Thin Layer Chromatographic Separation of the Components of Petunia Petals (Hibiscus Flower) Using Cassava/Corn Gel as Adsorbent

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ABSTRACT

Silica gel, cellulose, kieselghur alumina are the common materials used as a stationary phase on a solid support in thin layer chromatography (TLC). Thin layer chromatography is used most frequently to determine the purity of organic compounds. This study compares the efficiency of mixture of cassava and corn starch as stationery phase at three different mixing ratios of 50:50, 60:40 and 70:30 cassava and corn starch respectively using solvent mixture of Butanol, Acetic acid and distilled water at ratio 2:1:3 respectively to separate the colour components of red and white petunia petals (Hibiscus flower). The separations obtained were distinct, showing that cassava/corn gel does not enter into irreversible reaction with the analyte or the mobile phase. Hence, it was discovered that cassava/corn gel could separate effectively.
Keywords: *Thin layer, chromatographic separation, petunia petals, adsorbent*

INTRODUCTION

Chromatography is a laboratory technique for the separation of mixtures. It involves passing a mixture which contains analyte through a stationary phase. The stationary phase separates the analytes from other molecules in the mixture. Chromatography is a physical method of separation in which the component to be separated are distributed between the stationary phase and the mobile phase. The stationary phase may act by adsorption, partition, ion exchange or gel permeation. The branches of chromatography include gas chromatography which is divided into gas liquid and gas solid chromatography, liquid chromatography which is also divided into ion exchange and exclusion chromatography. It also divides directly to paper and thin layer chromatography (Edward and Robert, 1978). It involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, cellulose and kieselghur on a flat, inert substrate (Abere, Okeri and Okarfor, 2005). Thin layer chromatography was first introduced by Izmailov and Shraiber (Kamlesh, 2000). It is a form of chromatography useful for wide scale qualitative analysis screening and can also be used for quantitative analysis. It involves a stationary phase consisting of a thin,

ISSN: 2350-2169

uniform layer of about 0.1 – 0.25mm thick of a dry, finely powered material applied to a suitable support such as a glass plate or an aluminum foil or plastic. The mobile phase is allowed to move across the surface of the plate by capillary action and the process may depend upon adsorption, partition or a combination of both.

Properties of Silica Gel, Cassava and Corn Starch: Silica gel is a form of silicon dioxide (SiO_2) that contains hydroxyl groups (OH) and oxygen atom (O_2). The silicon atoms are joined via oxygen atoms in a giant covalent structure. At the surface of the silica gel, the silicon atoms are attached to –OH groups. So at the surface of silica gel you have Si – O - H instead of Si -O-Si. The surface of the silica gel is very polar and so can form hydrogen bond with suitable compounds. It has high viscosity and before optical quality is achieved, it requires successive melting (Michel, 2007). Silica gel is an amorphous form of SiO_2 with a very porous structure. Its properties depend critically in the conditions of preparation but typical samples have a pore diameter of 2200 – 2600pm. Surface area of 750 – 800m^2g^{-1} and bulk density of 0.67 – 0.75gm^{-3} hence its use as chromatographic support among others. It can absorb 40% of its own weight of water, chemically inert, non-toxic and dimensionally stable (Greenwood and Earnshaw, 1997).

Cassava, originated in South and Central America, brought to West African costal area by emancipated slaves. It has several varieties and species and it is propagated by stem cutting (International Institute for Tropical Agriculture 1990). Its leaves and tender shoot serves as vegetable. Cassava tuber produces ethanol, animal feed and starch and it consists mainly about 60 – 70% water as well as 35% carbohydrate (Ogunmola, Nwokocha and Oke, 2001). Maize is an important source of food and manufacturer's products like breakfast cereals, snacks, corn flour and starch syrup. It consists of about 13% moisture, 9.5% protein, 4.3% fat and energy 1.19MJ/100g (Pearson, 1997).

