

Chapter 2

Electrospun Polyvinyl Alcohol Ultra-Thin Layer Chromatography of Amino Acids

Abstract Electrospun crosslinked polyvinyl alcohol (PVA) was prepared and used as stationary phase for ultrathin layer chromatographic (UTLC). The nano-structured stationary phase shows greatly enhanced efficiency compared to conventional HPTLC plate. An example of using PVA-UTLC plate for the analysis of hydrolysis products of aspartame in diet coke, aspartic acid and phenylalanine, is included.

2.1 Introduction

Compared to HPLC, the range of stationary phase types is smaller for TLC. Many stationary phases can be readily developed by using electrospinning technique. In this study, polyvinyl alcohol (PVA) was used as the stationary phase for UTLC. PVA is a nontoxic, biodegradable, and a biocompatible polymer [1]. The hydroxyl groups render PVA as polar stationary phase. Because PVA nanofibers are soluble in water this would limit its use for the separation of polar compounds. Therefore efforts to enhancing the water resistance of the PVA were necessary. The most popular way of decreasing PVA's solubility in water is to crosslink the polymer. Crosslinking using high energy ionization radiation [2], and UV irradiation of PVA [3], has been used but these methods require special instrumentation or modification of the PVA polymer backbone. Crosslinking reactants such as glutaraldehyde [4, 5], glyoxal [6], formaldehyde [7], and maleic anhydride or maleic acid [7, 8], were used previously. Crosslinking of PVA nanofibers has also been accomplished after electrospinning by immersion into a solution of crosslinker [9, 10]. Another means of stabilizing PVA is to add the crosslinker to the electrospinning solution which allows electrospinning and crosslinking to occur in the same step [11].

The separation of amino acids is important in agriculture, food analysis and biomedical applications. HPLC, CE, and GC are used for the amino acids analysis [12]. However, TLC has the added benefits of simplicity, speed of analysis, and

cost-effectiveness compared to the instruments mentioned above. Therefore, TLC is also a useful and commonly-used technique for the amino acids analysis [13–16]. For example, Liu et al. [17] reported chiral separation of racemic amino acids on a TLC plate made by PVA film containing DNA. Simon et al. [18] recently compared the differences in the distribution of free amino acids in sanguine plasma for normal patients and those with different stages of diabetes, renal syndrome and hepatic cirrhosis using TLC analysis. A biodegradable UTLC device for such biomedical analyses would likely be advantageous for the diagnoses and monitoring of disease states. A small UTLC device could be more efficient and less organic solvent is needed.

The separation of amino acids using TLC has been challenging. The available stationary phases are limited as mentioned therefore complex mobile phase mixtures including a variety of organic solvents [19] are needed and additives such as acids and bases [20], metal ions [21], or surfactants [22] are usually required to improve the separation. The complex multicomponent mobile phases require time consuming optimization and some of the reagents are not usually available in regular labs. Glassy carbon UTLC has been used for amino acid separation with high efficiency [5]. However, the limitation is amino acids cannot be analyzed directly. Labeling with fluorescent dyes was required using glassy carbon UTLC. And the detection of labeled amino acids required an additional step: solvent extraction of the labeled amino acids. Otherwise the fluorescence is completely quenched by carbon. In this work we used electrospun PVA as a stationary phase which showed high efficiency and unique selectivity. With highly efficient separation, compounds can be separated even at lower selectivity which simplifies the optimization of selectivity using different mobile phases. In this work, the separation of amino acids using ternary mixtures of commonly-used organic solvents, butanol, methanol or ethyl acetate, and water, without any other additives was achieved.

Because amino acids cannot be visualized by exposure to UV light, a fluorescent label was attached to the amino acids before the separation [15]. Dye-labeled amino acids are colored and show fluorescence under UV light. The spots can be visualized during the separation and fluorescence can be detected for small quantities of the analytes. Therefore it is convenient to study the chromatographic behavior using the fluorescent labeled amino acids. Isothiocyanate derivatizing agents are commonly-used for visualization of amino acids by reacting with the amino acid to form fluorescent thioureas. Also, ninhydrin as a visualization reagent has been widely used for amino acids detection on TLC plate. We studied and separated fluorescein isothiocyanate (FITC) derivitized amino acids [23] and visualized amino acids with ninhydrin to detect amino acids on an electrospun PVA plate. The hydrolysis products of aspartame in diet coke, aspartic acid and phenylalanine, were also analyzed using PVA-UTLC plate.

