# CHAPTER VI

# ELECTROSPUN NANOFIBERS WITH FLUORESCENCE INDICATOR $UV_{254}$ FOR DETECTION OF UV-ACTIVE COMPOUNDS

## 6.1 Abstract

The development and application of electrospun polycrylonitrile nanofiber phases for ultra-thin layer chromatography (UTLC) are described. The devices use a nanofibrous stationary phase with fiber diameters of ~150–225 nm and thickness of ~25μm. Separations of mixtures of water-soluble food dyes were performed to illustrate the capabilities of these UTLC media. Analyses of the retention properties of the individual preservatives compounds are also studied by using the e-spun PAN nanofiber phase with fluorescence indicator $UV_{254}$. The complete analyses were found to require less development time and solvent than typical TLC methods. The efficiency of the separations was substantially improved compared to that determined using commercial phases.

(**Key-words**: thin layer chromatography, $UV_{254}$ indicator, separation of preservatives compounds, Nano-stationary phase, electrospinning)

## 6.2 Introduction

Thin layer chromatography or planar chromatographic technique was developed in the 1950s and was widely used for a variety of applications including identification of drugs and toxic substances in biological fluids, monitoring water supplies for pesticides, analysis of pharmaceutical products, and evaluation of the flavor potential of plant materials [1]. Normal phase silica gel is the best established and most widely used stationary phase [2. 3]. The most significant change came in the 1970s with the introduction of high performance TLC plates. Compared to TLC plates, HPTLC plates have thinner layers containing a smaller size of particle, providing shorter migrations distances, faster separations, and lower reagent and mobile phase consumption [2, 3].

Ultrathin layer chromatographic (UTLC) plates were introduced in 2001 to improve the efficiency of TLC and HPTLC plates in terms of sensitivity, analysis times and amount of consumables [4, 5]. UTLC plates are made by coating a glass substrate with a monolithic silica gel, creating a 10 μm thick sorbent layer characterized by 1–2 μm macropores and 3–4 nm mesopores. Separations on UTLC plates are faster and require smaller reagent and sample volumes than HPTLC and TLC. In most cases, UTLC plates have a lower limit of detection; however, they also exhibit lower resolution due to shorter development lengths and lower available specific surface area [5]. UTLC plates have been shown to provide a better interface in coupled TLC-mass spectrometry, where the thinner adsorbent layer on UTLC plates improved the sensitivity of TLC atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry (TLC-MALDI-MS) by 10–100 times over HPTLC plates [6].

Recently,[7] electrospinning has been shown to be an effective method for creating a electrospun polyacrylonitrile (PAN) nanofirous stationary phase for UTLC. Binder materials may have a negative impact on the chromatographic efficiency of TLC devices due to the introduction of heterogeneous interaction sites. Electrospinning of the stationary phase in UTLC allows the production of binder-free UTLC plates and gives the scientist control of stationary phase mat thicknesses

and chemical functionality present using a minimal amount of materials (~1mL of polymer solution)[8, 9].

Since most substances separated on modified plates, (Diol-,$NH_2$ , and CN-) are colorless, the majority of modified silica plates contain the blue fluorescent, acid-stable UV indicator $F_{254}s$ [10]. Samples that absorb shortwave UV at 254 nm are detected due to fluorescence quenching. In this work, a manganese-activated zinc silicate was used as fluorescent indicator ($UV_{254}$) by mixing directly into polyacrylonitrile solution prior electrospinning process [11]. The e-spun PAN nanofiber phase with $UV_{254}$ was used for study the separation of 7 preservatives and beverage sample and compared with a commercial silica TLC plate.

## 6.3 Experimental Details

### 6.3.1 Materials

Commercially-available PAN (weight-average molecular weight ≈ 55,500 Da, weight composition of methyl acrylate comonomer =8.6%; Thai Acrylic Fibre, Co., Ltd., Thailand) were used to prepare the e-spun PAN nanofiber phase by electrospinning process. Dimethylformamide (DMF, research grade of 99.98% purity; Sigma Aldrich) was used as the solvent of PAN solution. MeOH (gradient grade >99.8% for GC) was purchased from Sigma Aldrich. Toluene (SupraSolv®) was purchased from Merck (Darmstadt, Germany). Ammonium hydroxide ($NH_3$ 25%) was analytical grade and obtained from VWR (Darmstadt, Germany). For initial studied, 5 water-soluble food dyes were analyzed which are tartrazine (E102), quinoline yellow (E104), alpha-naphthol orange (E111), erythosine (E127), and brilliant blue FCF (E133). Fluorescent indicator $UV_{254}$ was supplied from Macherey & Nagel (Dueren, Germany). Acetonitrile (Chromasolv® for HPLC) were purchased from Sigma-Aldrich. HPTLC plate silica gel 60 and cyano-modified TLC plate (supplied by Merck) were used for comparison experiment. The analytes were 7 preservatives which are sorbic acid (So; E200), benzoic acid (B; E210), 4-hydroxybenzoic acid (pHB), methyl 4-hydroxybenzoate (ME; E218), ethyl 4-hydroxybenzoate (EE; E214), propyl 4-hydroxybenzoate (PE; E216) and butyl 4-

