาดเส้นผ่านศูนย์กลางภายใน 100 ไมโครเมตร และมีเส้นผ่านศูนย์กลางภายนอก 363 ไมโครเมตร ประสิทธิภาพของแพคแคปปิลารีได้แสดงให้เห็นโดยใช้แยกสารไนโตรฟูเรน 2 ตัว และได้มีการศึกษาการแยกสารพวกโพลีไซคลิกอะโรเมิกไฮโดรคาร์บอนบนแพคแคปปิลารี และพบว่าผลของการเกิดฟองอากาศต่อการแยกเป็นปัญหาหลักภายใต้สภาวะที่ใช้

คำหลัก Electrochromatography, packed capillary, frit

Abstract

Project Code TRG4580008

Project Title Determination of Optimal Conditions of Preparation of Packed Capillaries for Use in Capillary Electrochromatography and the Analysis of Polycyclic Aromatic Hydrocarbons

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Project Period 1 Year

Methods for preparing frits and packing in fused-silica capillaries as used for capillary electrochromatography are investigated. Nucleosil C_{18} is used as packing material. The frits and packing are performed on fused-silica capillaries with an inner diameter of 100 μm and an outer diameter of 363 μm. The frits produced showed mechanical stability under moderate pressure and high permeability. The performance of manufactured packed capillaries is demonstrated by separating two nitrofurans. The application of separating PAHs is also investigated. The effect of bubble formation on separation is a main problem under condition used.

Keywords Electrochromatography, packed capillary, frit

1. Introduction

Capillary electrochromatography (CEC) introduced in 1974 by Pretorious and co-workers is a high performance chromatography technique that combines aspects of both capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) [1]. CEC has attracted immense attention over recent years [2-7], with several review articles describing the subject [8-10]. CEC differs from capillary zone electrophoresis (CZE) in that the capillary is packed with a stationary phase used in LC.

The separation of uncharged analytes is achieved on the basis of differential partitioning with a stationary phase. The buffer is electrically pumped and all analytes are carried through the capillary by electroosmotic flow. CEC has several advantages over HPLC since the velocity flow profile in the capillary is near plug-like. This leads to improved efficiency and resolution. Moreover, CEC uses much less mobile phase than standard column HPLC.

At present, the main problem in CEC is the frits [11-12] that retain the stationary phase in the capillary during analysis. The frits can be fabricated by means of a paste of silica and sodium silicate that is sintered, and a fined tapered end. The paste of silica and sodium silicate protocol is a simple protocol to obtain the frits formed at the end of the capillary. There are some disadvantages for this protocol. These include non-uniformity of the stationary phase surface caused by the sintering process [13-14], the formation of bubbles caused by insufficiently degassed mobile phases and structural inhomogeneities inside the outlet frit [11], and the fragility of the detection window. All drawbacks in the paste of silica and sodium silicate method can be solved using a fined tapered end method or fritless packed capillary [15]. Generally, the fined tapered end is made using a laser-based

micropipette puller that is not a basic device in laboratory. The taper shape and tip size would be varied by several parameters. These include incident laser power, laser spot size, pull strength and pull velocity [16].

The studies presented in this report are primarily focused on the determination of optimal conditions of preparation of packed capillaries for use in CEC and the analysis of polycyclic aromatic hydrocarbons (PAHs). More specifically the research is divided into three distinct sections:

- First, a simple method of the preparation of packed capillary is investigated. The challenging objectives in this stage are to develop and optimize procedures for the frit fabrication and the slurry-packed capillary by a simple device.
- Second, the packed capillary is characterized in terms of its operation and efficiency through the electrophoretic analysis of simple molecules.
- Finally, the packed capillary is used to study the feasibility of analysis of PAHs in real sample by investigating to a standard mixture of PAHs.

2. Experiment

2.1 Materials

HPLC-grade acetonitrile and AR-grade acetone were purchased from Mallinckrodt Baker (Phillipsburg, USA) and BDH (Dorset, UK). AR-grade Nitrofurantoin and Furazolidone were purchased from Sigma (St. Louis, USA). All PAHs standards were purchased from BDH (Dorset, UK). Nucleosil 100-3 C_{18} was obtained from Liquid-Chromatographie (Macherey-Nagel, Germany). Tris-Hydroxymethyl methylamine was purchased from Univar (Seven Hills, Australia). Fused-silica capillaries with a transparent UV coating were obtained from Polymicro Technologies (Phoenix, USA). A CEC conditioning syringe pump was purchased from MicroSolv Technology (Long Branch, USA). Mobile phases were prepared by mixing acetonitrile and 5 mM Tris. High resistivity (18 MΩ) deionized water was used. The mobile phase was sonicated for 1 hour and filtered through 0.45 μm pore disposable membrane filter (Merck, Dorset, UK) before introduction into capillaries.

