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Examination of pH and the Electric Field in Simultaneous Chromatography and Electrophoresis

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Abstract

Thin layer chromatography, with its history as a simple and effective means to identify compounds in a mixture, has motivated research to improve upon its foundations and develop novel techniques to further increase its speed and resolution while maintaining its simplicity. Simultaneous chromatography and electrophoresis (SCE) is a novel two-dimension separation technique that combines an electric field orthogonal to capillary action on a TLC plate. The simplicity of TLC is retained and separation is improved by allowing compounds to be separated according to both their charge-to-mass ratio and polarity. This work investigates how pH of both the mobile and stationary phases alter SCE separations. Additionally, different apparatus designs used to introduce and control mobile phase flow are evaluated. The highest-quality separations are achieved by applying pressure to the TLC plate during analysis.

Keywords: Chromatography, Electrophoresis, Two-Dimensional Separation

1. Introduction

Thin layer chromatography (TLC) is useful as an inexpensive method to separate compounds from a mixture. Unique from other separation techniques, TLC can be used to simultaneously separate components of a known and an unknown sample for the purpose of visual comparison. Separation resolution can be increased for complex mixtures by adjusting mobile and stationary phase polarities. Yet, for many mixtures, these changes alone are unable to separate similar compounds. Two-dimensional separations further increase resolution for complex mixtures.[1] Because most of these techniques are performed in tandem, two-dimensional separations offer improvements at the expense of increased analysis times. Simultaneous chromatography and electrophoresis is a new two-dimensional separation technique that allows compounds to be separated according to their polarity and charge-to-mass ratio by applying an electric field orthogonal to the flow of the mobile phase. Improved separations can be performed without increase to analysis time.

It was recently shown that simultaneous chromatography and electrophoresis (SCE) improves compound separation of selected vitamins, amino acids, and dyes when compared with TLC alone.[2] The apparatus employed solely capillary action for chromatography in an open-apparatus design while an electric field was applied orthogonal to capillary action. Complete separation occurred in approximately 15 min. However, significant compound-spot streaking was apparent. In an attempt to further improve separation and reduce streaking, many alterations to the apparatus design and separation conditions (e.g., increasing the number of mobile phase reservoirs, altering the shape of the reservoir notches, adjusting electrolyte concentration, and modifying electric potential) were investigated. These changes moderately improved separation and decreased separation time to less than 10 min. Further evaluation of possible pH gradients and electric field inhomogeneities are required.

Recently, pressure was applied to a simultaneous chromatography and electrophoresis system in an attempt to improve separation. The technique was called orthogonal pressurized planar electrochromatography.[3] In a one-dimensional separation technique known as pressurized planar electrochromatography, application of pressure to a TLC plate with the electric field parallel to capillary action has demonstrated improved separation.[4,5] The electric field is applied to assist capillary action and introduce electroosmotic flow. For SCE, the use of pressure offers control of the mobile phase flow rate when compared to capillary action. According to the Van Deemter equation, controlling flow rate can potentially improve separation efficiency. This work attempts to conserve the simplicity of TLC, along with its unique property of comparing the unknown mixture with a standard mixture, while improving compound separation and reducing analysis time.

2. Methods

2.1 Chemicals

1-propanol and ammonia were purchased from Fisher Scientific (Fair Lawn, NJ). Phosphoric acid, glycine, sodium hydroxide, crystal violet, methylene blue, methyl red and bromocresol green were purchased from Sigma-Aldrich (St. Louis, MO). Tartrazine, allura red, erythrosine, and erioclaucine were purchased from McCormick & Co. Inc. (Hunt Valley, MD). Ethanol was purchased from Heath Link (Jacksonville, FL).

2.2 Apparatus

2.2.1 non-pressurized design

TLC silica gel plates were cut 7 x 6 cm with 0.5 cm diameter hemispheres located along the bottom of the plate at 1.5, 3.5, and 5.5 cm. Platinum electrodes (Sigma-Aldrich, St. Louis, MO) were placed 4 mm from the edge of the plate. TLC plates (Macherey-Nagel, Easton, PA) were secured by two acrylic plates with electrode notches filled with weather stripping to allow the copper electrodes to be pressed evenly against the TLC plate. Two rows of copper electrodes were inserted into the acrylic, flush with the inside, to measure voltage during separation. A solvent reservoir that consisted of four separate glass chambers to encourage current to flow through the TLC plate was placed under the apparatus (Figure 1).

