แผ่นเส้นใยอิเล็คโตรสปันนาโนพอลิอะคริโลไนไตรล์ที่มีการจัดเรียงตัว อย่างเป็นระเบียบ สำหรับการวิเคราะห์สีผสมอาหาร ด้วยเทคนิคโครมาโตกราฟีแบบชั้นบาง

THE ALIGNED ELECTROSPUN POLYACRYLONITRILE FIBER MATS USE AS ULTRATHIN-LAYER CHROMATOGRAPHY SUBSTRATES FOR FOOD DYES ANALYSIS

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บทคัดย่อ

แผ่นเส้นใยนาโนพอลิอะคริโลไนไตรล์ที่มีการจัดเรียงตัวอย่างเป็นระเบียบถูกพัฒนาขึ้นเพื่อปรับปรุงประสิทธิภาพการแยกสารของเฟสคงที่ในเทคนิคโครมาโตกราฟีแบบชั้นบาง แผ่นเส้นใยนาโนพอลิอะคริโลไนไตรล์ถูกเตรียมด้วยการละลายพอลิอะคริโลไนไตรล์ ความเข้มข้นร้อยละ 12 มวลต่อปริมาตร ในตัวทำละลายไดเมทิลฟอร์มาไมด์ สภาวะของกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตถูกตั้งค่าความความต่างศักย์ 16 กิโลโวลต์ ระยะทางในการเก็บเส้นใย 10 เซนติเมตร ความเร็วของตัวเก็บเส้นใยถูกควบคุมที่ 600 และ 3,300 รอบต่อนาที เพื่อให้ผลิตเส้นใยนาโนพอลิอะคริโลไนไตรล์ที่มีการจัดเรียงตัวอย่างไม่เป็นระเบียบ และเป็นระเบียบตามลำดับ จากการสังเกตลักษณะสัณฐานวิทยา พบว่าทั้งแผ่นเส้นใยนาโนพอลิอะคริโลไนไตรล์ที่มีการจัดเรียงตัวอย่างไม่เป็นระเบียบ และเป็นระเบียบมีพื้นผิวที่เรียบ โดยการจัดเรียงตัวที่เป็นระเบียบของแผ่นเส้นใยนาโนพอลิอะคริโลไนไตรล์ทำให้มีประสิทธิภาพในการแยกที่ดี โดยระยะการเคลื่อนที่ และเวลาที่ใช้ในการวิเคราะห์ที่ลดลงกว่าเมื่อเทียบกับแผ่นเส้นใยนาโนที่มีการจัดเรียงตัวอย่างไม่เป็นระเบียบ ดังนั้นแผ่นเส้นใยนาโนที่มีการจัดเรียงตัวอย่างเป็นระเบียบมีศักยภาพสำหรับประยุกต์ใช้เป็นเฟสคงที่ในเทคนิคโครมาโตกราฟีแบบชั้นบาง

คำสำคัญ: พอลิอะคริโลไนไตรล์ กระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิต เส้นใยที่มีการจัดเรียงตัวอย่างเป็นระเบียบ โครมาโตกราฟีแบบชั้นบาง สีผสมอาหาร

Abstract

The aligned electrospun polyacrylonitrile (PAN) fiber mats were developed by controlling the rotational speed of collector at 3,300 rpm to improve the separation efficiency of a stationary phase in ultra-thin layer chromatography. The electrospun PAN fiber mats were prepared by dissolving PAN at a concentration of 12% w/v in dimethylformamide. The electrospinning condition was set at an applied voltage of 16 kV and collection distance of 10 cm. The rotational speed of collector was controlled at 600 and 3,300 rpm to produce the non-aligned and the aligned electrospun PAN fiber mats, respectively. Based on morphological observation, both non-aligned and aligned electrospun PAN fiber mats had a smooth surface. The arrangement of the aligned electrospun PAN fiber mats was more orderly. This caused the aligned electrospun PAN fibers mats had higher separation efficiency with reduced migration distance, migration time and analysis time than the non-aligned electrospun PAN fiber mats. Thus, these fiber mats had the potential for use as the stationary phase of thin-layer chromatography.

