



Scholars Research Library

Archives of Applied Science Research, 2010, 2 (1) 165-175
(<http://scholarsresearchlibrary.com/archive.html>)



ISSN 0975-508X
CODEN (USA) AASRC9

Resolution of Quaternary Mixture of Ionic Dyes on Biphasic Alumina-Keiselguhr Thin Layer Plate: Synergistic Effect of Cationic-Nonionic Surfactants

Ali Mohammad* and Nazrul Haq

Department of Applied Chemistry, Aligarh Muslim University, Aligarh

Abstract

A new green thin layer chromatographic system comprising of biphasic alumina-keiselguhr (1:1) as stationary phase and equal volume mixture of 1% (w/v) aqueous solution of cationic surfactant (cetylpyridinium bromide) and nonionic surfactant (Brij-35) in 1:1 ratio as mobile phase has been proposed for the analysis of ionic (anionic and cationic) dyes. From the point of view of resolution of dyes, mixed alumina-keiselguhr has been found more effective than its individual components. In the same way, synergism between cationic and nonionic surfactant proves to be more efficient for the separation of dyes from their quaternary mixture than the individual surfactants. Effect of presence of heavy metal cations as impurities in the sample on the separation was examined. The limits of detection of bromocresol green, malachite green, rhodamine B and congo red were 0.27, 0.19, 0.21 and 0.23 μg respectively. Chromatographic parameters like ΔR_F , separation factor (α) and resolution (R_S) were calculated for the separation of bromocresol green- malachite green- rhodamine B- congo red. The proposed method is simple, rapid and free from the use of volatile organic solvents.

Key words: Thin layer chromatography, surfactant, green eluents, separation, dyes.

Introduction

Identification of a commercial dyestuff as a known dye or mixture of dyes is important for users of dyestuffs, public analysts and chemical examiners in customs laboratories. Because of the very large number of dyestuffs marketed under a much larger number of names, the problems of identification and analysis have become exceedingly complex.

Thin layer chromatography (TLC) has rapidly grown in its status in recent years and has emerged out as an indispensable analytical technique for rapid and efficient analysis of a variety of substances in complex matrices including agrochemicals [1,2], amino acids [3-5] and

synthetic dyes [6-8]. Amongst the methods available for the analysis of synthetic dyes, TLC (both normal and reversed phase) is the most useful technique due to (i) low cost, (ii) minimal sample clean up, (iii) wider choice of mobile phases, (iv) flexibility in sample detection, (v) high sample loading and (vi) easy handling.

Most of the reported TLC methods for the analysis of dyes involve the use of mixed organic solvent systems containing methanol, acetone, ethanol, butanol, ethyl acetate and hexane as one of the components whereas little work has been reported on the use of micellar mobile phases [9-11]. Surfactants modified TLC is a promising method and has found wider applications [12-19]. An interesting study on the use of aqueous micellar solutions as mobile phase systems in RP-TLC of fluorescein dyes was reported by Shtykov *et al.* [11]. Their study reveals the following facts.

- (a) Micellar mobile phases (MMP) based on ionic (cationic and anionic) surfactants are suitable for the separation of dyes.
- (b) Compared to use of conventional organic aqueous mobile phase, separation with MMP is quicker and more convenient.
- (c) Micellar mobile phases comprising of cetyltrimethylammonium bromide (cationic surfactant) and sodiumdodecyl sulphate (anionic surfactant) are very useful in promoting spot compactness and mobility of dyes enabling better separation of pairs of dyes.

In most commercial applications, mixtures of surfactants, rather than individual surfactants, are used. In some other cases, different types of surfactants are purposely mixed to improve the properties of the final product. Although the existence of synergistic relations between certain types of surfactants has been known and utilized for many years, the investigation of synergism in TLC is a recent development based upon a simple, convenient method for measuring molecular interactions between surfactants. All the mixtures of surfactants show more attraction (or less repulsion) after mixing. To our surprise, in spite of several advantageous features such as non-toxicity, non volatility, non-inflammability, non-aggressiveness, high-biodegradability and cost effectiveness, full potential of mixed surfactant system as an eluent for TLC analysis of dyes has not been exploited.

