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Influence of Surfactants on the Mobility and Separation of Galactose, Arabinose and Rhamanose on Cellulosic Surface.

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ABSTRACT

Different kinds of surfactants have been used for the study of mobility of three sugars. Mobility pattern of all three sugars viz, Galactose, Arabinose and Rhamanose has been studied in different aqueous and alcoholic eluents. An eco-friendly two-dimensional thin-layer chromatographic method was developed by using surfactants as eluents for the separation of Galactose, Arabinose and Rhamanose. Two different kinds of surfactants containing different types of charges were used for the study. Anionic surfactant (sodium cholate) was used in the first run and then the cationic surfactant (Cetyltrimethylammonium chloride) was used after rotating the plate at 90^{o}.

Keywords: Two-dimensional thin-layer chromatography; Surfactants; Mobility; Separation; Galactose; Arabinose; Rhamanose

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INTRODUCTION

Carbohydrates are energy source of all living beings and acts as biofuel in the living systems. Carbohydrates also perform numerous roles in living systems. In this study, we have developed a method for the separation of three simple carbohydrates viz. Galactose, Arabinose and Rhamanose. All the three carbohydrates are essential for the living systems and therefore their chromatographic study is also important. Several thin-layer chromatographic methods have been developed for the analysis of sugars [1-4]. Thin-layer chromatography is an essential analytical tool for the analysis of numerous environmental samples. On the other hand, 2D TLC provides resolution of complex mixtures (of closely related compounds) into their individual componentes [5-7].

Surfactants are the chemical compounds containing excellent resolving power for the isolation and separation of many environmental samples. One of the most exciting feature of the surfactants is the availability of these compounds with different charged head groups. Surfactants provide an unique chromatographic system either by using as solvent systems or as impregnants in stationary phases. Micellar liquid chromatography (MLC) has become most popular and widely applicable due to operational simplicity, cost effectiveness, less toxicity, and low aggressiveness. The selectivity of surfactants depends on the degree of solubilization of mixture components with micelles caused by complex electrostatic, hydrophobic, donor-acceptor and polarization interactions.

In this study, first anionic surfactant(Sodium Cholate) was used in one direction for the development of the chromatogram. And after rotating the plate at 90^0, second run was performed by cationic surfactant(CTAC). This combination provides us the resolution of three component mixture of sugars (Galactose, Arabinose and Rhamanose).

EXPERIMENTAL

All experiments were performed at 25±2^{o}C.

Chemical and reagents

Cellulose HPTLC aluminum foils (Merck, Darmstadt, Germany), Sodium cholate (Merck, Darmstadt, Germany), Sodium deoxycholate (Merck, Darmstadt, Germany), Cetytrimethyl ammonium chloride(Merck, Darmstadt, Germany), methanol, p-anisidine, phthalic acid and ethanol were used. All the reagents were of Analytical grade.

Sugars studied

Galactose, Arabinose and Rhamanose were purchased from Sigma-Aldrich, Chemie GmbH, Steinheim, Germany. All sugars were used as received.

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Test solutions

All the test solutions were prepared in distilled water, contained 0.5 %(w/v) aqueous solution of Galactose, Arabinose and Rhamanose.

Detection reagent

Spray with a solution of 1.23 g p-anisidine and 1.66g phthalic acid in 100ml 95% ethanol.

Mobile phase

The following solvent systems were used as mobile phases such as: Double distilled water (M_1), Methanol (M_2), 1% Sodium Cholate (M_3), 3% Sodium Cholate (M_4), 5% Sodium Cholate (M_5), 1% Aq. Sodium Cholate (M_6) and 1% Sodium Deoxycholate (M_7).

Procedure

Test solutions 1µL were applied on (20 cm x 20 cm) cellulose thin-layer plates (1 µg/zone) with the help of micropipette at about 2 cm above the lower edge of the plates. The solvent ascent was fixed to 10 cm in all cases for the determination of R_F value of individual sugars. Linear ascending development was carried out in a vapour-equilibrated TLC twin-trough chamber. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 2^{o}C). Subsequent to the development, plates were dried at room temperature. The plates were then detected by using detection reagent. The R_L (R_F of leading front) and R_T (R_F of trailing front) values of each spot were determined and the R_F value was calculated as

$$R_F = 0.5 (R_L + R_T).$$

In case of Two-dimensional thin-layer chromatography, the mixture of sugars was applied in one corner of a 20 X 20-cm HPTLC or 10 X10-cm HPTLC plate. Ascending development is carried out for the full length of the plate to achieve maximum resolution. The plate is removed from the chamber and air dried to remove solvent completely. The plate is then rotated at 90° angle and redeveloped, with a second solvent having different selectivity. The line of partly resolved components from the first development becomes the origin for the second development. All the experiments were carried out in three replicate measurements.

