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ORIGINAL RESEARCH ARTICLE

Polyaniline modified silica gel coupled with green solvent as eco favourable mobile phase in thin layer chromatographic analysis of organic dyes

Mahfoozurrahman Khan[1*], Ali Mohammad[2], Qasim Ullah[2], Faiz Mohammad[1]

[1] *Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh 202002, India. E-mail: mahfooz55@gmail.com*

[2] *Department of Chemistry, School of Sciences, Maulana Azad National Urdu University, Hyderabad 500032, India*

ABSTRACT

This article studies a new green eco-friendly TLC (thin layer chromatography) using silica gel and polyaniline modified silica gel as stationary phase in combination with ethyl acetate (EA), n-butyl acetate (BA) and butane-1-ol (BO) solutions as mobile phase for the comparative study of migration behaviour of organic dyes to identify the most suitable thin layer chromatographic system for the resolution of co-existing dyes. Better separation efficiency was observed by modifying silica gel with polyaniline as compared to pure silica stationary phase. Densitogrpahic presentation of separations achieved on polyaniline modified silica gel Pani@SG-EB1 was also presented. The thin layer chromatographic system comprising of polyaniline modified silica gel Pani@SG-EB1 as stationary phase and n-butyl acetate:DDW, 5:5 as green mobile phase was observed to be the most favourable for the separation of various combinations of three or four-component mixtures of organic dyes viz. methyl thymol blue, tartrazine, carmoisine, rose bengal, amidoblack 10B, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate. The effect of presence of cations and anions on separation trend was also examined and the limits of detection of the separated organic dyes were estimated. Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron micrograph (TEM) studies were undertaken to characterize silica gel and modified silica gel (stationary phase). The developed method has been successfully applied for the identification of carmoisine in Solvin cold DS syrup and tartrazine in MefastTM syrup.

Keywords: Organic Dyes; Modified Silica Gel; Densitometry; Pharmaceutical Syrup; Thin Layer Chromatography

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1. Introduction

Organic dye is a colour substance that has always attracted the person due to its attractiveness. Food colours are usually added to various commercial food products in order to make them more attractive and to achieve the desired colour. Food colours can be divided into four categories: natural, nature-identical, inorganic and synthetic[1]. For safety reasons there have been recent reductions in the number of permitted food colours but they are still being used all over the world because of their low price, effectiveness and stability. Moreover, the food processing industry uses all types of food colours, but to minimise potential toxicity the amounts of permitted synthetic colours used are strictly limited[2,3].

Synthetic dyes are widely used for improving the colour and enhancing the visual aesthetic appeal of some foods, and this effect is maintained throughout the production process and during storage. They present high stability to light, oxygen, and pH changes, and have lower prices compared to natural dyes[4,5]. Synthetic dyes can be classified into water soluble and fat soluble. Only water soluble dyes are permitted in foods. Some of the most commonly used are sunset yellow (E110), allura red (E129), brilliant blue (E133), erythrosine (E127), tartrazine (E102), amaranth red (E123), carmoisine, ponceau 4R (E124), and others. They are present in soft drinks, gelatins, snacks, yoghurts, ice cream, candies, puddings, chips, pickles, honey, mustard, gum, baked goods etc.

However, these dyes must be controlled because many of them have been related to health problems. It was observed that synthetic dyes present in foods may cause overactive behaviour in children, especially if they are consumed in excess. On the other hand, some dyes in the presence of analgesics like aspirin can induce allergic or asthmatic problems. Tartrazine and sunset yellow second and third most widely used food dyes, added to many food[6-8].

Dyes are very important in so many areas such as printings, paintings, textiles, cosmetics, pictures, food and screens[9–12]. Food dye additives are defined as dyes, pigments, or substances that impart colors into foods, drugs, or cosmetics. Originally, natural dyes present in foods are unstable and altered rapidly during food processing and storage. Hence, synthetic dyes that outperform natural ones at various aspects such as low price, high effectiveness, and excellent stability are widely used by food companies all over the world[13,14].

Many analytical methods for the determination of dyes have been reported, such as thin layer chromatography (TLC)[15–17], titrimetry[18], voltammetry[19,20], polarography[21], spectrophotometry, 12[22,23] high-performance liquid chromatography (HPLC)[24–26], capillary electrophoresis (CE)[27–29], ion chromatography[30], voltammetry, polarography, spectrophotometry and thin layer chromatography are simple and time saving methods been proposed.

Among all these techniques, thin layer chromatography (TLC) is one of the most cheapest, quickest and most versatile for the separation and identification of organics and inorganics chemical compounds for many applications like bio-medical and pharmaceutical field including dyes industries[31–33].

In the current study, silica gel and polyaniline modified silica gel layer has been used as stationary phase in combination of DDW, ethyl acetate (EA), n-butyl acetate (BA) and butane-1-ol (BO) as green mobile phase. Polymer modified silica gel enhances the selectivity and chemical stability of the stationary phases. Ethyl acetate, n-butyl acetate and butane-1-ol solvent have been used in TLC as a green solvent due to their versatility and environmental benefits[34]. The coupling of favourable properties of polyaniline (Pani) (e.g. good environmental stability, high electrical conductivity, electrochromism, reversible transformation of various oxidation states, catalytic, antistatic and anticorrosion activity) and silica gel (good heat resistance, high tensile strength, and hardness) together provide new stationary phase of improved structural and chromatographic characteristics[35,36].

In the current study, the polyaniline modified silica gel stationary phase Pani@SG-EB$_1$ = S$_2$ has been prepared by in situ oxidative polymerization of aniline to be used as stationary phase with several mobile phase for thin layer chromatographic analysis of seven organic dyes viz. tartrazine; carmoisine; rose bengal; amidoblack 10B; methyl thymol blue; bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate. Chromatographic parameters have been calculated for quaternary separations of organic dyes on Pani@SG-EB$_1$ = S$_2$ stationary phase in the presence of M$_6$ (n-butyl acetate:DDW, 5:5 by volume) as mobile phase. The use of green mobile phase as well as polyaniline modified silica gel as stationary phase is a novel approach to develop environmental friendly green TLC method. Thus prepared Pani@(SG)EB$_1$ = S$_2$ stationary phase has also been characterized by Fourier transform infrared (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron micrograph (TEM).

2. Materials and method

2.1 Apparatus

Glass plates (20 cm × 3.5 cm) coated with silica gel and polyaniline modified silica gel using TLC applicator (Toshniwal, India) were used as stationary phase. The micropipette (Tripette, Germany) was used for spotting of analytes and glass jars (24 cm × 6 cm) were used to perform TLC.

2.2 Experimental temperature

All the experiments were performed at 25 ± 2 ºC.

2.3 Chemicals and instrumentation

Silica gel (Fischer Scientific, India), methanol, ethanol, propanol-1, potassium persulphate, copper sulphate, zinc sulphate, manganese sulphate, nickel nitrate, HCl, n-butyl acetate and sodium salts of bromide, chloride, carbonate, acetate and nitrate (CDH, India); ethyl acetate, butane-1-ol (Fisher Scientific) and aniline (E-Merck India Ltd.) were used as received. All chemicals were of Analytical Reagent grade. The water used in these experiments was double distilled.

The fourier transform infrared spectra (FTIR) was recorded using Perkin-Elmer 1725 spectrometer operating in the 400-4000 cm^{-1} range. X-ray diffraction (XRD) data were recorded by using Bruker D8 diffractometer with Cu Kα radiation at 1.540 Å in the range of $5^o \leq 2\theta \leq 70^o$ at 40 kV. The morphology was observed by a JSM-6510LV system with a JEOL scanning electron microscope (SEM). Transmission electron micrograph (TEM) was done with the help of JEM 2100, JEOL.