hydroxybenzoate (BE). The structures of 7 preservatives compound were shown in Figure 6.1.

6.3.2 Electrospinning

A 10-mL glass syringe containing 5 mL of the PAN in DMF solution, attached with a stainless steel needle with an outer diameter 0.91 mm. The needle was connected to a high-voltage generator (Model ES30P-5W; Gamma High Voltage, FL, USA), operating in the positive DC mode. A tip-to-target distance of 10 cm was used. A rotating collector covered with clean aluminum foil was used to collect the electrospun products. The electrical potential was vary in range of 16-20 kV. The e-spun PAN nanofiber phases were collected continuously for 90 minutes. Prior to further uses, these fiber mats were placed *in vacuo* at room temperature (25 ± 1 °C) to remove as much solvent from them as possible [12].

Scanning Electron Microscopy observations were done with a Leica-Zeiss LEO 440 Microscope. Each specimen was coated with a thin layer of platinum prior to the SEM observation. The diameters of the individual fiber segments within each specimen were measured directly from the SEM images using ImageJ software. No less than fifty diameters were determined on different fiber segments and the average value was calculated.

After the electrospining process, the aluminum foil was cut into rectangular pieces approximately 3 cm x 4 cm. To obtain the uniform stationary phase in thickness, the plate was cut from middle part of the aluminum foil.

6.3.3 Standard Solutions

The 5 water-soluble food dyes were weighed (25 mg each of E127 and E133; 50mg each of E104, E111, and E102) and dissolved in 10 mL of methanol. The mixture solution was used for all experimental.

For the preservatives, dissolve the 7 preservations individually of 30 mg of benzoic acid and 2 mg each of sorbic acid, 4-hydroxybenzoic acid, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate and butyl 4-hydroxybenzoate in 1mL of ethanol.

6.3.4 Extraction of Samples

Liquid-liquid extraction technique was used to extract preservative compounds and concentrated them. 5 mL of beverage sample was put into 100 mL separating funnel. Added 2 mL of 20% sulfuric acid to protonate all acid components and became nonpolar ready to be extracted with nonpolar phase. Pour 20 mL of mixture of petroleum ether/methyl tert-butyl ether (1:1 v/v) into the funnel. Shake the separating funnel and drain only the lower layer into a beaker. Repeat with pouring 20 mL of the mixture. After that, save and combine the extracts after each extraction. Dry the solution by using the Rotary Evaporator. Re-dissolve the extracted with 1 mL of ethanol.

6.3.5 Application

The mixture of water-soluble food dyes solution was printed on the plate with the PRINTER as 3 mm bands (width in range of 0.1 -0.3mm) allowing maximal 5 tracks to be applied on the e-spun PAN nanofiber phase of 3 × 4 cm (distance from lower edge 5 mm, distance from the left side 5 mm, distance between bands 1.5 mm).

For the preservatives, each of solutions were spoted with the Automatic TLC Sampler 4 (ATS4, CAMAG, Muttenz, Switzerland) as ~1 mm in diameter for 8 tracks (distance from lower edge 5 mm, distance from the left side 5 mm, distance between spots 3 mm). Volume of 10 nL of each standard solution were applied on the plate.

6.3.6 Chromatography

Development of water-soluble food dyes solution on the e-spun PAN nanofiber phase was performed in the homemade chamber (4.5×1.5×5.5 cm) with a mixture of methanol, toluene and ammonium hydroxide 25% (40:57:3, v/v/v). The migration distance was 30 mm from the lower plate edge and the migration time was 8 min. After that, the e-spun PAN nanofiber phase was dried in a flow of air followed for 10 second. For documentation, the plates were documented by using the high resolution scanner (CanoScan 9000F; Canon). The Sorbflil TLC Video densitometer was used for evaluate spot areas in tracks on a TLC plate image with construction of a chromatogram (analog curve) on the deviation of track Intensity

from background Intensity and the subsequent quantitative evaluation of the chromatogram.

