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# On Plate Resolution of Three-Component Mixture of Cationic Surfactants with Mixed Aqueous-Organic Eluents Containing Formate Ion

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# On Plate Resolution of Three-Component Mixture of Cationic Surfactants with Mixed Aqueous-Organic Eluents Containing Formate Ion

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**Abstract:** The chromatography of three long chain aliphatic quaternary ammonium halides (cationic surfactants) was performed on kieselguhr layers using different solvent systems. The mutual separation of dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), and hexadecyltrimethylammonium chloride (HTAC) was achieved with the solvent system methanol: 10% aqueous sodium formate (30:70, v/v) on kieselguhr layer. The effect of nature of alkane chain length of alcohols on the mobility of DTAB, TTAB, and HTAC and also the effect of formate ion substituted by acetate ion and benzoate ion of sodium acetate and sodium benzoate respectively on the mobility of these surfactants were studied. The effect of the nature of different adsorbents (kieselguhr, silica gel, and alumina) on the mobility of DTAB, TTAB, and HTAC was also studied. The interference of metal cations as impurities on the resolution of mixture of DTAB, TTAB, and HTAC was also examined. The limits of detection of DTAB, TTAB, and HTAC estimated were 3.3, 3.1, and 2.8 μg/zone respectively. The developed method was utilized to separate these surfactants from different spiked water samples.

**Keywords:** Dodeyltrimethylammonium bromide, hexadecyltrimethylammonium chloride, kieselguhr, separation, tetradecyltrimethyammonium bromide, thin-layer chromatography

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## INTRODUCTION

Cationic surfactants are extensively used as antimicrobial, emulsifying, anticorrosion, and softening agents in pharmaceutical preparations, cosmetic formulations, cleansing products, disinfectants, and other industrial manufactures (1). The thin-layer chromatographic studies and the separation of trimethyl long chain quaternary ammonium salts ($C_{12}$ or dodecyl, $C_{14}$ or tetradecyl and $C_{16}$ or hexadecyl) is important because these are widely used as main active ingredients of lysing reagents for blood cell analyzers which perform white blood cells differential determination into two or more sub-populations by impedance analysis (2).

The aim of this study is to develop a new thin-layer chromatographic method for the separation of cationic surfactants on the basis of alkyl chain-length of the hydrophobic group attached to the nitrogen atom. Mangold and Kammereck (3) proposed a method for the separation of cationic surfactants according to the number of long-chain alkyl groups attached to the nitrogen atom on silica plates and used acetone and concentrated ammonia (9:1, v/v) as mobile phase. The ammonia used in the solvent system causes toxic effects after prolonged inhalation on the respiratory system, liver, kidneys, and bladder. The proposed method consists of kieselguhr as stationary phase and methanol:10% aqueous sodium formate (30:70, v/v) as mobile phase and hence the proposed method is environmental friendly. An extensive literature survey on TLC reveals a surprising fact that very little work has been reported (4) on the analysis of surfactants using kieselguhr as the stationary phase. Hence, the method comprising of kieselguhr as stationary phase is used for the present study. The eluent methanol was used in various combinations by chromatographers in various mixed solvent systems (5–9) for the TLC analysis of surfactants. The proposed new chromatographic system of kieselguhr and methanol:10% aqueous sodium formate (30:70, v/v) provides a unique environment for the separation of coexisting DTAB, TTAB, and HTAC on the basis of alkyl chain-length attached to the nitrogen atom of these cationic surfactants. The suggested TLC system does not involve the use of carcinogenic solvents. Furthermore, the proposed method is capable of identifying surfactants after their preliminary separation from the spiked aqueous systems. The fascinating feature of the present study is that it demonstrates the analytical usefulness of a weak adsorbent like kieselguhr (diatomaceous earth) in the separation of three long-chain cationic quaternary ammonium surfactants with alkyl chain containing 12, 14, and 16 carbon atoms that is homologues series. It is well known that kieselguhr has a very small surface area (1–5 $m^2/g$) compared to the most frequently used sorbent phase such as silica gel (>400 $m^2/g$) and alumina (150–20 $m^2/g$) and hence its use as layer material has been limited. This work is an example of demonstrating the versatility and excellent resolving power available from an adsorbent of low surface activity. Thus, a high selectivity can be achieved by optimal selection of stationary and mobile phases regardless of the specific surface area of the layer material.

## EXPERIMENTAL

All experiments were performed at 25 ± 2°C

### Chemicals and Reagents

Kieselguhr (CDH, New Delhi, India), Methanol (Merck, Mumbai, India), Ethanol (CDH, New Delhi, India), n-Propanol (Merck, Mumbai, India), sodium formate, sodium acetate, and sodium benzoate (CDH, New Delhi, India) were used.

### Surfactants Studied

Dodecyltrimethylammonium bromide (DTAB) was purchased from Sigma Aldrich (Steinheim, Germany), Tetradecyltrimethylammonium bromide (TTAB) from Merck (Mumbai, India) and Hexadecyltrimethylammonium chloride (HTAC) was obtained from Merck (Hohenbrunn, Germany).