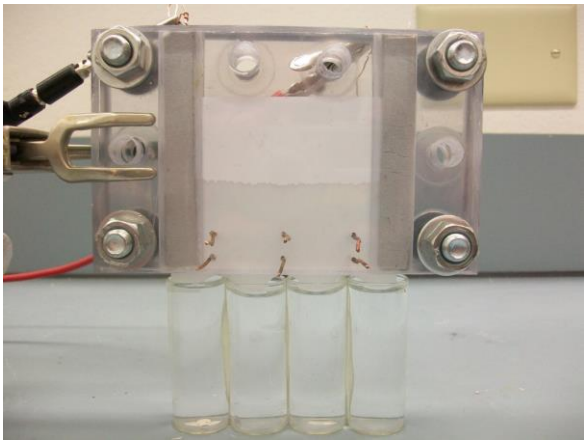


Figure 1. Non-pressurized SCE apparatus used in pH conditioning analyses and to measure electric potentials across the TLC plate.

2.2.2 *pressurized design #1*

Six injection ports were drilled into 2.5-cm acrylic plates to allow for solvent delivery. The ports were split between two syringes to prevent current from traveling through the solvent delivery system. An O-ring and putty seals were used to seal the bottom and side edges of the TLC plate allowing mobile phase to only exit the top edge of the apparatus. Two large electrode channels were filled with caulking to secure the electrodes. Eleven bolts were used around the bottom and side perimeter to apply uniform pressure. C-clamps were also used to apply pressure in the center of the plates (Figure 2).

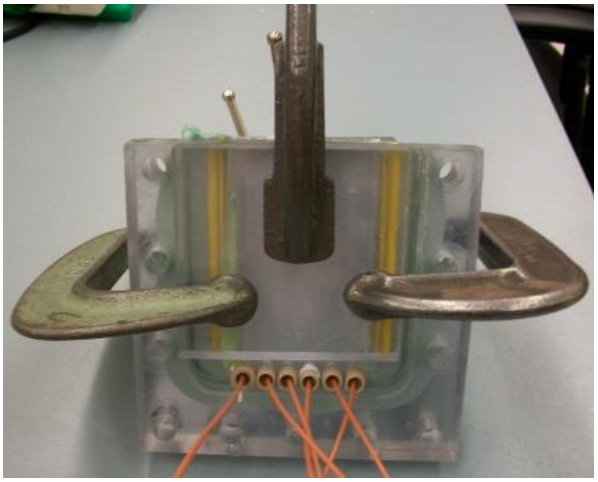


Figure 2. C-clamps used in the pressurized design #1 to apply pressure from the center.

2.2.3 pressurized design #2

Two injection ports were drilled into the side of the 2.5-cm acrylic plates; six equally spaced holes were drilled to the two injection ports for solvent delivery to the plates. A stronger O-ring was used to seal the TLC plate along the bottom and sides. Thin channels, slightly larger than the electrodes, were cut into the plates. Four wing-nut bolts were used to fasten the acrylic plates.

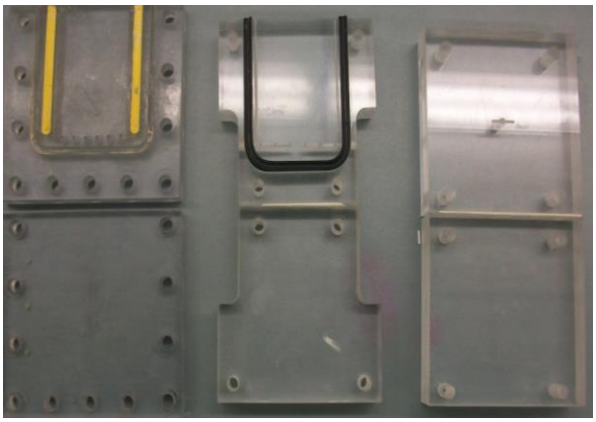


Figure 3. Pressurized apparatus designs #1, 2, and 3 from left to right, respectively.

2.2.4 pressurized design #3

A single injection site was drilled into the center of a 2.5-cm acrylic plate for solvent delivery. The injection port was extended by routing a 1.0-cm long and 0.16-cm deep notch. Compounds were spotted on all sides of the notch,

this allowed unknown and standard mixtures to be analyzed concurrently. Solvent was allowed to flow in all directions from the injection site. A hydraulic press (Carver, Inc., Wabash, IN) was used to apply pressure (Figure 4).