For the preservatives, the plate was developed with 1 mL of a mixture of water and acetonitrile (65:35) in 0.1M *tetra*-n-*butyl*-ammonium phosphate in aqueous solution. The migration distance was 40 mm from the lower plate edge and the migration time was about 12 min. The plate drying in a flow of air followed for 10 seconds.

Plate images were documented by DigiStore2 Documentation System (CAMAG) consisting of the illuminator Reprostar 3 and the Baumer optronic DXA252 digital camera.

## 6.4 Results and Discussions

### 6.4.1 Characterization of e-spun PAN with/without $UV_{254}$ Indicator

The morphology and size of the e-spun nanofibers depend on a number of factors, such as viscosity of polymer solution, evaporation rate of the solvent, electrical potential, and collection distance [13]. To obtain the optimum parameters, the concentration of polymer solutions were vary in range of 10-15% PAN, the electric field from 15-20 kV and collection distance from 5-20 cm. The selected conditions used for further studies were 12% of PAN in DMF, 16kV/10cm. The cross-sections of the neat PAN fibers were round and their surfaces were smooth. The diameters of these fibers were uniform with diameter of 224±65 nm [12, 14].

The resulting electrospun plates are mechanically stable and can be handled in the same manner as commercially available TLC devices. Most if not all commercially available TLC devices use a binder to ensure that the stationary phase particles remain attached to the substrate. Often times the presence of this binder limits the chromatographic performance of the devices. The electrospun PAN UTLC devices use no binder and thus are not limited by the chromatographic performance of a given binder.

The separation of preservatives compounds on e-spun PAN nanofiber phase and Nano-SIL CN cyano-modified silica are compared later. Since most of

preservatives compound are colorless and not visualized under UV light, the TLC device should fluoresce. Therefore, a manganese-activated zinc silicate was used as Fluorescent indicator ($UV_{254}$) with absorption maximum at 254 nm (green fluorescence). The amount of the fluorescent indicator was varying at 1, 5 and 10% $w/w_{of\ PAN}$ in PAN solution prior electrospinning. The sizes of individual fibers at different conditions were summarized in Table 6.1. The selected conditions used for further studies were 12% PAN with 10% fluorescent indicator $UV_{254}$, 18kV/10cm which is shown in Figure 6.2.

The thickness of the nanofiber phase was analyzed by placing the plate on a vertical sample holder to expose the cut edge. Images were taken and analyzed by measuring directly with ImageJ software. Figure 6.3 shows the variation of stationary phase thickness with electrospinning time. An approximate linear relationship is observed between electrospinning time and film thickness. TLC plates that were electrospun for 120 minutes was also examined but was found to have polymer beads present along with the nanofibers. This mixed morphology is not desirable for chromatography and thus only plates fabricated with times of 90 minutes with thickness of ~25 μm were used for analysis.

6.4.2 Separation of Water-soluble Food Dyes: Initial Study

A set of five water-soluble food dyes was used as a qualitative test set as an initial verification that thin layer chromatography was possible with electrospun fibers. The following dyes were examined: tartrazine (E102), quinoline yellow (E104), alpha-naphthol orange (E111), erythosine (E127), and brilliant blue FCF (E133). The retardation factor was calculated using Eq. (1) where $Z_s$ is the distance travelled by the analyte spot and $Z_f$ is the distance travelled by the solvent front:

$$hR_F = \frac{Z_s}{Z_f} \times 100 \qquad (1)$$

The retardation factors and standard deviation for replicate studies with, n = 5, for each laser dye are shown in Table 6.2. The mobile phase used for these studies was a mixture of methanol, toluene and ammonium hydroxide 25%

(40:57:3, v/v). Capillary action of the solvent through the electropun fibers was observed as well as different retardation factors for the dyes.

In TLC, plate number, N, is directly proportional to the square of the ratio of analyte (solute) migration distance, $Z_s$, to spot width, w (Eq. (2))[15].

$$N = 16\left(\frac{Z_s}{w}\right)^2 \qquad (2)$$

The commercially available silica plate was also used for comparison but this plate could not separate the set of 5 dyes under the same mobile phase system. **Figure 6.4** is a chromatogram of the separation of 5 water-soluble food dyes on the e-spun PAN nanofiber phase.