2.2 Experimental

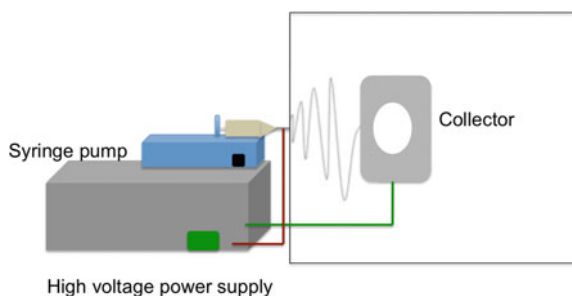
2.2.1 Materials

The polymer solution was prepared by dissolving PVA (99 + % hydrolyzed, M_w 89,000–98,000, Sigma, St. Louis, MO) in distilled water maintained at 80 °C. Glutaraldehyde (GA, 25 % in water, Baker) and hydrochloric acid (0.1 M) were used for crosslinking the PVA. Alanine (ala), methionine (met), aspartic acid (asp), glutamine (gln), arginine (arg) mono hydrochloride acid, tyrosine (tyr), phenylalanine (phe), histidine (his), and threonine (thr) were obtained from Sigma (St. Louis, MO). Cysteine (cys) and tryptophan (trp) were purchased from Aldrich (Milwaukee, WI). Merck silica HPTLC plates on glass plate with 4–8 μm diameter silica particles and 150–200 μm film thickness were obtained from EMD Chemicals (Gibbstown, NJ).

2.2.2 Electrospinning

Electrospinning of the polymer was accomplished using a previous reported procedure [11] with modifications and the instrumental setup is shown in Fig. 2.1. A syringe pump (KD Scientific, model: 780100), high voltage power supply (Spellman, Hauppauge, NY, model: CZE1000PN30), a stainless steel collector (11 cm \times 12 cm) covered with aluminum foil (super strength, Reynolds), and a Plexiglas enclosure. An 8 % (w/w) aqueous PVA solution was optimized to be the concentration for electrospinning. Before the electrospinning, glutaraldehyde (GA:PVA, mol:mol, 90:1) and HCl (HCl:GA, mol:mol, 1:5) were added to start the crosslinking reaction [12]. The feed rate of the PVA polymer solution was 0.5 mL/h. The voltage applied was 20 kV. The distance from the spinneret needle tip to the collector was optimized to be 20 cm. The relative humidity was controlled to 30 % or below by purging the plastic enclosure with dry N_2 while electrospinning and was monitored by VWR Traceable[®] digital hydrometer. At relative humidity levels higher than 30 %, bead formation on the top of the fibers was noted. The time of electrospinning for each polymer solution was 30 min.

Fig. 2.1 Instrument setup used for electrospinning



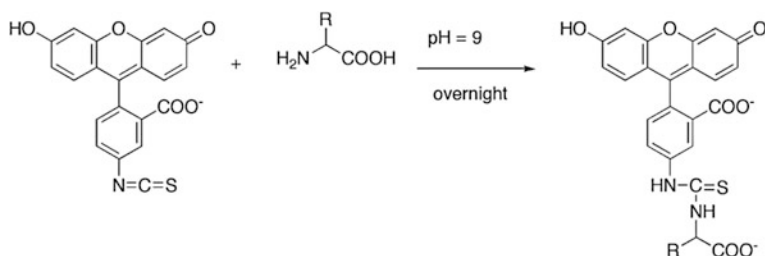


Fig. 2.2 Reaction scheme of the synthesis of FITC-labeled amino acids

2.2.3 FITC Labeled Amino Acids

The amino acids were labeled with FITC dye using the published reaction conditions [23, 24]. The reaction is shown in Fig. 2.2. 0.5 mL amino acid solution (2 mg/mL each, in 0.2 M pH = 9 carbonate-bicarbonate buffer) were mixed with 0.05 mL (60 $\mu\text{mol/mL}$ in acetone with trace amount of pyridine) FITC (Sigma, St. Louis, MO). The reaction solution was protected from light for overnight and was stored in the refrigerator. The FITC-labeled amino acids were used directly and were stable for analysis for 2 weeks. The concentration of the FITC-labeled amino acids was calculated from the amount of FITC added which was the limiting reagent in this reaction.

2.2.4 Thin Layer Chromatography

The electrospun PVA plate was cut into 7 cm \times 3 cm UTLC devices. The FITC labeled amino acid solutions were spotted onto the bottom of UTLC plate using fused silica capillary tubes (id: 50–250 μm , Polymicro Technologies, Phoenix, AZ). The volume of the solution that was spotted onto the plate was calculated from the volume difference in the capillary tube before and after spotting. About 1 mL of the freshly prepared mobile phase was used for each development. The development chamber was an 80 mL TLC chamber. Before each development, the mobile phase and plate were equilibrated for about 10 min. The method of the analysis of the results was reported previously [25]. Briefly, the resulting spots were visualized and analyzed by a digital documentation system from Spectroline (model CC-80). The images of FITC-labeled amino acids were recorded by taking a digital photograph which was then converted to chromatograms using a TLC analyzer. The separation using a Si-Gel HPTLC plate was conducted using the same method. For the separation of amino acids visualized by ninhydrin reaction, the amino acids (5 mg/mL aqueous solution) were spotted on the bottom of electrospun PVA plate. After development the ninhydrin solution (0.3 g in 100 mL of *n*-butanol with 3 mL of acetic acid) was sprayed evenly onto the plate

using a TLC reagent nebulizer (Kimble-Chase Vineland, NJ). After the plate was dry it was heated for 10 min at 110 °C [26]. Diet coke was heated and refluxed for 24 h. Then it was concentrated from 100 mL to ~1 mL. The analysis of treated diet coke was performed using the same method that has been used for unlabeled amino acid. Because the spots show different colors after ninhydrin reaction, a camera is not required to record the image. The image of the separation using ninhydrin as a visualization reagent was scanned using an EPSON GT-2500 scanner and the chromatogram was obtained using ImageJ 1.43u software. The chromatographic parameters were analyzed using PeakFitTM (version 4, SPSS Inc.). All of the results were based on at least three measurements.

