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Chromatographic analysis of food dyes using environmentally preferable solvents

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ABSTRACT

A schematic representation showing different interactions of the dyes with silica gel and aqueous methanol which arise during the separation of curcumin, erythrosine and amaranth from their mixture has been proposed. Effect of foreign substances on the achieved separation has been examined and the limits of detection of the separated dyes have also been calculated. The proposed method is applicable for the identification of these dyes in food samples and separation in spiked sample. Densitographic representation of achieved separation has also been included.

Keywords: Dyes; Separation; Densitometry; Food samples.

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INTRODUCTION

A dye is a distinct chemical that exhibits coloring power when it is dissolved. Water soluble dyes are added to beverages, dry mixes, confectionary, pet foods, baked and dairy goods and other products[1-3]. The dyes used may be carcinogenic in nature. So there is a need to test these products for the nature of colors that have been used.

Amaranth is an azo dye bearing fuctional group R-N=N-R' in which R and R' can be either alkyl or aryl groups. It is used as a food dye and in cosmetics. Erythrosine is a reddish-pink synthetic dye, most properly used as coloring agent and a host of other applications such as printing inks, a dental plaque disclosing agent. Because of the carcinogenic nature [4-5] of these dyes, this analysis is of great importance for the health of people.

A number of analytical techniques have been used for the analysis of food dyes which include HPLC[6-7], liquid chromatography[8], electrophoresis[9], thin layer chromatography [10-11] Among these, TLC proves to be most efficient for the qualitative analysis of dyes because of some advantageous features such as wider choice of stationary and mobile phases, open and disposable nature of TLC plates, reasonable resolving power, minimal sample cleanup, ability to handle a large number of samples simultaneously and reduced need of modern laboratory facilities.

In our study we have analyzed food dyes (amaranth, erythrosine and curcumin) and separated by the use of silica thin layer chromatography utilizing aqueous methanol as mobile phase. The separation is due to the different type of interaction of these dyes with aqueous methanol and silica gel because of different functional groups present in them.

MATERIALS AND METHOD

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

Apparatus

A TLC applicator (Toshniwal, India) was used for coating SIG on 20 cm × 3.5 cm glass plates. The TLC was performed in 24 cm × 6 cm glass jars. Micropipette (Tripette, Germany) (0.1-1.0 µL) was used for spotting of analytes. A glass sprayer (Borosil, India) was used to spray reagent on the plates to locate the positions of the spots of analytes.

Chemicals and reagents

All chemicals were of analytical reagent grade. Silica gel , methanol and acetone were purchased from Merck, India. Amaranth, erythrosine and curcumin dyes were purchased from Sigma, New Delhi, India. Water used in these experiments was double distilled water.

Test solutions

Test solutions of dyes (0.5% w/v) were prepared by dissolving 0.05 g of dye (i.e. amaranth, erythrosine or curcumin) in 10 mL of methanol.

Stationary phase and mobile phase

Silica gel G was used as stationary phase in all the separation experiments. The mobile phase used in all the experiments was distilled water (M1), methanol (M2) and 1, 5 or 10% aqueous methanol solutions (M3, M4 and M5).

Thin layer chromatographic separations

The details of preparation of TLC plates and chromatographic procedure can be seen elsewhere [14]. For the separation of mixture of dyes, equal volumes of dyes to be separated were mixed and an aliquot (0.1 µL) of the resultant mixture was loaded onto the activated TLC plate. The plates were developed with selected mobile phase, i.e. M5 (10% aqueous methanol), the spots were detected and subsequently R_F values of the separated dyes were calculated.

Limits of Detection

The limit of detection of food dyes (amaranth, erythrosine and curcumin) were determined by spotting 0.01 µL of the concerned dyes solutions onto the chromatographic plates, which were developed with mobile phase M5 and spots were visualized. This process was repeated with successive reduction of the concentration of dyes solution until no detection was possible. The amount of dyes just detectable was taken as detection limit. The detection limits of each dye were measured in four replicates.

Interference

To examine the effect of interference, an aliquot of 0.10 µL of foreign substance (cations and anions) was spotted on silica layer followed by the spotting of the mixture (0.10 µL) of dyes. After drying the spot, TLC was performed with mobile phase M5 and R_F values of separated dyes were calculated.

