Thin-Layer Electrophoresis

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Electrophoresis was being used as early as 1944 for the separation of amino acids (*1*), but techniques for performing thin-layer electrophoresis were not developed until the early 1960s (*2*, *3*). Since then, this method has largely been displaced by more quantitative, but much more expensive, techniques such as GC (*4*) and HPLC (*5*). Electrophoresis is an attractive technique to present in the teaching laboratory because it illustrates a principle that is comprehensible and useful with an apparatus that is simple and inexpensive. Inexpensive gel electrophoresis of amino acids has been reported in this *Journal* (*6*, *7*).

Theory

In contrast to chromatography, electrophoresis yields fundamental information about the molecule under study: namely, the charge. The charge on the molecule is directly proportional to the distance the molecule travels when an electric field is applied for a certain time. For the type of electrophoresis described in this article, the size of the molecule does not affect its mobility, presumably because the pore size of the medium is very much larger than the molecule. In order to determine the charge, the distance the sample has migrated is compared to the distance an internal standard of known charge has traveled. For accurate work, an uncharged internal standard is included in a run to correct for electroosmosis, which is the tendency of the solvent to migrate in an electric field. Thus the charge on the sample is

$$z_{\text{sample}} = \left(\frac{d_{\text{sample}} - d_{\text{uncharged}}}{d_{\text{standard}} - d_{\text{uncharged}}} \right) z_{\text{standard}} \qquad (1)$$

where z is charge and d is distance.

Note that electrophoresis measures the *net* charge on the molecule. For example, glycine at pH 7 has a protonated amino group (NH_3^+) and a deprotonated carboxyl group (COO^-), which cancel to yield no net charge. Additional information on amino acids may be found in our supplemental material[W] and at a comprehensive Web site (*8*).

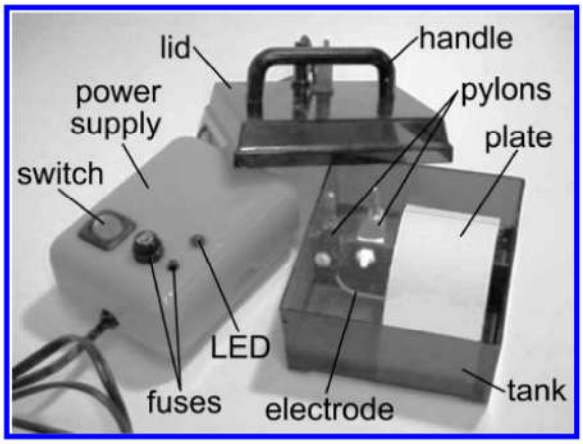

Figure 1. The thin-layer electrophoresis apparatus.

Apparatus

Figure 1 shows the electrophoresis apparatus, which consists of a power supply and a tank with an integral partition. This device is easily constructed from components costing about $50. A parts list and construction hints are included in the supplemental material.[W] The tank has an anodic and a cathodic buffer compartment into which the ends of a flexible cellulose-coated plate are immersed. When power is applied to the buffer, ions on the plate are impelled in the direction opposite their net charge.

Figure 2 presents the schematic electrical diagram. Because the apparatus produces about 275 volts peak-to-peak pulsed dc, several safety features have been incorporated into the design. The ac line voltage is isolated from the tank by a transformer, and both the primary and secondary circuits of this transformer have fuses. The connection to the tank is arranged so that removal of the tank lid disconnects the electrodes, and the power supply is sealed. There is no capacitor that could retain a charge after the unit is turned off. Note that this device is suitable for aqueous systems only; organic solvents

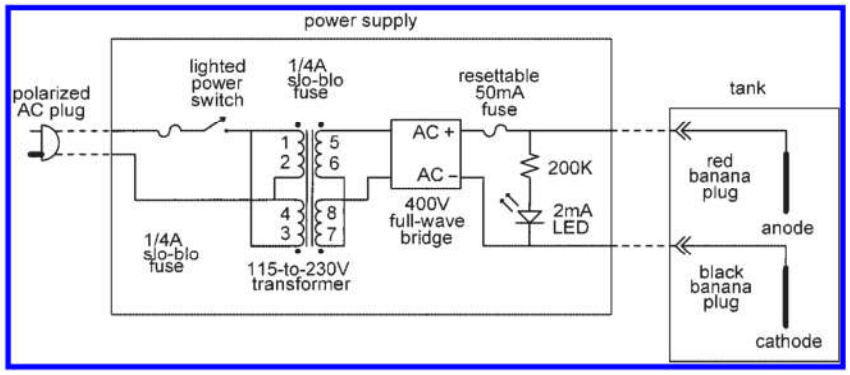

Figure 2. Diagram of electronic circuit for the thin-layer electrophoresis apparatus.

damage the tank and pose a fire hazard. Hundreds of high school and college students have used this device without incident.

Materials

The device is designed to accommodate 15 × 5-cm strips of Kodak Chromagram cellulose sheets, which give higher resolution than would a filter paper medium. Five strips can be obtained from each Chromagram sheet at a cost of approximately $1 per strip. Drummond MicroCaps or pulled capillary tubes may be used for sample spotting. Note: Wear gloves when handling the sheets and use only clean instruments (i.e., scissors and rulers), to avoid contamination (*9*).

A typical buffer is 0.1 M phosphate at pH 6. Ninhydrin solution is prepared by dissolving 0.3 g of ninhydrin and 3 mL of glacial acetic acid in 100 mL of *n*-butanol. This solution is usable for up to 3 weeks. A few milliliters of each of the ~0.1 M amino acid samples should be prepared. Some of the amino acids are not very soluble in water, so it may be necessary to add a few drops of ~6 M HCl and let the solution stand for a few hours with occasional stirring or shaking. If amino acid solutions are kept more than a day or two they should be refrigerated.