Maize and Cassava are mainly carbohydrate. Carbohydrates undergo various types of chemical reaction depending on which functional group(s) is involved. The hydroxyl groups (-OH) in the carbohydrate can hydrogen–bond with polar substance. Adsorbed water from the surface must be removed by oven drying at about 30^0C and 60^0C to prevent leaching. Okor, Uhumwango, Eichie and Gbenewei (2006) report that maize starch mucilage is 4 to 8 times more viscous than cassava mucilage*(tapioca)* at room temperature. They explain that at 50°C the viscosity of cassava *(tapioca)* mucilage increases slightly while that of maize starch decreases. Carbohydrates undergo various types of chemical reactions, depending on the functional groups involved. The hydroxyl groups (OH) in the carbohydrate hydrogen–bond with polar substances. However it must be air dried or over dried at lower temperature between 30°C and 60°C to prevent leaching of starch (Ogunmola, Nwokocha, Oke, 2001).

ISSN: 2350-2169

Theory of Thin Layer Chromatography (TLC): All forms of chromatography involve a dynamic and rapid equilibrium of molecules between the two phases. Molecules are moving backward and forward between the free states and the absorbed states depends on three factors:

1. The polarity and the size of molecules
2. The polarity of the stationary phase, and
3. The polarity of the solvent.

The solvent soak up the plated, the dissolve and the compounds in the spot on the base line. The compound present then tend to get carried up the plate as the solvent continues to move upwards. How fast the compounds get carried up the plate depends on two things:

1. The solubility of the compound in the solvent. This depends on the level of attraction that exists between the molecules of the compound and those of the solvent.
2. How much the compound sticks to the stationary phase, which is the level of attraction between the molecules of the compound and the silica gel.

If one of the spots contains two compounds, one of which can form hydrogen bond and the other are only to take part in weaker van der Waals interaction, the one that hydrogen-bond will stick to the surface of the silica gel more firmly than the other one. It means that it is adsorbed more than the other. Compounds only travel up the plate when it dissolve in the solvent but if it is adsorbed on the surface, it will stop temporarily, which simply means the more strongly a compound is adsorbed, the less distance it can travel up the plate.

Attractions between the compound and the solvent are more important than the attraction between the compound and the surface. There are over 220 varieties of the *Genus Hibiscus*. It grows in most tropical areas around the world, but certain hardy perennials can live anywhere, being able to weather freezing conditions and arising next year in bloom. Those found in the tropics cannot stand more than a few days of freezing weather and will die if such conditions persist. The flowers are of a magnificent variety of colors, some indicating their unique country of origin. There are many folk remedies attributed to hibiscus flowers, including help with stomach or digestive problems, and to help soothe the nerves. They are also the main ingredient in wonderfully refreshing teas made around the world, especially in Mexico, Latin America, and North Africa. Known as Agua de Jamaica, or simply Jamaica in Mexico, this tea is usually served chilled with copious amounts of sugar to sweeten the natural tartness of the hibiscus. Recently it has been added to many ready-made teas due to its high levels of anti-oxidants, and has even become the main ingredient in certain sodas. The Journal of Human Hypertension published an article that showed that drinking hibiscus tea can reduce the blood pressure in people with type 2diabetes. The tart taste of hibiscus is due to its contents of 15 to 30% plant acids, including citric, malic, and tartaric acids. The wine-red color of

ISSN: 2350-2169

the tea is the anthocyanins, including delphinidins and cyanidins (Mountain Rose Herbs, 2001). The fruity fleshy flowers can be used in a variety of different ways to add colour, taste and richness. In the food industry hibiscus is used in sauces, jellies, jams, cakes, ice-creams, sorbets, iced drinks, and herbal teas. It contains pectin – important in the marmalade, jelly and jam making industry. The tender leaves are emollient (soothing the mucus lining), diuretic (treating fluid retention), and refrigerant (cooling especially if you feel the heat on hot days) also have a sedative calming effect on the whole system. The fruits are anti-scorbutic, meaning they are high in vitamin C. (Nutrition with Sonia.com).