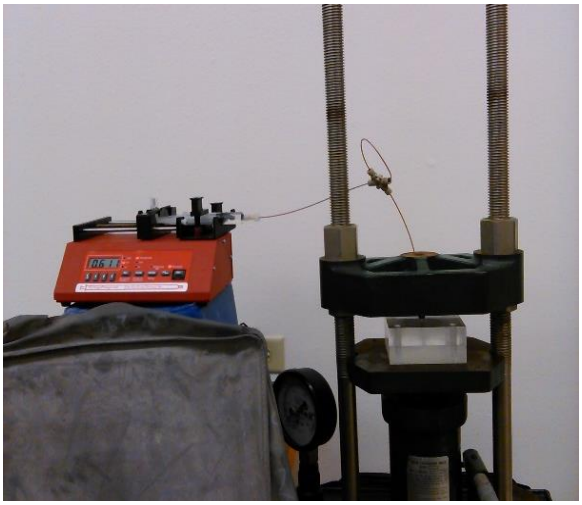


Figure 4. Hydraulic press used in design #3 to apply pressures around 1000 PSI to the apparatus.

2.3 Methodology

2.3.1 altering mobile phase pH (non-pressurized design)

Tartrazine, allura red, erythrosine, and erioclaucine were prepared by diluting 0.15 mL with 4.0 mL water. Crystal violet, and methylene blue solutions were prepared by dissolving 1.0 mg in 2.0 mL of water. Methyl red and bromocresol green were prepared by dissolving 1.0 mg in 0.75 ml of ethanol and 0.25 mL of water.

150-350 mM concentrations of glycine (pH 2.4), phosphoric acid (pH 6.5), and sodium hydroxide (pH 13) buffers were used in a volumetric ratio of 1:2 with *n*-propanol. A cut silica gel TLC plate was spotted with each of the eight dyes and then secured in the non-pressurized apparatus. The four chamber reservoirs were filled with mobile phase. The bottom of the TLC plate was lowered into the reservoirs. After 1 min., 500 V were applied for 14 min. The TLC plate was then immediately removed to dry. Universal indicator was sprayed on the plate to examine the pH.

2.3.2 altering stationary phase pH (non-pressurized design)

Cut silica gel TLC plates were conditioned by soaking for 30 min. in 350 mM concentrations of glycine at pH 2.4 or pH 9.6. A 1:2 mobile phase of the same buffer solution and propanol was prepared. Plates were dried in an oven and then spotted with the eight dyes. SCE procedure followed as described in section 2.3.1, except separations were shortened to <10 min.

2.3.3 pressurized design tests

Pressurized apparatus tests were conducted by pumping mobile phase solution through the pressurized apparatus. For flow analysis, dyes were injected, post TLC plate saturation, into the mobile phase to visualize flow. For compound separation, TLC plates were prepared by spotting one or more of the dyes on the TLC plate. Bolts, when used, were tightened equally. When using the hydraulic press, pressures of 500-1200 psi were applied to the TLC plate. Initially, no voltage has been applied when using the hydraulic press.

3. Results

3.1 Altering Mobile Phase pH (non-pressurized design)

The eight dyes travelled approximately the same distance across the TLC plate with glycine, phosphoric acid, ammonia (Figure 5). Similar movement was also seen with sodium hydroxide (not shown). Bromocresol green indicator showed that the majority of the plate had a pH of about 3.5, close to the pH of the silica gel (Figure 6). The inability of the mobile phase to alter the pH of the silica gel is likely due to the small amount of mobile phase flowing across the TLC plate due to capillary action. The basic region of the plate (left) and the acidic region (right) can be explained by electrolysis of water creating hydroxide and hydronium ions at the anode and cathode, respectively. The basic region along the bottom of the plate was due to the plates being submerged in the solvent reservoirs. Voltage measurements were generally the same regardless of the pH of the mobile phase.

