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Thin-layer chromatography of aromatic amines with hybrid CTAB-alcohol-water mobile phase: separation of indole from diphenylamine and *p*-dimethylaminobenzaldehyde

Ali Mohammad^a, Vineeta Agrawal^a

^a Analytical Research Laboratory, Faculty of Engineering, Aligarh Muslim University, Aligarh, India

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**THIN-LAYER CHROMATOGRAPHY OF
AROMATIC AMINES WITH HYBRID
CTAB-ALCOHOL-WATER MOBILE
PHASE: SEPARATION OF INDOLE FROM
DIPHENYLAMINE AND
p-DIMETHYLAМИНОБЕНЗАЛЬДЕХИД**

Ali Mohammad* and Vineeta Agrawal

Analytical Research Laboratory, Department of Applied
Chemistry, Faculty of Engineering, Aligarh Muslim
University, Aligarh 202002, India

ABSTRACT

Cationic and non-ionic surfactant-mediated systems have been used as mobile phases in thin-layer chromatographic separation of aromatic amines on silica gel layers. The effect of surfactant concentration below and above its critical micellar concentration on mobility of amines was examined. The influence of organic and inorganic additives such as alcohols, urea, NaCl and NaBr in micellar solutions on the mobility and separation efficiency of amines was also assessed. A mobile phase composed of 99.5:0.5 v/v water and (CTAB + methanol, 1:2 w/v) was identified as the best solvent system for rapid separation of indole from *p*-DAB and DPA in the presence of cations and phenols. The semiquantitative determination of *p*-anisidine by

*Corresponding author. E-mail: Mohammadali4u@rediff.com

spot-area measurement method was also attempted. The lower limits of detection of amines have been determined.

INTRODUCTION

The analysis of nitrogen-containing compounds has assumed importance because of their carcinogenic and pesticidal properties. As a result, several chromatographic techniques (1–6) have been used for their separation and identification at microgram level. Though gas chromatography with packed columns (specific for nitrogen compounds) has been used extensively for the more accurate quantitative analysis of complex mixtures of amines, thin layer chromatography (TLC) being inexpensive is more suitable for routine qualitative analysis of organic as well as inorganic substances. TLC separation of amines via charge transfer complexation with polynitro compounds has been achieved (7–9) with mixed organic mobile phases. Amines being n-donor (non-bonded electron donor), contribute electrons to the partially filled orbital of nitro groups resulting in the formation of charge-transfer complexes. The major drawback of this method is that the weak physical bond formed between amines and aromatic nitro compounds during charge transfer complexation is readily ruptured by polar solvents and adsorption forces. Alternatively, silica gel layers impregnated with various metallic salts have been tested for realizing mutual separations of aromatic amine isomers (10–12) using mixed organic eluants. The practical utility of metallic salt impregnated layers as stationary phase is restricted due to (i) formation of occasional tailed spots, (ii) lesser stability in acidic solvent systems, and (iii) tendency to absorb moisture.

On the other hand, TLC methods involving the use of mixed organic solvent systems containing benzene, carbon tetrachloride, dioxane or hexane (13,14) are not useful for routine analysis because of their toxic effect. It is, therefore, important to develop an inexpensive and reliable TLC system for rapid analysis of amines using relatively non-toxic mobile phases. Considering the advantageous features of surfactant-mediated mobile phases such as non-toxicity, unique separation selectivity, non-flammability and enhanced detection capability, an extensive investigation has been initiated in our laboratory to utilize the analytical potential of surfactant containing mobile phases with added small quantities of organic additives for rapid TLC separation of complex mixtures.

Despite evident advantages, surfactant mediated systems have not been fully utilized in TLC separation of organic compounds. The present communication describes a systematic approach for achieving improved



separations of aromatic amines using a cationic cetyl trimethyl ammonium bromide (CTAB) surfactant containing mobile phases.

EXPERIMENTAL SECTION

Apparatus

A TLC apparatus (Toshniwal, Mumbai, India), 20×3 cm² glass plates, and 24×6 cm² glass jars were used.

Chemicals and Reagents

Silica gel 'G', *n*-butanol [Qualigens, Mumbai, India], *N*-cetyl-*N,N,N*-trimethyl ammonium bromide (CTAB), amines, inorganic salts, methanol, ethanol, and 2-propanol [CDH, Mumbai, India], Brij-35, and Triton X-100 [Loba Chemie, Mumbai, India] were used. All other reagents were analytical reagent grade.