2.4 Dyes studied

Tartrazine and carmoisine (Roha Dyechem Pvt. Ltd., Mumbai, India), rose bengal (SD fine chemical), amidoblack 10B (CDH, India), methyl thymol blue (Merck), bromopyrogallol red (CDH, India) and 4-nitrobenzene dizonium tetrafluoroborate (Spectrochem, Pvt. Ltd. Bombay, India) were used.

2.5 Test solutions

Solutions 5 % (w/v, g/mL) of dyes were prepared in double distilled water (DDW).

2.6 Stationary phase

SG = S_1, Pani@SG-EB_1 = S_2 and Pani@SG-EB_2 = S_3 were used as stationary phase.

2.7 Mobile phase

Solvent systems used as mobile phase are listed in **Table 1**.

2.8 Composition of Mefast^{TM} P syrup and Solvin cold DS syrup

Table 1. The composition details of mobile phases

Mobile Phase Code	Mobile Phase
M_1	Double distilled water (DDW)
M_2	Ethyl acetate (EA)
M_3	n-Butyl acetate (BA)
M_4	Butane-1-ol (BO)
M_5	Ethyl acetate : DDW (5:5)
M_6	n-Butyl acetate : DDW (5:5)
M_7	Butane-1-ol : DDW (5:5)
M_8	Ethyl acetate : DDW (6:4)
M_9	n-Butyl acetate : DDW (6:4)
M_{10}	Butane-1-ol : DDW (6:4)
M_{11}	Ethyl acetate : DDW (4:6)
M_{12}	n-Butyl acetate : DDW (4:6)
M_{13}	Butane-1-ol : DDW (4:6)

The Mefast^{TM} P syrup from Zuventus Health Care Ltd. (A joint venture of Emcure^R Chandival Andheri (E), Mumbai, India) and Solvin cold DS syrup from Ipca Laboratories Ltd. (Selaqui, Dehradun; Regd. Off. 48 Kandivali, Mumbai, India) were studies to identify tartrazine and carmoisine organic dyes respectively. The pharmaceutical formulation of Mefast^{TM} P syrup contains mafenamic acid suspension IP 50 mg, paracetamol IP 125 mg and colouring agent tartrazine. The pharmaceutical formulation of Solvin cold DS contains paracetamol IP 250 mg, phenylephrine hydrochloride IP 5 mg, chlorpheniramine maleate IP 2 mg and colouring agent carmoisine.

2.9 Characterization

2.9.1 FTIR spectroscopic studies

The FTIR spectra of silica gel (SG) and Pani@SG-EB_1 stationary phases are shown in **Figure 1**. The main characteristic peaks of silica gel in curve are broad absorption band around 3462 cm^{-1} corresponds to Si-OH stretching vibrations, 1636 cm^{-1} for the Si-OH bending mode of vibration, the strong absorption band at 1099 cm^{-1} due to the asymmetric stretching vibration of Si–O–Si bond because of the formation of SiO_2 network, the absorption peak at 795 cm^{-1} represents Si-O-Si bending vibration[37-39].

In the case of Pani@SG-EB_1 stationary phases apart from the peaks of SG, the characteristic peak of 3454 cm^{-1} due to the free (non-hydrogen bonded) N-H stretching vibration is present, confirming the presence of Pani in Pani@SG[40,41]. 1643 cm^{-1} for the Si-OH bending mode of vibration, the strong absorption band at around 1087 cm^{-1} due to the asymmetric stretching vibration of Si–O–Si bond. Thus, it can be inferred that the incorporation of SG does not damage the backbone of Pani. It was observed that aromatic groups grafted onto the silica gel surface can be identified by their characteristic bands, which are distinguishable from those of the linear chain intermediate. A qualitative analysis of the organic groups immobilized on the silica gel surface can be carried out based on the very similar spectra of pure and supported aromatic groups.

2.9.2 X-Ray diffraction (XRD) studies

The x-ray diffraction patterns of silica gel and Pani@SG-EB_1 stationary phases are presented in **Figure 2**. It can be seen clearly that the silica retains a broad diffraction peak at about 22.3°, indicating that the silica is amorphous nature. Likewise, the diffraction peak of Pani@SG-EB_1 did not have an apparent shift[42–44]. However, there is no diffraction peak of Pani emerging in the patterns of Pani@SG-EB_1, confirming that the Pani is also amorphous and has been encapsulated in the pores and channels of the silica.

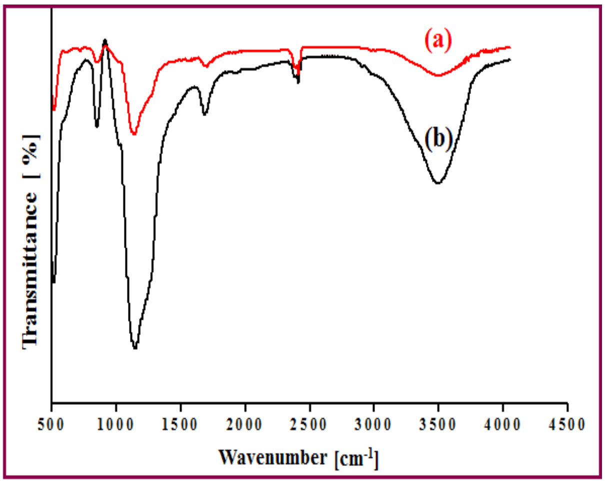

Figure 1. FTIR spectra of: (a) Pani@SG-EB_1 = S_2 and (b) silica gel = S_1 stationary phases.

2.9.3 Scanning electron micrographic (SEM) studies

The shape and surface morphologies of silica gel and Pani@SG-EB_1 stationary phases have been demonstrated in **Figure 3** (a-b). SEM images of silica nanoparticles shown in **Figure 3** (a) are irregular, porous and few are spheroidal shape with relatively smooth surface. In **Figure 3** (b), Pani@SG-EB_1 show rougher surface probably due to the polymerization of aniline on the surface of silica gel and porosity may increase due to presence of polyaniline[45,46]. However, both the micrographs are relatively similar and SiO_2 are mechanically very strong and not very distinct. The aniline has polymerized on the surface of silica gelas is evident from the FTIR analysis.

2.9.4 Transmission electron micrographic (TEM) studies

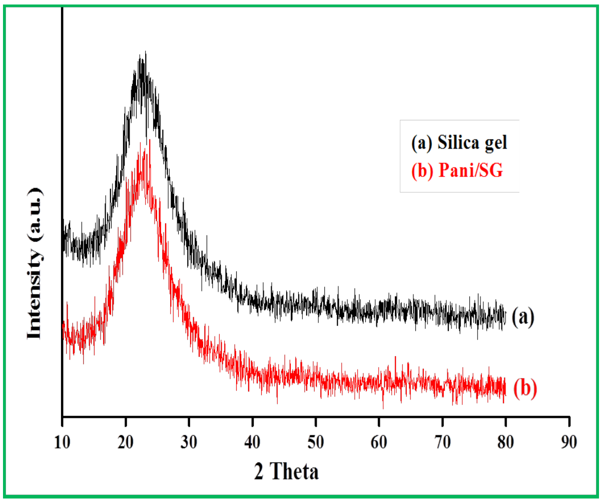

Figure 2. XRD spectra of: (a) silica gel = S_1 and (b) Pani@SG-EB_1 = S_2 stationary phases.

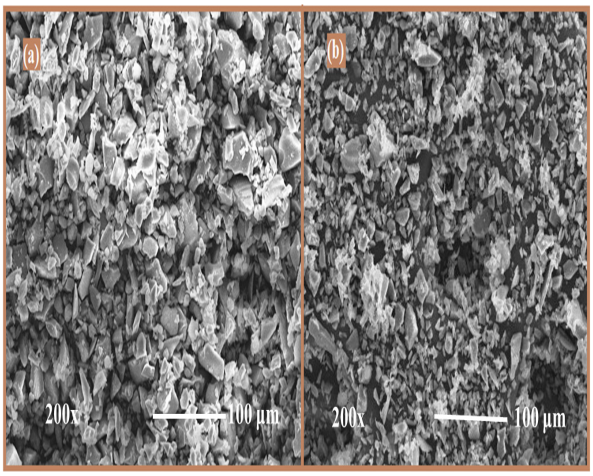

Figure 3. SEM micrographs of (a) silica gel = S_1 and (b) Pani@SG-EB_1 = S_2 stationary phases.