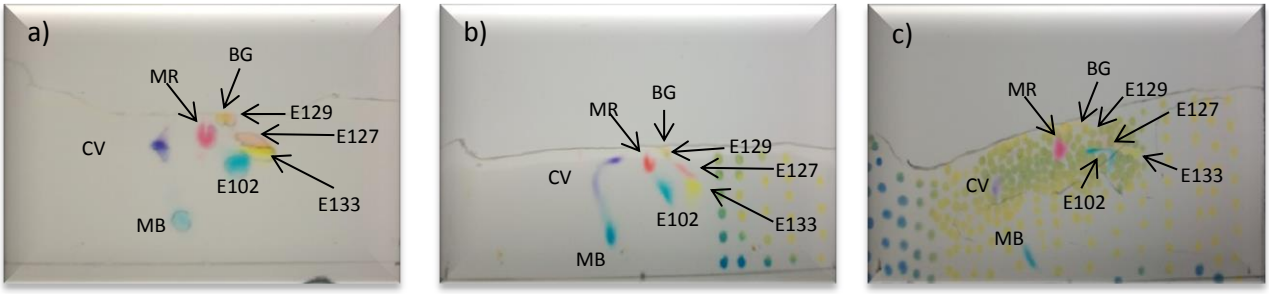


Figure 5. Separations achieved with (a) glycine at pH 2.3, (b) ammonia at pH 9.6, and (c) phosphoric acid at pH 6.5 mobile phase solutions. These separations are very similar. (b) and (c) are spotted with bromocresol green indicator to show pH.

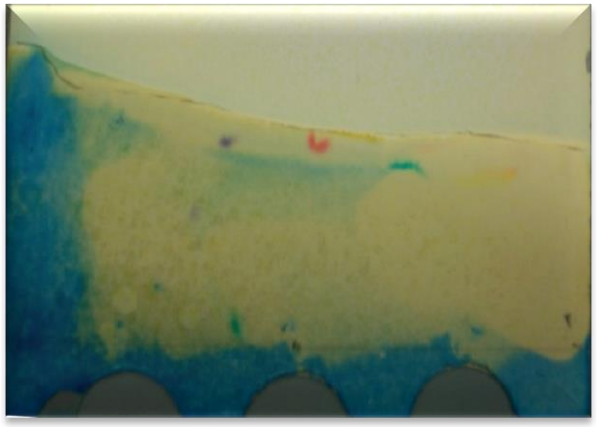
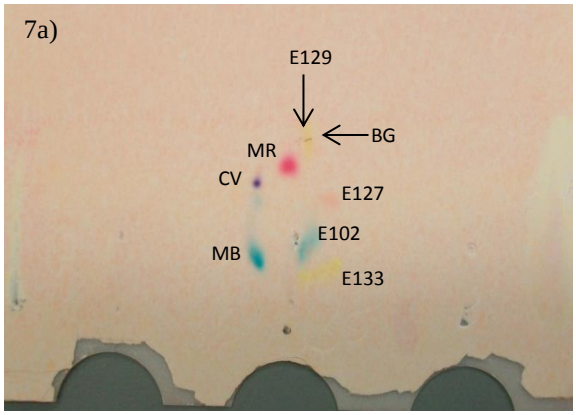


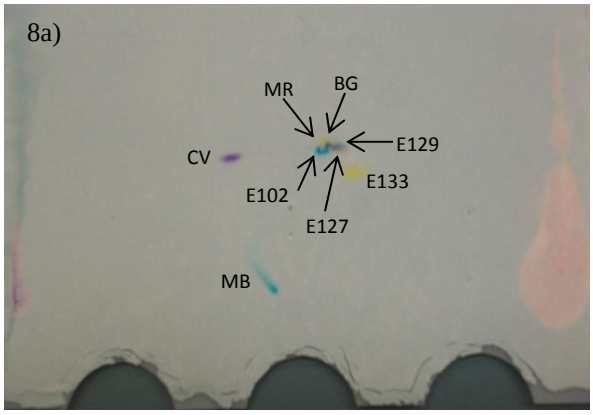
Figure 6. A non-conditioned TLC plate analysis with phosphoric acid mobile phase buffered to pH 6.5. The stationary phase was sprayed with bromocresol green indicator to visualize pH. Areas above the bottom of the plate and to the right of the anode were acidic (i.e., lower than pH 3.5) due to the small amount of buffer drawn up by capillary action.