Hazards

Acetic acid is corrosive and can cause eye and skin burns. It can also burn the digestive and respiratory tracts if ingested or inhaled, respectively. Hydrochloric acid is corrosive. It can cause irritation or burning of the nose and throat if inhaled. Ninhydrin can cause eye and skin irritation. Gloves should be worn when handling ninhydrin. Sodium hydroxide is a corrosive agent and an irritant. Avoid contact with the skin.

The electrophoresis apparatus produces less than 300 V at 50 mA. However, we recommend that this apparatus be handled with caution.

Experiment

A starting line is drawn across the middle of the cellulose strip with a pencil. Along the starting line three tick marks are made 1 cm apart. A plus sign is drawn at one end of the strip and a minus sign at the other. Using a Drummond MicroCap or a pulled glass capillary tube, 3 drops of sample are applied; the drops are allowed to dry after each application.

After all of the spots have dried, a Pasteur pipet is used to carefully drip buffer onto the cellulose plate. Buffer is applied at each end of the plate and allowed to spread from both ends so that the two buffer fronts meet exactly at the sample spots in the center.

A volume of ~35 mL of buffer is placed in each of the tank reservoirs. The cellulose plate is bent into an arc with the spots facing upward, and the ends of the plate are immersed in the buffer in the reservoirs. The end of the plate marked "+" is in the same reservoir as the red electrode pylon. After replacing the tank cover, the apparatus is plugged in and turned on, and the time is noted. After 45 min, the apparatus is turned off and unplugged. The strip is removed from the apparatus, sprayed with ninhydrin in the hood, and placed on a hot-plate to develop. The strip may also be developed by drying it in an oven at 90–100 °C.

The migration distance from the starting line to the center of each spot is measured. Equation 1 is used to calculate the charge.

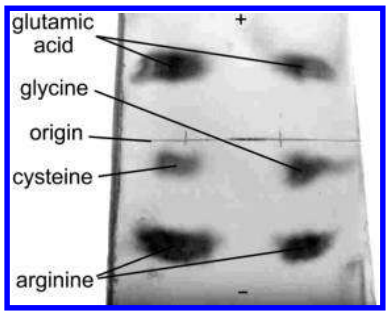

Figure 3. Result of a 45-min run in 0.1 M phosphate buffer, pH 6.0. Note that electroosmosis causes even glycine and cysteine, which are neutral at this pH, to be displaced from the origin.

Figure 3 shows a typical electrophoretic run. The distance the samples move permits the identification of unknown amino acids, whether they are single samples or mixtures. Cysteine can be identified by its unusual yellowish color. Testing at different pH values may permit the identification of more samples.

Amino acids that have nearly the same charge at every pH cannot be unambiguously distinguished by electrophoresis. Within this constraint, students can identify an unknown amino acid or the components of a mixture. An experiment in which the students are asked to determine which pair of amino acids in a set have had their labels switched has been part of our introductory lab for a number of years. The instructions for this experiment are included in the supplemental materials.[W] Twenty-four students equipped with 6 thin-layer electrophoresis devices are given 10 amino acids and 3 buffers (pH 4, 7, and 10). They have 8 hours to plan a strategy and make the necessary runs to discover which pair of amino acids has been mislabeled. Frequent difficulties include contamination from the hands, rulers, and scissors, buffer whose ionic strength is too high, over-wetted strip, and failure to compensate for electroosmosis.

Some other uses of the thin-layer electrophoresis apparatus are to determine the p*K* of a compound by performing multiple runs at several pH values clustered about the suspected p*K* and to sequence a small polypeptide (e.g., glutathione) by the partial hydrolysis method. Ionic materials other than amino acids may be run as long as there is a way to visualize them. Kodak Chromagram sheets with fluorescent indicator are convenient for detecting compounds (e.g., the B vitamins) that quench fluorescence.

Acknowledgment

Birute Williams performed many of the preliminary experiments.

[W]Supplemental Material

The following supplemental material for this article is available in this issue of *JCE Online*: an experiment separating amino acid mixtures that is appropriate for a high school lab, a simulation on mislabeled amino acids that is appropriate for an undergraduate lab, a chart of amino acids comparing charge and pH, a parts list, apparatus construction hints, and CAS registry numbers.

Literature Cited

1. Consden, R.; Gordon, A. H.; Martin, A. G. P. *Biochem. J.* **1944,** *38*, 224.
2. Honegger, C. G. *Helv. Chim. Acta* **1961,** *44*, 173.
3. Pastuska, G.; Trinks, H. *Chem.-Ztg.* **1962,** *86*, 135.
4. Husek, P.; Sweeley, C. C. *J. High Resolut. Chromatogr.* **1991,** *14*, 751.
5. Jarrett, H. W.; Cooksy, K. D.; Ellis, B.; Anderson, J. M. *Anal. Biochem.* **1986,** *153*, 189.
6. Hopkins, T. R.; Sreekrishna, K. *J. Chem. Educ.* **1987,** *64*, 279.
7. Emry, R.; Curtright, R. D.; Wright, J.; Markwell, J. *J. Chem. Educ.* **2000,** *77*, 1323.
8. Beavis, R. C. *Amino Acid Information*; *http://www.eng.uci.edu/~hslee/aminoacid.html* (accessed May 2001).
9. Hamilton, P. B. *Nature* **1965,** *205*, 284.