Keywords: polyacrylonitrile, electrospinning, aligned fiber, thin-layer chromatography, food dye

Introduction

Thin-layer chromatography (TLC) is the technique for separation based on differences in solubility of compounds in stationary and mobile phases moving in a specific direction. The stationary phase for TLC is normally solid, gel or liquid coated on solid substrate and mobile phase is liquid or gas (Nabi & Khan, 2003; Sherma & Fried, 2003). TLC is widely used to separate compounds, confirm and identify component because of its simple technique, low cost, less time consumption for analysis, high sensitivity, and high efficiency for separating mixture (Striegel & Hill, 1996). This technique is used for various applications such as the food industry (Sherma, 2000) and pharmaceutical industry (Ferenczi et al., 2011). In the 1970s, it was developed high-performance TLC (HPTLC) by reducing plate dimension in stationary phase. The various advantages such as shorter analysis time, shorter migration distance, and smaller reagent and mobile phase were found in HPTLC technique (Fried & Sherma, 1994). Later in 2001, ultrathin–layer chromatography (UTLC) was developed from TLC and HPTLC plate to improve the efficiency of stationary phases such as sensitivity, analysis time and minimal solvent. UTLC has a monolithic or nanostructure stationary silica gel phase coating on a glass substrate. Its layer thickness is reduced to 10 µm from 100-250 µm of TLC. The separation on UTLC plates is faster analysis time and uses smaller reagent and sample volume than that on HPTLC plates (Hauck et al., 2001).

Electrospinning process was first applied to prepare UTLC stationary phase. This process can prepare faster and inexpensive UTLC plate (Clark & Olesik, 2009;

Beilke et al., 2013; Kampalanonwat et al., 2013). It is used to produce ultrafine fibers with diameters in the nanometer or the micrometer range. In electrospinning process, a polymer solution held by surface tension at the end of a capillary tube is subjected to an electric field. The charge is induced on the liquid surface by an electric field. When an electric field is increased, the liquid at the tip of the capillary tube elongates to form a conical shape. Then, the liquid is ejected from the tip to collecting metal screen and travel through the air when the electric force overcomes the surface tension force. Finally, the fiber is laid on the collecting metal screen and the solvent is evaporated during ejecting (Doshi & Reneker, 1995; Reznik et al., 2004; Reneker & Yarin, 2008). Many polymers have been used in electrospinning such as Polyacrylonitrile (PAN) (Kampalanonwat et al., 2013), Polyvinyl alcohol (Koski et al., 2004), cellulose acetate (Rojanarata et al., 2013) and Polylactic acid (Li et al., 2006).

In this work, PAN was used to prepare a stationary phase for UTLC because it has strong chemical bond between the nitrile groups. Thus, it is resistant to most organic solvent, has good thermal stability and has potential for use as a stationary phase (Beilke et al., 2013; Kampalanonwat et al., 2013). To improve the separation efficiency of food dyes on the stationary phase, the aligned electro spun PAN fiber mats were prepared by controlling the rotational speed of collector at 3,300 rpm. The morphological appearance of the aligned electrospun PAN fiber mats was characterized by Scanning Electron Microscope (SEM). Two water-soluble food dyes including erythrosine (E127) and brilliant blue FCF (E133) were used to study the separation efficiency of the aligned electrospun PAN fiber mats in comparison with the non-aligned electrospun PAN fiber mats.

Materials and Methods

1. Materials

Polyacrylonitrile (PAN, M_W=55,500 Da) was obtained from Thai Acrylic Fiber, Bangkok, Thailand. The PAN contained 91.40 wt% acrylonitrile monomer (CH_2=CHCN) and 8.60 wt% methyl acrylate comonomer (CH_2=$CHCOOCH_3$). Dimethylformamide (DMF) was purchased from Labscan Asia, (Thailand). Methanol and toluene were purchased from Merck (Germany). Heptane was purchased from RCI Labscan (Thailand). Two water-soluble food dyes including erythrosine (E127) and brilliant blue FCF (E133) were obtained from Adinop Co., Ltd (Thailand). The TLC plate silica gel 60 F254 was purchased from Merck (Germany).