A Hewlett-Packard ^{3D}capillary Electrophoresis System (**Figure 1**) (Hewlett-Packard, Germany) with a diode array UV detector was used to obtain all data. Data were collected on a HP Vectra VE, Pentium II personal computer using the Hewlett-Packard ^{3D}CE Chemstation software. Separation conditions were stated in each experiment.

2.2 Frit formation and capillary packing

The UV-transparent polyimide coated silica capillaries used had 363 μm external diameter and 100 μm internal diameter. The capillary was cut with the length of 33 cm by means of fused-silica cutter. The inlet side was repeatedly dipped into dry bare silica until the silica was trapped into the capillary for approximately 1 mm. Then, the heat was applied to

the trapped silica using a torch for 10 seconds. The outlet side was then immersed into a C_{18} slurry reservoir and the inlet side containing the frit was connected to a vacuum pump. To obtain C_{18} slurry, C_{18} was weighed out in a small vial. A 100 µL of acetone was then added into the vial, and sonicated for 2 minutes. After the capillary was fully filled with the slurry, the vial containing the slurry was removed and the vacuum pump was turned off (**Figure 2**). The MicroSolvCEC syringe pump that can provide the moderate pressure (500 psi) was then connected to the outlet side using a polyethylene tubing (**Figure 3**). Pressure in the capillary was gradually raised by employing the syringe pump. The capillary was kept at moderate pressure for 4 hours before releasing the pressure drop to zero.