Application

The candy was dissolved in 10 mL of demineralized water. Aliquot (0.10 µL) of the resultant sample (i.e. dissolved candy) was loaded onto TLC plates and developed with mobile phase (M5). Similarly, turmeric powder was dissolved in 10 mL demineralized water and same TLC procedure was performed. The chromatography was performed on these spiked drugs samples also for the identification and separation of dyes in ternary mixture.

RESULTS AND DISCUSSION

Thin layer chromatography has been employed for the separation of amaranth, erythrosine and curcumin dyes. The different functionality of dyes makes them to interact differently with the mobile and stationary phases. The dye adhered strongly to the stationary phase has lower mobility while those with strong interaction with the mobile phase have high mobility. The involvement of different types of interactions leads to differential mobility which ultimately affects separation. Figure 1 shows the schematic representation of interactions which leads to separation of coexisting amaranth, erythrosine and curcumin dyes in mixtures. Results of the present study have been summarized in Tables 1-2 and Figures 1-5. From Table 1 it was observed that by using water (M1) and methanol (M2) as pure solvent for the analysis of food dyes on silica gel G static flat bed, there was no possibility of separation because of tailed spots in case of erythrosine and amaranth. Therefore, different combinations of these two solvents in the form of their mixtures were used for examining the migration behaviour of dyes and it was found that 10% aqueous methanol (M5) was most efficient for the separation of amaranth from erythrosine as well as from curcumin. Since methanol is capable of forming hydrogen bond with water molecule[12], the entire H-bonded methanol and water system has two different charges, a slight negative charge on oxygen atom and slight positive charge on H of water. These two centers are primarily responsible for interaction with dyes, hence resulting in their separation as curcumin (0.02), erythrosine (0.30) and amaranth (0.97) as shown in Figure 2. The values in bracket are the respective R_F values of dyes.

Table 1: Mobility of food dyes in terms of R_F values using different mobile phases

Food Dyes	Mobile Phases				
	M_1	**M_2**	**M_3**	**M_4**	**M_5**
Curcumin	0.08	0.95	0.07	0.10	0.02
Erythrosine	0.30T	0.50T	0.27	0.40T	0.30
Amaranth	0.53T	0.82	0.56	0.52T	0.95

Table 2: Effect of interferences on ΔR_F values of separated food dyes

Foreign substances		Curcumin-Erythrosine (0.28)	Erythrosine-Amaranth (0.67)
Cations	Mg^{2+}	0.25	0.65
	Zn^{2+}	0.30	0.62
	Mn^{2+}	0.24	0.65
	Cu^{2+}	-	-
Anions	CH_3COO^-	0.37	0.56
	CO_3^{2-}	0.33	0.52
	NO_3^-	0.32	0.58
	Br^-	0.39	0.51

The mutual separation of these three dyes on silica layer can be explained on the basis of their relative interactions with negatively charged silanol groups (SiO-) as well as interaction with mobile phase system. In case of amaranth dye, three negatively charged centers in its molecule offer strong repulsion to silanol groups and hence it moves with the solvent front showing highest R_F value. Compared to amaranth dye, the lower mobility of erythrosine is due to the presence of two negative centers instead of three negative centers in amaranth dye. Furthermore, the lone pair on oxygen is partially responsible for its lower R_F value. In cucurmin, the presence of two negative centers along with methoxy group seems responsible for further decrease in mobility. Because of lesser negativity and the stabilization of benzene ring, cucurmin molecule shows least repulsion by silinol groups. As a result, it is retained by silica gel showing minimum R_F value. The lowest possible detectable amounts of dyes by using M5 as mobile phase were found to be 0.10 µg/spot for curcumin, 0.01 µg/spot for both erythrosine and amaranth.

Effect of foreign substances such as metal cations, inorganic anions on separation of food dyes have been examined and chromatographic parameters were determined. Differences between RF values of two adjacent spots (ΔR_F) have been calculated for representative separations of food dyes (Tables 2). There was a marginal difference in the magnitude of ΔR_F in the presence of all interference ions except Cu2+. Thus, the separation is only hampered in the presence of Cu2+ because of its higher complex forming tendency with the dyes under study.