2. Preparation of non-aligned and aligned electrospun PAN fiber mats

First, 12% w/v PAN solution was prepared by dissolving 1.20 g of PAN in 10 mL of DMF. This solution was then added into a 10-mL glass syringe attached with a stainless steel needle. The needle was connected to the high voltage generator, operating in the positive DC mode. The applied voltage was fixed at 16 kV. A tip to collector distance was set at 10 cm. The arrangement of fibers was controlled by the rotational speeds of the collector. The rotational speeds of collector were controlled at 600 and 3,300 rpm to produce the non-aligned and the aligned electrospun PAN fiber mats, respectively. The electrospun PAN fiber mats were collected continuously for 90 minutes.

3. Morphology of non-aligned and aligned electrospun PAN fiber mats

The morphological appearance of the non-aligned and the aligned electrospun PAN fiber mats was characterized by a LEO 1450 VP SEM with operating voltage used 15 kV. Before morphological observation, each sample was coated with a thin layer of gold using a Polaron SC-7620 sputtering device. The fiber diameters were measured directly from SEM images using the SemAfore 5.21 software.

4. Preparation of standard solution and mobile phase

25 mg of food dyes (E127 and E133) was firstly dissolved in 10 mL of methanol. These solutions were further used for study the separation efficiency of the non-aligned and the aligned electrospun PAN fiber mats.

The various proportions of methanol and toluene (1: 9 to 9: 1 v/v) were varied to achieve the optimal mobile phase. The optimal mobile phase used in this study was methanol and toluene (2.5: 7.5 v/v) since this mobile phase showed the greatest separation of food dyes on the electrospun PAN fiber mats.

5. Chromatography

The pure E127, pure E133 and mixture of E127 and E133 were spotted on the bottom of the non-aligned and the aligned electrospun PAN fiber mats (size 3x4 cm). About 3 mL of the mobile phase (methanol and toluene (2.5: 7.5 v/v)) was used for each development. The development chamber was a homemade chamber with the following dimentions 50 mm x 10 mm x 60 mm. The migration distance was fixed at 30 mm from the lower plate edge. After chromatography, the plates were air dried and then documented by the high-resolution scanner.

Results and Discussion

1. Morphology of non-aligned and aligned electrospun PAN fiber mats

The electrospinning is an effective technique for producing a fiber with a diameter in the nanometer range. The condition used for the preparation of the electrospun PAN fiber mats was 12% w/v PAN in DMF, applied voltage of 16 kV, collection distance of 10 cm. The rotational speed of collector was controlled at 600 and 3,300 rpm to produce the non-aligned and the aligned electrospun PAN fiber mats, respectively. The morphology of the non-aligned and the aligned electrospun PAN fiber mats is shown in Figure 1. The results showed that both the non-aligned and the aligned electrospun PAN fibers had a smooth surface. The average diameters of the non-aligned and the aligned electrospun PAN fibers were 286±9 and 235±10 nm, respectively. Clearly, the arrangement of the aligned electrospun PAN fiber mats were more aligned than that of the non-aligned electrospun PAN fiber mats. Thus, the rotational speed of collector can control the arrangement of the fibers. Thickness of the obtained electrospun PAN fiber mats was about 200 µm.

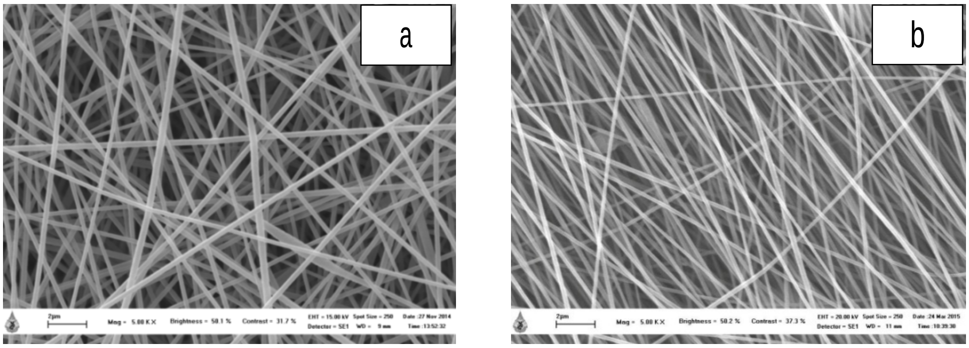

Figure 1 Selected SEM images of
a) non-aligned b) aligned electrospun PAN fiber mats