The brilliant colour found in fruit, vegetable and flower are as a result of the presence of a group of water soluble pigments called anthocyanin. The anthocyanin pigments are cyanidin-3-diglucoside-5-glucose and cyanidin-3-malonylglucoside-5-glucose. Simona Loana Vicas *et al* (2008) report that the TLC chromatography of purple petunia flower shows 3 spots at Rf values 0.51, 0.65, 0.77 with spot colours green-yellow, dark violet and yellow, the white and the violet shows 2 spots each at Rf values 0.67and 0.78, and 0.43 and 0.58 with spot colours yellow and violet. Yasuyuki Nakamura *et al* (1990) used two – dimensional cellulose thin layer chromatography (TLC) to separate the major anthocyanin in the flowers of Hibiscus *(Hibiscus rosa – sinensis L.)*. It was discovered that the anthocyanin in the precipitate were separated into the major three minor spots.

Abeere, Okeri and Okafor (2005) carried out a comparative test on cassava, guinea corn and Irish potato starches both in natural form and various modified forms to evaluate and determine suitability as adsorbents for thin layer chromatography. The result obtained proved starch to be a suitable adsorbent in its natural and modified forms in the separation of amino acids and steroids and it seems very promising for future use. Adeboy, Bolaji, Adeagbo, and Oyewole (2007) used cassava and corn at mixing ratio 3:2 using two different solvents mixture of 70/30 and 50/50 propyl alcohol/water as stationary phase in thin layer chromatographic separation of amino acids. It was discovered that cassava/corn gel could compare favourably with silica gel. Adeboye (2007) compares the effectiveness of the mixture of cassava/corn starch at ratio 1:1 and 2:3 and silica gel as stationary phase in the separation of dyes. It was discovered that cassava/corn gel has slightly higher Rf values that the silica gel. Adeboye (2010) also used cassava/corn starch at three different mixing ratios of 3:2, 1:1 and 2:1 to separate leaf pigment, it was discovered that the adsorbent could separate effectively.

METHOD

Preparation of Tlc Plates: The coated TLC plate was placed in the oven at about 50-60^{o}C for about 10 minutes to activate it. This is to dry off water molecules that bond to the polar site of the surface of the plate.

ISSN: 2350-2169

Preparation of Solvent: One solvent mixture was used for the separation process. The solvent comprises of the mixture of Butanol, Acetic acid and distilled water at ratio 2:1:3 respectively.

Extraction of Flower Pigments: About 150g of the flower pigment was weighted and the plant pigment was extracted using ethanol. Red pigments for red hibiscus flower and yellow pigment for the white hibiscus flower. The red pigment in the red hibiscus flower is anthocyanin and the yellow pigment in the white hibiscus flower is flavonoid.

Preparation of TLC Plate: The TLC plates were marked with a light pencil at about 2.0cm from the bottom of the TLC plates and at the top of the plate. The narrow end of a capillary tube was used to apply the sample two to three times. The solvent was allowed to evaporate complete from the spot before the development of the chromatograms.

Experimental Procedure: Red and white hibiscus flowers *(hibiscus rosa–sinensis)* were collected from a garden in Oyo town, Oyo State and was authenticated by the senior technologist in the Department of Biology, Emmanuel Alayande College of Education, Oyo. Three different mixing ratios of cassava–corn starch were used at the particle size of about 0.212mm (50:50, 60:40 and 70:30).

Preparation of Ratio 50:50 Mixture of Cassava and Corn: The slurry comprises of 50g cassava, 50g corn starch, and 15g calcium sulphate 90mls of distilled water was added to the mixture.

Preparation of Ratio 60:40 Mixture of Cassava and Corn: The slurry comprises of 60g cassava, 40g corn starch and 15g calcium sulphate 90mls of distilled water was also added to the mixture.