2.2.5 Instrumentation

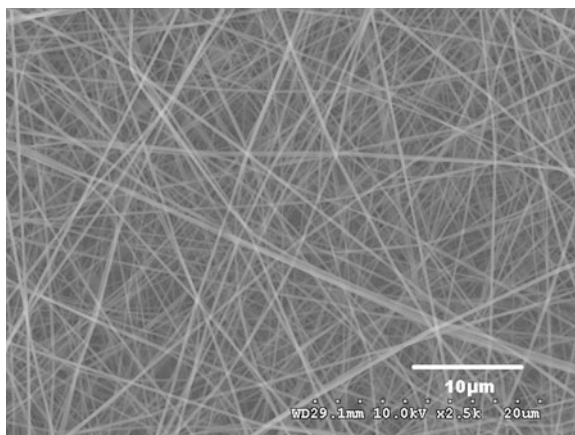
The micrographic images were obtained with a Hitachi S-4300 (Hitachi High Technologies, America, Inc., Pleasanton, CA) scanning electron microscope (SEM). Before the SEM analysis the sample was sputter coated with gold for 2 min at 10 μ A. Vertical SEM sample stage was used for mat thickness measurement. The thickness was measured from the image of the cross-section of the plate.

2.3 Results and Discussion

2.3.1 Electrospinning for Optimization of PVA Nanofibrous Mat Structure

Two methods of producing water resistant PVA nanofibers were tested: cross-linking during electrospinning and cross-linking after electrospinning. Tang et al. [11] studied in situ crosslinking of PVA while electrospinning previously. An optimal amount of the GA, crosslinker, and the HCl, catalyst, was determined in previously for the best water resistance and minimized swelling when the PVA fibers were exposed to water. Here we optimized the electrospinning concentration, 8 % PVA in water, to generate beads free fibers. The water resistance of in situ crosslinked PVA using these optimized conditions was compared to that using extensive crosslinking of PVA after electrospinning. For the post-electrospinning reaction, the PVA fibrous mat is immersed into a solution prepared by dissolving 75 % GA in acetone (1 %, v:v). The crosslinking reaction was stopped after 1, 3, and 5 h, respectively. Using the immersion method, the fibers partially dissolved in water which was the solvent of the crosslinker GA. On the other hand, the in situ crosslinked PVA nanofibers maintained the morphology quite well after crosslinking. Figure 2.3 shows the SEM image of the morphology of the in situ

Fig. 2.3 SEM image of crosslinked PVA nanofiber. The average diameter of the PVA nanofibers is 190 ± 50 nm



crosslinked electrospun nanofibers on the aluminum foil. The average diameter of the nanofibers is ca. 200 nm. The PVA nanofibrous stationary phase adheres strongly to the aluminum foil and it was easy to cut into desired sizes.

Figures 2.4, 2.5, 2.6 illustrates the enhanced-water stability gained through in situ crosslinking electrospinning. Figure 2.4 illustrates that noncrosslinked PVA nanofibers dissolve readily when soaked for 15 min in water; Fig. 2.5 shows markedly less dissolution for the cross-linked PVA when soaked in water for 15 min and Fig. 2.6 shows that minimal change in the morphology of the cross-linked fibers occurs when soaked in the optimized mobile phase mixture for the separation of the FITC-labeled amino acids for 15 min. Clearly the in situ crosslinking was needed and effective.

When electrospinning each polymeric solution for 30 min, a nanofibrous mat with a thickness of approximately 5 μm was produced. Due to the crosslinking reaction, after 30 min the polymeric solution became too viscous to electrospin. However, one layer, 5 μm thick, electrospun PVA phase was not thick enough and even the minimum amounts of analyte solution that can be applied would overload the plate. By electrospinning new PVA solutions for 30 min each the possibility of increasing the PVA layer thickness was provided. Figure 2.7 illustrates mat thickness increases when more layers of PVA were electrospun. Similar fibrous morphology was observed for one, two, and three layers (30 min each of electrospinning) mats. Compacting of the nanofibers beneath the upper layers was not observed for the thicknesses studied. However, minor beads were observed on the four-layered mat which is consistent with previous results [25].

Figure 2.8 illustrates the variation of solvent migration distance with the square root of time. The observed linear dependence ($r^2 > 0.999$) was anticipated by the Lucas–Washburn’s theory of capillary flow through porous media [27]. This dependence is similar to that observed with the other electrospun UTLC plates [25, 28].