2. Separation of water-soluble food dyes

The water-soluble food dyes including erythrosine (E127) and brilliant blue FCF (E133) were used to study the qualitative separation of the non-aligned and the aligned electrospun PAN fiber mats as stationary phases in thin layer chromatography. The mobile phase used to study the qualitative separation of the electrospun PAN fiber mats was a mixture of methanol and toluene (2.5: 7.5 v/v).

The position of the spot in stationary phase was characterized by the retention factor. The retention factor (hR_f) is defined as equation (1) where Z_s is distance that the color traveled, measured from center of the band of color to the point where the food color was applied and Z_f is total distance that solvent traveled:

$$hR_f = \frac{Z_s}{Z_f} \times 100 \qquad \text{.................................(1)}$$

Comparison between the non-aligned and the aligned electrospun PAN fiber mats, the hR_f range of the aligned electrospun PAN fiber mats (hR_f 61-83) was lower than that of the non-aligned electrospun PAN fiber mats (hR_f 42-74). The pure colors (E127 and E133) on the electrospun PAN fiber mats were used as the standard for comparing with the mixed color. The hR_f of mixed color (E127 and E133) on the aligned electrospun PAN fiber was 83±3 and 62±3 which were similar to the hR_f of the pure colors (E127; 83±4 and E133; 61±4). Beilke et al. (2013) compared the separation efficiency of a mixture of ß-blockers and steroidal compounds on the aligned electrospun PAN fiber mats (AE-UTLC) and the non-aligned electrospun PAN fiber mats (E-UTLC). The results showed that the range hR_f of AE-UTLC (hR_f 27-56) was lower than that of E-UTLC (hR_f 6-53) (Beilke et al., 2013).

To determine the separation efficiency of the stationary phase, the resolution (R) was calculated. The R is a qualitative analysis. The R values were used to measure how well two spots can be separated in a stationary phase. It was defined as the difference in retention of two adjacent spots 1 and 2, respectively (D_{R1} and D_{R2}), divided by the combined widths of two adjacent spot 1 and 2, respectively (w_1 and w_2). The R value was calculated using the following equation (2).

$$R = \frac{2(D_{R2}+D_{R1})}{(w_2+w_1)} \quad \text{..............................(2)}$$

The relative standard deviation (%RSD) is the measurement of the deviation of various terms from their averages in an observation as shown in equation (3). Where s is the standard deviation, and $\bar{x}$ is the mean.

$$\%RSD = \frac{s}{\bar{x}}\, x\, 100 \quad \text{..............................(3)}$$

Table 1 The resolutions between two colors of mixed food dyes on (a) non-aligned and (b) aligned electrospun PAN fiber mats (n=5)

Stationary phase	Mixed color	
	Resolution (R)	%RSD
(a) Non-aligned	0.3	15.4
(b) Aligned	1.3	8.2

From Table 1, the mean resolution of the aligned electrospun PAN fiber mats was 1.3, while the mean resolution of the non-aligned electrospun PAN fiber mats was 0.3. The aligned electrospun PAN fiber mats had higher resolution than the non-aligned electrospun PAN fiber mats. The aligned electrospun PAN fiber mats had high separation efficiency because the resolution value of these fiber mats was more than 1. Kampalanonwat et al. (2013) compared the mean resolution of 5 preservatives saparation between the electrospun PAN nanofiber phases with 10% UV_{254} and HPTLC plates silica gel CN. The mean resolution range of electrospun PAN nanofiber phases with 10% UV_{254} was 1.0 to 1.8, while the mean resolution range of HPTLC plates silica gel CN was 0.5 to 1.5. Thus, the electrospun PAN nanofiber phases showed better separation of preservatives than HPTLC plates silica gel CN (Kampalanonwat et al., 2013).

