Planar chromatography and electrophoresis are one-dimensional separation techniques. In this work, planar chromatography and electrophoresis were carried out simultaneously in two separate dimensions. Chromatography occurred via capillary action while an orthogonal electric field was simultaneously applied to promote electrophoresis. Using copper wires, a custom-made planar chromatography plate with a novel dual solvent chamber was used to apply the electric field. Several sample types were analyzed; i.e., hydrophilic vitamins, amino acids, and dyes.

Methods

Fluorescent thin-layer chromatography (TLC) plates pre-coated with silica gel 60 F_{254} were used for each sample tested after being activated at 110 °C for at least 30 minutes. Each TLC plate was cut to 78 mm x 100 mm. The copper wiring was threaded parallel to the longer sides 6 mm from the edge. Holes were manually introduced 7 mm apart for threading. The setup is shown in Figure 1. A series 225 power supply made by Barten Associates Inc. was used to provide voltage.

Four hydrophilic vitamins were analyzed: thiamin (B_{1}), riboflavin (B_{2}), nicotinic acid (B_{3}), and pyridoxine (B_{6}). These were purchased from Sigma-Aldrich (St. Louis). Separation occurred employing a 1-propanol/10 mM glycine (9 : 1) mobile phase for 30 min. The voltage was 750 V. All vitamins were visible under ultraviolet light of 254 nm except vitamin B_{6} which fluoresced at 365 nm.

Five amino acids were analyzed: alanine (A), glutamic acid (E), leucine (L), proline (P), and lysine (K). Separation occurred employing a 1-propanol/1.0 mM NH_{3} (7 : 3) mobile phase for 60 min. The voltage was 500 V. 0.1% ninhydrin in ethanol was used to develop the amino acids for visualization.

Eight dyes were analyzed: yellow 5 (E102), red 40 (E129), red 3 (E127), blue 1 (E133), crystal violet (CV), methylene blue (MB), bromothymol blue (BB), and methyl red (MR). Separation occurred employing a 1-propanol/100 mM glycine (2 : 1) mobile phase for 15 min. The voltage was 600 V.

Results

One-dimensional chromatographic separations and two-dimensional chromatographic/electrophoretic separations are shown to the right for each sample. Additionally, one- and two-dimensional separations (not shown) of each respective compound were performed for the purpose of identification.

Figure 2 shows a one-dimensional chromatographic separation of the hydrophilic vitamins. Figure 3 shows a simultaneous two-dimensional separation of the hydrophilic vitamins. Notice that upon applying voltage, two overlapping vitamins (i.e., B_{2} and B_{6}) separate. Figure 4 shows a one-dimensional chromatographic separation of five amino acids. Figure 5 shows a simultaneous two-dimensional separation of the amino acids. All amino acids were separated in Figure 5. Figure 6 shows a one-dimensional chromatographic separation of eight dyes. Figure 7 shows a simultaneous two-dimensional separation of the eight dyes. Upon applying voltage all dyes separated and were visible in only 15 minutes.

Discussion and Conclusion

Although planar chromatography and electrophoresis have been coupled in series, no reports have been found employing both techniques simultaneously. This work introduces a novel instrumental setup allowing for planar chromatography and electrophoresis to occur simultaneously in two separate dimensions. Vitamins, amino acids, and dyes were used to characterize the technique. Each sample showed improved separation utilizing the technique over a one-dimensional chromatographic separation. The feasibility of simultaneously employing chromatography and electrophoresis in two separate dimensions has been demonstrated.

References


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