Silica gel and Pani@SG-EB$_1$ stationary phase were characterized to identify size and shape of their particles by TEM studies as shown in **Figure 4**. Most of the silica gel particles were spheroidal or ellipsoidal with particle size ranging between 8–13 nm. However, some irregular particles were also observed[47]. In case of Pani@SG-EB$_1$ stationary phases, it appears that the Pani undergoes polymerization on the surface of silica gel leading to the formation of interconnected tubular and fibrous nanostructures containing silica gel in the core. However, the SG surfaces became rough and a large amount of amorphous films or particles were observed, indicating the formation of Pani over SG and thickness size range was 18-35 nm. Thus, the material particles belong to the nano size. The Pani is tightly bound to silica gel which is beneficial for its use in electronic and separation technique[48].

2.10 Preparation of polyaniline modified silica gel stationary phase

Polyaniline modified silica gel stationary phases were prepared by simple in-situ oxidative polymerization of aniline using potassium persulfate as an oxidizing agent in presence of different amounts of SG. Aniline (1 mL) was dissolved in 200 mL of aqueous solution of HCl (1M). Different amounts of silica gel were added in aniline solution. Later, potassium persulphate (5 g), dissolved in HCl (1M) was added drop-wise in the aniline solution for the polymerization. The reaction mixture was put under continuous stirring for 22 h. The resultant mixture containing emeraldine salt turned slowly into greenish black slurry which was filtered, washed thoroughly with DDW and undoped with 500 mL of 1M ammonia solution for making Pani@SG-EB. The resultant product washed with DDW and methanol to remove the impurities until filtrate became neutral and colourless. The prepared nanocomposites were dried at 70–80°C for 12 h in an air oven, converted into fine powders and were stored in a desiccator for further investigations as shown in **Table 2**.

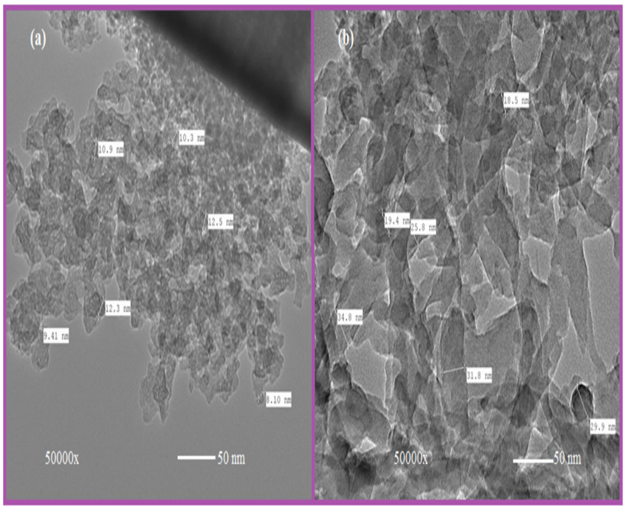

Figure 4. TEM micrographs of (a) silica gel = S$_1$ and (b) Pani@SG-EB$_1$ = S$_2$ stationary phases.

2.11 Thin layer chromatography

The preparation of TLC plates and chromatographic procedure were followed as reported earlier[49]. Aliquots (0.1 µL) of dyes were spotted on prepared TLC plates (S$_1$, S$_2$ and S$_3$) and the plates were developed with different mobile phases (M$_1$-M$_{13}$). After development, spots were visualised and the R$_F$ values were calculated for individual dyes. For mutual separations of dyes, equal volumes (1 mL each) of dyes (Tartrazine, carmoisine, rose Bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate) were mixed and 0.1 µL of the resultant mixture was spotted on the TLC plates. The plates were developed with selected mobile phase (M$_6$) and static phase (S$_2$), and the spots were detected and the R$_F$ values of the separated dyes were determined. The R$_F$ values were calculated from the R$_L$ (R$_F$ of leading front) and R$_T$ (R$_F$ of trailing front) values of the spot as given below.

Table 2. Preparation details of Pani@SG stationary phases

Mobile Phase Code	Volume of Aniline (mL)	Weight of $K_2S_2O_8$ (g)	Weight of Silica Gel (SG) (g)
Pani@SG-EB$_1$ = S$_1$	1.0	5.0	30.0
Pani@SG-EB$_2$ = S$_2$	1.0	5.0	60.0

$$R_F = 0.5\ (R_L - R_T) \qquad (1)$$

For the separation of dyes mixture, an equal volume of each dye were mixed and 0.1 µL of the resultant mixture was applied onto the TLC plates of silica gel or polyaniline modified silica gel. The TLC plates were developed with different mobile phases, the spots were detected, the R$_F$ values were determined and other studies were carried out on the separated spots of the dyes.

2.12 Separation

For the separation of the organic dyes mixtures, an equal volume of each organic dyes were mixed and 1 µL of the resultant mixture was applied onto TLC plate. The plate was developed with M_6 (n-butyl acetate, DDW, 5:5) by volume), the spots were detected and the R_F values of the separated spots of the organic dyes were calculated.

2.13 Ageing effect of mobile phase

To examine the ageing effect (stability) of mobile phase (M_6) on the separation of organic acids, the sample mixture was spotted on the activated Pani@SG-EB_1 TLC plates, developed with freshly prepared mobile phase (M_6) and the R_F values were calculated. The same process was repeated using previously prepared mobile phase (M_6) at different intervals of time for 24 h. Later on the R_F values were calculated and compared with the values obtained with the use of freshly prepared mobile phase.

2.14 Effect of foreign substances

For investigating the interference of metal cations and inorganic anions as impurities on the separation of the mixture, 0.1 µL of the standard test mixture of dyes solutions were spotted on the S_2 TLC plate followed by spotting of 0.1 µL of the metal cations or inorganic anions being considered as impurities. The plates were developed with M_6, detected and the R_F values of the separated dyes were calculated.

2.15 Limits of detection

The detection limits of the dyes were determined by spotting 0.1 µL of tartrazine, carmoisine, rose bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate of different concentrations on the S_2 stationary phase. TLC plates which were developed with the selected mobile phase M_6 and the spots were visualized. This process was repeated by successive reduction of the concentration of dye until the detection of dye was not possible anymore. The amount of dye just detectable was taken as the detection limit.

3 Results and discussion

SG was modified by in-situ oxidative polymerization of aniline. Separations of mixture of organic dyes (tartrazine; carmoisine; rose bengal; amidoblack 10B; methyl thymol blue; bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate was performed on three different stationary phases silica gel = S_1, Pani@SG-EB_1 = S_2 and Pani@SG-EB_2 = S_3 with thirteen different mobile phases of eco-friendly green organic solvent for obtaining a novel TLC system for the separation of dyes from their mixtures. Polyaniline modifies silica gel show more proficiently separation in comparison to SG. It can be understood that on modification of silica surface, there is a partial conversion of surface silanol groups to new organo-functional groups imparting considerably different set of properties to the surface than the original matrix[50].

3.1 Pani@SG stationary phases

SG was modified by in-situ oxidative polymerization of aniline over it. Separations of mixture of organic dyes were more efficiently achieved on Pani@SG in comparison to SG. It may be understood that on modification of silica gel surface, there is a partial conversion of surface silanol groups to new organo-functional groups imparting considerably different set of properties to the SG surface[50] (**Figure 5**).