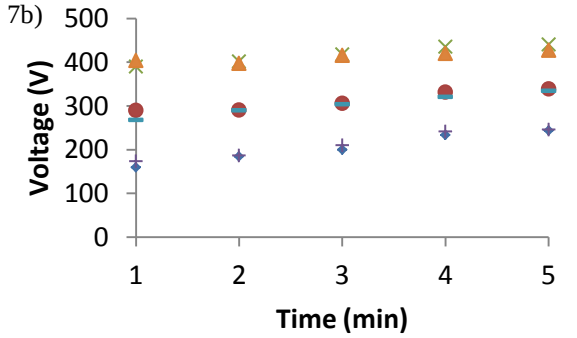
3.2 Altering Stationary Phase pH (non-pressurized design)

Universal indicator shows that conditioned TLC plates effectively change the pH of the silica gel (Figures 7 and 8). This change in pH altered the horizontal and vertical movement of many of the dyes. In particular, methyl red showed no horizontal movement at pH 3.5 (large dark red spot in Figure 7a) and moved significantly to the right at

pH of 9.6 (Large yellow spot in Figure 8a). This coincides with methyl red being neutral when protonated (no horizontal movement) and an anion above pH 5.1. Voltage measurements were recorded across the stationary phse every minute during the separation (Figure 7b and 8b). There were differences noted between plates run with acidic and basic mobile phase: the acidic tending towards higher voltage readings across the plate and increasing over time compared to basic which generally had lower readings and decreased over time.







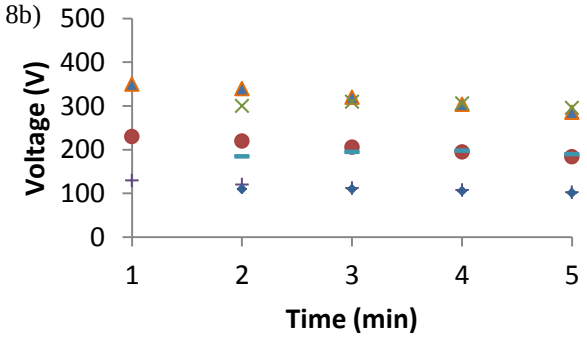


Figure 7. (a) Conditioned TLC plate. 200 mM glycine mobile phase (pH 2.4) at 500 V. Plate sprayed with universal indicator after separation. (b) Voltage versus time at six consistent copper electrode locations.

Figure 8. a) Conditioned TLC plate at pH 7. 200 mM glycine mobile phase (pH 9.6) at 500 V. Plate sprayed with universal indicator after separation. (b) Voltage versus time at six consistent copper electrode locations.

3.3 Pressurized Apparatus Design #1

A non-uniform solvent front was exhibited. This was likely due to uneven application of pressure causing solvent to travel faster along the electrode channels and O-ring seal. Lack of pressure and bowing were observed due to the partially compressed O-ring. This was solved by using putty instead of an O-ring as a seal (Figure 9). However, additional pressure, more than that supplied by the eleven bolts, was needed to fully compress the O-ring.

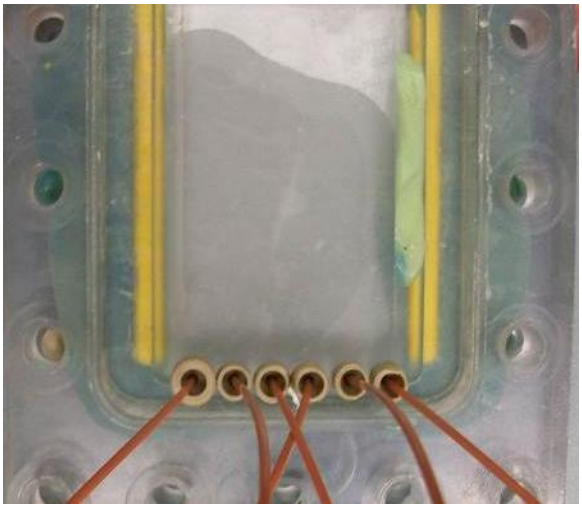


Figure 9. Design #1 flow study: water mobile phase with putty seal.

3.4 Pressurized Design #2

The pressure applied by the bolts was not enough to compress the new O-ring to seal the apparatus; thus, putty was required as a temporary fix. Bowing was observed across the apparatus, which resulted in a loss of analyte as mobile phase flowed quickly over the silica gel instead of through it (Figure 10). A similar uneven solvent flow to design #1 was seen (Figure 11).