Although both the commercial silica stationary phase and the electrospun polyacrylonitrile stationary phase are polar, differences between their chemical specificity obviously result in different retention behavior for identical analytes. These results show that the electrospun devices are feasible for thin layer chromatography.

6.4.3 Analysis of preservatives compound

Polar nitrile stationary phases are well suited for the analysis of preservatives compounds. Both polar and nonpolar mobile phases have been used with nitrile stationary phases to effectively separate preservatives. A chromatogram of the preservatives on the e-spun PAN nanofiber phase with $UV_{254}$ was shown in **Figure 6.5**. The 8 tracks on the nanofiber phase are sorbic acid (So; E200), benzoic acid (B; E210), 4-hydroxybenzoic acid (pHB), methyl 4-hydroxybenzoate (ME; E218), ethyl 4-hydroxybenzoate (EE; E214), propyl 4-hydroxybenzoate (PE; E216) and butyl 4-hydroxybenzoate (BE) and beverage sample, respectively. The mobile phase used for these studies was 1 mL of a mixture of water and acetonitrile (65:35) in 0.1M *tetra*-n-*butyl*-ammonium phosphate in aqueous solution. The $hR_f$ values for each of the preservatives were shown in **Table 6.3.**

To ensure the accuracy of quantitative analysis, the *R* value (Fig. 1) of the analyte peak with the adjacent peak must be larger than 1, unless otherwise specified. The *R* value is calculated by using the following equation;

$$R = \frac{2(D_{R2}-D_{R1})}{(w_2+w_1)} \quad (3)$$

where $D_{R1}$ and $D_{R2}$ = the retention distance of two adjacent peaks 1 and 2, respectively,

$w_1$ and $w_2$ = the widths of two adjacent peaks 1 and 2, respectively.

The resolution of separations are shown in Table 6.4

6.4.4 Comparison of Mobile Phase Velocities

One of the key advantages of the e-spun TLC plate is the decrease in immigration time of analysis due to faster mobile phase transport. The e-pun PAN nanofiber phase demonstrate faster mobile phase transport than commercial silica-based TLC devices.[8] This allows for a decrease in analysis time as well as a decrease in the volume of solvent needed to perform analyses, also while increasing the efficiency of the separations. Figure 6.6 shows a comparison in the mobile phase velocities of two commercial silica TLC plates and the e-pun PAN nanofiber phase. For analysis of individual analytes and for separation studies, the separation was performed on the TLC plates until the solvent front reached a distance of 30 mm. A significant decrease in separation time is observed for the e-pun PAN nanofiber phase to reach 30 mm when compared to both commercially available phases.

For porous media involving spherical particulate layers, the relationship between the position of the solvent front and time $t$ is given by the quadratic relationship shown in eqs 4 and 5:

$$Z_f^2 = \kappa t \quad (4)$$

$$\kappa = 2K_0 d_p \left(\gamma/\eta\right) \cos\phi \quad (5)$$

where $Z_f$ is the distance traveled by the solvent front, $t$ is the development time, and $\kappa$ is the velocity constant; $K_0$ is the permeability constant of the layer, $d_p$ is the average particle size, $\gamma$ is the surface tension of the mobile phase, and $\phi$ is the contact angle between the mobile phase and the layer.[16] This equation is related to the Washburn equation (eq 6)

$$Z_f = \frac{\gamma R t \cos\theta}{2\eta} \quad (6)$$

where the definition of the terms in eq 1 are the same and $R$ is the "equivalent" capillary radius. Washburn originally derived this equation to describe flow in capillaries of well-defined internal diameter. However, modifications of this equation were later used to correctly describe the rise of solvents in porous media, such as fibrous filter media, planar chromatographic media, even the wicking behavior of water in biscuits [17-20]. In comparison of eqs 1 and 2 to eq 3, they are equivalent if $(K_0 d_p) = R$[21]. The e-spun PAN phase use nanofibers instead of spherical particles. An expression describing the "effective" radius for that structure will need to be determined in the future. However, the experimental results show that the e-spun PAN nanofiber phase has larger migration distances than the commercially available phases for each development time examined. Washburn's equation describes well the relationship between distances traveled and $(\text{time})^{0.5}$ for both the commercial phases and the e-spun PAN nanofiber phase (linear curves in Figure 6.5 have $r^2 > 0.99$).