Thus, after modification of SG with Pani, both the positive and negative centers are developed on the stationary phases which are capable to selectively interact with the dyes in the mixture. The polarons of Pani interact with the oxygen atoms of the silanol groups via electrostatic interaction giving rise to a new organo-functional group with both positive and negative centers[51]. In aqueous medium, dyes molecule bears both positively and negatively charged centers along with other groups and hence can interact with the organo-functional groups of the stationary phase via electrostatic interaction. However, different dyes interact differently depending on their molecular structure. This difference in mobility due to different types of interactions of different dyes with organo-functional group of sta-

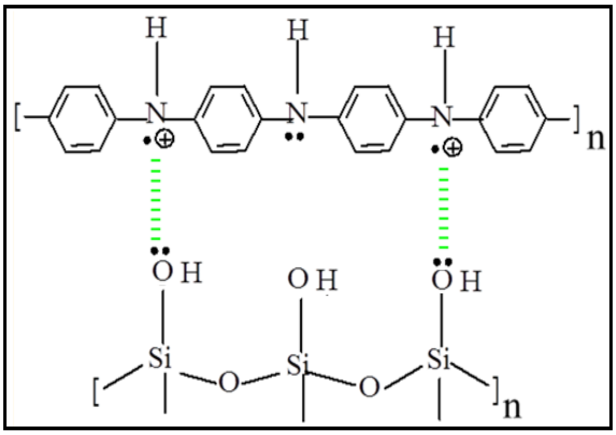

Figure 5. Schematic presentation of interaction between Pani and silica gel Pani@SG stationary phase.

tionary phase eventually leads to their separation.

Due to the polar nature of stationary phase, mobile phase and dyes molecules, electrostatic interactions between the anionic dye and the polymer, hydrogen bonding (between H, O and N atoms in dye molecule and N and H atoms in polymer), van der Waals forces (between the aromatic nature of polymeric matrix and the aromatic rings of the dye) are responsible for differential migration of dyes which eventually led to their separations. In the present case, 4-nitrobenzene dizonium tetrafluoroborate and methyl thymol blue are strongly retained by the stationary phase exhibiting very low R_F value (R_F ranges 0.05–0.06). It appears that steric hindrance due their bulkiness plays dominant role in case of MT to reduce the mobility of the dyes besides the strong electrostatic interaction between the dyes and stationary phase S_2.

In case of bromopyrogallol red, the steric hindrance is lesser than that in NT and MT therefore the mobility (R_F = 0.22) of this dye, while in case of rose bengal dye the steric hindrance and electrostatic interactions are less and therefore its R_F (0.40) is greater than other four dyes leading to a very good separation from others.

But in case of tartrazine and carmoisine dyes, the steric hindrance and electrostatic interactions are minimal and therefore R_F values are 0.95 and 0.70 respectively, which are greater than all other dyes leading to a very good separation from others. The chemical structures of dyes under study are illustrated in **Figure 6**.

Thin layer chromatography of dyes (tartrazine, carmoisine, rose bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate) was performed on three different stationary phases viz. (i) silica gel = S_1, (ii) Pani@SG-EB_1 = S_2 and (iii) Pani@SG-EB_1 = S_3 with thirteen different mobile phases of green solvent for obtaining a novel TLC system for the separation of dyes from their mixtures. Obtained results tabulated in Tables 3–7 and presented in Figures 5–9 are discussed below.

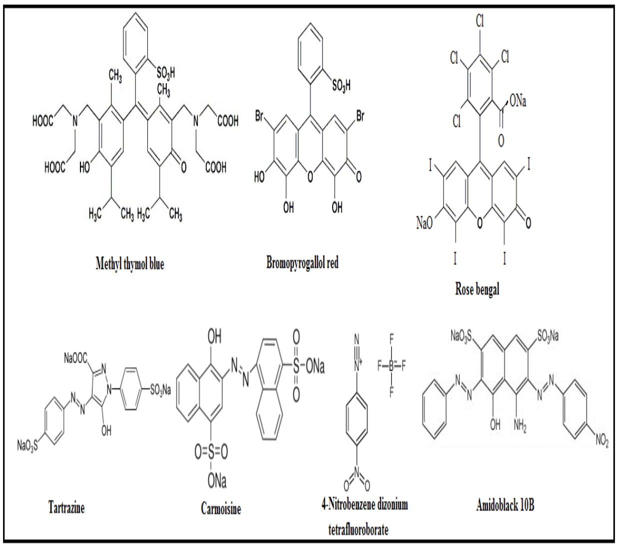

Figure 6. Structures of dyes: methyl thymol blue, bromopyrogallol red, rose bengal, tartrazine, carmoisine, 4-nitrobenzene dizonium tetrafluoroborate and amidoblack 10B.

3.2 Silica gel (S_1) system

Using double distilled water (M_1), on pure silica static phase (S_1), binary separation of carmoisine (CS) from Tartrazine (TZ), rose Bengal (RB), amidoblack 10B (AB) and methyl thymol blue (MT) from the mixture of azo dyes has been observed while bromopyrogallol red (BG) and 4-nitrobenzene dizonium tetrafluoroborate (NT) are not detected.

With ethyl acetate (M_2) and n-butyl acetate (M_3) on S_1 static phase TZ, CS, AB, MT and BG organic dyes shows strong interaction with silica static phase all are shows at the point of application exhibiting R_F values 0.01 and RB dyes shows broad (R_F = 0.22-0.25) spot on both M_2 and M_3 mobile phases while NT dyes shows double spot on M2 and in case of M_3 mobile phase at point of application. Hence, none of the organic dyes can be well separated from their mixture.

In M_4 (Butane-1-ol) mobile phase the selective binary separation of RB (R_F = 0.75) from TZ, CS, AB, MT, BG and NT (R_F = 0.01-0.12) on pure SG stationary phase.

In M_5-M_7 equal volume ratio (5:5) of two component mobile phase system using with the hope of better separation possibilities were used. In M_5 mobile phase TZ can be separated from CS, RB and AB dyes while MT and BG are tailed spot and NT having double spot. In M_6 binary separations were achieved (NT/TZ-CS-AB) while M_7 mobile phase (MT-BG/TZ-CS-RB-AB) occur.

With increasing volume ratio of organic solvent in DDW (M_8-M_{10}) in M_8 mobile phase TZ, CS, RB and AB (R_F = 0.82-0.87) exhibit high mobility and tailing in MT, BZ. In mobile phase M_9 and M_{10} consisting of TZ, CS, AB and MT shows strong interaction with silica gel static phase remain near the point of application (R_F = 0.01-0.09) while RB exhibit tailing spot and BG and NT dyes shows double spot, hence no separation were achieved.

In case of M_{11}-M_{13} mobile phase system on pure SG static phase the M_{11} mobile phase all spot near the point of application with broader range (R_F = 0.01-0.20) hence no good separation was achieved. In M_{12} mobile phase binary separation occur from TZ (R_F = 0.82) high mobility and CS, RB, AB and MT near the point of application (R_F = 0.02-0.25) while BG and NT not detected. With butanol:DDW ratio (4:6) v/v, in which M_{13} mobile phase occur binary separations occur TZ (R_F = 0.54), CS (R_F = 0.53) and AB (R_F = 0.48) exhibit high mobility and NT (R_F = 0.01) organic dyes remain the point of application as shown in **Table 3**.

3.3 Pani@SG-EB$_1$ (S$_2$) system

When pure double distilled water (M_1) is used as a mobile phase for examining migration behaviour of organic dyes on modified silica gel Pani@SG-EB$_1$, as stationary phase CS (R_F = 0.55) has high mobility, selected binary separation were occur TZ, RB, AB and MT remain at the point of application.

Using ethyl acetate (M_2) and n-butyl acetate (M_3) as the mobile phase on Pani@SG-EB$_1$) as stationary phase NT reach the maximum allowed distance (R_F = 0.91-0.92), while other near the point of application except RB which shows tailed spot binary separation achieved. In case of M_4 mobile phase RB (R_F = 0.77) separate out from all other organic dyes having low R_F values (RB/TZ-CS-AB-MT-BG-NT) binary separations were obtained with compact spots. Using M5 mobile phase all were found near the point of application except RB and NT. However RB produced tailed spot and NT was not detected.

