

# THIN LAYER CHROMATOGRAPHY

## SEPARATION AND IDENTIFICATION OF AMINO ACIDS

Chromatography is the process of separation and purification of homogenous, multicomponent mixtures using the differences in their affinity to the stationary and mobile phase. Variety of physicochemical properties (hydrophobicity, pKa, additional functional groups) differing amino acids make a chromatographic separation possible and, what more important very efficient. In our approach we will use a thin-layer chromatography (TLC) with a silica stationary phase. For the detection we will use a ninhydrin test - highly sensitive chemical reaction, allowing for detection of  $10^{-9}$  mol of amino acid (**NOTE !! Ninhydrin leaves indelible purple spots on the skin**).

### Equipment and materials:

- TLC silica gel plates
- pencil and ruler
- standards of amino acids ( Gly, Ala, Gln, Phe, Met) and capillaries
- chamber with a cover
- eluent (*n*-butanol-acetic acid-water 4:1:1)
- ninhydrin in acetone (to visualize the spots)

**PROCEDURE:** On a TLC silica gel plate (4 x 9 cm), lightly mark the start line with pencil (about 0.8 cm from the bottom of the plate). Draw the line carefully to avoid damaging the plate surface. Do not touch the gel surface with fingers – the method is very sensitive and your fingerprints may affect the elution and will be visible after ninhydrin-test. On the start line, select start points for reference amino acids (Gly, Ala, Gln, Phe, Met) and one for the analyzed sample (X). At the bottom of the plate, mark with pencil the numbers corresponding to the amino acids standards and the unknown sample (see figure). Use a capillary to introduce reference amino acids solutions onto the selected starting points of the TLC plate.

A single spot with a diameter of 0.3 mm for each amino acid is sufficient for experiment. Ask the instructor to spot the analyzed sample. Fill the developing chamber (a baker covered with Petri dish) with the eluent. The eluent level (about 0.5 cm) should be well below the start on the TLC plate) (see Figure 1). Put the beaker on a flat surface, put the prepared plate inside and

cover the chamber. To develop two plates in one chamber plates should be placed back to back, only the upper edges in contact (see Figure 1). The side edges of the plates should not touch the walls of the chamber. Do not move the beaker once the plate is inserted. The process takes about 40-60 min.

When the solvent front is close to the top of the plate, remove the plate from the beaker and mark the solvent front line (in pencil). Dry the plate in the fume hood using a hair dryer. Observe the plate in UV light and note the dark spots (visible only in the case of a plate with a fluorescent marker). Spray the plate with ninhydrin solution (use the box to reduce ninhydrin splatter, wear gloves to protect your hands). Dry the plate again and observe the development of spots.

Mark the spot borders on the plate, make notes about the colors. Fix the spots with  $\text{Cu}(\text{NO}_3)_2$  solution and dry the plate again; observe color changes. The residue of the eluent should be placed in the waste container. Measure the distances between the start line and centers of the spots, and calculate the retention factors ( $R_f$ ) (see Figure 1). Use the  $R_f$  values and characteristic colors to identify the amino acids in the unknown sample. The TLC plate could be attached to the report using a transparent tape covering the whole surface. Drawings or photographs are also acceptable as documentation of analysis.

**Positive Test:** All aminoacids form colourful derivative of ninhydrin in this test. The sensitivity of the reactions allows to detect even traces of amino acids.

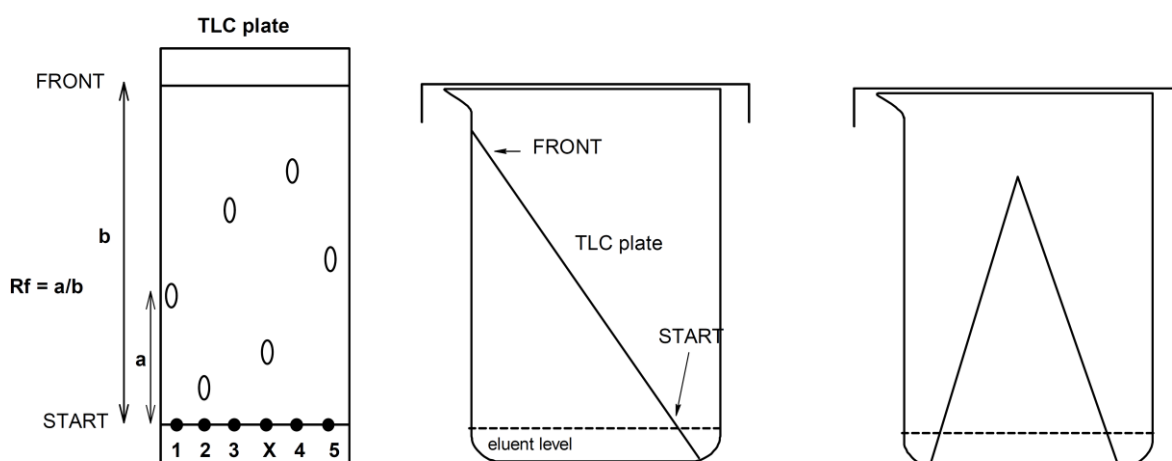


Figure 1. Schematic representation of TLC plate and chromatographic chamber.

# REPORT

## TLC - SEPARATION AND IDENTIFICATION OF AMINO ACIDS

**Observations:**.....  
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**Draw the obtained TLC plate and calculate R<sub>f</sub> values for each sample**



**Conclusions:**.....  
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*Instructor's signature*

*Date*