For the separation of mixture of sugars, equal volumes of all sugars were mixed and 1 µL of the resultant mixture was applied on cellulosic TLC plate.

RESULTS AND DISCUSSION

Thin-layer chromatography of three sugars (Galactose, Arabinose and Rhamnose) has been performed on the cellulosic flat bed with the alcoholic sodium cholate. Different solvent

systems were used for the chromatography of these compounds. First of all, double distilled water (M_1) and methanol (M_2) were used as solvent systems. And then different concentrations of methanolic sodium cholate were used for the study. The results presented in Table 1 clearly indicate that in double distilled water, mobility of all the three sugars is higher as compared to observed in methanol. Both the solvent systems, double distilled water and methanol were not able to provide any good results.

Table 1: R_f values of Galactose, Arabinose and Rhamnose on cellulosic plates using different solvent systems

Sugars	M_1	M_2	M_3	M_4	M_5
Galactose	0.87±0.04	0.36±0.02	0.32±0.02	0.35±0.02	0.43±0.02
Arabinose	0.88±0.04	0.45±0.02	0.42±0.02	0.43±0.02	0.45±0.02
Rhamnose	0.94±0.05	0.57±0.03	0.59±0.03	0.60±0.03	0.65±0.03

In order to achieve the useful mobilities for the separation of these sugars, water and methanol were replaced by methanolic sodium cholate (M_3). The interesting results were obtained by using the different concentrations of sodium cholate. From the results obtained, it is clear that with the increase in concentration of sodium cholate , mobility of all the three compounds increases. While in case of Galactose and Arabinose, the mobilities in the lower concentration of sodium cholate (M_3 and M_4) are not significantly comparable. From all the three different concentrations of sodium cholate (M_3, M_4 and M_5) used for the study, 1% sodium cholate (M_3) was useful for the chromatography. The R_f values clearly indicate that the resolution of a mixture of Galactose(R_f=0.32±0.02), Arabinose(R_f=0.42±0.02) and Rhamnose(R_f=0.59±0.03) is possible by using 1% sodium cholate. But, it was not possible to separate all the three compounds in a single run. Only two spots appeared on the plate because Galactose, Arabinose produced single long spot and the other one separated was Rhamnose. Therefore, ternary separation of the mixture was not achieved in 1% sodium cholate in a single run. In order to achieve the resolution of ternary mixture, the only absolute way to resolve the mixture is to rotate the plate at 90^0. Two-dimensional thin-layer chromatography provides the resolution of closely related compounds. Especially, when there is some overlapping or mixing of spots of two or more compounds. Therefore, the second run was performed by using 1% methanolic CTAC (M_4). As a result of this study, the Two-dimensional resolution (Fig. 1) of a mixture of Galactose, Arabinose and Rhamanose was achieved.

In another study, alc.sodium cholate (M_3) was replaced with the aq. sodium cholate (M_6), it was observed that the mobility (Fig. 2) of all the three sugars was higher in aq.sodium cholate as compared to alc. sodium cholate. And it was not also possible to achieve useful migration of all the three sugars for the separation. Almost all the three sugars migrated equally in aq. sodium cholate (M_6).

The comparison between the sodium cholate and sodium deoxycholate on the mobility behavior of all the three sugars was also performed. It is obvious from the results (Fig. 3) that sodium deoxycholate(M_7) did not provide any useful R_f values as obtained in the solvent system containing sodium cholate. Therefore, sodium cholate was used for the separation of

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Galactose, Arabinose and Rhamanose in the first run followed by the development by CTAC after rotating the plate at 90^0.

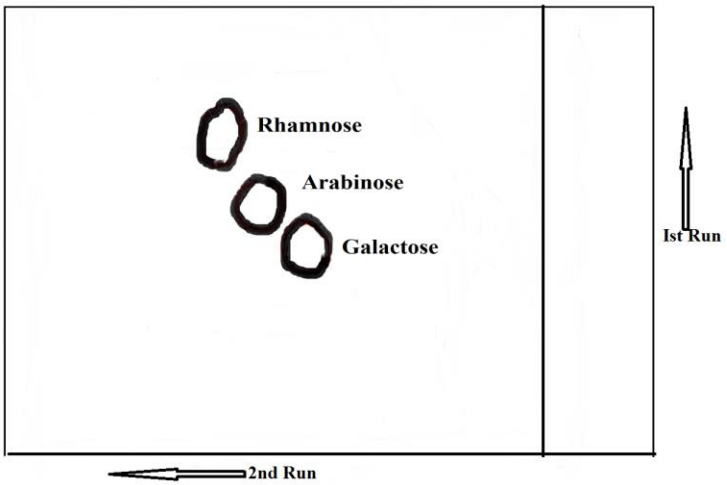

Figure 1: Chromatogram showing the 2D-TLC resolution of Galactose, Arabinose and Rhamnose.