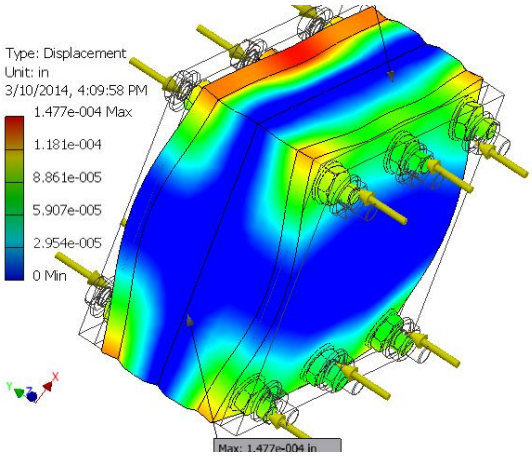


Figure 10. Computer simulation that shows bowing caused when pressure is applied along the edges with bolts.

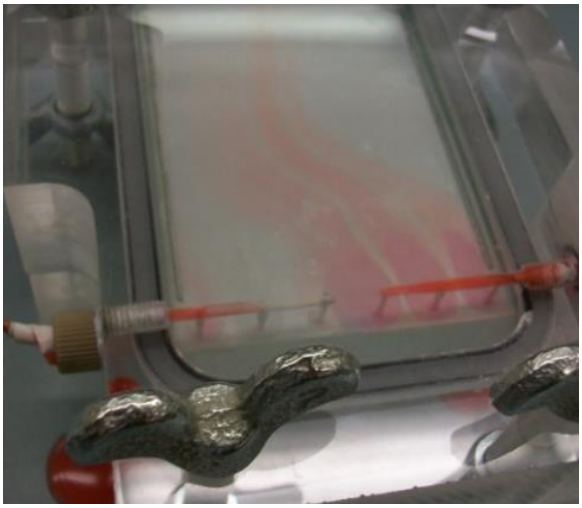


Figure 11. Design #2 flow study: O-ring seal, dye introduced post saturation.

3.5 Pressurized Design #3

The uniform pressure supplied by the hydraulic press removed bowing and the loss of analyte seen in previous designs. The single injection site decreased streaking and simplified the task of creating a uniform solvent front. Separations with electrophoresis at 500 V took 5 min. (Figure 12).

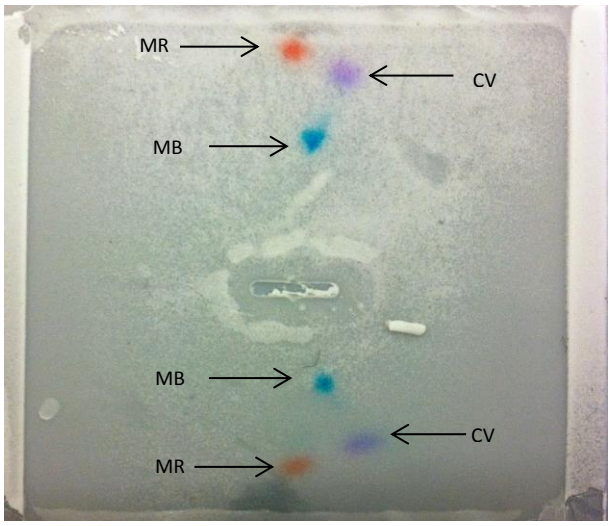


Figure 12. Design #3 under 12,000 pounds of pressure with 500 V and a mobile phase flow of 60ul/min applied for 4 min. Methyl red, crystal violet, and methylene blue were spotted.

4. Conclusion

Conditioning the TLC plates prior to SCE is effective at altering the pH of the plate and changing in the horizontal and vertical movement of selected dyes. This could allow an acidic or basic pH to be chosen for additional control over the compound separation.

The pressurized design with a single injection port in the center eliminated some of the uneven solvent problems when used with a hydraulic press. The bolts along the edges of each apparatus applied an insufficient amount of pressure to the apparatus to compress the O-rings. Applying pressure along the sides of the apparatus also lead to bowing which resulted in mobile phase traveling above the silica gel instead of through it. By removing the O-ring and evenly applying more pressure to the entire apparatus an even solvent front was obtained. The by spotting across from the injection port at the same distances this also allows two samples to be run concurrently for the purpose of comparison maintaining TLC simplicity. More work needs to be done to refine mobile phase flow, voltage, and pressure to compare the pressurized separations to the non-pressurized separations.

5. Acknowledgments

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6. References

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