Amines Studied

Diphenylamine (DPA), *o*-chloroaniline (*o*-CAL), *m*-chloroaniline (*m*-CAL), *p*-chloroaniline (*p*-CAL), α -naphthylamine (α -NPA), *p*-bromoaniline (*p*-BAL), *p*-anisidine (*p*-ASD), *p*-phenylenediamine (*p*-PND), aniline (AL), *N,N*-dimethylaniline (DMAL), *o*-toluidine (*o*-TLD), *m*-toluidine (*m*-TLD), *p*-toluidine (*p*-TLD), *o*-nitroaniline (*o*-NAL), *m*-nitroaniline (*m*-NAL), *p*-nitroaniline (*p*-NAL), indole (ID), *p*-dimethyl aminobenzaldehyde (*p*-DAB), and DL-tryptophan (DL-TTP).

Test Solutions

The test solutions (1% w/v) of all amines were prepared in methanol.

Detection

All amines were detected by exposing the TLC plates to iodine vapors. DL-tryptophan was detected with 0.5% (w/v) ninhydrin solution prepared in acetone.



Mobile Phases

The following solvent systems were used as mobile phases:

Symbol	Composition
M ₁	H ₂ O
M ₂	0.16% (w/v) CTAB in water
M ₃	0.32% methanol in water
M ₄	0.08 mL (CTAB + methanol)* + 99.92 mL water
M ₅	0.5 mL (CTAB + methanol) + 99.5 mL water
M ₆	1.0 mL (CTAB + methanol) + 99.0 mL water
M ₇	1.5 mL (CTAB + methanol) + 98.5 mL water
M ₈	2.0 mL (CTAB + methanol) + 98.0 mL water
M ₉	3.0 mL (CTAB + methanol) + 97.0 mL water
M ₁₀	0.5 mL (CTAB + ethanol) + 99.5 mL water
M ₁₁	1.0 mL (CTAB + ethanol) + 99.0 mL water
M ₁₂	1.5 mL (CTAB + ethanol) + 98.5 mL water
M ₁₃	2.0 mL (CTAB + ethanol) + 98.0 mL water
M ₁₄	0.5 mL (CTAB + propanol) + 99.5 mL water
M ₁₅	1.0 mL (CTAB + propanol) + 99.0 mL water
M ₁₆	1.5 mL (CTAB + propanol) + 98.5 mL water
M ₁₇	2.0 mL (CTAB + propanol) + 98.0 mL water
M ₁₈	0.5 mL (CTAB + butanol) + 99.5 mL water
M ₁₉	1.0 mL (CTAB + butanol) + 99.0 mL water
M ₂₀	1.5 mL (CTAB + butanol) + 98.5 mL water
M ₂₁	2.0 mL (CTAB + butanol) + 98.0 mL water
M ₂₂	0.5 mL (Triton X-100 + methanol) + 99.5 mL water
M ₂₃	0.5 mL (Brij-35 + methanol) + 99.5 mL water
M ₂₄	0.5 mL (CTAB + methanol) + 99.5 mL aq. NaBr (0.01–0.2 M)
M ₂₅	0.5 mL (CTAB + methanol) + 99.5 mL aq. NaCl (0.01–0.2 M)
M ₂₆	0.5 mL (CTAB + methanol) + 99.5 mL aq. urea (0.01–0.2 M)

* CTAB + organic solvent taken as 1:2, w/v in all cases and required volume of water or salt sol was added into the mixture of CTAB + organic solvent.

Preparation of Thin Layer Chromatography Plates

The TLC plates were prepared by mixing silica gel G with demineralized water in 1:3 ratio by weight with constant shaking to obtain a homogeneous slurry. The resultant slurry was applied on the glass plates with the help of an applicator to give a 0.25 mm-thick layer. The plates were first dried at room



temperature and then activated at $100 \pm 5^\circ\text{C}$ by heating in an electrically controlled oven for 1 hr. The activated plates were stored in a close chamber at room temperature until used.