Application

The proposed method was applicable for the identification of curcumin in turmeric (Figure 3) and amaranth dye in candy powder (Figure 4). It was also applied for the separation of amaranth (from candy), curcumin (from turmeric powder) and erythrosine from spiked sample as shown in Figure 5.

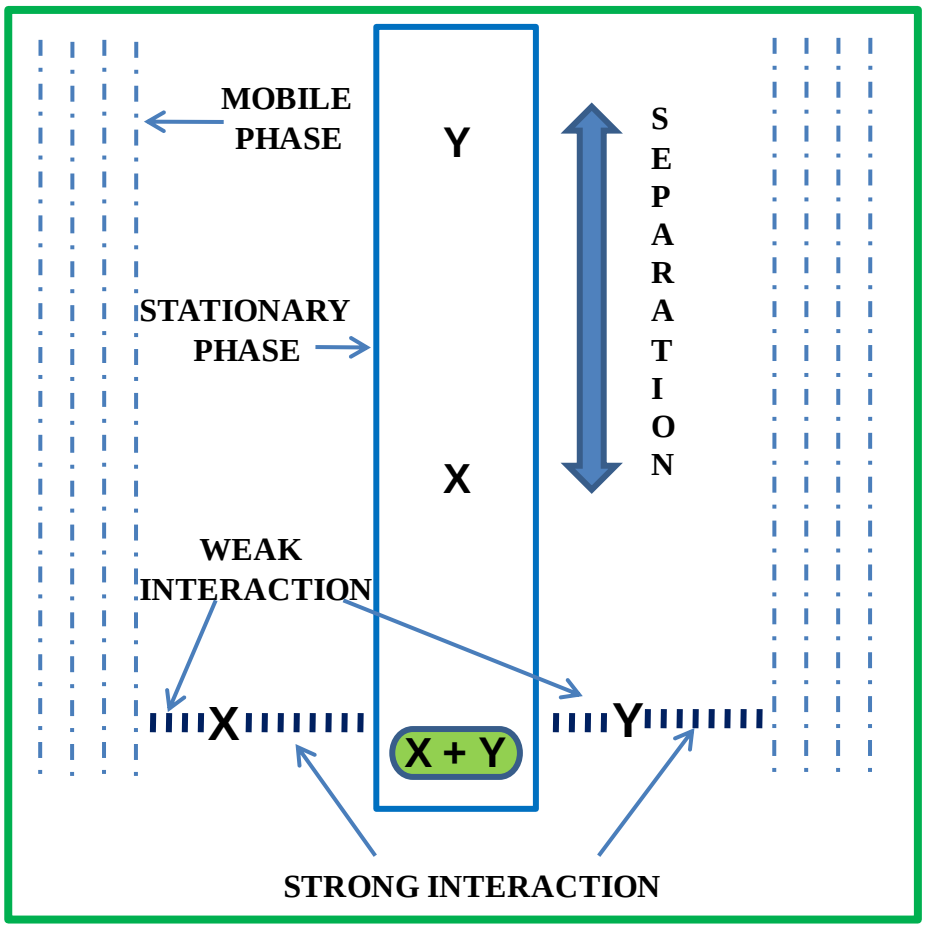

Figure 1: Schematic representation of interactions leading to separation of mixture of dyes