Fig. 2.4 SEM image of PVA nanofibers without crosslinking after soaked in water

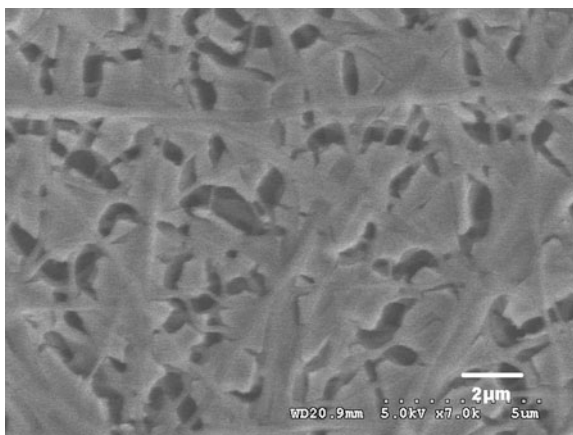


Fig. 2.5 SEM image of crosslinked PVA nanofibers after soaked in water

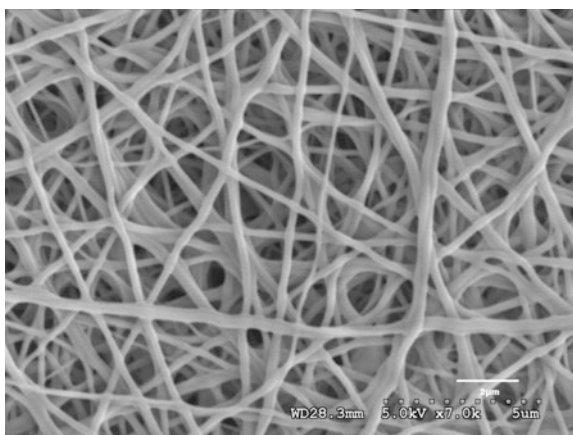


Fig. 2.6 SEM image of crosslinked PVA nanofibers after soaked in methanol, butanol and water (7:5:1, v/v/v)

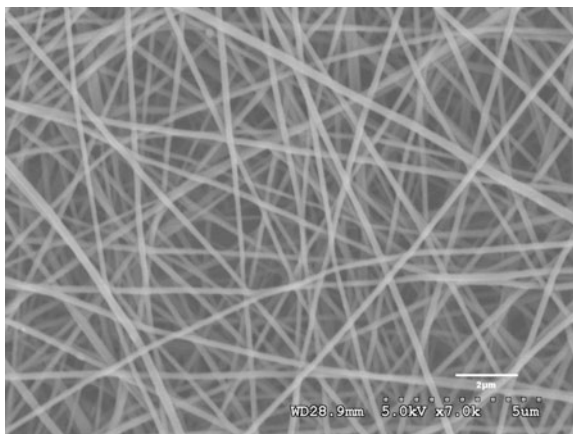


Fig. 2.7 Different mat thickness when multiple layers of electrosun PVA

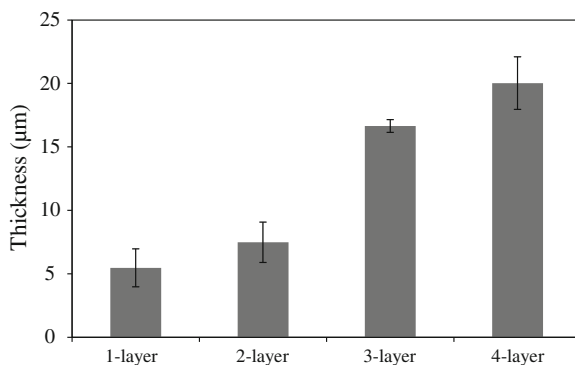
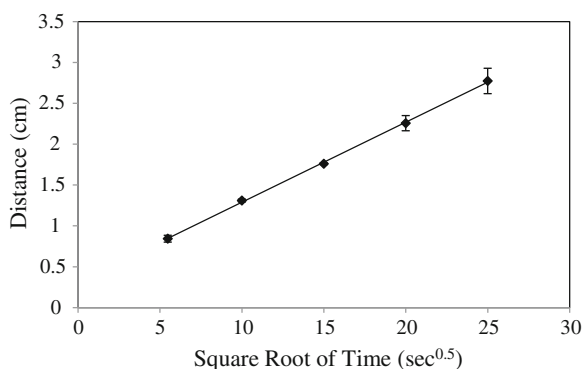


Fig. 2.8 Mobile phase (methanol/butanol/water, 7:5:1, v:v:v) migration rate on PVA plate



2.3.2 Selectivity

To optimize the mobile phase for the separation of amino acids, separations in methanol, ethanol, *n*-propanol, *i*-propanol, *n*-butanol, acetone, acetonitrile were evaluated. These mobile phases were not polar enough and all of the amino acids did not migrate up the plate. Water was added to the above solvents for further characterization. Different proportions of water were added but the selectivity among the amino acids was poor using the binary mobile phase system. Therefore, ternary mobile phase systems, including acetone/acetonitrile/water, methanol/butanol/water, ethanol/butanol/water, *n*-propanol/butanol/water, *i*-propanol/butanol/water, were tested to optimize the selectivity of the FITC labeled amino acids. Different ratios of each three components of the mobile phase were tested for all of the studied amino acids. The selectivity of the amino acids for these ternary mixtures was compared. Methanol/butanol/water (7:5:1, v:v:v) and ethanol/butanol/water (5:5:2.5, v:v:v) provided the better electivity for most of the FITC-