Procedure

About 10 μL of test solution was spotted on a thin layer plate with the help of micropipette. The plates were developed in the chosen solvent system by the ascending technique. The solvent ascent was fixed to 10 cm in all cases. After development, the plates were withdrawn from glass jars and dried at room temperature. The TLC plates were then exposed to iodine vapors for about 10 min. The amines were detected as yellowish brown spot. The R_L (R_F of leading front) and R_T (R_F of trailing front) values for each spot were determined and the R_F value was calculated

$$R_F = \frac{R_L + R_T}{2}$$

The R_F values reported in this manuscript were calculated for compact spots where the difference in R_L and R_T values is less than 0.3.

The limit of detection of various amines was determined by spotting different amounts of amines on the TLC plates, developing the plates and detecting the spot. The method was repeated, with successive lowering of the amount of amines, until no spot was detected. The lowest amount of amines detected on the TLC plates was taken as the limit of detection.

To study the effect of the presence of heavy metal cations and phenols on the separations of indole from *p*-DAB, a synthetic mixture was prepared by adding 0.5 mL of heavy metal or phenol solution (1.0% w/v) into 0.05 mL mixture of *p*-DAB and indole (1:1 v/v). About 0.01 mL of the resultant mixture was spotted on TLC plate and the spot was dried at 30°C . The plates were developed, the spots were detected and the R_F values of the separated amines were determined from their R_L and R_T values.

For semiquantitative determination by spot-area measurement method, 0.01 mL from a series of standard solution of *p*-anisidine (0.5–2%) were spotted on silica layers. The plates were developed with M_5 . After detection the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated.

RESULTS AND DISCUSSION

The mobile phases were prepared by the addition of water (different volumes) into 3 mL (approximately) of alcoholic CTAB obtained by dissolving



1 g CTAB in 2 mL alcohol. Compared to pure water (M_1), higher mobility of most of the amines was observed with aqueous CTAB (M_2), water+MeOH mixture, 99.68:0.32 v/L (M_3) and methanolic CTAB+water mixture (M_5) mobile phases (Table 1). The trends of mobility of *o*-, *m*-, and *p*-chloroanilines in M_1 and M_3 are in the order of *o*-CAL > *m*-CAL > *p*-CAL and *m*-CAL > *p*-CAL > *o*-CAL respectively. This behavior is possibly due to the coordination between methylene oxygen and amino hydrogen causing the association between the two and increasing the bulk around the substituents. Though such association is also possible in case of *p*- and *m*-CAL but no corresponding steric phenomena arise in their cases. Thus, the steric factor in the case of *o*-CAL is responsible for reversing the order of its mobility when M_1 is substituted by M_3 . The higher mobility of *m*-CAL, *p*-CAL and ID in M_3 compared with their corresponding mobilities in M_1 may be due to increased solubilizing tendency of methanol compared to water. The R_F values (i.e., mobility) of amines differ marginally

Table 1. Mobility (i.e., R_F) of Amines in the Presence and Absence of Surfactant in the Mobile Phase

Amines	R_F values			
	M_1	M_2	M_3	M_5
DPA	0.44	0.55	0.61	0.59
<i>o</i> -CAL	0.82	0.62	0.67	0.73
<i>m</i> -CAL	0.75	0.77	0.90	0.85
<i>p</i> -CAL	0.67	0.68	0.84	0.73
α -NPA	0.67	0.70	0.80	0.70
<i>p</i> -BAL	0.75	0.72	0.87	0.80
<i>p</i> -ASD	0.77	0.70	0.75	0.78
<i>p</i> -PND	0.70	0.74	0.75	0.70
AL	0.77	0.77	0.82	0.82
DMAL	0.03	0.03	0.07	0.08
<i>o</i> -TLD	0.80	0.75	0.75	0.63
<i>m</i> -TLD	0.85	0.80	0.72	0.67
<i>p</i> -TLD	0.72	0.75	0.75	0.67
<i>o</i> -NAL	0.65	0.75	0.67	0.81
<i>m</i> -NAL	0.70	0.86	0.78	0.78
<i>p</i> -NAL	0.82	0.85	0.85	0.80
ID	0.67	0.78	0.80	0.90
<i>p</i> -DAB	0.49	0.67	0.58	0.59
DL-TTP	0.88	0.92	0.81	0.89



when M_3 (alcoholic water without surfactant) is replaced by M_2 (aqueous CTAB) or M_5 (CTAB in alcoholic water) and the development time increases from 13 (M_1) to 17 min (M_5). The higher mobility of ID ($R_F = 0.9$) compared to its mobility in M_1 – M_3 facilitates a good separation of ID from *p*-DAB and DPA (Table 1). This separation is important from the analytical point of view as *p*-DAB forms colored products with both ID and DPA in solution (15). On the other hand these amines react with 2,4-dinitrotoluene (DNT) in the solid state to produce yellow products (16) and hence the presence of *p*-DAB or DPA hampers the detection of ID with DNT.

