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SIMULTANEOUS SEPARATION OF NITROANILINE ISOMERS WITH A WATER-IN-OIL MICROEMULSION

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SUMMARY

Thin-layer chromatography of nineteen amines has been performed on silica gel layers with a water-in-oil microemulsion (sodium dodecyl sulphate + water + heptane + *n*-pentanol, 8.0 g + 8.0 mL + 160.0 mL + 25.0 mL) as mobile phase. The effect of the basicity of the amines on their R_F values was examined. The proposed thin layer chromatographic method was used to separate isomers of nitroaniline from their mixture.

INTRODUCTION

Analysis of nitrogen-containing compounds is important because of their industrial, pharmaceutical and pesticidal properties. As a result several chromatographic techniques [1–10] have been used for their separation and identification at microgram levels. Although gas chromatography with packed columns (specific for nitrogen compounds) has been extensively used for the most accurate quantitative analysis of complex mixtures of amines, thin-layer chromatography (TLC) is more suitable for routine qualitative analysis of organic and inorganic substances because of its rapidity, reasonable sensitivity, and capability of handling a large number of samples simultaneously. TLC separation of amines by charge-transfer complexation with polynitro compounds has been achieved by several workers [11–14]. In addition to plain silica gel, silica gel impregnated with aqueous solutions of a variety of sodium salts [15], ammonium cerium(IV) nitrate [16], or copper sulphate [17], bonded or reversed phase silica gel [18–20], mixed silica phases [21, 22], and silica gel treated with cationic surfactant [23] in combination with mixed organic or aqueous–organic mobile phases have been used to achieve separations of aromatic amines. In most of this

work toxic organic solvents such as benzene, carbon tetrachloride, and chloroform, etc., were used as mobile phase components.

To substitute undesirable organic mobile phases with environmentally friendly aqueous mobile phases, efforts have been initiated in our laboratory to use systems mediated with relatively non-toxic surfactants as mobile phases for analysis of organic [24–26] and inorganic substances [27,28].

In this study a water-in-oil microemulsion was introduced as a novel mobile phase for achieving simultaneous separation of isomers of nitroaniline. The term microemulsion was first used by Hoar and Schulman [29] to describe the transparent and thermodynamically stable systems formed spontaneously when an oil and water were mixed with relatively large amounts of an anionic surfactant and a cosurfactant (medium chain alcohol, e.g. pentanol or hexanol). Among microemulsion systems, water-in-oil microemulsions comprising an ionic surfactant, a medium-chain-length alcohol as cosurfactant, water, and a hydrocarbon have been the systems most extensively studied by physical chemists [30–32]. The analytical potential of microemulsion systems, however, has not been fully explored. The use of microemulsion systems for TLC separation of amino acids has been reported recently by our laboratory [33,34]. Here we report the usefulness of a water-in-oil microemulsion containing SDS, *n*-pentanol, water, and heptane as a mobile phase for TLC analysis of aromatic and aliphatic amines on silica layers. Reliable and rapid separation of nitroaniline isomers has been achieved.

The aim of the study was to separate *o*-, *m*-, and *p*-nitroanilines from their mixture by TLC technique. Separation of the isomers of nitroaniline is very important because commercially produced aromatic amines are usually contaminated by their isomers (one or two of the *o*, *m*, and *p* isomers) as unavoidable by-products.

EXPERIMENTAL

All experiments were performed at 30 ± 1°C

Chemicals and Reagents

Amines, heptane, and iodine were from CDH (India), silica gel 'G' and sodium dodecyl sulphate, SDS, from Merck (India), and *n*-pentanol from Fluka (Switzerland). Other reagents were of analytical reagent grade.

The amines studies were diphenylamine (DPA), *o*-chloroaniline (*o*-CAL), *m*-chloroaniline (*m*-CAL), *p*-chloroaniline (*p*-CAL), *o*-nitroaniline (*o*-NAL), *m*-nitroaniline (*m*-NAL), *p*-nitroaniline (*p*-NAL), *o*-toluidine (*o*-TLD), *m*-toluidine (*m*-TLD), *p*-toluidine (*p*-TLD), aniline (AL), quinoline (QL), trimethylamine (TMA), methylamine (MA), *tert*-butylamine (TBA), ethylamine (EA), dimethylamine (DMA), triethylamine (TEA), and diethyl amine (DEA). Test solutions (1%) of all the amines were prepared in methanol.

Chromatography

Preparation of Silica Gel TLC Plates

Silica gel with water were mixed in 1:3 ratio with constant shaking until a homogeneous slurry was obtained. This slurry was applied to 20 cm × 3.5 cm glass plates, by means of a Toshniwal (India) applicator, to give 0.25 mm layers. The plates were dried in air at room temperature and then activated by heating for 1 h at 100 ± 5°C in an electrically controlled oven. The activated plates were stored in a closed chamber at room temperature until used.