The aim of this study was to develop new environmental friendly TLC systems comprising of surfactants as green eluents for studying the mobility pattern of dyes and selection of the best possible combination of stationary and mobile phases for the resolution of dyes from their binary, ternary and quaternary mixtures. Surfactants solutions at concentration level above their critical micellar value (CMC) were deliberately used because above CMC value, surfactants form micelles which selectively solubilize hydrophobic and hydrophilic compounds.

Materials and Methods

Experimental

All experiments were performed at $30 \pm 1^\circ\text{C}$.

2.1 Apparatus:

A thin layer chromatographic applicator (Toshniwal, India) was used for coating layer materials (silica gel, alumina and keiselguhr and their binary mixtures) onto 20 cm x 3 cm glass plates. The chromatography was performed in 24 cm x 6 cm glass jars. Tripette (Werthlin, Germany) was used for spotting of analyte.

2.2 Materials and Methods:

Anionic dyes (Xylenol orange, D1; bromocresol green, D2; congo red, D3; alizarin red S, D4; pyrocatechol violet, D5), and cationic dyes (malachite green, D6; methylene blue, D7; brilliant green, D8; rhodamine B, D9; crystal violet, D10) were purchased from CDH, India. Silica gel 'G', alumina (neutral), keiselguhr 'G', sodium deoxycholate (NaDC), Brij-35, Brij-78, cetylpyridinium bromide (CPB), cetylpyridinium chloride (CPC), cetyltrimethyl ammonium bromide (CTAB), acetone, butanol, and formic acid were from Merck, India.

2.3 Test solutions:

All dyes except xylenol orange 0.2% (w/v) were used as 0.1% (w/v) solutions in water plus methanol (1:1).

For metal cations (Zn^{2+} , Cd^{2+} , Ni^{2+} , Co^{2+} , Fe^{2+}) – 1% (w/v) solutions of their chloride, nitrate, or sulphate salts were used.

2.4 Stationary phases:

Code	Composition
S1	Silica gel 'G'
S2	Alumina neutral 'G'
S3	Keiselguhr 'G'
S4	Alumina + Keiselguhr (1:1)

2.5 Mobile phases:

Code	Composition
M1	1% (w/v) Aqueous CPB
M2	1% (w/v) Aqueous NaDC
M3	1% (w/v) Aqueous Brij-35
M4	M1 + M3 (1:1)
M5	1% (w/v) Aqueous CPC + M3 (1:1)
M6	1% (w/v) Aqueous CTAB + M3 (1:1)
M7	1% (w/v) Aqueous CPB + Brij-78 (1:1)
M8	M4 + acetone (1:1)
M9	M4 + formic acid (1:1)
M10	M4 + butanol (1:1)

2.6 Preparation of TLC Plates:

The desired stationary phase (20 g) was homogenized with 60ml double distilled water by constant shaking for 5 min. and the resulting slurry was coated immediately onto 20 cm x 3 cm glass plates as 0.25 mm thick layer by means of a TLC coater (Toshniwal, India). The plates

were dried at room temperature. After drying, the plates were activated by heating at $100 \pm 1^\circ\text{C}$ for 1h in an electrically controlled oven. After activation, the plates were cooled to room temperature and then stored in a closed chamber (30°C) before use.

2.7 Procedure:

Thin layer chromatography was performed on plates of silica gel, alumina, keiselguhr and their mixtures in different ratios in glass jars. Test solutions ($0.050 \mu\text{l}$) were applied by means of a tripette (Werthlin, Germany) 2 cm above the lower edge of the plates. The spots were dried at room temperature (30°C). Chromatography was performed in 24 cm x 6 cm glass jars with lids. Chambers were presaturated with mobile phase vapour for 10 min. before introducing the plates for development. The plates were developed in the chosen solvent system by the ascending technique. The solvent ascent was fixed 10 cm from the point of application in all cases. After, development, the plates were withdrawn from glass jars and dried at room temperature, dyes were detected visually according to their original colour. The retention sequences of dyes by different stationary phases under different experimental conditions were measured in terms of retardation factor (i.e. R_F value). For determining R_F value, the R_L (R_F of leading front) and R_T (R_F of trailing front) values of each spot were determined and overall R_F value was calculated as:

$$R_F = (R_L + R_T) \times 0.5$$

2.8 Separation:

For the mutual separation, equal volumes (1 ml each) of dyes to be separated were mixed and $0.05 \mu\text{l}$ of the resultant mixture was loaded onto the selected stationary phase. The plates were developed in aqueous solution of surfactants as mobile phase (M1-M4), the spots were self detected due their inherent colour and the R_F values of the separated dyes were determined.

2.9 Effect of metal cations:

Dyes being amphipathic molecules, their chromatographic behaviour is supposed to change in the presence of metal cations. Therefore, it was felt worthwhile to observe the effect of heavy metal cations (Zn^{2+} , Cd^{2+} , Ni^{2+} , Co^{2+} , Fe^{2+}) as an additive on the separation of quaternary mixtures of dyes. For investigating this, an aliquot ($0.05 \mu\text{L}$) of additives (1% aqueous solution) was spotted along with the mixture ($0.050 \mu\text{L}$) of dyes and chromatography was performed as described earlier. The spots were self detected and the R_F values of separated dyes were determined.

3.0 Chromatographic Parameters:

The stability of the colour intensity of dye spots on the chromatogram was observed visually. The developed chromatoplates were protected from light and the spot intensities were compared visually after every 12 h for a period of 100 h.

To investigate the stability (ageing effect) of mixture of dyes under study, the mixture (binary, ternary and quaternary) was chromatographed as described earlier after every 24 h for the duration of 10 days under similar experimental conditions. The R_F values of dyes so obtained after every 24 h interval were compared with the values obtained from the freshly prepared mixture of dyes.

The reproducibility of R_F values was checked by determining the R_F of the same sample by three different analysts and by the same analyst on different days under identical experimental conditions, in the same laboratory, using same apparatus.

3.1 Effect of replacement of CPB with other cationic surfactants:

In order to examine whether the charge of the surfactant or any specific moiety of surfactant is playing role in the separation of dyes, CPB in M4 was replaced by CPC and CTAB and the resultant mobile phase (M5 and M6 respectively) were used to chromatograph using S4 as stationary phase.

3.2 Effect of replacement of Brij-35 with other anionic surfactant:

To study the role of carbon chain length of the nonionic surfactant in the separation process, Brij-35 was substituted by higher member of the same series i.e. Brij- 78 (M7) in the mobile phase and chromatography of the mixture of dyes was performed as described above.

3.3 Role of organic additives in the mobile phase:

Dyes, being organic molecules, their chromatographic behavior are supposed to change in the presence of organic solvents in the mobile phase. Therefore, to ascertain this, organic solvents like acetone, formic acid and butanol was added in the mobile phase M4 in equal proportion and the resultant solutions M8, M9 and M10 respectively were used as eluent to study their effect on the separation of quaternary mixture of dyes.

3.4 Limit of Detection:

The limits of detection of bromocresol green, malachite green. Rhodamine B and congo red was determined by spotting different amounts of these dyes on S4 TLC plates, developing the plates with mobile phase (M5) and detecting the corresponding spots visually. The method was repeated with successively decreasing amounts of bromocresol green, malachite green, rhodamine B and congo red until no spot was detected. The minimum detectable amounts of these dyes were taken as limits of detection.

Results and Discussion

The results of this study have been summarized in Tables 1-4. The interesting features of this study are:

- i) Exploitation of the chromatographic property of mixture of two different adsorbents.
- ii) Enhanced separation efficiency of the eluent due to the synergistic effect of the mixture of surfactants.
- iii) Absence of corrosive, volatile and toxic organic chemicals in mobile phase thus making the TLC study 'green'.
- iv) Mutual separation of dyes from their multicomponent mixtures including the quaternary separation of bromocresol green-malachite green- rhodamine B-congo red.
- v) Study of various chromatographic parameters.
- vi) Application of the proposed chromatographic system.

