

Retention behaviour of synthetic dyes on coconut oil impregnated egg shell layers in pure and mixed solvent systems

Aditya Kumar Misra*

Department of Chemistry, NMSN Dass (P.G.) College, Budaun 243 601, India
Email: akmisra1234@rediffmail.com

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The retention behaviour of twenty four anionic, cationic, sulphonated and amphoteric synthetic dyes has been examined on plain and coconut oil impregnated egg shell layers using pure methanol, pure acetonitrile and methanol-water, acetonitrile-water, acetonitrile-methanol and acetonitrile-acetone mixed mobile phases. The mobile phase composition and the effect of solubility of dyes on hR_f values have been discussed. The mechanism of migration is explained in terms of partition on impregnated egg shell layers and the polarity of the mobile phase used.

The acetonitrile-acetone (75:25) system was found to be most effective and gave several quaternary separations of dyes. A large number of separations of same coloured dyes as well as having approximately similar structural formula have been achieved. The separation of acidic dyes from basic dyes; acidic dyes from acidic dyes; and acidic dyes from amphoteric dyes are of special interest.

Keywords: TLC, Synthetic dyes, Impregnated layers, Coconut oil, Mixed solvent systems

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Synthetic dyes are most widely used in toys, food stuffs, medicines, cosmetics, textiles, leather colouring, some of which are prohibited in various countries and may affect the human health, so their separation and identification are of great importance. The separation of dyes is difficult due to similar structure, spot tailing and close hR_f values. To encounter this problem organic compounds and metal salts^{1,2} have been used to impregnate the layers in planar chromatography. In our laboratory, the ion exchangers^{3,4}, egg shell layers^{5,6} and mixed sorbent layers^{7,8} have been successfully utilized for the same purpose in planar chromatography. The oil soluble coal tar dyes in cosmetics have been separated by RPTLC⁹.

Many adsorbents are available^{2,10} for the reverse phase thin layer chromatography (RPTLC), each is better for some applications than the others. No perfect adsorbent has been found, some has great retentive power, whereas others hold the adsorbent loosely and the commercial layers available in the market are very expensive. The chromatographic adsorbents most often used are silica gel and cellulose. Alumina and starch are used less frequently. Although, reverse phase planar chromatography is one of the most popular analytical techniques, the

processes responsible for the solute retention are still not fully understood. The RPTLC technique is gaining interest due to the easily available non polar substances and impregnation is too easy. Sharma *et al.*^{11,12} used home made reverse phase layers for the separation of amino acids using tributyl amine, tributyl phosphate and silicon oil DC200 as impregnants. The egg shell layers have shown high potential in planar chromatography for the separation of metal chlorosulphates¹³, phenols¹⁴, dyes⁵ and metals ions⁶. The carboxylic acid herbicides have been separated on barium sulphate and calcium sulphate coatings, using coconut oil¹⁵ as impregnant in reverse phase thin layer chromatography.

This study is an attempt to achieve the analytically difficult separation of the synthetic dyes on coconut oil impregnated egg shell layers by RPTLC. On the basis of R_f values, several binary, ternary and quaternary separations of dyes have been achieved. The mechanism of migration is explained in terms of partition.

Experimental Procedure

Reagents and Chemicals

Coconut oil (Tata Chemicals), methanol, acetone, and acetonitrile were obtained from E.Merck (India)

and chicken egg shells were collected from different natural sources. The dyes studied were from BDH (Poole) and Merck (Germany).

Test solution and detection

The solutions of synthetic dyes (10^{-3} M) were prepared in ethanol-water (4:1) (v/v). Dyes are self visualized compounds. The dyes studied were: Crystal violet (CrV), Gentian violet (GnV), Hematoxylin (Hm), Bismark brown (Bb), Lacmoid (La), Resorcinol blue, (Rb), Fuschin basic (Fb), Amiodoswartz (As), Curcumin S (CrS), Eriochrom black T (EBT), Bromophenol blue (BrB), Brilliant green (BrG), Safranin (S), Orange G (OrG), PanS (PS), Methyl Red (MR), Naphthol green B (NgB), Eosin (E), Bromothymol Blue (BtB), Indigocarmine (IC), Rhodamine B (Rh), Methyl violet (MV), Alizarin Red S (ArS) and Sudan Black (SB).