Table 2.1 hR_F of FITC labeled amino acids on PVA and Si-gel plate

PVA plate	Si-gel HPTLC plate	
	hR_F	hR_F
Phe	34 ± 2	80 ± 2
His	24 ± 2	37 ± 7
Asp	33 ± 3	42 ± 7
Cys	11 ± 1	55 ± 1
Met	40 ± 2	77 ± 2
Thr	21 ± 2	69 ± 1
Trp	38 ± 3	27 ± 1
Gln	35 ± 3	62 ± 2
Ala	39 ± 2	73 ± 1
Tyr	28 ± 1	77 ± 2
Arg	37 ± 2	56 ± 1

labeled amino acids than the other mobile phase systems. For the solvent system of methanol/butanol/water to travel about 3 cm on the PVA plate, 10 min was required while ethanol/butanol/water mixtures took 15 min to travel the same distance. Therefore, by comparing the speed of the mobile phase moving up on the PVA plate, methanol/butanol/water was chosen to shorten the analysis time, and this solvent mixture was used for all the other studies. The mobile phase for the Si-Gel plate was optimized similarly and ethanol/butanol/water (7:5:0.5) was selected as the preferred mobile phase. Six to seven minutes were required for the mobile phase to travel about 3 cm on the Si-Gel plate. The hR_F values using mobile phase methanol/butanol/water (7:5:1, v:v:v) are listed in Table 2.1. Also, Table 2.1 shows the hR_F values for the optimized mobile phases for the Si-Gel plate.

The selectivity on a PVA UTLC plate was compared with the selectivity on Si-Gel with the respective optimal mobile phase (Figs. 2.9 and 2.10). Although both PVA and silica gel have hydroxyl groups which make them highly polar stationary phases, the retardation factors on the silica gel plate are slightly higher

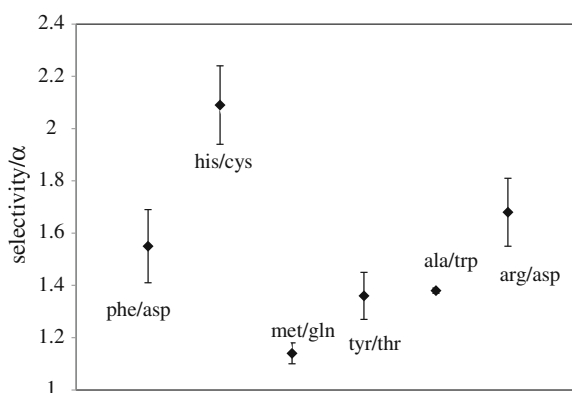
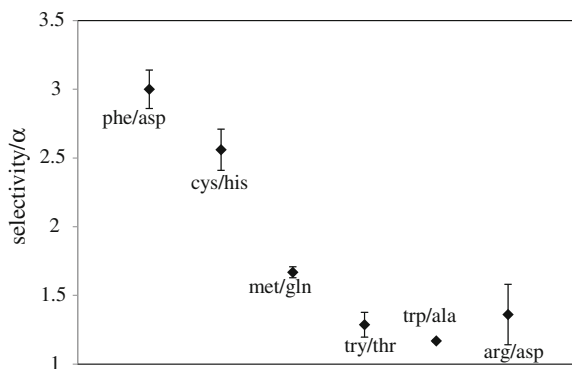
Fig. 2.9 Selectivity of FITC labeled amino acids on PVA plate (mobile phase methanol/butanol/water, 7:5:1, v:v:v)

Fig. 2.10 Selectivity of FITC labeled amino acids on Si-gel HPTLC plate (mobile phase ethanol/butanol/water, 7:5:0.5, v:v:v)



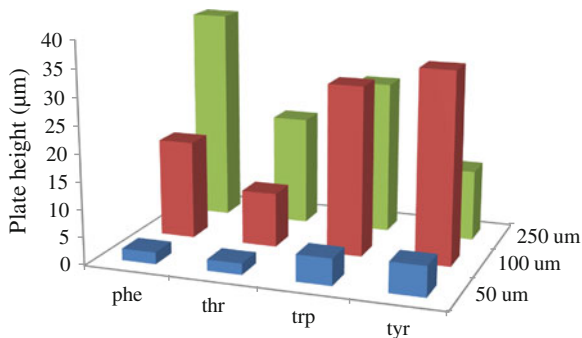
than that on the PVA plate using the optimized mobile phase system. For the pair of cys and his, the retardation factor of his is larger than that of cys on PVA plate while cys has larger retardation factor on Si-Gel plate. A similar trend was observed for trp/ala. The selectivity of tyr/thr and arg/asp was higher on the PVA plate, while phe/asp showed higher selectivity on the Si-Gel plate. The selectivity of met/gln on these two types of plates is similar. In summary, the selectivity of FITC-labeled amino acids is markedly different for most of the studied amino acids on PVA plates and Si-Gel plates even though both have hydroxyl functionalization.