With the use of equal volume ratio n-butyl acetate: DDW, (M_6) mobile phase on Pani@SG-

Table 3. Mobility of dyes in terms of R_F values on S_1 (silica gel) stationary phase with different mobile phases

Mobile Phase	**R_F Value**						
	TZ	**CS**	**RB**	**AB**	**MT**	**BG**	**NT**
M_1	0.18	0.55	0.01	0.04	0.04	ND	ND
M_2	0.01	0.01	0.22	0.01	0.01	0.01	0.10 and 0.94 DS
M_3	0.01	0.01	0.25	0.01	0.01	0.01	0.02
M_4	0.01	0.12	0.75	0.12	0.05	0.01	0.02
M_5	0.05	0.62	0.50	0.58	0.31T	0.32T	0.03 or 0.78 DS
M_6	0.94	0.82	0.35T	0.80	0.31T	0.38T	0.03
M_7	0.85	0.70	0.82	0.95	0.25	0.02	ND
M_8	0.87	0.83	0.85	0.82	0.41T	0.40T	0.03 or 0.63 DS
M_9	0.01	0.01	0.34T	0.01	0.01	0.02 or 0.86 DS	0.03 or 0.88 DS
M_{10}	0.01	0.03	0.31T	0.08	0.09	0.04	0.01 or 0.66 DS
M_{11}	0.18	0.20	0.19	0.19	0.18	0.20	0.01
M_{12}	0.82	0.10	0.25	0.02	0.12	ND	ND
M_{13}	0.54	0.53	0.35T	0.48	0.22	0.28	0.01

TZ, CS, RB, AB, MT, BG, and NT represent tartrazine, carmoisine, rose bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate respectively. DS, ND and T stand for double spot, not detected and tailed spots respectively.

T=tailed spot, (R_L - $R_T \geq 0.3$)

EB_1 stationary phase showed differential migration behaviour TZ (R_F = 0.95), CS (R_F = 0.70), RB (R_F = 0.40), AB (R_F = 0.78), MT (R_F = 0.06) BG (R_F = 0.22) and NT (R_F = 0.05) and hence this TLC system was considered most favourable for quaternary separations such as TZ-CS-RB-MT, TZ-CS-RB-NT and ternary separation RB-AB-MT and RB-AB-NT; good separation was obtained hence this mobile phase most efficient for the separation of these organic dyes (**Table 4**). This system was selected for further detailed studies.

On S_2 stationary phase, the mobility of RB dyes with mobile phases M_7 (R_F = 0.70) can be separated from CS, AB, MT and BG R_F (0.02, 0.10, 0.10 0.02 and 0.02 respectively), hence binary separation achieved while NT and TZ organic dyes was not detected.

When TLC plates were developed with M_8, M_9 mobile phases, with M_8 mobile phase all are found near the point of application (R_F = 0.01-0.11) except, RB tailing spot and NT, TZ were not detected on Pani@SG-EB_1 stationary phase. On M_9 binary separation (TZ-CS/MT) of these organic dyes achieved. And other dyes were not detected.

In M_{10}-M_{13} mobile phase with S_2 static phase with M_{10} mobile phase RB (R_F = 0.44) dyes separated from all other dyes which are near the point of application, thus binary separation was achieved. With mobile phase M_{11}, M_{12} and $M_{13,}$ some could not detected on the use of Pani@SG-B_1 and other remain at the point of application, thus no any separation was achieved; so this system was not favourable.

3.4 Pani@SG-EB_2 (S_3) system

On Pani@SG-EB_2 stationary phase using double distilled water (M_1) mobile binary separation of CS (R_F = 0.55) from TZ, RB, AB and MT their R_F = 0.18, 0.01, 0.04, 0.04 respectively while BG and NT were not detected.

With S_3 static phase with ethyl acetate (M_2) and n-butyl acetate (M_3) mobile phase NT organic dyes reach the maximum allowed distance, showed RF in the range of 0.91-0.92. Other organic dyes near the point of application except RB which showed tailed spot. Thus binary separation achieved (NT/TZ-CS-AB-MT-BG) in both mobile phase M_2 and M_3 as shown in **Table 5**.

RB (R_F = 0.70) exhibit high mobility and all organic dyes (TZ, CS, AB, MT, BG, NT) remain at the point of application (R_F = 0.01-0.10) as S_3 stationary phase. Binary separations of these organic dyes were achieved.

With equal volume of two component mobile

Table 4. Mobility of dyes in terms of R_F values on Pani@SG-EB_1 = S_2 stationary phase with different mobile phases

Mobile Phase	R_F Value						
	TZ	CS	RB	AB	MT	BG	NT
M_1	0.18	0.55	0.01	0.04	0.04	ND	ND
M_2	0.01	0.01	0.45T	0.01	0.01	0.01	0.92
M_3	0.01	0.01	0.46T	0.01	0.01	0.01	0.91
M_4	0.01	0.05	0.77	0.06	0.01	0.01	0.01
M_5	0.12	0.12	0.45T	0.01	0.05	0.02	ND
M_6	0.95	0.70	0.40	0.78	0.06	0.22	0.05
M_7	ND	0.10	0.70	0.10	0.02	0.01	ND
M_8	ND	0.10	0.40T	0.10	0.08	0.01	ND
M_9	0.36	0.30	ND	ND	0.01	ND	ND
M_{10}	0.02	0.10	0.44	0.10	0.03	0.02	0.01
M_{11}	0.17	0.10	ND	ND	ND	0.20	ND
M_{12}	ND	ND	ND	ND	0.08	ND	ND
M_{13}	0.13	0.21	ND	ND	0.05	ND	ND

TZ, CS, RB, AB, MT, BG, and NT represent tartrazine, carmoisine, rose bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate respectively. DS, ND and T stand for double spot, not detected and tailed spots respectively.

T = tailed spot, (R_L - $R_T \geq 0.3$)

phase ethyl acetate:DDW. In M_5 mobile phase TZ ($R_F = 0.45$) and CS ($R_F = 0.40$) can be separated from BG and NT dyes. In case of AB and MT lack of clarity of coloured spot and RB shows tailing spot.

In M_6 mobile phase TZ, CS and AB showed high mobility, their R_F values in the range of (0.75-0.93), other RB, MT and NT dyes show strong interaction with stationary phase so low R_F value detected, hence binary separations were achieved (TZ-CS-AB/RB-MT-NT) while BG dye not detected. On M_7 mobile phase binary separation occur (RB-AB-MT/TZ-BG-NT). CS showed broader and not clear spot.

With M_8 mobile phase of organic solvent, the analysis of organic dyes on Pani@SG-EB_2 stationary phase all are exhibited near the mid point of application, thus no any good separation was achieved due to broadness of spot. In case of M_9 mobile phase, the strong interaction occur between static phase and TZ, CS organic dyes. Hence, it shows high RF values near mid and above the mid point of application, hence mutual binary separation of these dyes TZ ($R_F = 0.70$), CS ($R_F = 0.50$), RB ($R_F = 0.05$), AB ($R_F = 0.01$) and NT ($R_F = 0.02$) while BG not detected.

RB and AB ($R_F = 0.82$-0.87) exhibit high mobility and tailing in MT, BZ. In mobile phase, M_9 and M_{10} consisting of TZ, CS, AB and MT showed strong interaction with silica gel static phase remained near the point of application ($R_F = 0.01$-0.09), while RB exhibit tailing spot along with BG and NT dyes showed double spot. Hence, no separation was achieved.

In case of M_{10} mobile phase system on S_3, none of the analyte move along the mobile phase all organic dyes remained near the point of application ($R_F = 0.01$-0.10). Hence, no separation was found. While in M_{11} mobile phase, mutual binary separation was achieved (TZ-CS-AB/NT) on S_3 static phase while RB showed tailed spot and MT broader spot not compact. In M_{12} mobile phase RB, MT, BG and NT ($R_F = 0.01$-0.05) at the point of application and other not detected in this case no separation occur while M13 mobile phase TZ, CS, AB, RB and MT shows broader spot near the point of application and other organic dyes BG, NT were not detected, thus no good separation could be achieved hence mobile system M_{12} and M_{13} are not suitable for separation of dyes as shown in **Table 5**.