Preparation of chicken egg-shell powder

Broken pieces of egg shell were first washed with boiling water, then distilled water and then soaked in 10% NaOH solution for 20-30 h to remove the protein content. The cleaned egg shell pieces were then washed several times with distilled water to ensure complete removal of NaOH, dried at 100°C, ground and sieved to furnish 150-250 mesh powder. The resulting egg shell powder was used to prepare slurry for coating onto TLC plates without addition of binder.

Preparation of TLC plates

The 15 g sorbent and 2 mL coconut oil were mixed with demineralised water (30 mL) with constant shaking to furnish an homogenous slurry. This slurry was applied to 3.0×15 cm glass plates as 0.25 mm layer by means of Toshniwal (India) TLC applicator. The plates were dried at room temperature and then activated at $80 \pm 2^\circ\text{C}$ by heating in an electrically controlled oven for ca. $\frac{1}{2}$ h. The activated plates were stored in a closed chamber at room temperature until used.

Method

The test solutions (500 ng) were applied to TLC plates ca 1.5cm above the layer edge by means of a micropipette. The spots were air dried and plates were developed with suitable mobile phases by ascending technique in 5×20 cm glass jars presaturated with the mobile phase. The solvent ascent was fixed to 10 cm above the point of application. After development, the

plates were removed from the jars, dried at room temperature (25°C). The spots were self visualized.

Solvent systems used

The following solvent systems were used:

- | | |
|----------------------------------|----------------------------------|
| 1.Methanol | 2.Methanol water (80:20) |
| 3.Methanol water (60:40) | 4.Methanol water (40:60) |
| 5.Methanol water (20:80) | 6.Acetonitrile |
| 7.Acetonitrile water (80:20) | 8.Acetonitrile water (60:40) |
| 9.Acetonitrile methanol (25:75) | 10.Acetonitrile methanol (50:50) |
| 11.Acetonitrile methanol (75:25) | 12.Acetonitrile acetone (25:75) |
| 13.Acetonitrile acetone (50:50) | 14.Acetonitrile acetone (75:25) |

Results and Discussion

Studies to develop new sorbent phases using unconventional materials revealed the possibility of using chicken egg shell powder as an effective sorbent layer for the separation of transitional metals, rare earths¹², dyes⁵, phenols and amines¹³. The results obtained were promising, despite the limitation that egg shell powder is unstable under acidic medium. The spots at more than 3 cm have been treated as tailed spots. At least 1.5 cm distance between the R_L of one spot and R_T of the other spot has been used to resolve the dye. R_T and R_L are the rear and front limits of the spot of dye respectively.

$$R_f = \frac{(R_L + R_T)/2}{10} \quad \text{and} \quad hR_f = R_f \times 100$$

The composition of the egg shell layer may slightly vary from place to place due to the different nutritive environment of the hens. Therefore, the egg shells have been collected from Biyala, Kolkata; Thane, Maharashtra; Mansarovar, Jaipur, Rajasthan; Nainital, Kumaun, Uttarakhand and Gwalior, Datia and Bhopal districts of Madhya Pradesh and the retention behaviour has been tested for some cationic and amphoteric dyes. The R_f values were found to vary not more than $\pm 2\%$. Therefore, further studies have been carried out by using these layers.

Table 1 gives an account of the R_f values of different types of dyes in fourteen pure and mixed solvent systems containing methanol, acetonitrile and acetone while the binary, ternary and quaternary separations achieved have been given in Table 2.

Table 1 — hR_f values of synthetic dyes on egg shell layer impregnated with coconut oil in pure and mixed solvent systems

