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Institute of Applied Sciences and Techniques -ISTA
1st year LMD,
Module: Analytical Tools of Biochemistry

PW N°5: Extraction and Identification of Some Amino Acids Derived from Casein by TLC

Purpose: Hydrolysis of casein in an acidic medium and identification of some amino acids present.

I. Hydrolysis of Casein

Casein is a protein that, through acid hydrolysis, yields the different amino acids that constitute it.

- Place about 0.5 g of chemical casein in a flat-bottomed Erlenmeyer flask, then add two pumice stones and approximately 10 mL of distilled water.
- Attach parafilm to the neck of the Erlenmeyer flask and proceed under a fume hood to handle concentrated hydrochloric acid.
- Under the hood, pierce the parafilm with scissors and gently pour 10 mL of concentrated hydrochloric acid through the opening, stirring manually.
- Return to your workstation and set up the reflux assembly (support stand, heating plate, Erlenmeyer flask with mixture, water condenser). Secure the Erlenmeyer flask and the condenser using two clamps and grease the ground glass joints.
- Heat for about 35 minutes (starting from when the solvent begins to boil in the flask). The mixture will turn brown during heating.
- Once the heating time is complete, turn off the heating plate and allow the solution to cool at room temperature for 10 minutes, then in a cold-water bath for another 10 minutes (keep the Erlenmeyer flask attached to the condenser).
- Add three spatulas of activated carbon and a magnetic stir bar to the reaction mixture. Reattach the Erlenmeyer flask to the condenser and stir using a stirring plate for 10 minutes.
- Filter the solution using a funnel and filter paper. Secure the funnel on a ring stand using a clamp. The hydrolyzed solution is now obtained.

II. Identification of Amino Acids from Casein

You will separate the amino acids by thin layer chromatography (TLC).

- In a chromatographic tank (250 mL beaker), precisely add: 7 mL of ethyl acetate, 2 mL of formic acid, and 1 mL of water. Cover the tank with a small crystallizer and let it stand for 15 minutes.
- Three amino acid solutions in water are prepared: aspartic acid (denoted As), alanine (denoted Al), and phenylalanine (denoted P).
- Prepare a TLC plate by making four evenly spaced deposits along the application line: one drop of the As solution, one drop of the Al solution, one drop of the P solution, and one deposit of the hydrolyzed casein solution (H). For the H solution, make three successive deposits, drying with a hair dryer between each application.
- Label each deposit with the corresponding letter using a pencil.
- Dry the deposits thoroughly with a hair dryer, then place the plate in the tank and cover it with the small crystallizer. Do not move the tank during elution.

- When the solvent front is approximately 1 cm from the top of the plate, remove the plate and immediately mark the solvent front with a pencil. Dry the plate with a hair dryer.
- Under a ventilated fume hood, wearing large gloves and safety goggles, use tweezers to immerse the face of the TLC plate in a tank containing ninhydrin. Drain excess ninhydrin on filter paper.
- Still under the fume hood, place the plate on a slightly heated hot plate. Purple spots will appear quickly. Do not allow the spots to turn brown.