An important quaternary and ternary separation mixture of organic dyes consisting of Tartrazine (R_F =0.95), carmoisine ($R_F = 0.70$), rose Bengal ($R_F = 0.40$), amidoblack 10B ($R_F = 0.78$), methyl thymol blue ($R_F = 0.06$), bromopyrogallol red ($R_F = 0.22$) and 4-nitrobenzene dizonium tetrafluoroborate (R_F

Table 5. Mobility of dyes in terms of R_F values on Pani@SG-EB_2 stationary phase with different mobile phases

Mobile Phase	R_F Value						
	TZ	**CS**	**RB**	**AB**	**MT**	**BG**	**NT**
M_1	0.18	0.55	0.01	0.04	0.04	ND	ND
M_2	0.01	0.01	0.40T	0.01	0.01	0.01	0.91
M_3	0.01	0.01	0.45T	0.01	0.01	0.01	0.90
M_4	0.01	0.10	0.70	0.10	0.01	0.01	0.02
M_5	0.45	0.40	0.55T	0.22	0.12	0.05	0.02
M_6	0.93	0.75	0.15	0.80	0.25	ND	0.02
M_7	0.13	0.21	0.60	0.43	0.53	0.02	0.02
M_8	0.60	0.57	0.76	0.45	0.60	0.38	0.03 or 0.76 DS
M_9	0.70	0.50	0.05	0.01	0.40T	ND	0.02
M_{10}	0.07	0.06	0.10	0.06	0.10	0.01	0.01
M_{11}	0.42	0.41	0.55T	0.34	0.20	ND	0.02
M_{12}	ND	ND	0.05	ND	0.01	0.05	0.01
M_{13}	0.26	0.25	0.25	0.17	0.10	ND	ND

TZ, CS, RB, AB, MT, BG, and NT represent tartrazine, carmoisine, rose bengal, amidoblack 10B, methyl thymol blue, bromopyrogallol red and 4-nitrobenzene dizonium tetrafluoroborate respectively. DS, ND and T stand for double spot, not detected and tailed spots respectively.

T = tailed spot, (R_L - $R_T \geq 0.3$)

= 0.05) has been achieved on S_2 with M_6 (**Figure 7**). Hence, TLC system comprising of Pani@SG-EB_1 as stationary phase and [n-butyl acetate:DDW, 5:5] (M_6) as mobile phase has been most favourable for resolution of multi-component mixtures of organic dyes (two-three or four component mixtures).

3.5 Effect of modification of silica gel with polyaniline as a stationary phase

Silica gel was replaced by modified silica gel with the hope of getting improved separations. It was observed that in case of silica gel, separations of rose Bengal, methyl thymol blue and bromopyrogallol red was not obtained with silica gel due to tailing of spots. The chromatographic performance of the stationary phases was in the order: Pani@SG-EB_1 > Pani@SG-EB_2> silica gel. Thus, better performance of Pani@SG-EB_1 as stationary phase is probably due to its large surface area as compared to other stationary phases.

3.6 Aging effect of mobile phase

Stability (aging effect) of mobile phase M_6 on the separation was insignificant since slight variation in their R_F values was observed during the separation of organic dyes from their quaternary mixture with the use of freshly prepared mobile phase and the use of the same mobile phase after storing it to 24 h. Hence, it can be concluded that the composition of eluent M_6 remains unaltered for several hours and suitable for chromatographic analysis.

3.7 Effect of foreign substances

Effect of metal cations, anions on the magnitude of separation factor (α) and resolution parameter (Rs) and ΔR_F values for separation of four-component mixtures consisting of tartrazine, carmoisine, rose bengal and methyl thymol blue has been examined and the results are presented in **Table 6**. From the results, it is clear that magnitude of these parameters is marginally influenced (increases or decreases) in the presence of these foreign substances, but separation was always possible in each case. The minor change in the value of these parameters was due to the slight increase in spot size of the analyte because of certain interactions of organic dyes with these foreign substances.

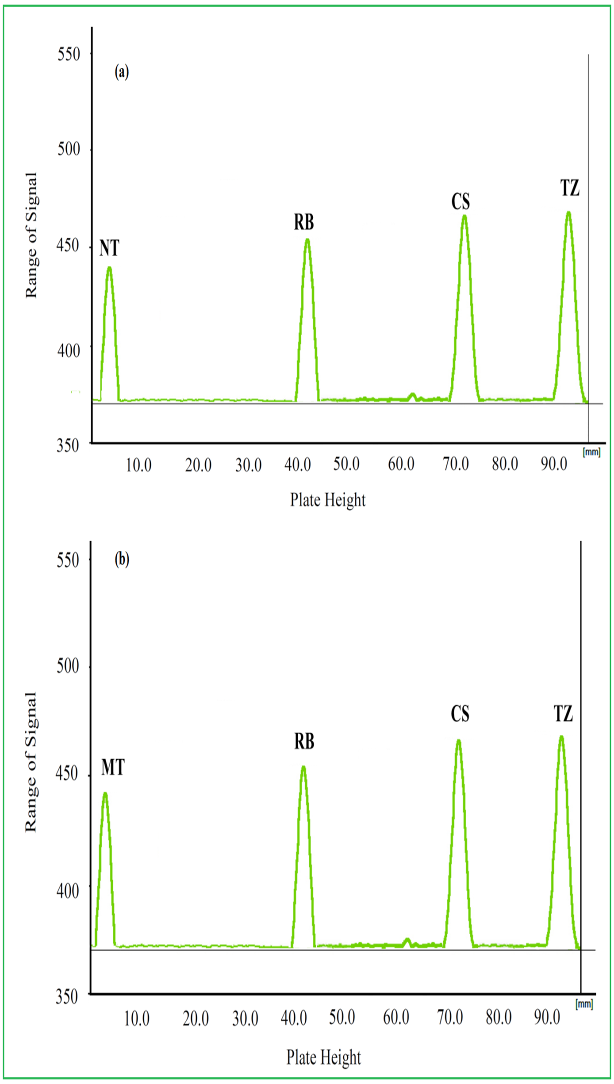

Figure7. Densitographic presentation of representative separations of four- component mixtures of dyes achieved with M_6 mobile phase on S_2 stationary phase (a) (NT-RB-CS-TZ) and (b) (MT-RB-CS-TZ).

3.8 Limits of detection

The lowest possible detectable amounts (µg spot^{-1}) of rose bengal (1.0), amidoblack 10B (1.3), carmoisine (0.75), tartrazine (1.0), methyl thymol blue (1.25), bromopyrogallol red (0.90) and 4-nitrobenzene dizonium tetrafluoroborate (1.0) showing reasonable sensitivity of the proposed method for on spot detection of these organic dyes of different concentrations on the Pani@SG-EB_1. TLC plates which were developed with the selected mobile phase M_6 and the spots were visualized. This process was repeated by successive reduction of the concentration of organic dyes until the detection of organic dyes was not possible anymore.

4. Applications

The proposed thin layer chromatographic method, comprising of polyaniline modified silica gel Pani@SG-EB$_1$ as stationary phase and aqueous M$_6$ (n-butyl acetate:DDW, 5:5) by volume as mobile phase, is applicable for the identification of carmoisine in Solvin cold DS syrup and tartrazine in Mefast^{TM} P syrup (**Table 7** and **Figures 8–9**).