2.3.3 Efficiency

The plate number (N) and plate height (H) describe the efficiency of separations TLC. In this work N was determined by PeakFitTM software, using statistical moment analysis of the chromatographic band and H was obtained with the equation $H = N/L$.

Different in the internal diameter of the capillary tubes used for the spotting the analyte solution on the ULTC plat can impact the sizes of the initial spots and

Fig. 2.11 Plate height of different capillary size



therefore the efficiency (Fig. 2.11). A larger i.d. capillary applies a larger original spot, which can lower the efficiency of the separation. Both 250 and 100 μm capillary showed lower efficiency than the 50 μm capillary. Therefore 50 μm capillary tube was selected for the remainder of the optimization studies.

The loading capacity of the three-layered plates was investigated using a 50 μm capillary tube and three-layer mat. Figure 2.12 shows that 7 mM solutions of analytes could be applied with minimal impact on the band dispersion. Significant band dispersion was noted for when 14 mM solutions were applied to the plate. The plate is clearly overloaded at this concentration. Comparing to a PVA-UTLC plate, 250 μm capillary tube and 7 mM solutions of analytes were used for the Si-Gel HPTLC plate without overloading. The HPTLC plate is much thicker than the UTLC plate. Therefore the loading capacity of HPTLC plate is larger. However if a 50 μm capillary tube was used for the HPTLC plate no fluorescent spot could be detected under UV light, which indicates that UTLC is a more sensitive technique than conventional TLC.

Sample volumes applied onto the plate also impact the efficiency (Figs. 2.13 and 2.14) with a 50 μm capillary tube and three-layered mat. For both 7 mM and 4 mM analyte concentration the smaller sample volume showed higher efficiency.

Fig. 2.12 Plate height of different analyte concentration

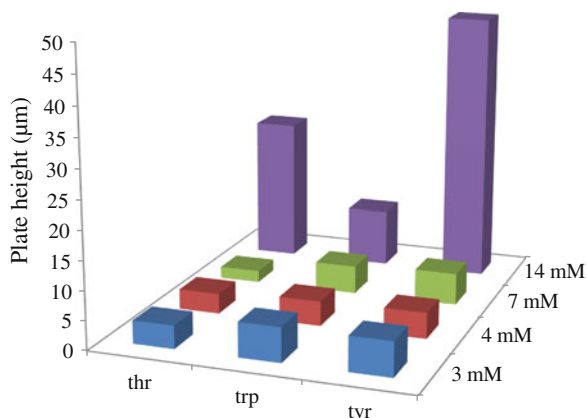


Fig. 2.13 Plate height of different analyte volume using 7 mM analyte

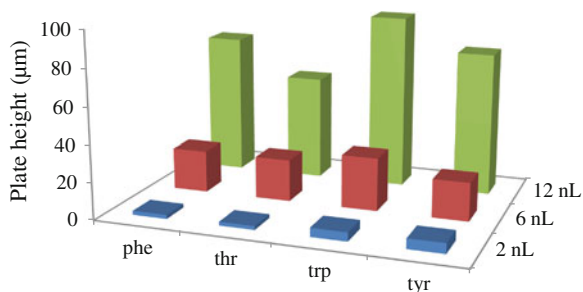


Fig. 2.14 Plate height of different analyte volume using 4 mM analyte

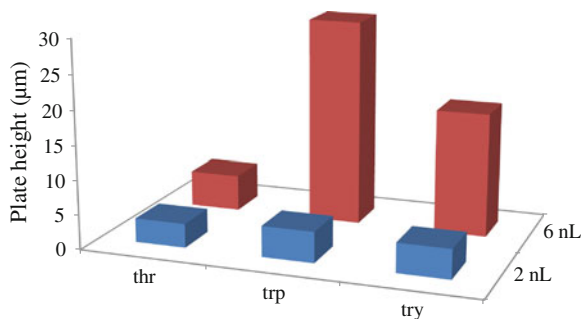
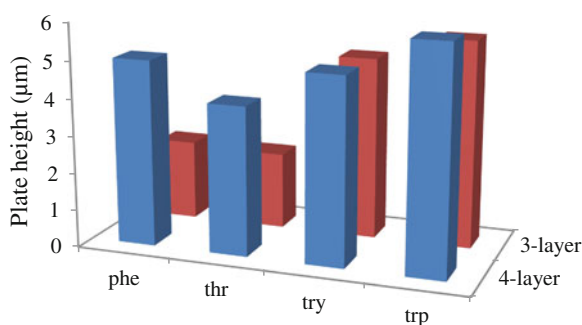


Fig. 2.15 Plate height of different mat thickness



Therefore, the minimum sample volume was applied each time to get the optimal efficiency for the currently used concentration range.

The mat thickness of the TLC plate can also impact the efficiency of the separation [25]. We studied the efficiency on PVA plates of different mat thickness (Fig. 2.15) with a 50 μm capillary tube and 7 mM solution. The one-layer and two-layered plates were overloaded because the mats are too thin as mentioned in Sect. 2.3.1. Also the application volume and analyte concentration used for this analysis are those optimized for the three layered thick mat. However, interestingly, it was observed that the efficiency on the four-layered thick plate is slightly lower than that on the three-layered thick plate. The reason could be the beads that form on the PVA plate as mentioned previously, may cause the heterogeneity of the plate, and therefore lower the efficiency.