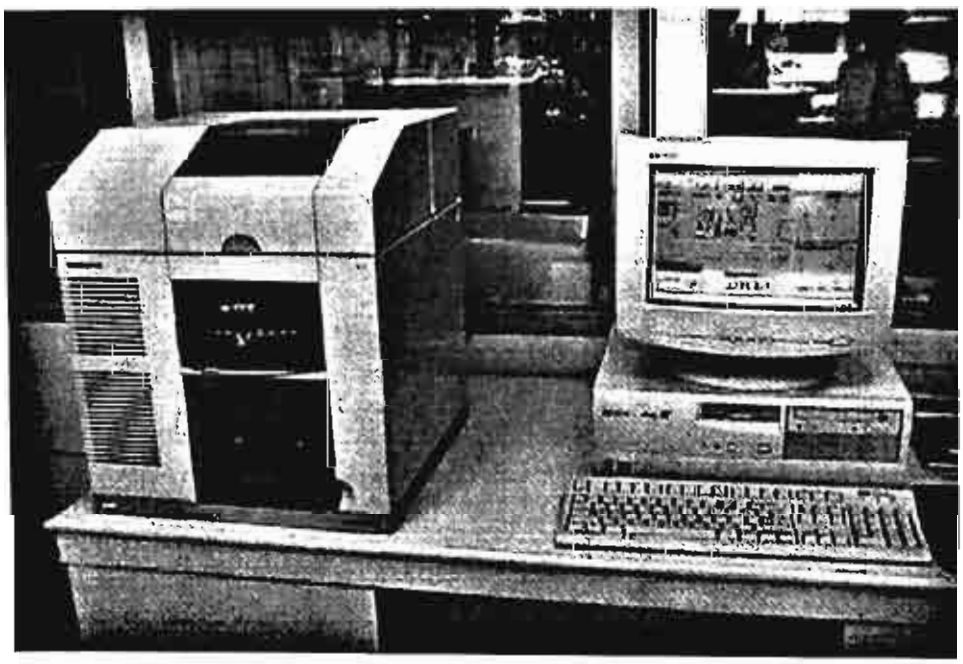


Figure 1 A Hewlette-Packard ^{3D}CE System

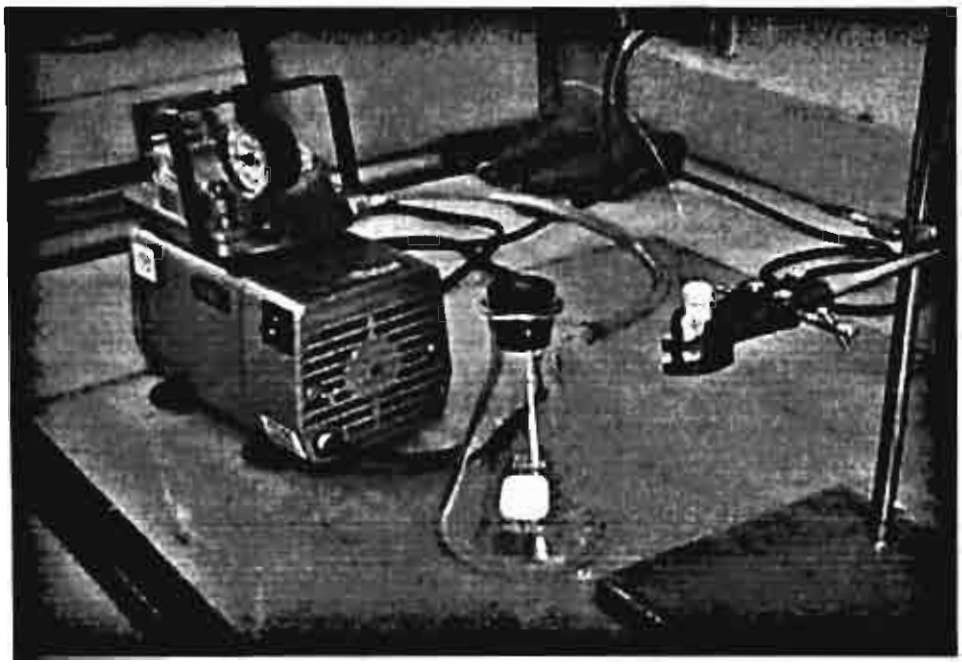


Figure 2 Illustration of the instrumental setup for the slurry introduction into the capillary

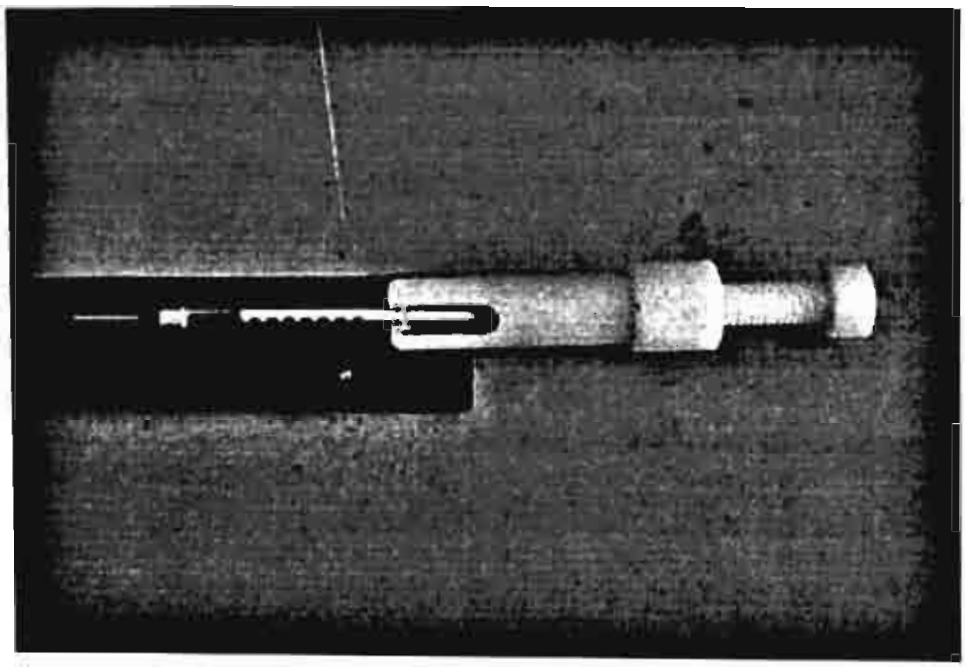


Figure 3 A MicroSolvCEC Syringe Pump

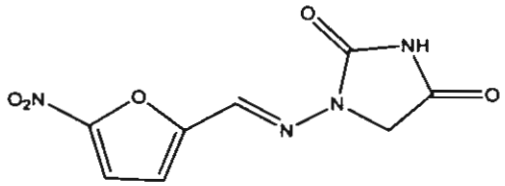
2.3 Relationship between the amount of C_{18} and the packing length