In TLC, the number of theoretical plates (N) is used to determine the performance and the efficiency of the stationary phase. When comparing between the non-aligned and the aligned electrospun PAN fiber mats (Table 2), the results showed that the aligned electrospun PAN fiber mats had higher plate number in both the pure color and the mixed color cases. Thus, the separation efficiency of the aligned electrospun PAN fiber mats was higher than that of the non-aligned electrospun PAN fiber mats. Because the higher plate number resulted in the reduced migration time and migration distance. Kampalanonwat et al. (2013) compared the electrospun nanofiber phases with UV_{254} indicator with HPTLC plates silica gel CN. The electrospun nanofiber phases with UV_{254} indicator showed higher plate number than HPTLC plates silica gel CN resulting in the reduced migration time and migration distance of the electrospun nanofiber phases with UV_{254} indicator (Kampalanonwat et al., 2013).

Table 2 The number of plates (N) values for food dyes on (a) non-aligned and (b) aligned electrospun PAN fiber mats (n=5)

Food dyes		**(a) Non-aligned**		**(b) Aligned**	
		N	**%RSD**	**N**	**%RSD**
Pure color	E127	1278	7	1738	7
	E133	1328	14	3377	13
Mixed color	E127	1765	14	2019	7
	E133	2184	6	3919	13

3. Comparison of mobile phase velocities

The mobile phase velocities were compared between the non-aligned electrospun PAN fiber mats, the aligned electrospun PAN fiber mats and the TLC plate silica gel 60 F_{254}. The Lucas-Washburn equation (4) was used to describe the capillary flow through porous media. When mobile phase velocities were increased, the analysis time was decreased while the efficiency was increased.

$$Z_f^2 = \frac{\gamma R t \cos\theta}{2\eta} \quad \text{..............................(4)}$$

In this equation, Z_f is the distance traveled by the solvent front, t is the corresponding time, γ and η are the surface tension and viscosity of the mobile phase, θ is the contact angle of the mobile phase with the stationary phase, and R is the effective pore radius. From the Lucas–Washburn equation, the porous media was measured by using heptane for nonpolar solvents. The velocity constant (k) is often used to describe mobile phase transport in TLC and replaced the term $\gamma R/2\eta$. This factor is used to describe mobile phase velocity in stationary phase. k is related to Z_f and t according to equation (5).

$$Z_f^2 = kt \qquad (5)$$

From Figure 2, the aligned electrospun PAN fiber mats showed the fastest mobile phase migration. Since the average fiber diameter of the aligned electrospun PAN fiber mats was smaller than that of the non-aligned electrospun PAN fibers. Moreover, the alignment of the aligned electrospun PAN fibers could increase the mobile phase velocities. Beilke et al. (2013) compared the mobile phase velocities of AE-UTLC plates, E-UTLC plates and commercial cyano TLC plates using heptane as the mobile phase. The AE-UTLC plates showed faster mobile phase velocities than others (Beike et al. 2013).

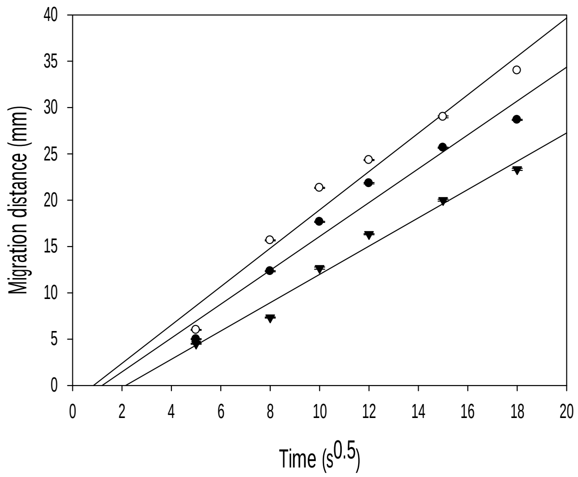

Figure 2 The comparison of mobile phase velocities of (•) non-aligned, (○) aligned electrospun PAN fiber mats and (▼) TLC plate silica gel 60 F_{254}