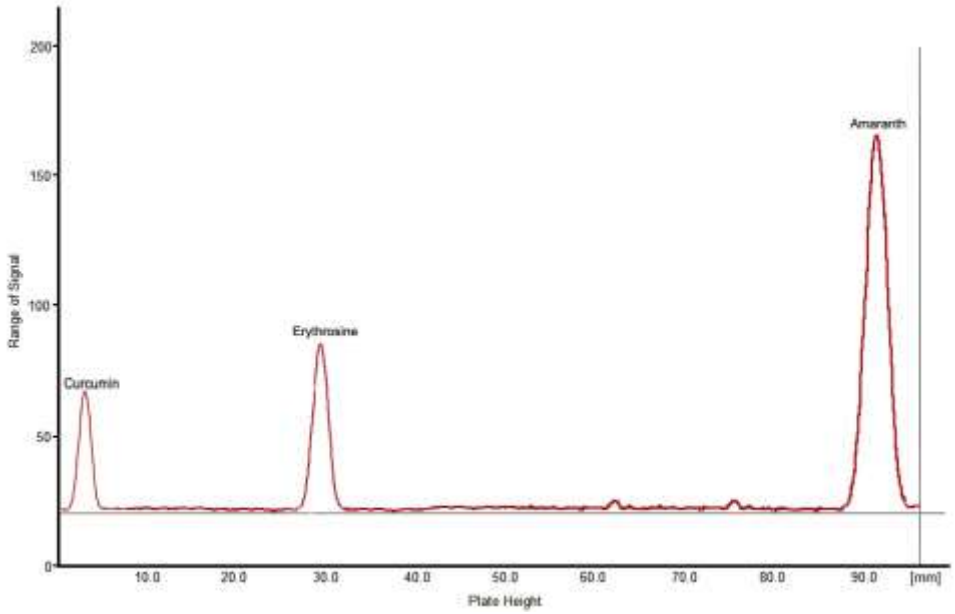

Figure 2: **Densitographic presentation of separation of three-components mixture of dyes**

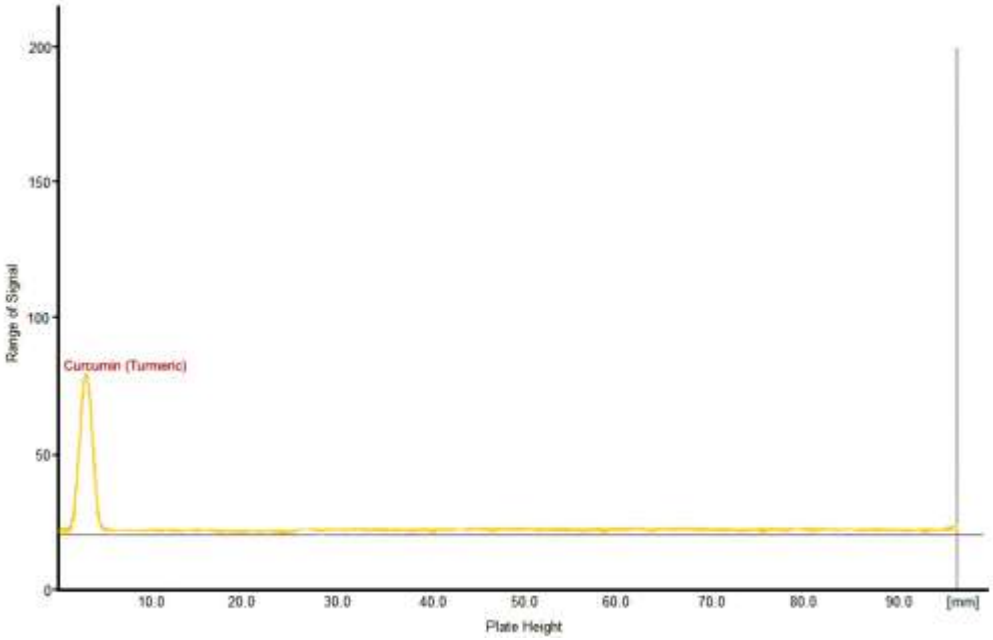

Figure 3: Densitographic presentation of identification of curcumin in turmeric powder