In order to examine the mobility sequence of dyes on adsorbents of different/varying nature, dyes were chromatographed on plain silica gel 'G' (S1), plain alumina 'G'(S2), and plain keiselguhr 'G' (S3) using 1% (w/v) aqueous solutions of cationic (CPB, M1), anionic (NaDC, M2) and nonionic (Brij-35, M3) surfactants as eluents. Results obtained, shown in Table 1, were as follows:

1) **Silica gel (S1):** When silica gel was employed to play the role of stationary phase, except bromocresol green, all dyes are strongly adsorbed and remained at the point of application irrespective of the charge of the eluent (M1-M3). Bromocresol green showed reasonable mobility with M2 ($R_F = 0.51$) and M3 ($R_F = 0.43$). Hence, for further studies silica gel was not used except for some separations.

2) **Alumina (S2):** On alumina, all anionic dyes except bromocresol green remained near the point of application. Bromocresol green produced tailed spot with M1 ($R_F = 0.64T$) and compact spots with M2 ($R_F = 0.69$) and M3 ($R_F = 0.83$) showing reasonable mobility and hence it can be separated selectively from other anionic dyes using M2 or M3 as eluent. As far as cationic dyes are concerned, all showed R_F in the range 0.30-0.63 with M1 as eluent. Except rhodamine B, all other cationic dyes produced tailed spot either with M3 or with both M2 and M3.

3) **Keiselguhr (S3):** On S3-M1 TLC system, all dyes were showing differential migration behaviour in which xylenol orange, bromocresol green and pyrocathocol violet achieved maximum mobility whereas malachite green, brilliant green and rhodamine B showed intermediate mobility and the congo red and alizarin red S remained at the point of application. However, methylene blue and crystal violet exhibits tailing. With M2 as the mobile phase, all anionic dyes and one cationic dye (rhodamine B) moved significantly. Malachite green, brilliant green and crystal violet produced tailed spot while methylene blue moved slightly. With M3 rhodamine B ($R_F = 0.53$) can be easily separated from xylenol orange or bromocresol green ($R_F = 0.95$) as well as from all other dyes ($R_F \sim 0.07-0.29$) listed in Table I.

Table I: R_F values of dyes on different stationary phases (S1-S3) with different mobile phases(M1-M3)

Dyes	R_F								
	Silica gel (S1)			Alumina (S2)			Keiselguhr (S3)		
	M1	M2	M3	M1	M2	M3	M1	M2	M3
D1	0.02	0.23T	0.25T	0.03	0.02	0.04	0.93	0.96	0.95
D2	0.03	0.52	0.43	0.64T	0.69	0.83	0.93	0.97	0.95
D3	0.05	0.20T	0.08	0.05	0.06	0.04	0.07	0.77	0.12
D4	0.04	0.05	0.05	0.04	0.03	0.03	0.10	0.87	0.29
D5	0.07	0.06	0.08	0.06	0.07	0.05	0.86	0.88	0.13
D6	0.03	0.04	0.05	0.63	0.36T	0.27T	0.61	0.24T	0.12
D7	0.03	0.07	0.06	0.42	0.29T	0.15T	0.36T	0.12	0.10
D8	0.06	0.04	0.08	0.45	0.80	0.25T	0.51	0.39T	0.11
D9	0.08	0.06	0.04	0.46	0.81	0.78	0.56	0.83	0.53
D10	0.04	0.03	0.06	0.30T	0.30T	0.37T	0.29T	0.27T	0.07

On the basis of the above results, it is clear that the preferred order of stationary phases from separation point of view of dyes is keiselguhr (S3) > alumina (S2) > silica gel (S1). For achieving maximum chromatographic performance, mixture of alumina and keiselguhr in various ratios as stationary phase were used for separation purpose using mixed surfactants as eluents. Thus, alumina was homogenized with keiselguhr in varying proportions (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) and the resultant slurry was used as stationary phase. Similarly, aqueous solutions (1% w/v) containing CPB: Brij-35 and NaDC: Brij-35 in varying ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) were used to examine the mobility trend of all dyes. From this study, alumina:keiselguhr (1:1, S4) and CPB: Brij-35 (1:1, S5) was selected as the best possible combination of stationary phase and mobile phase for the resolution of quaternary mixture of dyes into its individual components. When mixture containing bromocresol green-malachite green-rhodamine B-congo red was chromatographed with this most favourable TLC system, all four components migrated differentially resulting in their separation. (Fig.I)