Conclusion

The electrospun PAN fiber mats were successfully fabricated by electro spinning for use as a stationary phase in ultrathin-layer chromatography (UTLC). The development of the aligned electrospun PAN fiber mats as a stationary phase to improve separation efficiency was studied. Both the non-aligned and the aligned electrospun PAN fiber mats had a smooth surface. The average diameter of the non-aligned was higher than that of the aligned electrospun fibers. The aligned electrospun PAN fibers mats showed better values of hR_f, resolution, plate number and mobile phase velocities. Thus, the aligned electrospun PAN fibers mats had higher separation efficiency with reduced migration distance, migration time, and analysis time than the non-aligned electrospun PAN fibers mats and could be used as stationary phase for food dyes analysis.

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References

Beilke, M.C., Zewe, J.W., Clark, J.E., & Olesik, S.V. (2013). Aligned electrospun nanofibers for ultra–thin layer chromatography. **Analytical Chimica Acta**, 761, 201-208.

Clark, J.E., & Olesik, S.V. (2009). Technique for ultrathin layer chromatography using an electrospun, nanofibrous stationary phase. **Analytical Chemistry**, 81, 4121–4130.

Doshi, J., & Reneker, D.H. (1995). Electrospinning process and applications of electrospun fibers. **Journal of Electrostatics**, 35, 151-60.

Ferenczi, F.K., Vègh, Z., & Renger, B. (2011). Impurity profiling of pharmaceuticals by thin-layer chromatography. **Journal of Chromatography A**, 1218, 2722-2731.

Fried, B., & Sherma, J. (1994). **Thin-Layer Chromatography: Techniques and Applications**. New York: Marcel Dekker, Inc.

Hauck, H.E., Bund, O., Fischer, W., & Schulz, M. (2001). Ultra-thin layer chromatography (UTLC)—A new dimension in thin-layer chromatography. **Journal of Planar Chromatography**, 14, 234-236.

Kampalanonwat, P., Supaphol, P., & Morlock, G.E. (2013). Electrospun nanofiber layers with incorporated photoluminescence indicator for chromatography and detection of ultraviolet-active compounds. **Journal of Chromatography A**, 1299, 110-117.

Koski, A., Yim, K., & Shivkumar, S. (2004). Effect of molecular weight on fibrous PVA produced by electrospinning. **Materials Letters**, 58, 493-497.

Li, D., Frey, M.W., & Baeumner, A.J. (2006). Electrospun polylactic acid nanofiber membranes as substrates for biosensor assemblies. **Journal of Membrane Science**, 279, 354-363.

Nabi, S.A., & Khan, M.A. (2003). Selective TLC separation of lysine and threonine in pharmaceutical preparations. **Acta Chromatographica**, 13, 161–171.

Reneker, D.H., & Yarin, A.L. (2008). Electrospinning jets and polymer nanofibers. **Polymer**, 49, 2387-2425.

Reznik, S.N., Yarin, A.L., Theron, A., & Zussman, E. (2004). Transient and steady shapes of droplets attached to a surface in a strong electric field. **Journal of Fluid Mechanics**, 516, 349-377.

Rojanarata, T., Plianwong, S., Su-uta, K, Opanasopit, P. & Ngawhirunpat, T. (2013). Electrospun cellulose acetate nanofibers as thin layer chromatographic media for eco-friendly screening of steroids adulterated in traditional medicine and nutraceutical products. **Talanta**, 115, 208-213.

Sherma, J. (2000). Thin-layer chromatography in food and agricultural analysis. **Journal of Chromatography A**, 880, 129-147.

Sherma, J. & Fried B. (2003). **Handbook of Thin-Layer Chromatography**. 3rd ed. New York: Marcel Dekker, Inc.

Striegel, M.F., & Hill, J. (1996). **Thin-layer Chromatography for Binding Media Analysis (Tools for Conservation)**. Los Angeles: The Getty Conservation Institute.