The data presented in Table 2 demonstrate how the mobility of amines is controlled by the composition of a three-component (i.e., water–MeOH–CTAB) mobile phase. The concentration of each component, i.e., MeOH, CTAB or water was simultaneously varied in mobile phases (M_4 – M_9) keeping total volume constant (i.e., 100 mL). Depending upon the composition of the mobile phase, the development time for 10 cm ascent was 15–35 min. It is clear from Table 2 that a

Table 2. Mobility of Amines on Silica Layer Developed with CTAB–MeOH–H₂O Mobile Phase

Amines	R_F values					
	M_4	M_5	M_6	M_7	M_8	M_9
DPA	0.55	0.59	0.57	0.60	0.59	0.56
<i>o</i> -CAL	0.70	0.73	0.65	0.75	0.76	0.80
<i>m</i> -CAL	0.85	0.85	0.77	0.77	0.75	0.74
<i>p</i> -CAL	0.74	0.73	0.66	0.77	0.73	0.77
α -NPA	0.74	0.70	0.73	0.80	0.75	0.75
<i>p</i> -BAL	0.78	0.80	0.72	0.75	0.82	0.82
<i>p</i> -ASD	0.75	0.78	0.69	0.75	0.75	0.82
<i>p</i> -PND	0.70	0.70	0.77	0.80	0.74	0.75
AL	0.75	0.82	0.65	0.72	0.70	0.80
DMAL	0.10	0.08	0.04	0.05	0.05	0.07
<i>o</i> -TLD	0.70	0.63	0.81	0.77	0.78	0.76
<i>m</i> -TLD	0.67	0.67	0.75	0.72	0.77	0.70
<i>p</i> -TLD	0.68	0.67	0.70	0.77	0.74	0.79
<i>o</i> -NAL	0.80	0.81	0.68	0.78	0.78	0.78
<i>m</i> -NAL	0.60	0.78	0.78	0.79	0.76	0.75
<i>p</i> -NAL	0.75	0.80	0.78	0.80	0.75	0.75
ID	0.75	0.90	0.78	0.80	0.65	0.76
<i>p</i> -DAB	0.59	0.59	0.55	0.54	0.48	0.48
DL-TTP	0.87	0.89	0.90	0.89	0.88	0.85



good separation of indole from *p*-DAB and DPA can be achieved with M_5 containing 0.16% CTAB. At other CTAB concentration levels, i.e., very low (M_4) or higher (M_8 – M_9) relatively poor separation of indole from *p*-DAB and DPA is possible. As the reported critical micellar concentration (CMC) value of CTAB (17) in water is 0.46%. It is therefore concluded that hybrid mobile phases composed of a cationic surfactant (CTAB), methanol and water are most suitable for separation of aromatic amines if the CTAB concentration in the mobile phase is kept below or just above to its CMC value. The separation possibility of ID from *p*-DAB and DPA is increased as a result of some specific interactions of cationic CTAB monomers with π electrons of aromatic rings and the non-bonding (i.e., lone pair) electrons residing on nitrogen atom of indole, *p*-DAB and DPA. As evident from Table 2 the separation of indole from *p*-DAB or DPA is also possible at CTAB concentration higher than its CMC value (M_9). In such case, the separation is again controlled by the interactions of n or π electrons with ionic head group of CTAB micelles.

Taking into consideration of better separation possibilities, spot compactness and detection clarity of amines on silica gel layer, M_5 containing 0.16% CTAB was selected as mobile phase for detailed investigations. At this concentration level, CTAB is present as cationic monomer.

In order to demonstrate the effect of type of alcohol on the mobility (R_F values) of amines, results obtained with M_5 , M_{10} , M_{14} and M_{18} mobile phases which are composed of CTAB– H_2O –alcohol (methanol, ethanol, propanol, or butanol) were plotted as R_F Vs amines in Fig.1. From this figure following trends are noticeable.

