A Simple, Rapid and Inexpensive Procedure to Distinguish Amino Acids and their Esters

Introduction

Chromatography is one of the several separation methods based on differential migration. Originally conceived as a method to separate plant pigments, it has undergone numerous modifications each adapted to a particular problem. In adsorption chromatography, pigments extracted from plants are separated into discrete bands each corresponding to a different pigment. In this technique, the plant extract is percolated through a glass tube containing finely powdered calcium carbonate and then washed with light petroleum. The separation of pigments here is due to their differences in affinity for the calcium carbonate, which is acting as an adsorbent (see [1]). Pigments that strongly adsorb migrate more slowly than those adsorbed weakly. Differences in adsorption in this case, result in differences in migration rates. Hence it is possible to collect each pigment separately, if the column is continually washed with the solvent.

Two phases can be distinguished in a chromatographic process, a stationary phase represented in the above example by calcium carbonate and a mobile phase represented by the solvent travelling down the column. The same basic conditions apply in all the other forms of chromatography.

Paper chromatography is a type of partition chromatography. The stationary phase in partition chromatography is water held by any type of material with a great capacity for hydration. The mobile phase on the other hand is an organic solvent saturated with water. Resolution of substances in this type of chromatography results from differences in the distribution coefficient (partition coefficient (Box 1)) of the individual components of a mixture between the stationary and mobile phases. Partition chromatography was conceived and developed in 1941 by the British scientists A J P Martin and R L M Synge.

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Box 1. Definition of Partition Coefficient

When a solute at constant temperature is added to two immiscible solvents in contact with each other, the solute gets distributed between the two solvents with different equilibrium concentrations. For example, when iodine is added to water and carbon tetrachloride, it distributes in such a way that at equilibrium the ratio of the concentrations of iodine in the two solvents is constant at any given temperature, if $C_A$ and $C_B$ are the concentrations of iodine in water and carbon tetrachloride, then

$$K_D = \frac{C_A}{C_B}$$

The constant $K_D$ is called the distribution or partition coefficient of the solute between the two solvents at the given temperature. The value of $K_D$ depends on the nature of the solute and the solvent pair.

In paper chromatography the stationary phase is water retained by the cellulose fibers of the filter paper. The mobile phase is generally a solvent poorly miscible with water but saturated with water before use.

In this article, we present a simple and novel method to separate and distinguish amino acids and their esters by circular paper chromatography. It is the simplest, most inexpensive and less time consuming of all the paper chromatographic techniques. In addition, a simple method to prepare amino acid esters is also presented.

All amino acids and their carboxyl group derivatives like esters and amides including smaller peptides produce a purple colour with the classical ninhydrin reagent. This reagent was modified by adding cupric ion to the reagent, in order to distinguish qualitatively the carboxyl group derivatives from their amino acids by paper chromatography [2]. Amino acids produced a pink colour and their carboxyl derivatives produced a yellow colour with this reagent. We have used this method to identify the amino derivatives from the young leaf extracts of many plants [3]. Recently we have also used this technique in the identification of amino acid esters synthesized by an esterase enzyme in non-aqueous medium.
While conducting the laboratory class the first part deals with the preparation of reagents and the second part with the execution of the experiment.

**Preparation of Amino Acid Esters**

The amino acid esters used in the experiment were prepared by passing dry HCl gas for 5 mins into any one alcohol (ethylene glycol/ethanol/methanol) solution containing 50 mM amino acid at ambient temperature. Under these conditions the amino acid partially gets esterified resulting in a mixture of amino acid and its ester. The equation for the reaction with ethylene glycol is shown below.

\[
\begin{align*}
R - \text{CH} - \text{COOH} + \text{HO-CH}_2\text{NH}_2 & \xrightleftharpoons{H^+} R - \text{CH} - \text{CO-O-CH}_2\text{NH}_2 + \text{HO-CH}_2\text{NH}_2 + \text{H}_2\text{O} \\
\text{Amino acid} & \text{Ethylene glycol} & \text{Amino acid ethylene glycol ester}
\end{align*}
\]

**Preparation of the Cu (II)-ninhydrin Reagent**

The Cu (II)-ninhydrin reagent is prepared by dissolving cupric nitrate (25 mmol/L), ninhydrin (1% w/v) (see Box 2) in a minimum quantity of water:glacial acetic acid mixture (3:1 v/v) and diluted with a small amount of acetone.

**Solvent System**

Isopropanol: water (4:1 v/v) system

**Chromatography**

The mixture containing amino acid and its ester is spotted on a circular Whatman No. 1 filter paper at the centre. Depending upon the number of samples the paper may be demarcated. The diameter of the sample spotted is restricted to 0.5 cm by intermittent use of a hot air dryer. The sample spotting may be done for 5 to 10 times to ensure sufficient concentration of the amino acid and its ester. The chromatography is carried out in...
an isopropanol:water (4:1 v/v) system, by connecting a filter-paper wick to the solvent system through a hole made at the centre of the circular paper. After the run, which takes about 30-45 mins, the chromatogram is air dried at ambient temperature for 30 mins. The air dried chromatogram is developed by spraying uniformly with the Cu(II)-ninhydrin reagent followed by drying at 65°C for 10 min. A sample chromatogram is shown in Figure 1.

Discussion

The method discussed here is a novel one because no other methods presently used can form two different coloured products with a single developing reagent. Amino acids produce a red colour and their esters produce a yellow colour with Cu(II)-ninhydrin reagent. It is also a general procedure to distinguish all amino acids and their carboxyl group derivatives like amino acid esters, amino acid amides and even oligopeptides containing up to five amino acid residues [2] except for the derivatives of L-proline and L-hydroxyproline, which do not produce any coloured products. The minimum structural requirement for the formation of a yellow chromophore is given in Box 3.

Even though many other forms of chromatography like gel permeation chromatography,
gas chromatography (GLC) and high performance liquid chromatography (HPLC) work by the same principle, the principle cannot be demonstrated vividly, because, the separated components cannot be distinguished by simple procedures. Moreover all these methods need expensive equipment, chemicals and maintenance.

**Suggested Reading**