### Test Solutions

Solutions of the surfactants were prepared in methanol to give concentrations of 0.5% (w/v).

### Detection Reagent

Modified Dragendorff reagent prepared as described below was used to detect all the surfactants.

Dragendorff reagent comprised of two solutions A & B. Solution A was prepared from the two solutions:

1. Solutions of bismuth subnitrate ($BiONO_3.H_2O$, 1.7 g) dissolved in acetic acid (20 mL) and diluted 100 ml water.
2. Solution of Potassium Iodide (65 g in 200 mL water).

Both solutions were transferred into a standard flask of 1 L capacity. Acetic acid (200 mL) was added and the contents were diluted one liter with water (solution A).

Solution B was prepared by dissolving barium chloride dihydrate ($BaCl_2.2H_2O$, 290 g) in 1 L water. Solutions A and B were mixed in the volume ratio 2:1 and the resultant solution was used for the detection of all surfactants.

## Metal Ions

The aqueous solutions (1% w/v) of metal ions ($Bi^{3+}$, $VO^{2+}$, $Co^{2+}$, $Cr^{6+}$, $Cd^{2+}$, $Hg^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Cr^{3+}$, $Mo^{6+}$) were prepared from their chlorides, nitrates or sulphate salts, potassium dichromate ($Cr^{6+}$), and sodium molybdate ($Mo^{6+}$) were used for interference studies.

## Stationary Phase

Kieselguhr ($S_1$), Silica gel G ($S_2$), Alumina ($S_3$).

## Mobile Phases

The following solvents systems (Table 1) were used as mobile phases.

**Table 1.** Solvent Systems

| Symbol | Composition |
|---|---|
| $M_1$ | 1% aqueous sodium formate |
| $M_2$ | 5% aqueous sodium formate |
| $M_3$ | 10% aqueous sodium formate |
| $M_4$ | Methanol |
| $M_5$ | Methanol + 10% aqueous sodium formate (10:90, v/v) |
| $M_6$ | Methanol + 10% aqueous sodium formate (20:80, v/v) |
| $M_7$ | Methanol + 10% aqueous sodium formate (30:70, v/v) |
| $M_8$ | Methanol + 10% aqueous sodium formate (40:60, v/v) |
| $M_9$ | Methanol + 10% aqueous sodium formate (50:50, v/v) |
| $M_{10}$ | Methanol + 10% aqueous sodium formate (60:40, v/v) |
| $M_{11}$ | Methanol + 10% aqueous sodium formate (70:30, v/v) |
| $M_{12}$ | Methanol + 10% aqueous sodium formate (80:20, v/v) |
| $M_{13}$ | Methanol + 10% aqueous sodium formate (90:10, v/v) |
| $M_{14}$ | Ethanol + 10% aqueous sodium formate (30:70, v/v) |
| $M_{15}$ | Propenol + 10% aqueous sodium formate (30:70, v/v) |
| $M_{16}$ | Methanol + 10% aqueous sodium acetate (30:70, v/v) |
| $M_{17}$ | Methanol + 10% aqueous sodium benzonate (30:70, v/v) |

## Preparation of TLC Plates

TLC plates were prepared by mixing kieselguhr with double distilled water in 1:3 ratio. The resultant slurry was mechanically shaken for 5 min and then coated onto glass plates with the help of a TLC applicator to give a layer of 0.25 mm thickness. The plates were first air dried at room temperature and then activated by heating at 100°C for 1 h. After activation, the plates were kept in an air tight chamber until used.

## Procedure

Test solutions (0.01 mL) were applied on kieselguhr thin-layer plates (50 μg/zone) with the help of micropipettes at about 2 cm above the lower edge of the plates. The solvent ascent was fixed to 10 cm in all cases for the determination of $R_F$ value of individual surfactants. Linear ascending development was carried out in a vapour-equalibrated TLC twin-trough chamber. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 2°C). Subsequent to the development, TLC plates were dried at room temperature. The plates were then detected by using Dragendorff reagent and all the studied surfactants are visualized as orange colored spots. The development time was 25–30 mins for 10 cm ascent. The $R_F$ value and capacity factor were calculated for the surfactants.

For the separation of the surfactant mixture, an equal volume of surfactants was mixed and 0.01 ml of the resultant mixture was applied on TLC plates. The plate was developed with $M_7$, the spots were detected, and the $R_F$ values of the separated spots of surfactants were calculated.

In order to examine the effect of carbon chain length of alcohols on the mobility of DTAB, TTAB, and HTAC, the methanol in $M_7$ was substituted with ethanol and n-propanol and the resultant mobile phase systems $M_{14}$ (ethanol:10% aqueous sodium formate, 30:70, v/v) and $M_{15}$ (n-propanol:10% aqueous sodium formate, 30:70, v/v) were used as eluents. The affinities of carbon chain length of these alcohols were assessed by capacity factor values of these surfactants.