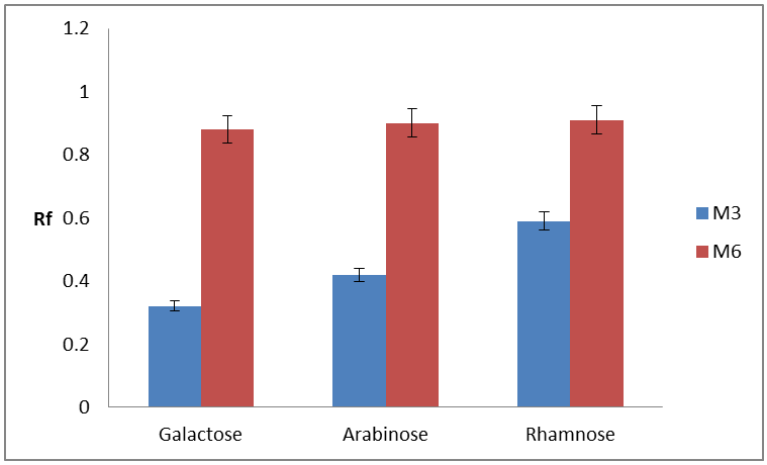

Figure 2: Mobility of Galactose, Arabinose and Rhamnose in alc.sodium cholate (M_3) and aq.sodium deoxy cholate(M_6).(Number of replicates, $n_= 3$)

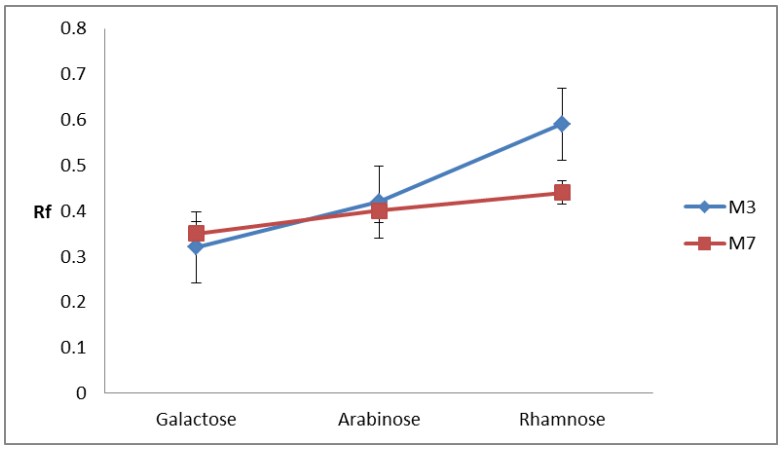

Fig 3: Mobility of Galactose, Arabinose and Rhamnose in sodium cholate (M_3) and sodium deoxy cholate(M_7). (Number of replicates, $n_= 3$)

A simple and eco-friendly method was developed for the separation of Galactose, Arabinose and Rhamanose. A number of highly sophisticated analytical instruments have been used for the analysis of carbohydrates [8-10]. Most of the methods are expensive, involve lengthy sample preparation steps, require large volume of sample etc. Therefore, these methods cannot meet our needs for monitoring performance on a small scale. Among all the chromatographic techniques, thin layer chromatography (TLC) has been most popular for routine analysis due to its simplicity. A simple 2D-thinlayer chromatographic method was developed for the separation. In this study surfactants are used as solvent systems. Surfactants are most preferred solvents in place of other toxic organic solvents .Now-a-days surfactants are extensively used in the separation technology because of their excellent solubilization tendency for various kinds of solutes. Keeping in view of their importance in modern separation science, we have selected surfactants as our solvent systems. In this developed method two different kinds of surfactants (Sodium cholate and CTAC) have been utilized for the seaparation of three sugars (Galactose, Arabinose and Rhamanose) on cellulosic surface.

CONCLUSION

The most important role played in this separation is obviously because of the enormous potential of surfactants in resolving complex mixtures. In this study, two different kinds of surfactants with different charges on their hydrophilic head groups were used. In the first run anionic surfactant (sodium cholate) was used for the development of plate and after rotating the plate at 90^0 cationic surfactant (CTAC) was used to resolve the mixture of three (Galactose, Arabinose and Rhamanose) components.

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