To obtain the relationship between the grams of C_{18}, the various grams of C_{18} was weighed out in the small vial, and 100 µL of acetone was added. The slurry was sonicated for 2 minutes before use. The slurry was introduced into the capillary with the length of 33 cm using the vacuum pump until the capillary was fully filled with the slurry. The slurry reservoir was removed and the vacuum pump was turned off. The syringe pump was connected to the outlet side of the capillary. Pressure in the capillary was slowly developed by tightening the handle on the syringe pump. The packing length was measured.

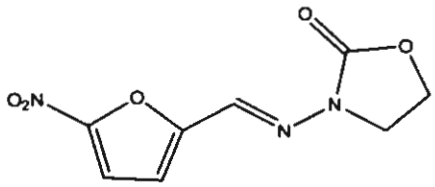
2.4 The performance of the packed capillary

The performance of manufactured CEC capillaries was characterized using the Hewlett-Packard ^{3D}CE discussed in section 2.1. Preliminary experiment was performed to investigate the behavior of the packed capillary during electrophoresis. For this purpose, a mixture of nitrofurans consisting nitrofurantoin and furazolidone was studied. The chemical structures of these compounds are presented in **Figure 4**. Before running experiment, the packed capillary was preconditioned. The syringe pump was slowly filled with a mobile phase consisting of acetonitrile and Tris-buffer, and then connected to the outlet of the capillary with polyethylene tubing. The handle on the syringe pump was tightened to generate a moderate pressure. The capillary was preconditioned for 2 hours. After 10 minutes when the air bubbles were completely removed, the pressure on the syringe pump was released by loosening the handle on the pump. After preconditioning, the packed capillary was installed in the cassette and then equilibrated. The equilibration of the packed capillary was performed by running the packed capillary on the ^{3D}CE instrument at a low voltage, 4 kV for 15 minutes

with the mobile phase. After that, the voltage was increased to the desired value of the method. The voltage injection at 5kV for 3 seconds was chosen for sample introduction.



Nitrofurantoin



Furazolidone

Figure 4 Chemical structures of Nitrofurantoin and Furazolidone

2.5 The study of the feasibility for applying the manufactured packed capillary to PAHs analysis

The purpose of studies in this section was to investigate the feasibility of the manufactured packed capillary by analyzing a standard mixture of PAHs by CEC technique. The effect of bubble formation on separation was also investigated.

3. Results and discussion

3.1 Frit formation and capillary packing

After fabricating the frit, the mobile phase (acetonitrile/5 mM Tris (pH 8.5) 70/30) was flushed through a capillary with one frit and no silica particles present using the MicroSolvCEC syringe pump. It was found that there was no increase in the backpressure observed as compared with the capillary without the frit. From the results, it can be concluded that the frit made by this procedure does not significantly increase the flow resistance of the system. Furthermore, the inlet frit can withstand a moderate pressure from the MicroSolvCEC syringe pump used during the capillary packing because no particles packing were found to pass through the frit pores during the packing procedure.

3.2 Relationship between the amount of C_{18} and the packing length

The relationship between the amount of the packing C_{18} and the packing length was investigated. To obtain the relationship, the weight of the packing C_{18} was varied (0.001-0.020 g.) in 100 µL filtered acetone so called slurry. The slurry was introduced into the capillaries that had length 33 cm by the vacuum pump. As soon as the capillaries were completely filled with the slurry, the vacuum pump was disconnected and the reservoir of the slurry was removed. The pressure was applied on the capillary by connecting the MicroSolvCEC syringe pump to the outlet side of the capillary with polyethylene tube and tightening the handle on the syringe pump. The capillary was left until the acetone was completely removed and no backpressure was observed, and the packing length was measured.

Figure 5 shows the relationship between the amount of packing C_{18} and the packing length. As can be seen, changes in the grams of packing C_{18} result in changes in the packing length in the capillaries. The packing length was observed to increase as the amount of the packing C_{18} in 100 µL was increased. It can be seen that the relationship between the amount of packing C18 and the packing length is linear with $R^2 = 0.9958$.