5. Conclusions

It may also be concluded from this work that the use of thin layer chromatographic system consisting of polyaniline modified silica gel as stationary phase with aqueous M$_6$ (n-butyl acetate:DDW, 5:5) as eco-friendly mobile phase is most favourable for the identification and separation of four-component mixtures of rose bengal, tartrazine, carmoisine,

Table 6. Effect of interference on ΔR_F, separation (α) and resolution (R_S) factors of the separated ternary mixtures of amino acids

Foreign Substance	**Quaternary Separations**								
	Tartrazine (TZ), Carmoisine (CS), Rose bengal (RB) and Methyl thymol blue (MT)								
	TZ and CS			**CS and RB**			**RB and MT**		
	ΔR_F	**α**	**R_S**	**ΔR_F**	**α**	**R_S**	**ΔR_F**	**α**	**R_S**
	(0.23)	**(6.394)**	**(65.74)**	**(0.31)**	**(3.676)**	**(56.363)**	**(0.34)**	**(10.444)**	**(56.666)**
Cations									
Cu^{2+}	0.23	5.693	51.111	0.31	3.649	47.692	0.34	12.148	48.571
Zn^{2+}	0.22	6.091	55.00	0.32	3.858	53.333	0.34	10.444	52.307
Mn^{2+}	0.17	3.552	37.777	0.32	3.928	53.333	0.35	9.626	53.846
Ni^{+}	0.21	6.674	46.666	0.35	4.452	46.666	0.31	7.352	36.470
Li^{+}	0.21	5.793	46.666	0.32	3.891	45.714	0.35	10.886	46.666
Mg^{2+}	0.21	5.165	46.666	0.33	4.022	50.769	0.32	8.494	45.714
Anions									
CO$_3^{2-}$	0.21	5.165	46.666	0.31	3.701	47.692	0.35	10.886	46.666
NO$_3^{-}$	0.22	7.026	44.00	0.30	3.583	42.857	0.35	8.679	46.666
Br^{-}	0.21	5.793	60.00	0.30	3.583	50.00	0.34	7.639	42.50
Cl^{-}	0.18	4.428	36.00	0.34	4.317	48.571	0.34	9.232	42.50
CH$_2$COO^{-}	0.20	6.332	40.00	0.36	4.692	51.428	0.32	8.494	45.714

Table 7. Identification of carmoisine (in Solvin cold DS syrup) and tartrazine (in Mefast^{TM} P syrup) according to their R_F values

Organic Dyes	**R_F Value**	
	Standard Sample	**Drug Sample**
Carmoisine	0.70	0.67
Tartrazine	0.95	0.90

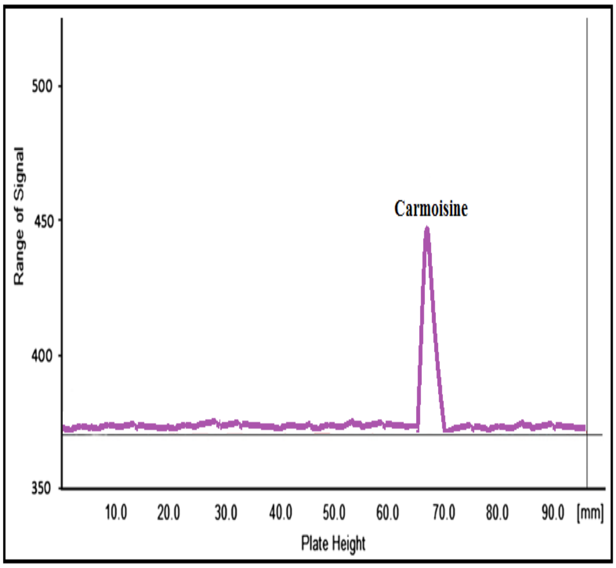

Figure 8. Densitographic illustration of identification of carmoisine in Solvin cold DS syrup on S$_2$ with M$_6$.

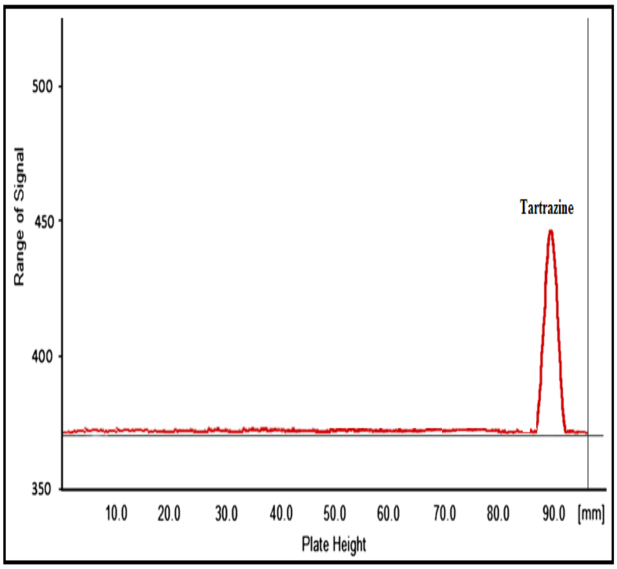

Figure 9. Densitographic illustration of identification of Tartrazine in Mefast^{TM} P syrup on S$_2$ with M$_{6}$.

methyl thymol blue or 4-nitrobenzene dizonium tetrafluoroborate. The chosen TLC system is also applicable for the identification of these food dyes in pharmaceutical products (Solvin cold DS syrup and Mefast^{TM} P syrup). The use of aqueous solutions of n-butyl acetate as green mobile phase system makes the proposed TLC system as environment friendly and paves the way for the development of green chromatography.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Aberoumand A. A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. World Journal of Dairy & Food Sciences 2011; 6: 71–78.
2. Huang D, Ou B, Prior RL. Journal of Agricultural and Food Chemistry 2005; 53: 1841–1856.
3. Hajimahmoodi M, Afsharimanesh M, Moghaddam G, *et al.* Determination of eight synthetic dyes in foodstuffs by green liquid chromatography. Food Additives & Contaminants: Part A 2013; 30: 780–785.
4. Alves SP, Brum DM, de Andrade e CM, *et al.* Determination of Synthetic Dyes in Selected Foodstuffs by High Performance Liquid Chromatography with UV-DAD Detection. Food Chemistry 2008; 107: 489–496.
5. Llamas NE, Garrido M, Di Nezio MS, *et al.* Second order advantage in the determination of amaranth, sunset yellow FCF and tartrazine by UV–vis and multivariate curve resolution-alternating least squares. Analytica Chimica Acta 2009; 655: 38–42.
6. Larsen JC. Legal and illegal colours. Trends in Food Science & Technology 2008; 19: S64–S69.
7. Nevado J, Flores JR, Llerena MJV. Adsoptive stripping voltammetry of Tartrazine at the hanging mercury drop electrode in soft drinks. Fresenius' Journal of Analytical Chemistry 1997; 357: 989–994.
8. Silva MLS, Garcia MBQ, Lima JL, *et al.* Voltammetric determination of food colorants using a polyallylamine modified tubular electrode in a multicommutated flow system. Talanta 2007; 72: 282–288.
9. Amini M, Arami M, Mahmoodi NM, *et al.* Dye removal from colored textile wastewater using acrylic grafted nanomembrane. Desalination 2011; 267: 107–113.
10. Harrelkas F, Azizi A, Yaacoubi A, *et al.* Treatment of textile dye effluents using coagulation–flocculation coupled with membrane processes or adsorption on powdered activated carbon. Desalination 2009; 235: 330–339.
11. Chen AH, Chen SM. Biosorption of azo dyes from aqueous solution by glutaraldehyde-cross-linked chitosans. Journal of Hazardous Materials 2009; 172: 1111–1121.
12. Charumathi D, Das N. Packed bed column studies for the removal of synthetic dyes from textile wastewater using immobilised dead C. tropicalis. Desalination 2012; 285: 22–30.
13. Ma M, Luo X, Chen B, *et al.* Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high-performance liquid chromatography–diode array detection–electrospray mass spectrometry. Journal of Chromatography A 2006; 1103: 170–176.
14. Vachirapatama V, Mahajaroensiri J, Visessanguan W. Identification and determination of seven synthetic dyes in foodstuffs and soft drinks on monolithic C18 column by high performance liquid chromatography. Journal of Food and Drug Analysis 2008; 16: 77–82.
15. Young ML. Rapid identification of color additives, using the C18 cartridge: Collaborative study. Journal of Association of Official Analytical Chemists 1988; 71: 458–461.
16. Steele JA. High performance thin layer chromatographic identification of synthetic food dyes in alcoholic products. Journal of Association of Official Analytical Chemists 1984; 67: 540–541.
17. Oka H, Ikai Y, Kawamura N, *et al.* Simple method for the analysis of food dyes on reversed-phase thin-layer plates. Journal of Chromatography 1987; 411: 437–444.
18. Horwitz W, Chichilo P, Reynolds H. Official Methods of Analysis of the Association of

Official Analytical Chemists. Washington, DC, USA: Association of Official Analytical Chemists; 1970.

