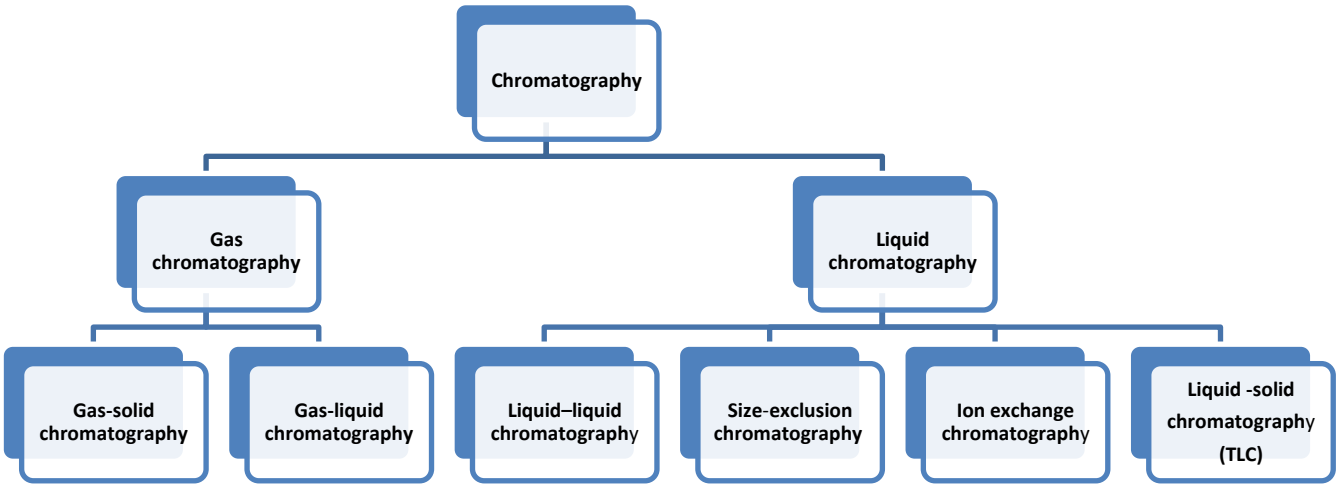
EXPERIMENT NO: 6

CHROMATOGRAPHIC SEPARATION AND QUANTITY DETERMINATION METHODS APPLIED IN TOXICOLOGY

A) General Information:

- Chromatography, technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase.
- The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.The different behavior of the various compounds between the stationary and mobile phases causes the paths they take on these phases to be different. This allows separation of the mixtures or recognition of the compounds making up the mixture.
- There are 4 main physicochemical mechanisms that enable chromatographic separation. These are ion exchange, surface adsorption, partition, and size exclusion.
- One of these mechanisms in different types of chromatography is the main factor, but other mechanisms also affect the chromatographic separation in varying proportions. Therefore, in the classification of chromatography types, segmentation according to the physical properties of the stationary and mobile phases is better rather than classifications such as adsorption chromatography or partition chromatography. Accordingly, we divide the chromatography types into gas and liquid chromatography according to the mobile phase. The next division is based on the nature of the stationary phase:



THIN LAYER CHROMATOGRAPHIC DETECTION AND IDENTIFICATION OF THE ORGANOPHOSPHORUS PESTICIDE

TLC is one of the most common methods in analytical toxicology because of its rapid results and high sensitivity. Glass plates are coated with adsorbents such as silicagel, aluminum oxide, cellulose. These adsorbents are suspended in water; the glass plates are coated with suitable means. The thickness of the adsorbent varies between 0.2-2 mm.In analytical studies, the optimum thickness was found to be 0.25 mm. Plates with thicknesses up to 2 mm are used in preparative studies. In order to activate the plates, the water is removed in the incubator heated to 105-110 ° C after plating. In this way, liquid-solid chromatography may be carried out; where the active power is more adsorption.When the drying process is carried out at lower temperature, the water does not move away at all. During separation, the sample is dispersed between the water retained by the adsorbent and the developing solvent. This application can be interpreted as partition chromatography. The sensitivity in the TLC can drop to 0.1µg.

B) Experimental Procedure:

Identification of organic phosphorus insecticides by thin layer chromatography

1. Plates are prepared in 250 µm thickness with silicagel G and activated at 105 ° C for 1 hour.
2. Samples and standards are applied to the plate.
3. The plates are placed in the saturated tank by adding a mixture of hexane: acetone (4: 1). The developing solution is expected to rise until 10 cm from the start, the elapsed time is recorded.
4. The plates removed from the tank are dried at room temperature.
5. Plates are sprayed with color reagent to make stains on the chromatogram visible. Organic phosphorous insecticides on blue background gives violet color. For this color to become apparent, 1% citric acid is sprayed to remove blue.
6. The Rf values of the chromatograms are determined and compared to the standards and the type of organic phosphorus insecticides is determined.

Color reagent:

a) 1% $AgNO_3$ (3: 1 mixture of water and acetone)

b) 0.5% bromphenol solution (in acetone)

When in use, 100 mL (a) of the solution is mixed with a solution of 10 mL (b).