## 6.5 Conclusions

The experimental results show that electrospun PAN nanofibers are effective as the stationary phase for thin layer chromatography. The devices have been shown to decrease time of analysis and volume of solvent needed. The visualization of preservatives on the stationary phase was easily done by put $UV_{254}$ indicator directly into polymer solution. In addition to enhance chromatographic performance, e-spun PAN nanofibers phase is also cost and time efficient and can be applied for different surface selectivity by changing the type of polymer.

## 6.6 Acknowledgements

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## 6.7 References

[1] C.F. Poole.Thin-layer chromatography: challenges and opportunities, Journal of Chromatography A, 1000 (2003) 963-984.

[2] C.F. Poole. Planar chromatography at the turn of the century, Journal of Chromatography A, 856 (1999) 399-427.

[3] A. Siouffi. From Paper to Planar: 60 Years of Thin Layer Chromatography Separation and Purification Reviews, 34 (2005) 155-180.

[4] H.E. Hauck, O. Bund, M. Fischer, M. Schulz. Ultra-Thin Layer Chromatography (UTLC)—A New Dimension in Thin-Layer Chromatography, Journal of Planar Chromatography, 14 (2001) 234–236.

[5] H.E. Hauck, M. Schulz, Ultrathin-Layer Chromatography, J Chromatogr Sci, 40 (2002) 550-552.

[6] Piia K. Salo, Hannele Salomies, Kirsi Harju, Raimo A. Ketola, Tapio Kotiaho, Jari Yli-Kauhaluoma, R. Kostiainen, Analysis of Small Molecules by Ultra Thin-Layer Chromatography-Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry, Journal of the American Society for Mass Spectrometry, 16 (2005) 906-915.

[7] J.E. Clark, S.V. Olesik, Technique for Ultrathin Layer Chromatography Using an Electrospun, Nanofibrous Stationary Phase, Anal. Chem. , 81 (2009) 4121–4129.

[8] L.W. Bezuidenhout, M.J. Brett, Ultrathin layer chromatography on nanostructured thin films, Journal of Chromatography A 1183 (2008) 179–185.

[9] J.E. Clark, S.V. Olesik, Electrospun glassy carbon ultra-thin layer chromatography devices, Journal of Chromatography A, 1217 (2010) 4655-4662.

[10] N.D.E. Kruijf, M.A.H. Rijk, L.A. Pranoto-Soetardhi, A. Schouten, Thin-layer chromatographic procedure for the identification of preservatives in cosmatic products, Journal of Chromatography A, 410 (1987 ) 395-411.

[11] B. Tobias, R.C. Strickler, Photoactivated zinc silicate in thin layer chromatography plates: A potential cause for error in liquid scintillation counting, Steroids, 37 (1981) 213-221.

[12]P. Kampalanonwat, P. Supaphol, Preparation and Adsorption Behavior of Aminated Electrospun Polyacrylonitrile Nanofiber Mats for Heavy Metal Ion Removal, ACS Applied Materials & Interfaces, 2 (2010) 3619-3627.

[13]J. Sutasinpromprae, S. Jitjaicham, M. Nithitanakul, C. Meechaisue, P. Supaphol, Preparation and characterization of ultrafine electrospun polyacrylonitrile fibers and their subsequent pyrolysis to carbon fibers, Polymer International, 55 (2006) 825-833.

[14]P. Kampalanonwat, P. Supaphol, Preparation of Hydrolyzed Electrospun Polyacrylonitrile Fiber Mats as Chelating Substrates: a Case Study on Copper(II) Ions, Industrial & Engineering Chemistry Research, 50 (2011) 11912-11921.

[15]C.F. Poole. The Essence of Chromatography, Elsevier Science, Amsterdam, 2003.

[16]C.F. Poole, S.K. Poole. Multidimensionality in planar chromatography, Journal of Chromatography A, 703 573-612.

[17]E.W. Washburn, The dynamics of capillary flow, Phys. Rev., 17 (1921) 273-283.

[18]B.J. Mullins, R.D. Braddock, G. Kasper, Capillarity in Porous, Fibrous Filter Media: relationship to media properties, Chemical Engineering Science, 62 (2007) 6191-6198.

[19]R.J. Good, N.J. Lin, Rate of penetration of a fluid into a porous body. , J. Colloid Interface. Sci. , 54 (1976 ) 52-58.

[20]E. Chibowski, F. Gonza´lez-Caballero, THEORY AND PRACTICE OF THIN-LAYER WICKING Langmuir 9(1993) 330–340.