- (a) Mobility of amines is influenced by the nature of the alcohol used. Generally, most amines move faster in MeOH-containing mobile phases. Conversely, mobility of amines was lower in ethanol containing mobile phases.
- (b) Compared to the mobility in MeOH and ethanol containing mobile phases, amines show intermediate mobility in mobile phases containing propanol or butanol.
- (c) *N,N*-dimethyl aniline is more strongly retained by stationary phase ($R_F \approx 0.08$) irrespective of the type of alcohol used in mobile phase and hence it can be selectively separated from all other amines studied. Conversely, tryptophan shows almost no interaction with stationary phase and migrates with mobile phase giving R_F of about 0.9. Thus, tryptophan can easily be separated from amines having lower R_F values.
- (d) Indole ($R_F = 0.9$) can be very well separated from most of amines in MeOH containing mobile phase.

To understand the migration trend, amines were chromatographed on silica layer using mobile phases containing different concentrations of ethanol (M_{11} – M_{13}), propanol (M_{15} – M_{17}) and butanol (M_{19} – M_{21}). The magnitude of R_F value



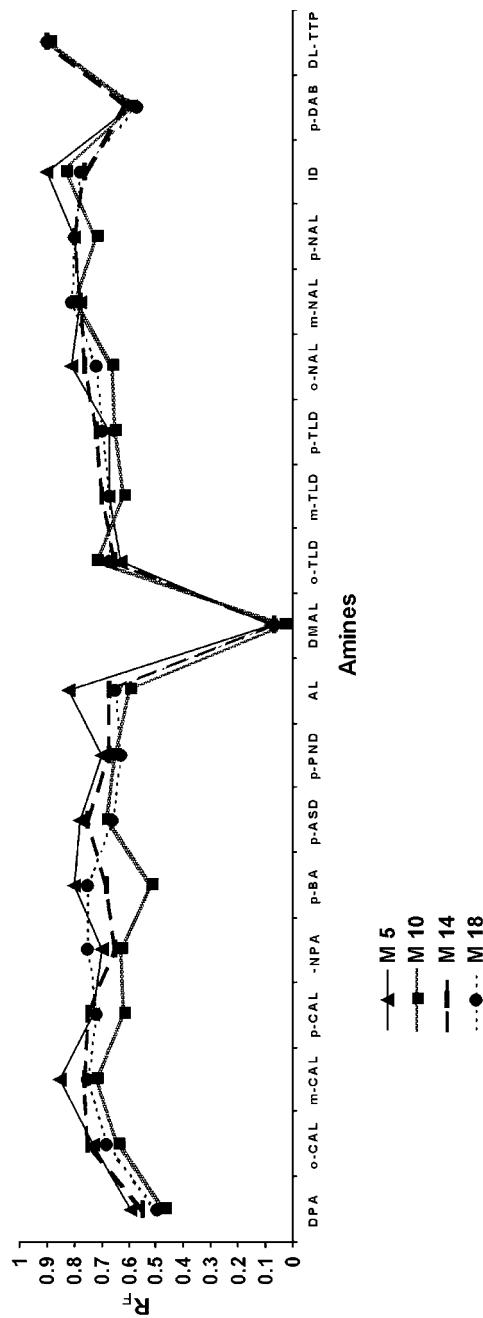


Figure 1. Effect of type of alcohol in the mobile phase on the mobility of amines.



Table 3. Mobility of Amines as a Function of Their Basicity

Mobile Phase	R _F Values					
	AL	DMAL	<i>o</i> -CAL	<i>o</i> -NAL	<i>m</i> -CAL	<i>m</i> -NAL
M ₆	0.65	0.04	0.67	0.68	0.77	0.78
M ₇	0.72	0.05	0.75	0.78	0.77	0.79
M ₈	0.70	0.05	0.76	0.78	0.75	0.76
M ₁₀	0.60	0.03	0.64	0.66	0.72	0.80
M ₁₁	0.77	0.08	0.77	0.80	0.78	0.79
M ₁₂	0.78	0.07	0.81	0.82	0.79	0.79
M ₁₃	0.78	0.05	0.80	0.80	0.82	0.85
M ₁₄	0.67	0.07	0.75	0.76	0.76	0.78
M ₁₅	0.75	0.05	0.76	0.78	0.77	0.80
M ₁₆	0.73	0.12	0.74	0.76	0.75	0.79
M ₁₇	0.65	0.10	0.68	0.72	0.75	0.81
M ₁₈	0.70	0.07	0.70	0.79	0.70	0.75
M ₂₀	0.75	0.05	0.76	0.78	0.80	0.75
M ₂₁	0.75	0.07	0.76	0.82	0.77	0.78