Preparation of Ratio 70:30 Mixture of Cassava And Corn: The slurry comprisesof 70g cassava, 30g corn and 15g calcium sulphate. 90mls of distilled water was added to the mixture. The resultant slurry was thoroughly mixed by a glass rod to homogenize the mixture. The TLC plate was prepared by coating the plate. The solvent was poured into a Shandon chromatographic tank which is about 4-5 mm deep. The spotted plate was carefully placed in the developing tank. The plate was left, for about 30 minutes to allow the solvent front to rise by capillary action to the top pencil line. The plate was removed and was placed on a flat clean dry surface to allow the solvent evaporate.

Measurement of Rf Values: The retention factor (Rf) value was measured between the bottom and the top pencil lines.

$$Rf = \frac{\text{Distance moved by sample}}{\text{Distance moved by solvent}}$$

ISSN: 2350-2169

RESULTS AND DISCUSSION

Table 1: Rf Values for Red Hibiscus Flower

Pigment Colour	**Rf Values (cm)**
50:50	
Violet	0.23
Pink	0.14
60:40	
Violet	0.16
Pink	0.15
70:30	
Violet	0.11
	Nil

Source: Fieldwork, 2012

Table 2: Rf Value for White Hibiscus flower

Pigment Colour	Rf Values (cm)
50/50	
Yellow	0.14
Light Yellow	0.17
60/40	
Yellow	0.15
Light Yellow	0.20
70/30	
Yellow	0.12
	NIL

Source: Fieldwork, 2012

Table 3: Reviewed Rf values for Purple, white and Red hibiscus flower

Types of Petunia	**Rf Values (cm)**	**Colour of Spot**
Purple	0.51	Green-yellow
	0.65	Dark violet
	0.77	Yellow
White	0.67	Light Yellow
	0.78	Yellow
Violet	0.43	Yellow
	0.58	Violet

Source: Vicas, Purcarea, Ruszakai and Laslo (2008)

From table 1, the separation for red hibiscus at 50:50 mixing ratio shows two colours at Rf values of 0.23 and 0.14, at 60:40, it also shows two colours at Rf values of 0.16 and 0.15 while mixing ratio 70:30 shows only one colour at Rf value of 0.11. Table 2, shows the Rf values for white hibiscus flower, for 50:50 mixing ratio there are two different colour Rf values of 0.14 and 0.17 while 60:40 mixing ratio also shows two colours at Rf values of 0.15 and 0.20 and 70:30 shows only one colour at Rf value of 0.12. From tables 1 and 2 there is an indication that the mixing ratio of 70:30 cassava/corn is not suitable for separation in hibiscus flower.

ISSN: 2350-2169

Table 3 shows that the observed values in the fieldwork could compare with the reviewed work, although the Rf values in the reviewed work were higher compared to that of the field work (Simona, Purcarea, Ruszakai and Laslo, 2008). The separations obtained were distinct, showing that cassava/corn gel does not enter into irreversible reaction with the analyte or the mobile phase. Furthermore, the cassava/corn gel is not toxic like silica gel as reported by (Greenwood, and Earnshaw, 1997). The viscosity of tuber crops as reported by (Ogunmola, Nwokocha and Oke, 2001) contributes to the observed performance of cassava/ corn gel as adsorbent in thin layer chromatography.

CONCLUSION

This study compares the efficiency of mixture of cassava and corn starch as stationary phase at three different mixing ratios of 50:50, 60:40 and 70:30 cassava and corn starch respectively using solvent mixture of Butanol, Acetic acid and distilled water at ratio 2:1:3 respectively to separate the colour components of red and white petunia petals (Hibiscus flower). The experimental result shows that cassava and corn have high potential for use as adsorbent appropriate mixing ratio, and seems very promising as adsorbent in thin layer chromatography.

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ISSN: 2350-2169