	YILDIZ TECHNICAL UNIVERSITY **BIOMEDICAL ENGINEERING DEPARTMENT** **BME2901- BIOCHEMISTRY COURSE** **2020-2021 FALL SEMESTER**

EXPERIMENT 3
PAPER CHROMATOGRAPHY

3.1. PURPOSE OF THE EXPERIMENT: To understand concepts of chromatography and to learn identification of aminoacid by using paper chromatography.

3.2. THEORETICAL KNOWLEDGE

Chromatography is the physical separation of a mixture into its individual components. In chromatography methods, the components to be separated are distributed between two phases, a stationary and mobile phase. A mixture which contains the solutes is separated into pure components by passing it over the stationary phase (an insoluble material) to which the substances stick to varying degrees. The mobile phase, solvent (liquid or gas) is carrying the solutes over the stationary phase.

Separation by the chromatography is based on the different interactions of the compounds with the two phases. The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases: Substances that stick tightly to the stationary phase move very slowly, while those that stick loosely or do not stick at all move rapidly.

Chromatography steps separate the individual components of a complex mixture into *fractions* based on the properties of the protein—such as size, shape, or electrical charge.

There are different types of chromatography. However, most of them are based on column chromatography where a porous solid material with appropriate chemical properties (**the stationary phase**) is held in a column, while buffered solution (**the mobile phase**) carries the molecule of

interest through it. According to the properties of the column which constitutes the stationary phase, compounds in a mixture are separated based on their size, shape, affinity or electrical charge.

Paper Chromatography is one of the chromatographycal methods which uses an adsorbent material usually paper as the stationary phase. Since it uses paper as the stationary phase this technique is generally called Paper Chromatography. It separates the compounds by taking advantage of their different rates of migration across sheets of paper. It is an inexpensive but powerful analytical tool that requires very small quantities of material.

In this technique, the compounds to be separated are applied on the paper as spots. Then the side of the paper where the sample is applied is immersed in a solvent which constitutes the mobile phase. The solvent penetrates the paper by capillary action and, in passing over the sample spot, carries along with it the various components of the sample. Thus, the compounds in the mixture migrate through the pores of the paper with a velocity depending on the differences in their affinity towards stationary and mobile phases under the capillary action of pores in the paper.

The distance a compound travels up the paper depends on a competition between how strongly the compound is attracted to the solvent and how strongly the compound is attracted to the stationary phase (i.e. paper). This attraction is related to the relative polarities of the sample, the paper and the solvent. Paper chromatography is especially useful in characterizing amino acids. The different amino acids move at differing rates on the paper because of differences in the polarities of their R groups.

When amino acids are analyzed using paper chromatography, they separate into colorless spots that are not visible to the eye. After the separation the paper will be treated with ninhydrin to make the spots visible. Ninhydrin reacts with the amino groups in the amino acids in the presence of heat and turns the spots purple.

The separated components of a mixture can be identified by comparison of their relative positions on the paper with the positions of known reference samples. The ratio of the distance traveled by a particular compound to the distance traveled by the solvent is called the Rf value of that particular compound (and is unique to that compound for the particular paper and solvent used).

Rf value = distance traveled by the compound / Distance traveled by the solvent

Rf values of aminoacids when isopropanol : water (70 : 30) (v / v) solution is used as the mobile phase are given in the table below:

Aminoacid	Rf value
Glycine	0.32
Alanine	0.37
Valine	0.45
Leucine	0.55
Isoleucine	0.53
Serine	0.35
Threonine	0.37
Aspartic acid	0.33
Asparagine	0.14
Glutamic acid	0.35
Glutamine	0.15
Lysine	0.03
Histidine	0.20
Arginine	0.02
Phenyl alanine	0.58
Tyrosine	0.57
Tryptophan	0.62
Cysteine	0.38
Cystine	0.32
Methionine	0.51
Proline	0.26
Hydroxy proline	0.34