The efficiency, plate number and plate height of the separation was compared with that on a Si-Gel HPTLC plate (Figs. 2.16 and 2.17). The efficiency on the PVA plate using the optimal condition mentioned above, 50 μm capillary tube, 3-layered mat, and 7 mM solution was studied. The distance traveled by the mobile phase on both PVA UTLC plate and Si-Gel plate is 3 cm. The result showed that the efficiency on the PVA plate is much higher than the efficiency on the Si-Gel HPTLC plate. The Si-Gel often has tailed bands when it is used to separate polar compounds, including amino acids. Therefore, PVA as a stationary phase provided an alternative for the separation of the polar compounds.

Fig. 2.16 Comparison of the plate number on PVA plate (*black*) and Si-gel HPTLC plate (*blank*). The error bars represent the standard deviation of three replicates

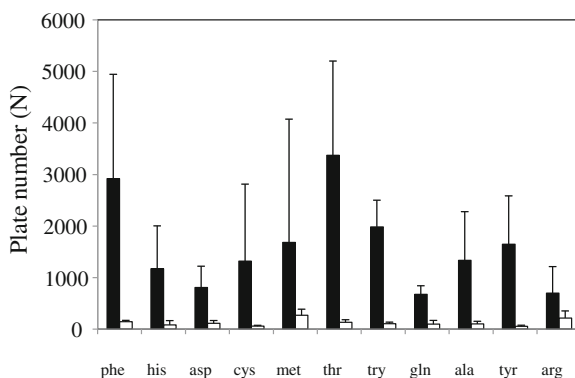
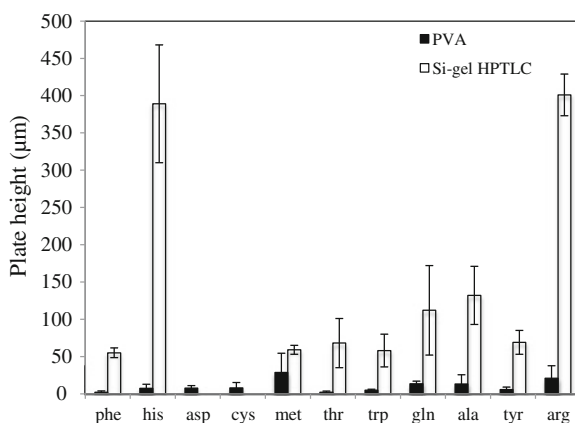


Fig. 2.17 Comparison of the plate height on PVA plate (*black*) and Si-gel HPTLC plate (*blank*). The error bars represent the standard deviation of three replicates



The plate height of asp and cys are not shown for Si-gel HPTLC due to significant tailing.

2.3.4 Separation of Amino Acids Using Ninhydrin as Visualization Reagent

Ninhydrin is the most widely-used detection agent for the visualization of amino acids by TLC. This visualization method was also studied with the electrospun PVA plates based on the optimized conditions for FITC-amino acid separations. Three-layered thick mats and a 50 μm i.d. capillary were therefore used. The ninhydrin reagent solution was sprayed onto the plate after development and the colored spots showed up after heating. No UV light or fluoresce detector are needed. The spots can be directly visualized after the ninhydrin reaction. The

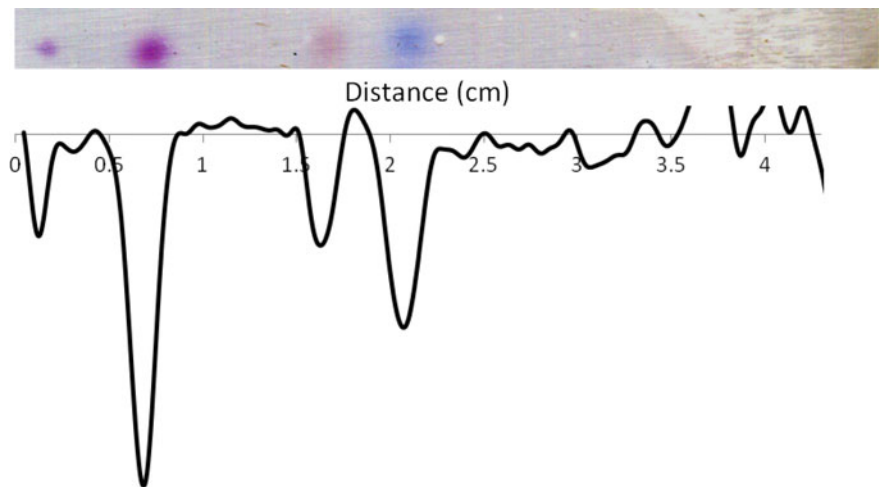
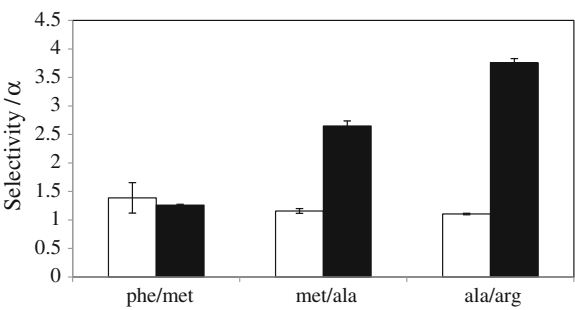