[21]D.I. Dimitrov, A. Milchev, K. Binder, Capillary rise in nanopores: molecular dynamics evidence for the Lucas-Washburn equation, Phys. Rev. Lett, 99 (2007) 054501–054504.

**Table 6.1** Mean diameters of the individual fibers of electrospun PAN nanofiber phases and of its formulations with different amounts of fluorescence indicator $UV_{254}$

| Applied voltage | Diameters of individual fibers ± SD (nm) | | | |
|---|---|---|---|---|
| | - | 1% $UV_{254}$ | 5% $UV_{254}$ | 10% $UV_{254}$ |
| 16 kV | 224±65 | 177±48 | 179±50 | 181±40 |
| 18 kV | 181±58 | 155±53 | 161±48 | 165±45 |
| 20 kV | 160±43 | 149±60 | 152±38 | 154±48 |

**Table 6.2** $hR_F$ values and number of plate ($N$) of water soluble food dyes on e-spun PAN nanofiber phase (n=5)

| | $hR_f$ | %Sd | N |
|---|---|---|---|
| E102 | 25 | 1 | 8345 |
| E111 | 33 | 4 | 8286 |
| E133 | 57 | 14 | 22885 |
| E127 | 73 | 16 | 9596 |
| E104 | 90 | 1 | 9497 |

**Table 6.3** Comparison of $hR_F$values and number of plates ($N$) for 7 preservatives and an extracted non-alcoholic beverage sample on (A) electrospun PAN nanofiber phases with 10% $UV_{254}$ and (B) HPTLC plate silica gel CN phases (n=5)

| | A) Electrospun PAN nanofibers with 10% $UV_{254}$ | | | B) HPTLC plate silica gel CN | | |
|---|---|---|---|---|---|---|
| | $hR_F$ | *%RSD* | N | $hR_F$ | *%RSD* | N |
| BE | 17 | 1.3 | 9044 | 40 | 0.10 | 3937 |
| PE (E216) | 24 | 1.5 | 6745 | 46 | 0.65 | 4161 |
| EE (E214) | 31 | 2.1 | 3822 | 54 | 0.86 | 6568 |
| ME (E218) | 40 | 1.5 | 6005 | 58 | 0.40 | 5112 |
| B (E210) | 45 | 2.2 | 3711 | 65 | 0.19 | 4136 |
| So (E200) | 48 | 2.5 | 4984 | 76 | 0.40 | 3783 |
| pHB | 56 | 3.9 | 3997 | 83 | 0.55 | 5263 |
| Beverage | 86 | 1.0 | 2214 | 96 | 1.16 | 3139 |
| Sample | 47 | 2.6 | 6233 | 76 | 0.47 | 3025 |

**Table 6.4** Comparison of resolutions between zones of a mixture of 7 preservatives on (A) electrospun PAN nanofiber phases with 10% $UV_{254}$ and (B) HPTLC plate silica gel CN phases (n=5)

| Stationary phase | BE/PE | PE/EE | EE/ME | ME/B | B/So | So/pHB |
|---|---|---|---|---|---|---|
| A) Electrospun PAN nanofibers with 10% $UV_{254}$ | 1.04 | 1.26 | 0.97 | 1.34 | 1.82 | 1.16 |
| B) HPTLC plate silica gel CN | 1.45 | 1.41 | 1.33 | 0.49 | 0.56 | 1.43 |

