

Electrophoresis of Amino Acids

Introduction:

Electrophoresis is a separation technique based on the movement of charged ions under the influence of an electrical field. This technique is primarily used for the separation of amino acids and peptides on the basis of their charge. All amino acids contain ionizable groups that cause the amino acids, in solution, to act as charged polyelectrolytes that can migrate in an electric field. The amino acids with a net positive charge will migrate toward the negative electrode. Those with a negative net charge will move toward the positive electrode. An inert substance such as paper or gel is used as a supporting medium for the conducting liquid in most electrophoretic methods. In this experiment of separating amino acids, a phosphate buffer (pH 6) will be used as the conducting liquid and cellulose as the supporting medium.

Purpose:

The purpose of this experiment is to determine the effect of an electrical field on charged particles and to use this information to identify the amino acids present in an unknown sample.

Equipment/Materials:

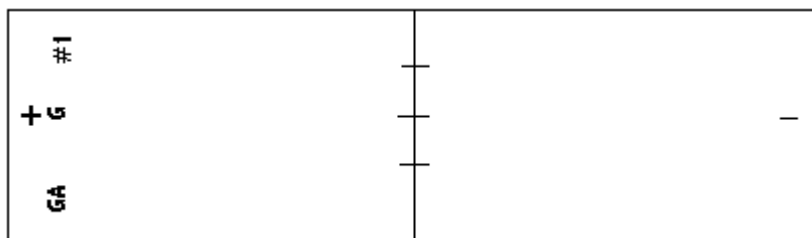
Electrophoresis apparatus	Ninhydrin spray
Graduated cylinders, 100 mL	Pasteur pipets/bulbs
Hot plates	Phosphate buffer pH 6
Kimwipes	Pulled capillary tubes
Kodak Chromogram™ cellulose sheets with fluorescent indicator	Scissors
Metric ruler	Standard amino acid solutions, ~0.1M
	Unknown amino acid solutions

Safety:

- Goggles must be worn at all times.
- Ninhydrin must be used under the hood.
- Make sure the electrophoresis apparatus is off when inserting and removing the plate.
- Wear gloves when handling plates and ninhydrin.

Procedure:

1. Obtain a strip of a cellulose chromagram sheet that is approximately 5cm wide and 15cm long. **REMEMBER: wear gloves.**
2. Using a pencil, mark one end of the plate with a plus sign and the other end with a minus sign. (See the diagram on the following page.)



3. Divide the two ends by drawing a line through the middle of the plate. Wipe the ruler with a Kimwipe before it touches the surface of the plate.
4. Place a tic mark on the line for each sample. Samples should be at least 1cm apart and 1cm from the edge. Place 3 samples on a sheet.
5. Label on one end of the plate what samples are on each tic mark.
6. Fill each electrode compartment with ~35mL of buffer.
7. Obtain 2 standard amino acid solutions and an unknown solution.
8. Use pulled capillary tubes to apply small volumes of the samples to the plate. Draw sample into the capillary tube by inserting the pointed end of the tube into the sample bottle. Use a different capillary tube for each sample. **Do not contaminate samples by using a capillary tube from a different sample.** Apply the sample to the plate by touching the end of the capillary tube to the proper mark on the line. Touch the plate lightly and quickly; the spots should be small. Reapply the same sample twice more to the same spot, allowing the plate to dry between applications.
9. Once the samples have dried, apply buffer to the plate using a Pasteur pipet. Begin by dropping the buffer at one end of the plate and then at the other end, allowing it to move toward the sample spots in the center. Continue in such a way that the buffer meets exactly at the middle line to reduce the migration of sample spots.
10. Carefully place the plate in the apparatus so that the plus corresponds to the positive electrode (red jack) and the minus side to the negative electrode (black jack). **Be careful not to tilt the plate in any direction to avoid migration of the sample.**
11. Turn on the apparatus and record the time.
12. Allow the sample to run for 45 minutes, or as long as possible, given time constraints. Each plate in the class must run for the same amount of time.
13. Turn the hot plate on low a few minutes before removing the plate.
14. Once the time has expired, turn off the apparatus and carefully remove the plate. **Be sure to wear gloves.**
15. **Under the hood**, spray the plate with ninhydrin.

16. Place the plate on the hot plate until it dries and spots appear.

17. Identify the contents of the unknown solution by comparing to standard amino acid samples. Groups will need to share the results of their standard amino acids.

Data:

Standard Amino Acid	Rate of Migration	Direction (+/-)

Unknown # _____

Component	Rate of Migration	Direction	Identity
1			
2			
3			

Calculations:

Calculate the rate of migration for each standard and component in the unknown.

$$\text{Rate of Migration} = \frac{\text{Distance Component Migrated}}{\text{Time of Electrophoresis}}$$