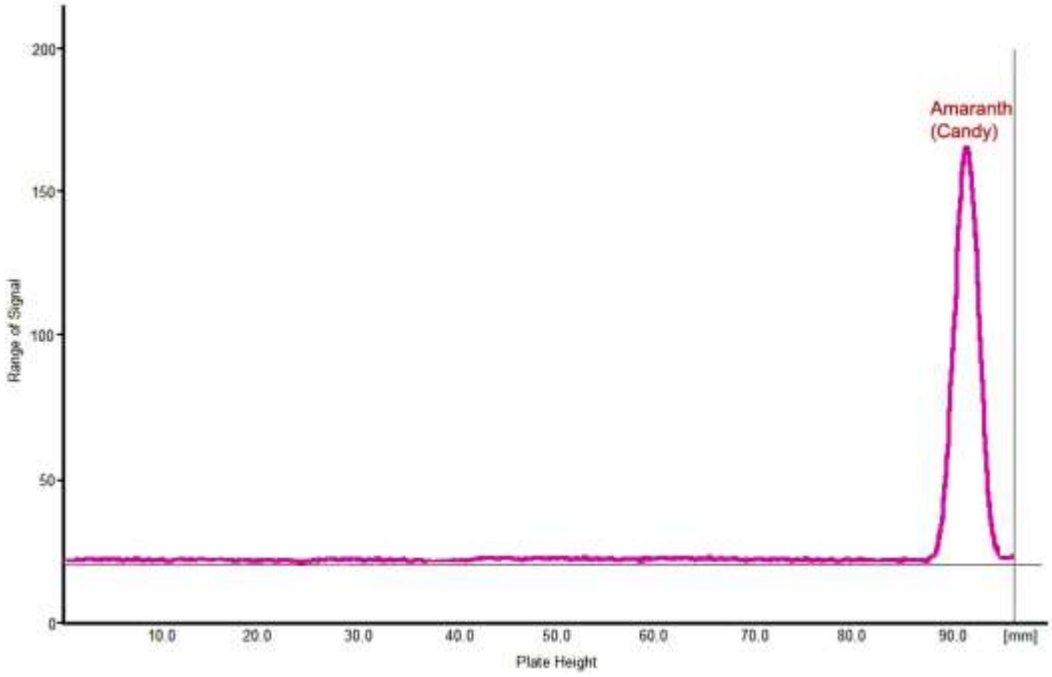

Figure 4: Densitographic representation of identification of amaranth in candy

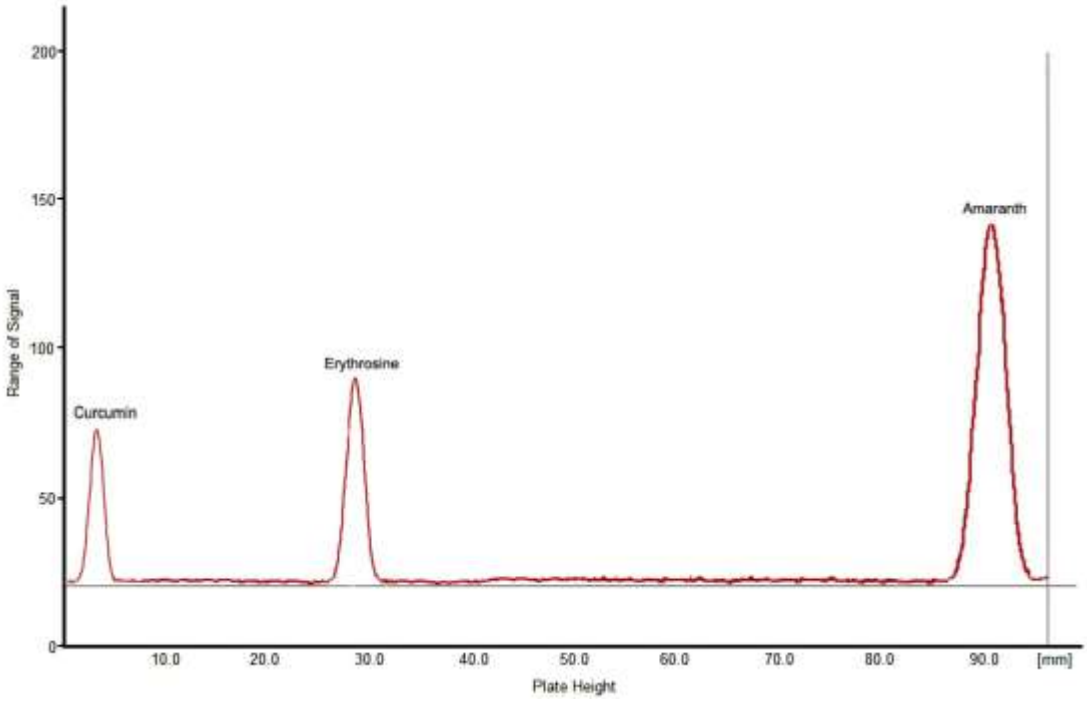

Figure 5: Densitographic representation of separated dyes in spiked sample

CONCLUSION

The different modes of interaction of curcumin, erythrosine and amaranth with silica gel and the selected mobile phase leads to separation of these dyes from there mixture. The method is also applicable for the identification and separation of these in dyes spiked sample.

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