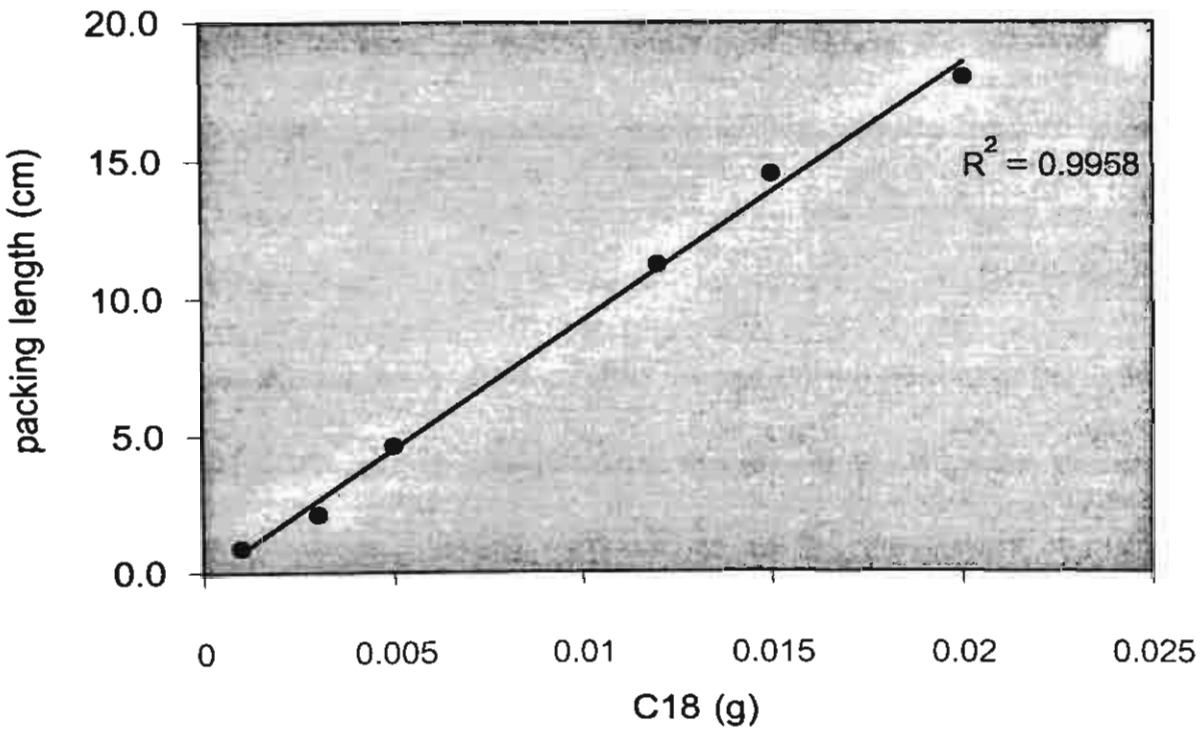


Figure 5 The relationship between grams of C_{18} and packing length

3.3 Optimization of the electric field strength during separation

As stated, the bubble formation is a practical problem in CEC. The bubble formation results in a very noisy baseline and spike peaks in the electrochromatogram. Furthermore, the bubbles cause interruption of electrical current and consequently of the electroosmotic flow leading to an abortion of the electrochromatographic process. At present, the bubble formation can be minimized by applying the pressure both inlet and outlet sides during separation. Since the CE instrument used in this project has no external pressure to apply at the inlet and outlet sides. To avoid the bubble formation, therefore, the optimization of the electric field strength used in the separation needs to be investigated. To obtain the optimized electric field strength, the current was observed at different voltages applied to the capillaries.

Figure 6 shows the influence of the applied voltage on the current of the system. As expected, an increased in the applied voltage causes the current of the system to increase. It can be seen that as the applied voltage increases to 14 kV, the current of the system decreases from about 11 μA to 3 μA after 8 minutes. In other words, when the applied voltage is greater than 14 kV the bubble formation becomes significant leading to interruption of electrical current. **Figure 7** illustrates the electrochromatograms at different voltages used to investigate. As expected, the instability of the current of the system causes noisy baseline.

The results of both studies suggest that the maximum of applied voltage should not be greater than 13 kV to avoid the bubble formation.