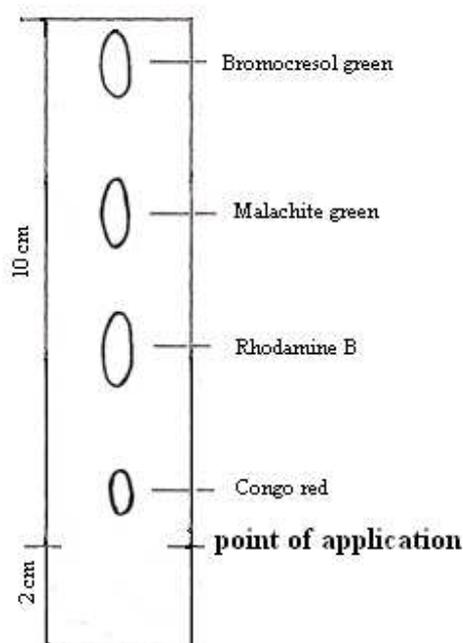


Fig. I. Separation of dyes from their mixture (bromocresol green-malachite green-rhodamine B-congo red) on biphasic alumina-keiselguhr (1:1) as stationary phase and equal volume mixture of 1% (w/v) aqueous solution of cetylpyridinium bromide and Brij-35 in 1:1 ratio as mobile phase.

Various chromatographic parameters for this separation, such as ΔR_F , α (separation factor) and R_S (resolution) were calculated using the following equations:

$$\Delta R_F (\text{bromocresol green} - \text{malachite green}) = R_F (\text{bromocresol green}) - R_F (\text{malachite green})$$

$$\Delta R_F (\text{malachite green} - \text{rhodamine B}) = R_F (\text{malachite green}) - R_F (\text{rhodamine B})$$

$$\Delta R_F (\text{rhodamine B} - \text{congo red S}) = R_F (\text{rhodamine B}) - R_F (\text{congo red S})$$

$$\alpha = K_{(\text{bromocresol green})}/K_{(\text{malachite green})}$$

where $K_{(\text{bromocresol green})} = (1-R_{F(\text{bromocresol green})})/R_{F(\text{bromocresol green})}$

and

$$K_{(\text{malachite green})} = (1-R_{F(\text{malachite green})})/R_{F(\text{malachite green})}$$

$$\alpha = K_{(\text{malachite green})}/K_{(\text{rhodamine B})}$$

where $K_{(\text{rhodamine B})} = (1-R_{F(\text{rhodamine B})})/R_{F(\text{rhodamine B})}$

$$\alpha = K_{(\text{rhodamine B})}/K_{(\text{congo red S})}$$

where $K_{(\text{congo red S})} = (1-R_{F(\text{congo red S})})/R_{F(\text{congo red S})}$

$$R_s = X/0.5 (d_{(1)} + d_{(2)})$$

where $X = \Delta R_F \times 1$, 1 is the migration distance of mobile phase $d_{(1)}$ and $d_{(2)}$ were the spot diameters of two consecutive spots.

Table II list the various parameters calculated for this separation and from the table, it is clear that satisfactory separation has been achieved when compared with the standard values.

Table II: Values of different chromatographic parameters calculated for three pairs of the quaternary separation (Data taken from Fig. I)

Dyes	ΔR_F	α	R_s
Bromocresol green – malachite green	0.19	6	2.1
Malachite green – rhodamine B	0.25	3	1.9
Rhodamine B – congo red S	0.31	4	1.8

In addition to the quaternary separation, important separations of binary and ternary mixtures of cationic and anionic dyes have been achieved using different combinations of stationary phases (S1-S3) and mobile phases (M1-M3). Some of such separations are listed in Table II and III.