Basicity ($\text{kb} \times 10^{10}$ value) of amines given in parenthesis are: AL (4.2), DMAL (11.7), *o*-CAL (0.05), *o*-NAL (6 \times 10⁻⁴), *m*-CAL (0.3), *m*-NAL (0.03), *p*-CAL (1.0) and *p*-NAL (10⁻³). Data from Ref. (8).



of individual amine shows a variation of ± 0.16 on simultaneous increase in the concentration of alcohol and CTAB in the mobile phases (M_{11} – M_{13} , M_{15} – M_{17} , and M_{19} – M_{21}) irrespective of the type of alcohol (ethanol, propanol or butanol) used. For example, the difference of 0.24 in R_F values was noticed in the case of *p*-DAB on using M_{12} or M_{13} mobile phase instead of M_{10} . Similarly, *p*-bromoaniline shows a difference of 0.35 if TLC plate is developed with M_{12} instead of M_{10} .

In order to examine the effect of nonionic surfactant on the mobility of amines, CTAB in M_5 was substituted with Triton X-100 or Brij-35. The mobility of almost all amines lowered marginally in mobile phases containing Triton X-100 or Brij-35 compared to their mobility in CTAB. The lowering in the mobility of amines with non-ionic surfactants exerts an adverse effect on the separation of amines. Our observation is in consonance with earlier findings where non-ionic surfactants have been found unsuitable for TLC separation of fluorescein (18), pesticides (19) and metal diketonates (20) because of their poor eluant strength.

In consonance with earlier studies on TLC of amines with non-surfactant mediated mobile phases (8), the R_F values of certain amines in the present case were also found to decrease with the increase in their basicity. From the data summarized in Table 3 the increasing order in R_F values of amines, e.g., $AL < o\text{-}CAL < o\text{-}NAL$; $AL < m\text{-}CAL < m\text{-}NAL$; $AL < p\text{-}CAL < p\text{-}NAL$; $o\text{-}CAL < m\text{-}CAL$; $o\text{-}NAL < m\text{-}NAL$; $o\text{-}NAL < p\text{-}NAL$, and $DMAL < AL$ was just opposite to the basicity order of amines (21), DMAL being a stronger base than aniline, and substituted aniline has the minimum mobility.

The results presented in Fig. 2 (Plot of R_F Vs amines) show that the mobility of amines is significantly influenced by the presence of electrolyte (NaCl or NaBr) and non-electrolyte (urea) in the hybrid (CTAB–H₂O–MeOH) mobile phase. Generally the presence of salt or urea in the mobile phase (M_{24} – M_{26}) leads to reduction of mobility of amines as a result of their enhanced affinity to stationary phase. However, the decrease in R_F value is more pronounced in the case of urea compared to NaCl or NaBr. Several factors such as greater affinity of silanols for CTA⁺ than for Na⁺ (22), capability of urea to decrease hydrophobicity effect (23), decrease in CMC value, and increase in micelle size (24) on the addition of these additives are responsible for the alteration of the retention pattern of amines on silica layer. The same behavior is seen in pure aqueous mobile phase, 5–95% v/v methanol–water phases, and 0.1 mol/L NaCl phases on both stationary phases. Minor changes in mobility of individual amines were noticed on substitution of NaCl by NaBr in the mobile phase. This effect may be attributed to common ion effect.