3.3. MATERIALS AND METHODS:

3.3.1. Materials to be used in the experiment: Precision balance, Spatula, Distilled water, Graduated cylinder, L-cysteine, L-hydroxy proline, Amino acid mixture, Unknown amino acid, Phosphate buffered saline (pH = 7.4), Isopropanol, Distilled water, Ninhydrin solution (0.25% in acetone), Whatmann Paper No:1, 500 mL Beaker

3.3.2. Experimental Method:

1. Dissolve amino acids in PBS solution with a concentration of 200 mM.

2. Prepare a mixture of isopropanol : distilled water (70 : 30) (v / v).
3. Pour the solution into a 500 mL beaker.
4. Use a pencil to mark the Whatmann paper 1.5 cm from the sides and 2 cm from the bottom. Mark 4 spots on the bottom line for loading amino acids. Spots should be at least 1 cm apart from each other.
5. Load 2 µL of each amino acid and amino acid mixture to each spot.
6. Let the spots to dry.
7. Place the paper in the beaker. The solvent level should be below the line of spots.
8. Let the chromatogram to develop for 60 min.
9. After 60 min, mark the distance that the solvent travelled. Take the whatman paper from the set up.
10. Let chromatogram dry in the oven at 100^{o}C for 30 min.
11. Spray ninhydrin solution on the paper to visualize the amino acids.
12. Let the colour to develop in the oven at 100^{o}C for 5-10 min.
13. Measure the distance travelled by the solvent and by each amino acid.
14. Identify each amino acid on the chromatogram, calculate the Rf values of the amino acids.
15. Determine the unknown amino acid.

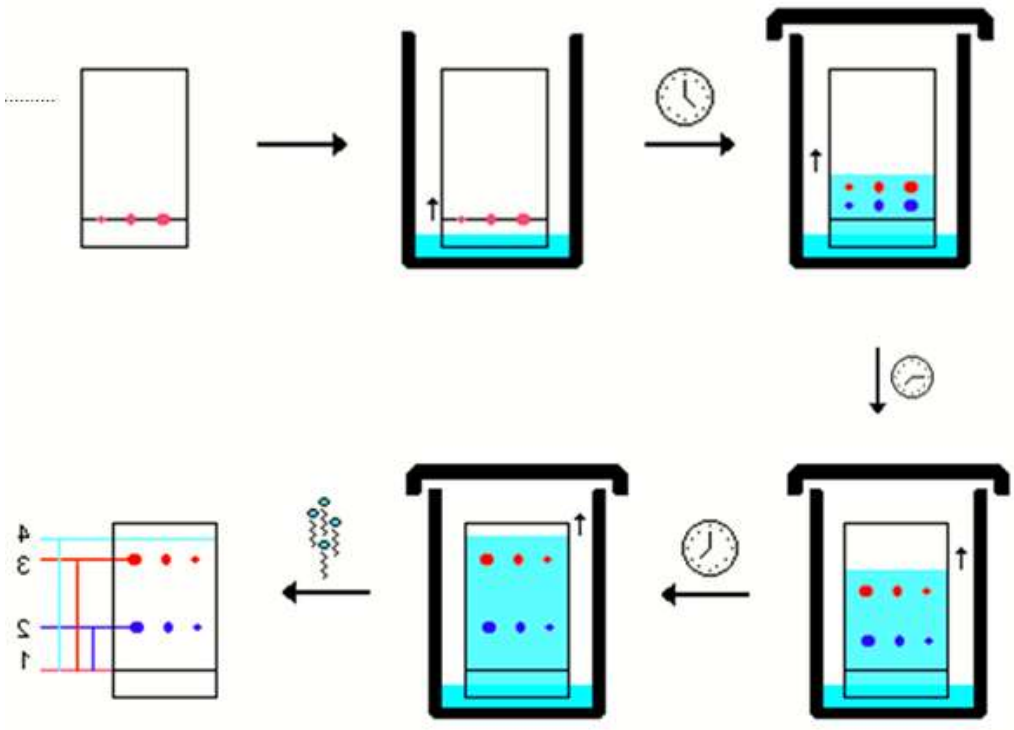

Figure 3.1 Experimental Procedure