Mobile Phase

A water-in-oil microemulsion of SDS, heptane, *n*-pentanol, and water was used as mobile phase. It was prepared at 30°C by titrating a coarse emulsion of heptane (160 mL), water (8 mL), and SDS (8 g) with *n*-pentanol (25 mL). The microemulsion system was transparent, optically clear and remained stable at 30°C for several weeks.

Procedure

Test solutions (approx. 10 μL) were applied approximately 2.0 cm above the lower edge of the TLC plates by means of micropipettes. The spots were dried, and the plates were developed in 24 cm × 6 cm glass jars by the ascending technique. Before chromatography the glass jars containing the mobile phase were covered with a lid for approximately 10 min to enable pre-saturation with mobile phase vapour. The mobile phase migration distance was always 10 cm from the start line. After development the plates were withdrawn from the glass jars and dried at room temperature. All the amines were then detected by exposing the plates to iodine vapour in a closed chamber for approximately 10 min. The amines were detected as yellow–brown spots. The R_F of the leading and trailing edges of the spots (R_L and R_T, respectively) were measured and R_F was calculated.

For specific separation of amines equal amounts of *o*-NAL, *m*-NAL, and *p*-NAL were mixed and 20 μL of the resulting mixture was applied to a TLC plate. The plate was developed, the spots were detected, and the R_F values of the separated amines were determined.

Limits of Detection

The identification limits of *o*-NAL, *m*-NAL and *p*-NAL were determined by spotting different amounts of the compounds on the plates. The process was repeated with successive reduction of the amounts of *o*-NAL, *m*-NAL and *p*-NAL applied until detection was not possible. The amount of amine just detectable was taken as the detection limit.

Semiquantitative Determination by Spot Area Measurement

For semiquantitative determination by spot area measurement 10 μL of each of a series of standard solutions (0.5–3.0%) of *o*-NAL, *m*-NAL, and *p*-NAL were applied to the plates. The plates were developed and the spots were detected, and copied on to tracing paper from the plates. The area of each spot was then calculated.

RESULTS AND DISCUSSION

The thin-layer chromatographic system comprising of silica gel as stationary phase and water-in-oil microemulsion as mobile phase was chosen for analysis of aliphatic and aromatic amines for several reasons. First, silica is acidic and is, therefore, good for separation of basic analytes (e.g. amines). Second, the range of solubility of amines in water is very wide. Water-in-oil microemulsions preconcentrate hydrophilic, water soluble amines and so separations are possible between very soluble, less soluble and insoluble amines. Third, the tendency of the water-in-oil microemulsion to preconcentrate the analyte results in the formation of more compact and more easily detectable spots of the amines.

The mobility of amines on silica gel developed with the water-in-oil microemulsion is shown in Table I, which also includes the K_b values of the amines. From Table I it is clear that all the aromatic amines have either high or intermediate mobility whereas aliphatic amines have either no mobility ($R_F = 0.0$, DEA) or very low mobility ($R_F \leq 0.21$). This trend of chromatographic behaviour of amines can be explained on the basis of their relative basicity. Aliphatic amines are stronger bases than aromatic amines because of the presence of electron-releasing alkyl groups. The

alkyl group increases the availability of the lone pair of electron around the nitrogen atom, which helps it interact more strongly with the proton of silica gel, and hence mobility is low ($R_F \leq 0.21$). In aromatic amines the lone pair of electrons is partly shared with the benzene ring and is less available for sharing with the proton of silica gel, resulting in higher mobility ($R_F \geq 0.40$). Thus, the greater tendency of aliphatic amines to form intermolecular hydrogen bonds with the silanol groups of silica gel [–N·····OH–Si≡] is responsible for their lower mobility (greater retention) on silica layers. It is also clear from Table I that the mobility of aromatic amines depends on the nature of substituent groups on benzene ring. For example, nitroanilines are more mobile than chloroanilines and toluidines. NO_2, an electron-withdrawing group, reduces the basicity of aniline whereas the CH_3 group (electron-releasing) in toluene increases the basicity of aniline. Thus the higher the basicity of the amine the lower its mobility.

Table I

K_b values of the amines and the R_F values obtained on silica gel developed with water-in-oil microemulsion

Amine	K_b [35]	R_F
Aromatic		
o-NAL	0.00006×10^{-10}	0.92
DPA	0.0006×10^{-10}	0.76
p-NAL	0.001×10^{-10}	0.72
m-NAL	0.029×10^{-10}	0.54
o-CAL	0.05×10^{-10}	0.53
m-CAL	0.3×10^{-10}	0.49
p-CAL	1.0×10^{-10}	0.48
o-TLD	2.6×10^{-10}	0.48
AL	4.2×10^{-10}	0.55
m-TLD	5×10^{-10}	0.47
QL	6.3×10^{-10}	0.46
p-TLD	12×10^{-10}	0.40
Aliphatic		
TMA	0.6×10^{-4}	0.21
MA	4.5×10^{-4}	0.15
TBA	5.0×10^{-4}	0.08
EA	5.1×10^{-4}	0.08
DMA	5.4×10^{-4}	0.06
TEA	5.6×10^{-4}	0.05
DEA	10.0×10^{-4}	0.00

AL, however, deviates from this trend. It is a stronger base than *o*-TLD but has a greater R_F value (0.55) than *o*-TLD (0.48). The higher R_F of AL is because AL is approximately three times more soluble in water than *o*-TLD [36]. A plot of R_M (= log $(1 - R_F)/R_F$) against pK_b is approximately linear (Fig. 1). The plot reflects the effect of the basicity of amines on their migration behaviour on silica layers.