Code*	Solvent systems													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
CrV	97	34 ^T	39	11	00	89	92	19	97	98	98	82	84	76
GnV	98	73 ^T	28 ^T	11	00	90	97	60	98	42	97	92	95	83
Hm	48 ^T	27 ^T	28 ^T	12	06	00	10	08	14	31 ^T	00	10	08	13
Bb	50 ^S	21 ^T	26 ^T	00	00	08	97	12	95	97	96	50 ^S	90	22 ^T
La	15 ^T	22 ^T	08	00	00	05	95	65	88	80	90	49	05	10
Rb	03	00	05	00	00	00	00	00	00	00	00	00	05	00
FB	98	84	55	23	00	52 ^T	85	48	98	98	96	43	95	63
AS	00	00	04	00	00	00	18	09	00	00	00	00	00	00
CrS	00	00	07	05	00	00	11	35 ^T	05	03	00	00	00	00
EBT	16 ^T	12	21 ^T	00	00	00	05	05	11	07	00	06	07	00
BrB	06/32	05	12	05	05	00	18	24	08	10	05/81	23	00	20
BrG	59 ^T	09	19 ^T	05	00	96	77	61	95	97	98	86	95	93
S	95	29 ^T	63	00	00	91	98	33	97	98	98	72	98	99
OrG	29	61	23 ^T	04	00	10	71 ^T	48	90	93	58	20 ^T	59	17 ^T
PS	72	21	10	00	00	98	75	12	89	89	87	83	81	89
MR	69	37/77	17	00	19	15	89	40 ^T	42	91	95	95	72	99
NgB	08	24 ^T	14	15 ^T	00	00	19 ^T	06	23 ^T	16 ^T	19 ^T	00	13	00
E	04	30 ^T	18 ^T	00	00	00	80	13	34	86/05	05/97	90	04/57	30
BtB	35 ^T	41 ^T	43 ^T	10	20	00	20 ^T	65 ^T	13	12	06	00	06	00
IC	09	13	22 ^T	05	00	00	14	00	05	05	00	00	00	00
Rh	97	94	43 ^T	24	00	00	70	57	96	95	96	90	45	67
MV	90	28 ^T	59	10	05	95	60	27	90	97	97	90	97	96
ArS	00	19	00	00	00	00	00	00	00	00	00	00	00	00
SB	06	12	00	00	00	00	00	00	07	05	05	89	00	75

A – Pure methanol, B – Methanol:Water (80:20), C – Methanol:Water (60:40), D – Methanol:Water (40:60), E – Methanol:Water (20:80), F – Pure Acetonitrile, G – Acetonitrile:Water (80:20), H – Acetonitrile:Water (60:40), I – Acetonitrile:Methanol (25:75), J – Acetonitrile:Methanol (50:50), K – Acetonitrile:Methanol (75:25), L – Acetonitrile:Acetone (25:75), M – Acetonitrile:Acetone (50:50), N – Acetonitrile:Acetone (75:25), * – As per experimental, T – Tailing, S – Streak

Table 2 — Binary, ternary and quaternary separations of synthetic dyes achieved on egg shell layer impregnated with coconut oil in pure and mixed solvent systems

	Separations	Solvent systems
I	Mutual separation of anionic dyes	
1	NgB/E/IC ----- MR	Methanol
2	BrB/IC ----- MR	Acetonitrile-Water (80:20)
3	NgB/E ----- OrG	Acetonitrile-Water (60:40)
4	IC/BrB/BtB ----- OrG	Acetonitrile-Methanol (50:50)
5	BrB/NgB/BtB/IC ----- E	Acetonitrile-Acetone (25:75)
6	NgB/IC/BtB/BrB ----- E ----- MR	Acetonitrile-Acetone (75:25)
II	Mutual separations of cationic dyes	
1	AS/EBT/ArS/SB ----- FB/Rh/OrG	Acetonitrile-Methanol (50:50)
2	Hm/Rb ----- MV/La/FB	Acetonitrile-Methanol (75:25)
3	Hm/La/Rb/AS/CrS/ArS/SB ----- Rh ----- BrB/FB ----- MB	Acetonitrile-Methanol (75:25)
III	Mutual separations of amphoteric dyes	
1	Bb ----- CrV/BrG/S/MV	Acetonitrile
2	CrV ----- BrG	Acetonitrile-Water (60:40)
IV	Separations of anionic dyes from cationic dyes	
1	NgB/E/IC ----- FB/Rh	Methanol
2	BrB ----- FB	Methanol-Water (60:40)
3	MR ----- Rb/FB/AS/EBT/Rh/ArS/SB	Methanol-Water (20:80)
4	BrB/BtB/IC ----- Bb/La/FB/BrG/S/OrG/Rh	Acetonitrile-Methanol (50:50)
5	BrB/NgB/BtB/IC ----- FB/BrG/S/Rh/SB	Acetonitrile-Acetone (25:75)

Dyes code used as per experiment.