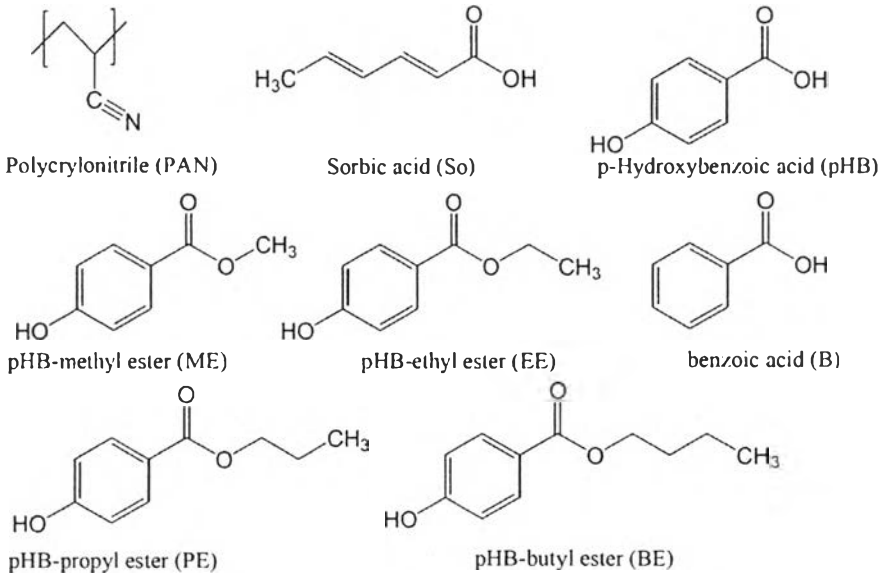


**Figure 6.1** Structures of polycrylonitrile and 7 preservatives

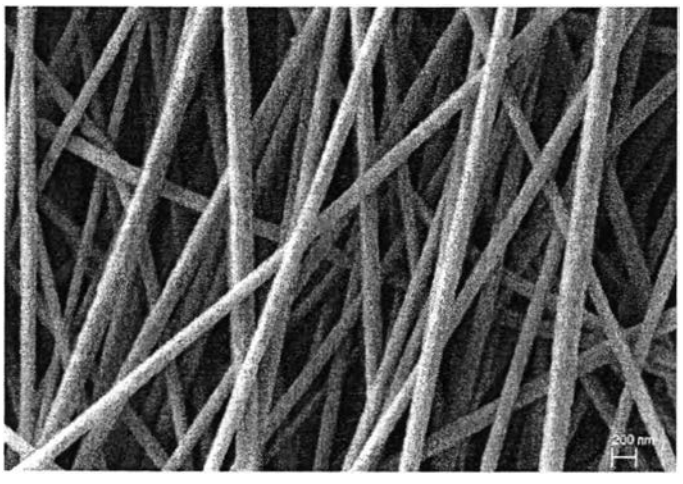


**Figure 6.2** Selected SEM image of an electrospun PAN nanofiber phase with $UV_{254}$ prepared from 12%PAN in DMF with 10%of $UV_{254}$ at 18kV/10cm.

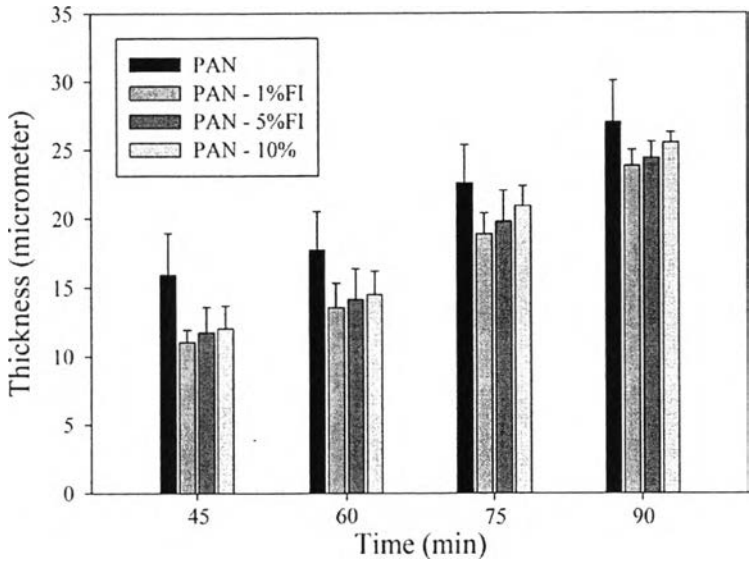


**Figure 6.3** Thickness of electrospun PAN nanofiber phases and and its formulations with different amounts of fluorescence indicator $UV_{254}$ (1%, 5% and 10%) at different collecting times (45-90 min)