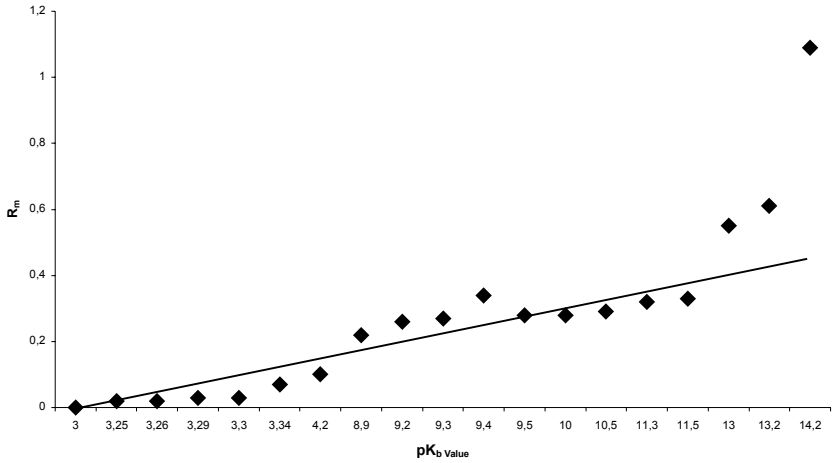

Fig. 1

Plot of R_M against pK_b for the amines

The formation of highly compact amine spots in chromatography with water-in-oil microemulsions facilitates their separation from multi-component mixtures. We have successfully separated several important amines on single silica gel TLC plate, as shown in Table II. Furthermore, aromatic amines can be easily separated from aliphatic amines. The propo-

Table II

Separations of amines achieved experimentally on plain silica layers developed with a water-in-oil microemulsion

No.	Amines separated (R_F value)
1	DPA (0.76) – AL (0.55) – QL (0.44) – MA (0.16) – DEA (0.0)
2	*o*-NAL (0.91) – *p*-NAL (0.71) – *m*-NAL (0.50) – TMA (0.20) – DMA (0.05)
3	*o*-NAL (0.90) – *p*-NAL (0.71) – *m*-NAL/*o*-CAL/*m*-CAL/*p*-CAL/*o*-TLD/AL/*m*-TLD/QL/*p*-TLD (0.50) – TMA (0.20) – TEA (0.05)/DEA (0.0)

sed method is most suitable for selective separation of *o*-NAL (R_F = 0.92) from other amines. Separation of mixtures of *o*-, *m*-, and *p*-nitroaniline is very important, and the proposed method produces excellently resolved spots for all three isomers of nitroaniline (Fig. 2).

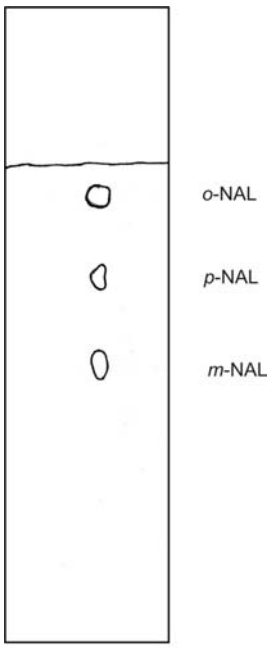

Fig. 2

Separation of a mixture of *o*-NAL, *m*-NAL, and *p*-NAL on silica gel with a water-in-oil microemulsion as mobile phase

The limits of detection for *o*-, *m*-, and *p*-NAL were 0.125, 0.10, and 0.08 μg, respectively, indicating that this system is highly suitable for sensitive detection of nitroanilines. Semi-quantitative determination *o*-, *m*-, and *p*-NAL by spot-area measurement was also attempted. Linear relationships was obtained between spot areas and amounts of the amines in accordance with the equation $\xi = Km$, where ξ is the spot area, m is the amount spotted, and K is a constant. Linearity was maintained for quantities of the nitroanilines up to 0.3 mg/spot. At higher concentrations deviation from linear law was observed. Precision and accuracy was within the range 8–10 % for all the nitroaniline isomers determined.

CONCLUSIONS

The method is useful for (i) separation of aromatic amines from aliphatic amines, (ii) sensitive detection of isomers of nitroanilines, and (iii) simultaneous separation and semiquantitative estimation of isomers of nitroanilines.

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