WEEK 2 WORK FROM HOME 28 March 2020

SET UP2

Chromatography is a common technique for separating chemical substances. The prefix “chroma,”comes from the word “color,” and the technique is named so because **colours** develop which help in identification of separated molecules. In this experiment you will use chromatography to separate and identify amino acids, the building blocks of proteins.

The proteins of all living things are composed of 20 different amino acids. Chromatography is partially characterized by the **medium** on which the separation occurs. This medium is commonly identified as the **“stationary phase”**. Stationary phases that are typically used include paper (as in this experiment), thin plates coated with silica gel or alumina, or columns packed with the same substances. The **“mobile phase”** is the medium that accompanies the analyzed substance as it moves through the stationary phase. Both liquids and gases can be used as mobile phases depending on the type of separation desired. To refer to gas or liquid chromatography, chemists often use the abbreviations **GC** or **LC,** respectively. The compositions of the stationary and mobile phases define a specific chromatographic method. Indeed, many **different combinations** are possible. However, all of the methods are based on the rate at which the analyzed substances migrate while in simultaneous contact with the stationary and mobile phases. The **relative affinity** of a substance for each phase depends on properties such as molecular weight, structure and shape of the molecule, and the polarity of the molecule. In this experiment, very small volumes of solutions containing amino acids will be applied (this process is sometimes called **“spotting”)** at the bottom of a rectangular piece of Whatman paper. For ready comparison of each component, it is vital that each solution be applied on the same **starting line** or **loading line.** After the solutions have been applied, the paper will be rolled or suspended into a cylinder and placed in a beaker that contains a few milliliters of the liquid mobile phase. For this separation, a solution containing **n-propanol, water and ammonia or BAW** – n butanol, glacial acetic acid and water in a particular ratio is the optimum mobile phase. As soon as the paper is placed in the mobile phase, the solution (sometimes called the eluting solvent) will begin to rise up the paper. This phenomenon is called **capillary action.** As the mobile phase rises on the paper it will eventually encounter the “spots” of amino acids. The fate of each amino acid in the mixture now depends on the affinity of each substance for the mobile and stationary phases. **If an amino acid has a higher affinity for the mobile phase than the stationary phase,** it will tend to travel with the solvent front and be relatively unimpeded by the filter paper. In contrast, if the amino acid has a higher affinity for the paper than the solvent, it will tend to “stick” to the paper and travel more slowly than the solvent front. This will lead to their separation on the paper. The affinities of these amino acids for the mobile phase can be correlated to the **solubility** of the different amino acids in the solvent (i.e., an amino acid that is highly soluble in the eluting solvent will have a higher affinity for the mobile phase than an amino acid that is less soluble in the solvent). When the **solvent front** comes near the top of the filter paper, the paper is removed from the beaker and allowed to dry. At this point, the various amino acids are

invisible. The acids can be visualized by spraying the paper with a compound called **ninhydrin** which reacts with amino acids to form a blue-violet compound. Therefore, the sprayed filter paper should show a number of spots, each one corresponding to an amino acid. The further the spot from the starting line, the higher the affinity of the amino acid for the mobile phase and the faster its migration. The relative extent to which solute molecules move in a chromatography experiment is indicated by **Rf values**. The Rf value for a component is defined as the ratio of the distance moved by that particular component divided by the distance moved by the solvent. Measurements are made from the line on which the original samples were applied to the center of the migrated spot.

Experimental Procedure

- Saturate the jar in which chromatogram is to be run, with the solvent. This is done by pouring the solvent in it that acts as the mobile phase (in your case it is BAW). Leave it aside taking care that it is left covered with a lid.
- Obtain a sheet of Whatman paper, and draw a faint pencil line about 1 to 2 cm from one of the long edges and parallel to that edge. This will be the bottom of the chromatogram.
- Mark off equally spaced points along this line as many as the number of amino acids to be loaded. (They should be separated by about 1cm). Label each spot (with pencil and below the starting line) to indicate its identity.
- You will be provided with four known amino acids and an unknown sample that will contain one or more amino acids (not known to you).
- The samples can be applied to the paper by using a narrow capillary tube.
- Dip the open end of a clean capillary into the solution to draw up a small volume of the solution into the tube.
- Lightly and briefly touch the tube to the paper and allow the sample to transfer.
- The spot should be about 2-3 mm in diameter. Loading of each sample should be done at least 4-5 times to get them concentrated. Each spot has to be dried before reloading another one.
- Be careful not to contaminate either the solutions or the spots. When you have finished spotting your paper, allow it to dry by waving it in the air or using a hair dryer. (Don't get it too hot.)
- When the sample spots have dried, roll the paper into a cylinder, with the short sides almost touching. Use a bit of "Scotch" tape along the top of the paper to hold the cylinder together.
- Evenly lower the paper cylinder, sample side down, into the beaker.
- The solvent will wet the paper, but the sample spots should not be immersed.
- In addition, the paper should not touch the walls of the beaker.
- At this point, cover the jar with the lid. When the solvent front travels about $2/3^{rd}$ of the paper (in perhaps about 2 hrs), remove the paper, use a pencil to mark the solvent front at several points, unroll the cylinder, and let the chromatography paper dry in air or use a drier (normal cool air).
- When the paper is dry, spray it with ninhydrin reagent. Allow the paper to dry, this time using the hair dryer (hot air), heat lamp, or an oven at about 40-50◦. When the chromatographic paper has fully dried, outline the spots, mark the centers of each of the spots, and note their colors. (Not if all amino acids give

the same color with ninhydrin).

- Measure and record the distance travelled by the solvent and each of the amino acids traveled from the origin.
- Use these distances to calculate Rf values for each sample.
- Comparison of the spots should enable you to identify the amino acid(s) present in your unknown sample.