

An Inexpensive Low Voltage Paper Strip Electrophoresis Apparatus

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The paper strip type of electrophoresis is a simple laboratory method of separating and identifying small quantities of chemical compound mixtures. The technique is based on the principles of chromatography in that separation is accomplished by the variety of migration rates of different compounds. Electrophoresis employs a high potential difference to cause the migration, while other forms of chromatography achieve this by solvent flow. The need for a source of high voltage and current regulation makes electrophoresis more costly and complicated than thin-layer or paper chromatography. My interest in the technique resulted in the construction of a simple, inexpensive electrophoresis apparatus with which successful separations of amino acids were performed.

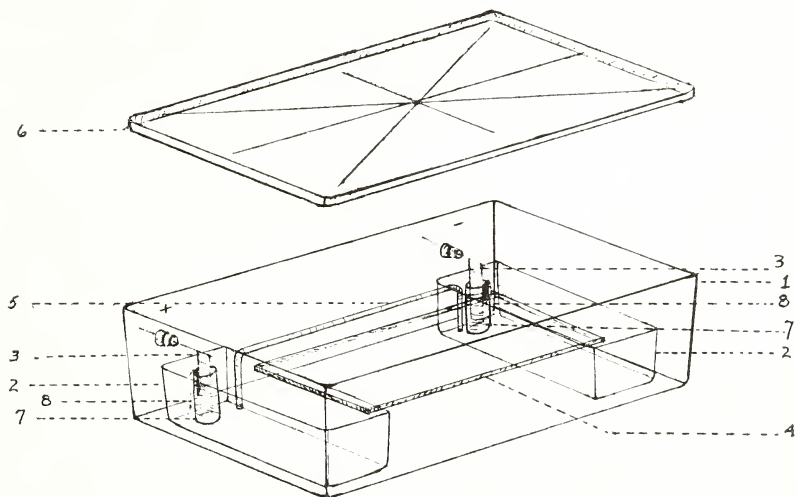


Figure 1. ELECTROPHORESIS APPARATUS

TANK	1	U-TUBE BRIDGE	5
BUFFER RESERVOIRS	2	LID	6
ELECTRODES	3	ELECTRODE CONTAINERS	7
GLASS PLATE SUPPORT	4	PAPER WICKS	8

The solvent containers of the instrument were a plastic shoe box and two plastic butter dishes. The main tank was a transparent box with dimensions of 30.5 x 17 x 9 cm. The transparent lid had a 2 cm. lip overlapping the top of the tank when in place. Within the tank there were two clear plastic reservoirs, one placed at each end and perpendicular to the length of the main vessel. These were 14.5 x 5.5 x 4 cm. and were used to hold the buffer solution. A 15 ml. analytical weighing bottle was placed in each of the reservoirs and contained the electrodes.

A strip of filter paper, 1 cm. wide and 7 cm. long, folded over the lip of the weighing bottle was saturated with the buffer solution and made electrical contact between the electrolyte in the reservoir and the electrode. In order to prevent a flow of the buffer through the chromatographic paper strip, a leveling U-tube (5mm-O.D.) was filled with electrolyte and placed so that the ends of the tube projected below the surface of the buffer in each reservoir (3). Two holes were melted into the same side of the tank with a heated 10 mm. glass tube. Each hole was situated about 3 cm. from the top and 4 cm. from the end of the tank. Small cork stoppers were inserted into these holes and platinum wires were pushed through the stoppers, projecting about 3 cm. into the tank. To these projections were attached platinum foil electrodes (1 x 5 cm.). A glass plate (10 x 20 cm.) was used as a support on which the paper strips were laid. This plate was supported on each end by the top edge of the buffer reservoir.

The power supply consisted of a Lab-Volt transformer, model 250B. It produced a voltage of 300 volts DC with a maximum current of 100 ma. The voltage gradient in a strip of paper 28 cm. long with approximately 1 cm. of each end immersed in buffer solution was about 12 volts per cm. A milliammeter (0-20 ma. range) was connected in series with the tank and the transformer. To prevent more heat from being generated than could be dissipated by evaporation of buffer solution from the paper strips, the amperage was controlled by a 2.5 watt, 37.5 kilo-ohm resistor in series with the circuit. This prevented the current from becoming greater than 9 ma. and could be read directly from the milliammeter.

