

# Good essay about thin layer chromatography of carbohydrates

[Psychology](#), [Behaviorism](#)



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## **Introduction**

TLC or Thin-layer chromatography refers to a technique that is applied in the separation of mixtures that are not volatile (Reich & Schibli, 2007). The process is done on a sheet of plastic, glass, or aluminum foil that has been coated using a thin layer of material that is adsorbent such as aluminum oxide, silica gel and cellulose (Sherma & Fried, 2003; University of Colorado, 2014). This adsorbent layer is referred to as the stationary phase. The sample is usually applied using a capillary tube on the plate, and the sample is taken up the plate through capillary by a solvent or a mixture of solvents (MIT, 2014). The solvent is thus referred to as the mobile phase. Separation process is attained as a result of the difference in the rate of ascending the plate by the analyte (Wall, 2005).

Thin-layer chromatography has been used to achieve different purposes in different fields such as monitoring of reaction progress, compound identity, as well as determination of substance purity (Pramod, 2008). Determination of compounds using thin-layer chromatography mainly uses the retention factor ( $R_f$ ) of an unknown substance and comparing it with the known ones (Larsen, 2014).

## **This experiment aimed at generating and examining a Thin Layer Chromatogram of different carbohydrates to determine their relative behavior.**

### **Method**

The side of the TLC plate covered with silica gel was used to make a line about 1.5 cm above the lower part of the plate. Four sites were marked along the line for the samples to be added. Supplied samples included lactose, fructose, unknown 2 and unknown 3. The samples (5% carbohydrate solution in water) were applied using capillary tubes and the silica gel allowed to draw out the sample by capillary action. Sufficient sample was applied by repeated spotting and drying the spot. After sample application, the chromatographic separation was carried out by placing the TLC plate in a covered beaker containing the mobile phase. The mobile phase consisted of butan-1-ol: acetone: phosphate buffer (pH 5) in the ratio (40: 50: 10). The solvent was allowed to run up to within about 1 cm of the top of the plate and the plate removed from the beaker. The plate was left to dry in a fume cupboard and the plate visualized by dipping in a mixture of anisaldehyde with 0.5% concentrated sulphuric acid and heated it in the oven at 100°C for several minutes.

## **The R<sub>f</sub> values of the carbohydrate spots were calculated.**

### **Results**

The Thin Layer Chromatogram obtained as was shown in Figure 1 below. Unknown 3 recorded the highest height at 4.2 cm followed by fructose at 3.5 cm, unknown 2 at 2.7 cm and finally lactose at 1.9 cm.

Figure 1: Thin Layer Chromatogram for the four samples after running in the solvent system

**The retention factor values for the four samples were calculated using the following formula**

Retention Factor=  $\frac{\text{Distance travelled by compound from application point}}{\text{Distance travelled by solvent from application point}}$

**For the fructose, the Rf was calculated as**

Retention Factor=  $\frac{3.57}{8}$

= 0.45

**For the lactose, the Rf was calculated as**

Retention Factor=  $\frac{1.97}{8}$

= 0.24

**For the unknown 2, the Rf was calculated as**

Retention Factor=  $\frac{2.77}{8}$

= 0.35

**For the unknown 3, the Rf was calculated as**

Retention Factor=  $\frac{4.27}{8}$

= 0.54

## Discussion

The techniques applied in thin-layer chromatography are usually simple and easy operate. The technique as has a high rate of sensitivity as well as resolution and has been used as the primary tool for the analysis of sugars

and other related compounds for ages. Due to its simplicity, TLC has also replaced other procedures that are sophisticated in the analysis of sugars such as gas-liquid chromatography, as well as the ion-exchange chromatography. The TLC process as a technique for the identification of sugars is also considered to be superior compared to other techniques due to the high differential reactions and the utilization of other characteristics such as colour differences and  $R_f$  values (Ghebregzabher, et al., 1976).

The current experiment aimed at generating and examining a Thin Layer Chromatogram of different carbohydrates to determine their relative behavior. The compounds investigated include lactose and fructose and two unknown carbohydrates. Fructose is ketose sugar and exists as a monosaccharide (Ophardt, 2003). The single molecule of fructose means that it has a small molecular weight and thus able to move faster in the TLC plate. The OH groups on the fructose molecule also makes it soluble in the mobile phase, and it thus spends much of the time in the mobile phase than in the stationary phase. This interaction with the mobile phase enhances the movement of the compound up the TLC plate. The fructose molecule is also in a chain form rather than in a ring structure. The molecule of lactose is a disaccharide consisting of a galactose molecule and a molecule of glucose (NCBI, 2013). The two molecules makes lactose have a higher molecular weight compared to fructose and thus not able to move as fast as fructose on the TLC plate. Lactose also has fewer OH groups and thus not as soluble as the fructose. This makes it spend less time in the mobile phase and more time on the stationary phase and this results in reduced movement up the TLC plate. Another factor is the fact that lactose exists in a ring structure

making it more difficult to move up the plate.

The unknown 2 has a lower Rf compared to fructose, but a higher one compared to lactose. The compound may be a monosaccharide similar to fructose but in a ring structure. Ring structure makes it harder for the unknown 2 to move up the plate due to the shape and also a reduction in solubility to the mobile phase. On the other hand, the unknown 3 has a much higher Rf value compared to the other three carbohydrates. The unknown 3 may be a carbohydrate that exists as a chain made up of 5 carbons. The reduced number of carbons makes the molecule lighter in weight compared to the other samples and hence the highest Rf value recorded.

In conclusion, experiment aimed at generating and examining a Thin Layer Chromatogram of different carbohydrates to determine their relative behavior. Unknown 3 gave the highest Rf value followed by fructose unknown 2 and finally lactose. It was concluded that unknown 2 was a monosaccharide existing in a ring structure while unknown 3 was a 5 carbon monosaccharide that existed in chain form.

## References List

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