Stability is an important and essential aspect of validation in thin layer chromatographic analysis. Highly sensitive samples should not decompose during development of the chromatogram and should be stable in-solution and on the adsorbent. The intensity of spots on the chromatogram should be constant at least for 1 h. In our case, no change in the intensities of the spots was noticed during the storage of developed plates, protected from light for periods upto of 72 h, indicating good stability of the mixture.

Because no difference in the R_F values of dyes was observed when their R_F values obtained from the freshly prepared solutions were compared with those obtained on consecutive thirty days, it was concluded that the mixture of dyes were sufficiently stable.

Another important property of the method is its reproducibility, defined as the precision under different conditions, for example when the method was performed by different analysts and by the same analyst on different days. The variation in R_F values of individual dyes measured by three different analysts and by the same analyst on three different days did not differ by more than $\pm 5\%$ from the average R_F value indicating a good reproducibility.

When CPB was replaced with CPC, no change in the mobility pattern of four dyes in their mixture was observed but in the case of CTAB, mobility of malachite green and rhodamine B increased resulting in the loss of quaternary separation/. This indicated that pyridinium group was playing a major role in separation of dyes.

The substitution of Brij-35 by Brij-78 does not bring change in the migration behaviour of these dyes. It indicates that the effect of length of hydrophobic chain of nonionic surfactant is ineffective.

As a result of enhanced solubility, organic additives increased the mobility of all dyes. This factor reduces the magnitude of differential migration of dyes and thus the separation possibilities are reduced.

Anionic dyes bromocresol green and congo red possibly formed complexes with all the metal cations, as a result of which their R_F values decreased while R_F values of cationic dyes remained unchanged.(Table IV)

Table IV: R_F values of dyes in the presence of metal ions as impurities

Dyes	R_F Values			
	Bromocresol green	Malachite green	Rhodamine B	Congo red
Zn ²⁺	0.69	0.74	0.49	0.16
Cd ²⁺	0.47	0.71	0.54	0.06
Ni ²⁺	0.50	0.73	0.48	0.13
Co ²⁺	0.63	0.76	0.52	0.11
Fe ²⁺	0.36T	0.79	0.53	0.10

The limits of detection of bromocresol green, malachite green, rhodamine B and congo red were 0.27, 0.19, 0.21 and 0.23 μg respectively.

Table III: Resolution of two component mixtures of dyes into individual components.

S.P	M.P	Binary Separations Achieved
S1	M2	Selective separation of bromocresol green from all other dyes except xylenol orange and congo red
	M3	Bromocresol green – congo red
S2	M1	Bromocresol green – xylenol orange, malachite green - brilliant green, Separation of malachite green or methylene blue or brilliant green or rhodamine B from mixture of xylenol orange + congo red + alizarin red S + pyrocatechol violet
	M2	Brilliant green - methylene blue, rhodamine B - crystal violet.
	M3	Rhodamine B - methylene blue, rhodamine B - malachite green, rhodamine B - brilliant green
S3	M1	Selective separation of xylenol orange from all cationic other dyes, xylenol orange – congo red, xylenol orange – alizarin red S, congo red - pyrocatechol violet, congo red - brilliant green, congo red - rhodamine B, congo red - malachite green
	M2	Congo red - crystal violet, congo red - methylene blue, alizarin red S - crystal violet, alizarin red S - methylene blue, alizarin red S - malachite green, pyrocatechol violet - crystal violet, pyrocatechol violet - methylene blue, pyrocatechol violet - malachite green, malachite green - rhodamine B, methylene blue - rhodamine B
	M3	Alizarin red S - rhodamine B, pyrocatechol violet - rhodamine B, brilliant green - rhodamine B, rhodamine B- crystal violet.

Table IV: Resolution of three component mixtures of dyes into individual components.