Fig. 2.18 Chromatogram of the separation of amino acids on electrospun PVA plate using ninhydrin as a visualization reagent

Table 2.2 hR_F , plate height, and color of spots of amino acids separated on PVA plate and plate height of conventional TLC using ninhydrin as a visualization reagent

	PVA UTLC		Color	Literature HPTLC [30]
	hR_F	h (plate height/ μm)		h (plate height/ μm)
Arg	1 ± 1	70 ± 40	Purple	83
Ala	14 ± 1	80 ± 10	Pink	96
Met	36 ± 1	30 ± 7	Orange	104
Phe	45 ± 1	30 ± 2	Blue	134

Fig. 2.19 Comparison of the selectivity of the FITC-labeled (*blank*) and unlabeled (*black*) amino acids on PVA plate



separation of four amino acids were shown in Fig. 2.18 and the hR_F and efficiency were calculated (Table 2.2).

The amino acids are arg, ala, met, and phe from left to right.

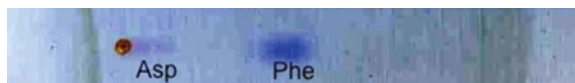


Fig. 2.20 A PVA-UTLC plate showing the separation of the hydrolysis products, asp and phe, of aspartame in diet coke in 2 cm

The mobile phase used to separate amino acids was optimized with butanol/ethyl acetate/water (5:5:1.5, volume ratio) showing the best selectivity. The amino acids without FITC labeling showed better selectivity compared to the FITC labeled amino acids on a PVA plate (Fig. 2.19). Four amino acids were baseline separated within 4 cm (Fig. 2.18) due to both improved selectivity and efficiency using a ternary mobile phase in composition of nontoxic organic solvents and water without any other additives. It is worthy to mention that the amino acids studied in this paper showed different colors (Table 2.2), which adds another dimension for the identification in addition to hR_F . Although the separation efficiency of amino acids is not as high as the FITC labeled amino acids on an electrospun PVA plate the efficiency of amino acids visualized by ninhydrin is higher than the efficiency of silica-gel monolithic UTLC that has been commercialized (80–100 μm) [29]. Flieger et al. [30] have reported high efficiency HPTLC separation of amino acids with ninhydrin as visualization reagent using salting-in TLC recently. The efficiency using PVA-UTLC and HPTLC [30] was compared in Table 2.2. The PVA-UTLC plate showed much higher efficiency for met and phe than that using HPTLC.

2.3.5 Analysis of Aspartame in Diet Coke

Analysis of aspartame, an artificial sweetener, and its breakdown products is important in the food industry since it is widely used in diet food and drinks as a sugar substituent. Change in pH or temperature can affect its stability and it degrades and forms asp, phe and methanol [31]. HPLC and TLC have been used for the analysis the breakdown products [32, 33]. In this work we used a PVA-UTLC plate to separate and identify the hydrolysis products, asp and phe. Diet coke was heated to reflux for one day to achieve a high yield of asp and phe [31]. The TLC mobile phase condition and ninhydrin treatment were similar to the method used in Sect. 2.3.4. The result is shown in Fig. 2.20. Asp and phe were well separated and could be readily identified from their color when compared to the color of their standards on PVA-UTLC plate after ninhydrin treatment. The colored ingredients in diet coke stayed in the origin and did not cause any inference. Therefore, a PVA-UTLC plate can be potentially used for aspartame analysis in food industry as a simple, fast, and low cost technique.

Butanol:ethyl acetate:water (5:5:1.5, volume ratio) was used as mobile phase.

2.4 Conclusions

A crosslinked PVA UTLC plate was fabricated and the fiber morphology was well maintained after soaked in water and the mobile phase used. The selectivity of FITC-labeled amino acids studied on a PVA plate was different from that on a Si-Gel plate except for met/gln. The efficiency depending on the mat thickness, capillary size, analyte concentration, and analyte volume was studied and optimized. For the three layered thick electrospun ULTC plates, smaller capillary tube and smaller analyte volume applied showed higher efficiency. Analyte concentrations below or equal to 7 mM showed similar efficiency, but the 14 mM analyte concentration showed much lower efficiency. The measured efficiency on the PVA ULTC plate was much higher than the efficiency on Si-gel plate. The separation of unlabeled amino acids showed higher selectivity and four amino acids were baseline separated using a simple and nontoxic ternary mobile phase. The hydrolysis products, asp and phe, of aspartame in diet coke were successfully separated and identified using a PVA-UTLC plate. This preliminary study of amino acid analysis also indicates the potential use of the PVA plate in the separations of other biomolecules.

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