19. Fogg AG, Summan AM. Differential-pulse polarographic monitoring of permitted synthetic food colouring matters and ascorbic acid in accelerated light degradation studies and the spectrophotometric determination of the ammonia and simpler amines formed. Analyst 1983; 108: 691–700.
20. Ni Y, Bai J, Jin L. Simultaneous adsorptive voltammetric analysis of mixed colorants by multivariate calibration approach. Analytica Chimica Acta 1996; 329: 65–72.
21. Combeau S, Chatelut M, Vittori O. Identification and simultaneous determination of Azorubin, Allura red and Ponceau 4R by differential pulse polarography: application to soft drinks. Talanta 2002; 56: 115–122.
22. Graichen C. Quantitative determination of FD&C colors in foods. Journal of Association of Official Analytical Chemists 1975; 58: 278–282.
23. Vidotti EC, Cancino JC, Oliveira CC, *et al.* Simultaneous determination of food dyes by first derivative spectrophotometry with sorption onto polyurethane foam. Analytical Sciences 2005; 21: 149–153.
24. Yang Y, Yin J, Shao B. Simultaneous determination of five aluminum lake dyes in chewing gum by HPLC with photodiode array detection. Food Additives & Contaminants: Part A 2011; 28: 1159–1167.
25. Sun S, Wang Y, Yu W, *et al.* Determination of sudan dyes in red wine and fruit juice using ionic liquid-based liquid–liquid microextraction and high-performance liquid chromatography. Journal of Separation Science 2011; 34: 1730–1737.
26. Liu R, Hei W, He P, *et al.* Simultaneous determination of fifteen illegal dyes in animal feeds and poultry products by ultra-high performance liquid chromatography tandem mass spectrometry. Journal of Chromatography B 2011; 879: 2416–2422.
27. Huang HY, Shih YC, Chen YC. Determining eight colorants in milk beverages by capillary electrophoresis. Journal of Chromatography A 2002; 959: 317–325.
28. Ishikawa R, Oishi M, Kimura K, *et al.* Determination of synthetic food dyes in food by capillary electrophoresis. Journal of the Food Hygienic Society of Japan 2004; 45: 150–155.
29. Prado MA, Boas LFV, Bronze MR, *et al.* Validation of methodology for simultaneous determination of synthetic dyes in alcoholic beverages by capillary electrophoresis. Journal of Chromatography A 2006; 1136: 231–236.
30. Chen Q, Mou S, Hou X, *et al.* Determination of eight synthetic food colorants in drinks by high-performance ion chromatography. Journal of Chromatography A 1998; 827: 73–81.
31. Tesic Z, Opsenica DM. Inorganic ion exchangers in paper and thin-layerchromatographic separations. In: Inamuddin, Luqman M (editors). chapter 15, Ion Exchange Technology II: Application, Springer Netherlands. Springer Netherlands; 2012. p. 365–389.
32. Dhote SS, Deshmukh L, Paliwal L. Heavy metal ions separation on thin layer of impregnated carbamideformaldehyde polymer. Journal of Chromatography & Separation Techniques 2012; 3(2): 1–3.
33. Soponar F, Moţ AV, Sarbu C. Quantitative determination of some food dyes using digital processing of images obtained by thin-layer chromatography. Journal of Chromatography A 2008; 1188: 295–300.
34. Mohammad A, Inamuddin, Siddiq A, *et al.* Green solvents in thin-layer chromatography. In: Mohammad A, Inamuddin (editios). Green Solvents I. Springer, Dordrecht; 2012. p. 331–361.
35. Huang S, Xiao Q, Li R, *et al.* A simple and sensitive method for L-cysteine detection based on the fluorescence intensity increment of quantum dots. Analytica Chimica Acta 2009; 645: 73–78.
36. Ge H, Wallace GG. High-performance liquid chromatography on polypyrrole-modified silica. Journal of Chromatography A 1991; 588: 25–31.
37. Behera D, Satapathy H, Bhanthia AK. Synthesis, purification and curing properties of vinyl ester network. Pigment & Resin Technology

2006; 35: 319–325.

38. Kamistos EI, Patsis AP, Kordas G. Infrared-reflectance spectra of heat-treated sol-gel-derived silica. Physical Review B 1993; 48: 12499.
39. Wood DL, Rabinovich EM. Study of alkoxide silica gels by infrared spectroscopy. Applied Spectroscopy 1989; 43: 263–267.
40. Ansari MO, Yadav SK, Cho JW, *et al.* Thermal stability in terms of DC electrical conductivity retention and the efficacy of mixing technique in the preparation of nanocomposites of graphene/polyaniline over the carbon nanotubes/polyaniline. Composites Part B: Engineering 2013; 47: 155–161.
41. Ansari MO, Mohammad F. Thermal stability of HCl-doped-polyaniline and TiO_2 nanoparticles-based nanocomposites. Journal of Applied Polymer Science 2012; 124: 4433–4442.
42. Shan Y, Gao L. Synthesis, characterization and optical properties of CdS nanoparticles confined in SBA-15. Materials Chemistry and Physics 2005; 89: 412–416.
43. Yu MR, Suyambrakasam G, Wu RJ, *et al.* Preparation of organic–inorganic (SWCNT/TWEEN–TEOS) nano hybrids and their NO gas sensing properties. Sensors and Actuators B: Chemical 2012; 161: 938–947.
44. Zheng C, Chen W, Ye X. Study on Au nanoparticles, TiO_2 nanoclusters, and SiO_2 nanoshells coated multi-wall carbon nanotubes/silica gel-glass. Optical Materials 2012; 34: 1042–1047.
45. Goren M, Qi Z, Lennox RB. Selective templated growth of polypyrrole strands on lipid tubule edges. Chemistry of Materials 2000; 12: 1222–1228.
46. Guo J, Gu H, Wei H, *et al.* Magnetite–polypyrrole metacomposites: Dielectric properties and magnetoresistance behavior. The Journal of Physical Chemistry 2013; 117: 10191–10202.
47. Wu CM, Lin SY, Chen HL. Structure of a monolithic silica aerogel prepared from a short-chain ionic liquid. Microporous and Mesoporous Materials 2012; 156: 189–195.
48. Chen M, Du C, Wang L, *et al.* Silicon/graphite/polyaniline nanocomposite with improved lithium-storage capacity and cyclability as anode materials for lithium-ion batteries. International Journal of Electrochemical Science 2012; 7: 819–829.
49. Mohammad A, Mobin R. Resolution of a three-component mixture of methyl group substituted cationic surfactants by use of a green eluent: A thin-layer chromatographic approach. Journal of Planar Chromatography – Modern TLC 2015; 28: 17–23.
50. Shimada T, Aoki K, Shinoda Y, *et al.* Functionalization on silica gel with allylsilanes: A new method of covalent attachment of organic functional groups on silica gel. Journal of the American Chemical Society 2003; 125: 4688–4689.
51. Ahmad S, Sultan A, Raza W, *et al.* Boron nitride based polyaniline nanocomposite: Preparation, property, and application. Journal of Applied Polymer Science 2016; 133.