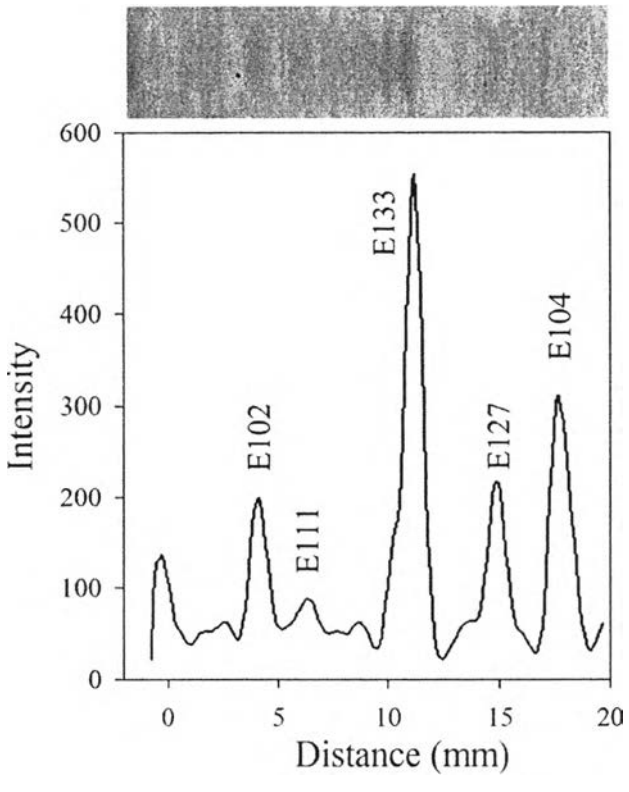


**Figure 6.4** Digital image and chromatogram of the separation of a mixture of 5 water soluble food dyes on an e-spun PAN nanofiber phase

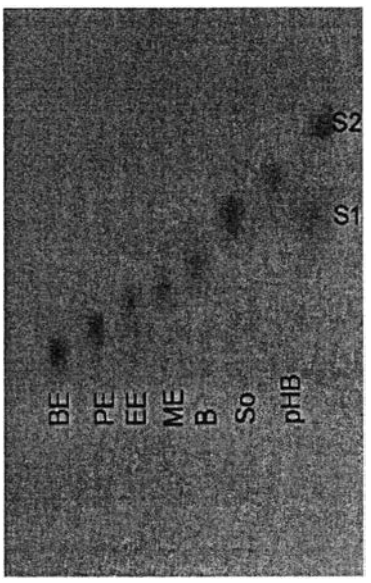


**Figure 6.5** Digital image of the separation of preservatives and non-alcoholic beverage sample on electrospun PAN nanofibers with 10% $UV_{254}$ with a development distance of 2.0 cm

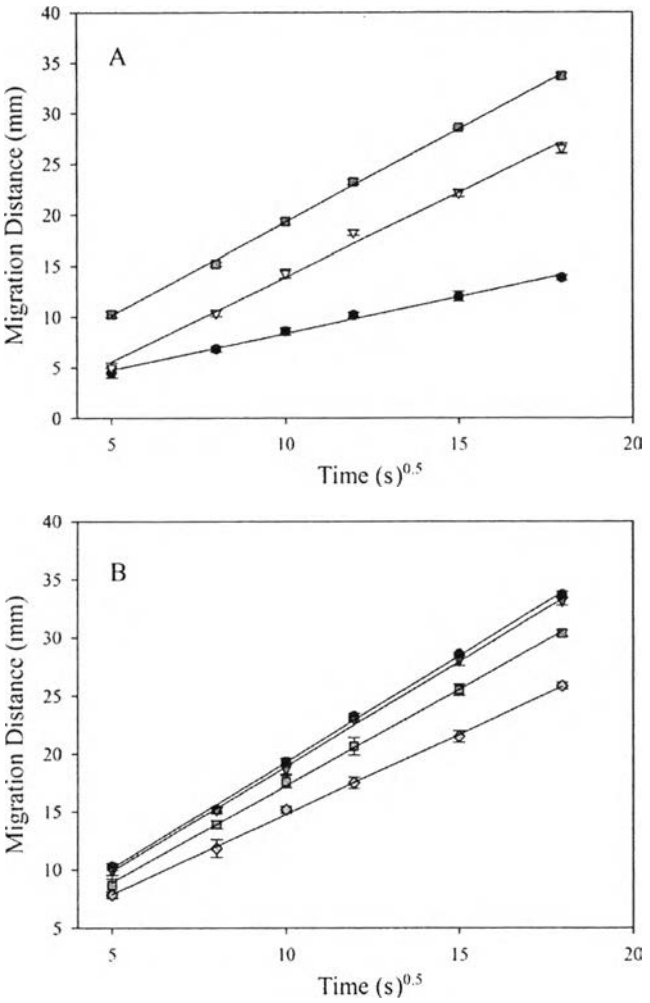


**Figure 6.6** Comparison of mobile phase velocities for different (A) layer types: HPTLC plates silica gel 60 ( ●), HPTLC plates silica gel CN ( ▼) and electrospun PAN nanofiber phase ( ▣) and for (B) different amounts of fluorescence indicator $UV_{254}$: 10% ( ◈), 5% ( ⊡), 1% ( ▼), and none ( ●)