An attempt has also been made for the semiquantitative determination of *p*-anisidine by spot area measurement method. For this purpose, 0.01 mL of standard solutions of *p*-anisidine (0.05–2%) were spotted on silica thin layer. The plates were developed and anisidine spot was detected on TLC plate. The spots



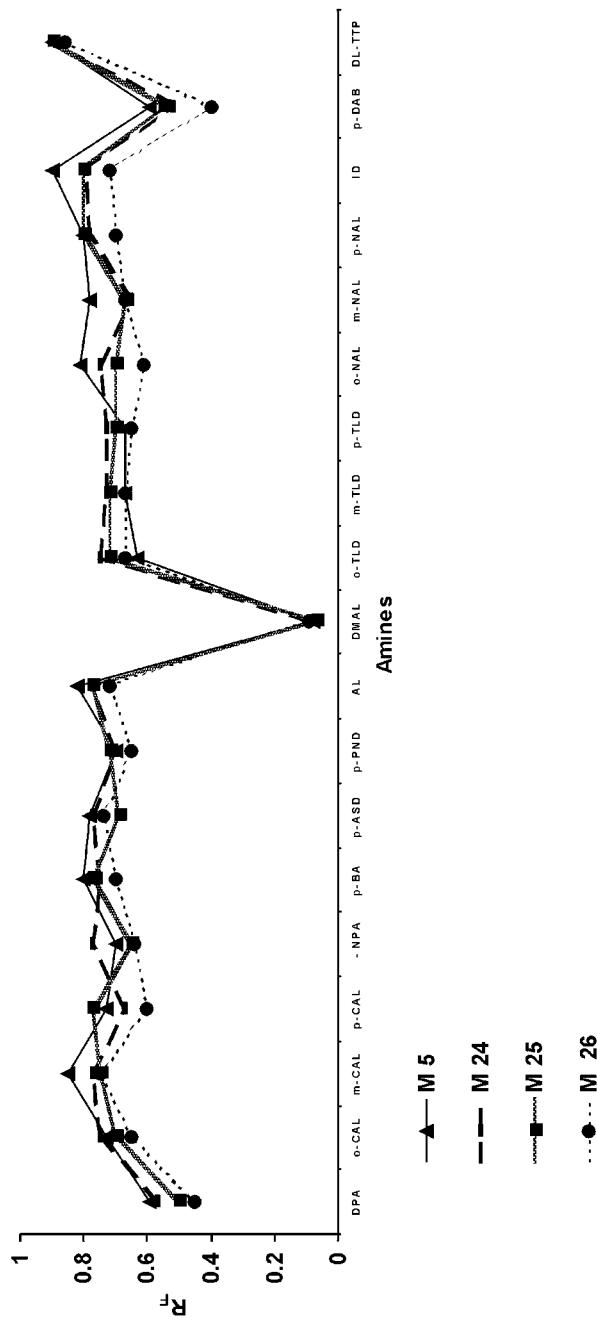


Figure 2. Effect of presence of electrolyte (NaCl and NaBr) on non-electrolyte (urea) in the mobile phase on the mobility of amines.



obtained were copied directly on tracing paper from the chromatoplates and the spot area was measured. A relationship between the spot area and microgram quantities of *p*-anisidine follows the empirical equation, $A^3 = km$ where A is the spot area, m is the spotted amount and k is a constant. The linearity is maintained up to 200 $\mu\text{g}/\text{spot}$ of *p*-anisidine. At higher concentration, a negative deviation from linear law was noticed. The precision and accuracy was $\pm 15\%$.

The lowest possible amounts of amines in micrograms (given in parentheses) along with dilution limit detectable on silica gel layer were *p*-DAB (0.05, 1: 2×10^5), ID (0.14, 1: 7.1×10^4), *o*-CAL (0.10, 1:1 $\times 10^5$), *m*-CAL (0.08, 1:1.2 $\times 10^5$), α -NPA (0.11, 1:9.09 $\times 10^4$) and *p*-TLD (0.07, 1:1.4 $\times 10^5$). The present method is superior for sensitive detection of *p*-DAB and indole to the reported method (25).

The effect of some heavy metal cation and phenol additives ($\approx 20 \mu\text{g}$) on the separation efficacy of indole from *p*-DAB was examined. Heavy metal cations do not cause any deleterious effect on the separation of indole from *p*-DAB. In fact, an improved separation of indole from *p*-DAB was realized in the presence of Cd^{2+} . Interestingly, the separation of indole from *p*-DAB is not hampered by the presence of resorcinol, orcinol or phloroglucinol ($R_F \approx 0.98$) despite the fact that these phenols move with the solvent front ahead of indole during the development of TLC plates. It is an important observation in the light of the fact that these phenols interfere in the detection of indole with most of chromogenic reagents in solution phase (26).

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