This apparatus was used to separate three amino acids: glycine, arginine, and aspartic acid. Four strips of Whatman No. 1 chromatography paper, 2.5 cm. wide, were cut to a length of 28 cm. These were saturated with the buffer solution and placed on the glass plate so that 4 cm. of the paper extended over the ends of the plate into the buffer solution reservoirs. The buffer solution was made up by mixing 100 ml. of 0.1N potassium acid phthalate and 40.6 ml. of 0.2N hydrochloric acid, diluted to 200 ml. with water (4). The electrode containers were filled with the solution and the remaining amount was divided equally between the two reservoirs using the U-tube to balance the levels. A blotter of folded chromatography paper was used to dry a 2.5 cm. wide space on the strips at the point of application of the amino acids. The acids were applied to the strips with an applicator made by folding a strip of filter paper around a rectangular wire frame. After about ten minutes, the buffer solution had flowed back to the point of application, producing a narrow band of the amino acids. Voltage (300 v) was applied to the completed circuit to produce an initial current of 2.5 ma. After four hours the current had risen to 7 ma. The strips were taken out and dried by evaporation at room temperature. The dry strips were sprayed with a 0.2% solution of ninhydrin in acetone and placed in a drying oven at 80-90 degrees C for about 3 minutes to develop the color. The relative positions of the amino acids could be seen by the various purple bands. Arginine (isoelectric point, 10.8) moved about 6 cm. in the direction of the cathode. Since the pH of the buffer

was 3.0, arginine was acidic and carried an excess of positive charges. Glycine moved only 3 cm. from the point of application toward the cathode. Because its isoelectric point is 6.1, it was less acidic and less positive than arginine. The third amino acid, aspartic acid, moved only slightly from the point of application because its isoelectric point is 3.0 and it was electrically neutral in the buffer solution (2).

A second experiment was conducted on the same amino acids. All conditions remained the same except for the change in the buffer solution. This was replaced with 10.4 ml. of 0.1 N sodium hydroxide, 100 ml. of 0.1N potassium dihydrogen phosphate and sufficient water added to make 200 ml. The final pH was 6.0. Paper strips were prepared in the same manner as before. The potential difference was 300 volts, and the current ranged from 2.5 ma. at the start to 7.0 ma. at the end of the experiment. The strips were removed after four hours and treated as before. The amino acids appeared in the same sequence as before but the rates of movement were different. Arginine, still acidic in this buffer solution, moved approximately 4 cm. toward the negative electrode. Glycine moved only 1 cm. in the same direction since the buffer solution with a pH of 6.0 was very near the isoelectric point of glycine. Aspartic acid, which was basic in this buffer, moved toward the anode (3 cm.) from the point of application.

The distances that the acids migrated from the point of application usually varied when the same buffer solution was used for the second time. There was an increase in the current flowing through the circuit. This was probably the result of water loss and an increase in salt concentration in the buffer solution due to evaporation and decomposition at the electrodes. The latter accounted for only a minor change in pH because of the separation of the electrodes from the buffer reservoirs achieved by the separate electrode containers. As a result of this change, the acids moved farther from the point of application. Exact reproducibility was difficult unless one used freshly prepared buffer solution for each experiment.

Other factors that greatly affected the degree of separation were the amount of the amino acids applied and the distribution of the sample on the strip. The streaking of the zone was another factor. If the zone applied was too wide or too much acid was applied, the zones did not separate into distinct spots. This streaking may have resulted from uneven application of the amino acids, or possibly from improper drying of the area before the acids were applied.

This type of laboratory method for the separation of chemical compounds is especially advantageous when working with ampholytic compounds such as proteins and amino acids (1). Other chromatographic techniques depend on the rate of adsorption of the compound to the resolving medium. Open strip electrophoresis can produce different separations when such conditions as temperature, voltage, pH of the buffer solution, and the time factor are controlled (3). Although the acids could not be identified by the absolute distance they had migrated, the number present and their approximate isoelectric points could be determined by their relative positions on the strip. One could make

positive identification of each zone by knowing the acids that were present in the mixture and the pH of the buffer.

This simple, inexpensive electrophoresis apparatus was constructed from regular laboratory equipment and some plastic containers. It was successfully used to separate amino acids. The results were reproducible within experimental error limits.

Literature Cited

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