A close examination of the data (Table 1) reveals that the dyes show compact spots and highest movement in pure methanol (PI-6.6) and generally the slight decrease in R_f values of dyes takes place in pure acetonitrile (PI-6.2). As the polarity index of the acetonitrile (6.2), methanol (6.6) and acetone (5.4) are very close and dyes have different solubilities in coconut oil, acetonitrile, methanol and acetone, therefore, an inspection of the solvent systems used, led to the following conclusion:

Methanol – Water system

CrV, GnV, FB, S, MV, Rh, PS and ArS are highly soluble or readily soluble in methanol and show high R_f values. Only ArS shows the differential behaviour and does not move while Bb, Hm, La, BrB, BrG and MR are less soluble and show movement. Tailing has been seen in some cases.

However, the addition of water causes tailing in some more cases due to high solubility of these dyes in water. Further addition of water causes compact spots and sharp decrease in R_f values. RB, CrS and SB are either insoluble or very slightly soluble in methanol as well as in water and show no movement in general. As the percentage of water increases further, the R_f values decrease continuously and most of the dyes show no movement in methanol-water (20:80) system, as the hydrophobic character is strong and the repulsive forces are higher than the capillary forces, the transport of elements with higher water content is not possible in layer.

Acetonitrile – Water system

CrV, GnV, FB, BrG, S, PS and MV are highly soluble in acetonitrile and show high R_f values, whereas other dyes were either insoluble or form insoluble complexes. The addition of water into acetonitrile further increases the R_f values of the dyes in general, as the majority of dyes are readily soluble in acetonitrile-water (80:20) mixture with the exception of BrG, PS and MV. However, R_f values of dyes were less than the R_f values in methanol system. Increasing the percentage of water in acetonitrile more than twenty percent, the R_f values show further decrease to a large extent due to the higher repulsive forces than the capillary forces. Since, some dyes are highly soluble in water, hence in presence of low percentage of water, show some movement.

Acetonitrile-Methanol system

The acetonitrile and methanol have a little difference in their polarity index, the addition of

acetonitrile in methanol gave similar R_f values as in the case of methanol. The spots were too compact. The methanol-acetonitrile (1:3) solvent system is useful to achieve the clean and faster separations of dyes. CrV, GnB, FB, BrG, S, PS and methyl violet are highly soluble in acetonitrile and methanol both and show increase in R_f values when acetonitrile is added to methanol. However, Hm, Bb and MR show decrease in movement. It is interesting that La, OrG and E which have small R_f values in both pure solvents have high R_f values in the mixed solvent systems. Conclusively, increase in the percentage of acetonitrile in methanol show a general trend of increase of R_f values of the majority of dyes. In pure acetonitrile, some dyes *viz.*, crystal violet, brilliant green, safranin, pan S etc show highest movement. However, in acetonitrile-methanol (1:1) system, dyes show maximum variation in R_f values i.e. optimization.

Acetonitrile-Acetone system

Addition of acetonitrile in acetone increases the R_f value as the polarity index of the acetone (PI-5.4) is less than the polarity index of the acetonitrile, however, the maximum R_f value has been found in acetonitrile-acetone (1:1) solvent system. CrV, E, GnV, BrG, MR, MV, S and SB are very soluble in acetone¹⁶ and gave high R_f values. Spots were very compact.

The sulphonated dyes move independently on non-polar matrix, it may be due to the differential solubility of dyes in coconut oil and solvent systems and cause fluctuated R_f values.

On the basis of R_f values, a large number of difficult binary, ternary and quaternary separation of dyes have actually been obtained and omitted for the sake of brevity. Some separations of special interest have been shown are represented in Table 2.

Above results indicate that polarity index of the solvent systems and the solubility of the dyes play an important role in the migration process of dyes. The adsorbent is a waste material and coconut oil is easily available and inexpensive to develop the suitable non-polar surface having proper hydrophobic residue and surface density suitable for dyes' separation.

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