S.P	M.P	Ternary Separations Achieved
S2	M1	Mixture of (malachite green + methylene blue) or (malachite green + brilliant green) separated from xylenol orange, congo red, alizarin red S or pyrocatechol violet
S3	M1	Xylenol orange - malachite green - - alizarin red S, xylenol orange - malachite green - congo red, bromocresol green - malachite green - - alizarin red S, bromocresol green - malachite green - congo red, pyrocatechol violet - malachite green - congo red, pyrocatechol violet - malachite green - alizarin red S
	M2	Xylenol orange - congo red - methylene blue, xylenol orange - congo red- crystal violet , bromocresol green - congo red- methylene blue
	M3	Xylenol orange - alizarin red S - crystal violet, bromocresol green - alizarin red S - crystal violet, xylenol orange - rhodamine B - congo red, xylenol orange - rhodamine B - alizarin red S, xylenol orange - rhodamine B - pyrocatechol violet , xylenol orange - rhodamine B - malachite green, xylenol orange - rhodamine B - methylene blue , xylenol orange - rhodamine B - brilliant green, xylenol orange - rhodamine B - crystal violet, xylenol orange - alizarin red S - methylene blue, bromocresol green - alizarin red S - methylene blue.

Conclusion

Thin layer chromatographic system comprising of alumina:keiselguhr (5:5) as stationary phase and mixture of 1% aqueous solutions of CPB and Brij-35 in 1:1ratio as mobile phase is most suitable for the resolution of quaternary mixture of cationic and anionic dyes. Furthermore, to make the process environmentally benign green eluent like aqueous solution of surfactants has been suggested for use in separation science.

References

- [1] J. Sherma, *J. Planar Chromatogr. –Mod., TLC*, **1994**, 7, 126.
- [2] H. S. Rathore, T. Begum, *J. Chromatogr.*, **1993**, 643, 321.
- [3] V. D. Krasikov, I. I. Malakhova, E. V. Degterev, B.V. Tyaglov , *J. Planar Chromatogr. Mod., TLC*, **2004**, 9, 113.
- [4] R. Bhusan, M. Arora, *Biomed. Chromatogr.*, **2001**, 15, 433.
- [5] S.D. Sharma, H. Sharma, and S.C. Sharma, *Chem. Environ. Res.*, **2002**, 11, 179.
- [6] V.G. Berezkin, E.V. Kormishkina, *J. Planar Chromatogr. –Mod. TLC*, **19**, (2006), 81.
- [7] M. Lederer, H. K. H. Nguyen, *J. Chromatogr. A*, **1996**, 723(2), 405.
- [8] T. N. Konstantinova, R. A. Lazarova, P. P. Milandinova, A. Y. Venkova, *J. Planar Chromatogr. Mod., TLC*, **2004**, 17(6), 444.
- [9] D. W. Armstrong, K. H. Bui, *J. Liq. Chromatogr.*, **1982**, 5(6), 1043.
- [10] D. W. Armstrong, G. Y. Stine, *Anal. Chem.*, **1983**, 55(14), 2317.
- [11] S. N. Shtykov, E.G. Sumina, E. V. Smushkina, N. V. Tyurina, *J. Planar Chromatogr. – Mod., TLC*, **1999**, 12(2), 129.
- [12] D.W. Armstrong, *J. Liq. Chromatogr.*, **1980**, 3, 895.
- [13] D.W. Armstrong, K.H. Bui, *J. Liq. Chromatogr.*, **1982**, 5, 1043.
- [14] A. Mohammad, S. Hena, S.A. Bhawani, *Indian J. Chem.*, **2006**, 45A, 1663.
- [15] D.W. Armstrong, G.Y. Stine, *Anal. Chem.*, **1983**, 55, 2317.
- [16] R.W. Williams, Z. Fu, W.L. Hinze, *J. Chromatographic Sci.*, **1990**, 28, 292.
- [17] M.G. Khaledi, *Trends Anal. Chem.*, **1988**, 7, 393.
- [18] A. Mohammad, V. Agrawal, *Sepn. Sci. Technol.*, **2004**, 35, 363.
- [19] A. Mohammad, V. Agrawal, S. Hena, *Adsorption Sci. Technol.*, **2004**, 22, 89.