For the study of the effect of the formate anion ($HCOO^-$), on the mobility of DTAB, TTAB, and HTAC, the formate anion ($HCOO^-$) of sodium formate was substituted with other carboxylate anions such as acetate anion ($CH_3COO^-$) of sodium acetate and benzoate anion ($C_6H_6COO^-$) of sodium benzoate and the resultant mobile phase systems $M_{16}$ (methanol: 10% aqueous sodium acetate, 30:70, v/v) and $M_{17}$ (Methanol:10% aqueous sodium benzoate, 30:70, v/v) were used as eluents.The calculated $R_F$ and capacity factor values were used for the comparative study.

For the study of the effect of the nature of adsorbents (Kieselguhr, silica gel, and alumina) on the mobility of DTAB, TTAB, and HTAC, the comparative study of these adsorbents were performed using $M_7$ as mobile phase and the affinity of these adsorbents with the surfactants were also examined by calculating the $R_F$ and the capacity factor values.

For investigating the interference due to the presence of metal cations as impurities on the resolution of DTAB, TTAB, and HTAC from their mixture, 0.01 ml of a standard test mixture of surfactant solutions was spotted on the plate followed by spotting of 0.01 ml of the cations being considered as impurities. The plates were developed with $M_7$, detected and $R_F$ values of the separated surfactants were calculated. This aspect of the study is important because of the tendency of metal to form complexes with cationic surfactants (10) which can either impair the separation or can alter the separation sequence.

The limits of detection of surfactants were determined by spotting different amounts of solutions of the DTAB, TTAB, and HTAC on the plates. The plates were then developed and the corresponding spots were detected. The method was repeated with successive lowering of the amounts of DTAB, TTAB, and HTAC until no spot was detected. The minimum amount of surfactants that could be detected was taken as the limit of detection for accuracy this was carried out in six replicate measurements.

The robustness which is one of the validation parameter was studied for the resolution of the mixture (DTAB, TTAB, and HTAC) in six replicate $R_F$ value measurements at a concentration of 50 µg/spot ot of the mixture.

## RESULTS AND DISCUSSION

The chromatography of three of cationic surfactants of varying chain length ($C_{12}$–$C_{16}$) was performed on kieselguhr layers using different solvent systems. The results have been presented in the form of retardation factor ($R_F$) and capacity factor (K) which were calculated as, $R_F = 0.5 \times (R_L + R_T)$ and $K = (1/R_F) - 1$. $R_L$ and $R_T$ represent the leading and trailing fronts respectively for the detected spot on the TLC plate. The capacity factor (K) is used to indicate the relative affinity of a compound between the solid substrate and the solvent. Strong affinity for the substrate produces high capacity factor, low capacity factor is indicative of weak substrate compound interactions as well as the higher relative affinity of the compound for the solvent system. The results of this study are presented in Table 2. In a monocomponent sodium formate, the mobility of these surfactants changes with the concentration of sodium formate.

**Table 2.** $R_F$ value of surfactants on kieselguhr plates developed with different mobile phase

| Surfactants | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $M_6$ | $M_7$ | $M_8$ | $M_9$ | $M_{10}$ | $M_{11}$ | $M_{12}$ | $M_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DTAB | 0.58 | 0.55 | 0.53 | 0.80T | 0.64 | 0.71 | 0.75 | 0.8 | 0.82 | 0.89 | 0.93 | 0.95 | 0.97 |
| TTAB | 0.14 | 0.1 | 0.06 | 0.77T | 0.1 | 0.19 | 0.48 | 0.72 | 0.75 | 0.87 | 0.92 | 0.93 | 0.96 |
| HTAC | 0 | 0 | 0 | 0.60T | 0 | 0.05 | 0.1 | 0.5 | 0.7 | 0.8 | 0.9 | 0.92 | 0.94 |

In lower concentration of sodium formate (1%), the DTAB ($R_F = 0.58$) gives higher mobility as compared to TTAB ($R_F = 0.14$) and HTAC ($R_F = 0.00$). As the concentration of sodium formate is increased from 5–10%, the mobility of DTAB ($R_F = 0.53$) and TTAB ($R_F = 0.06$) is decreased as compared in 1% aqueous sodium formate, while the HTAC ($R_F = 0.00$) remains at the point of application. The 10% aqueous sodium formate is selected for further study as all the three surfactants gave compact spots in comparison to the other concentration. In methanol, the mobilities of DTAB ($R_F = 0.80$T), TTAB ($R_F = 0.77$ T), and HTAC ($R_F = 0.60$T) are higher as compared in the aqueous sodium formate, this may be due to the hydrophilic nature of methanol. All the three surfactants produce badly tailed spots in methanol. The mobility of these surfactants is based on the extent of adsorption on the adsorbent layer. The increased hydrophobicity ($C_{12}$–$C_{16}$) results in the increase of the adsorption by decreasing the mobility of these surfactants.

The aim of this study is to select a useful solvent system to achieve the separation of coexisting DTAB, TTAB, and HTAC. In this attempt we have used a mixed solvent system consisting of methanol and 10% aqueous sodium formate in order to achieve a useful chromatographic system. It has been extensively discussed (11,12) that a mobile phase which consists of a mixture of different solvents that vary in polarity produces a better chromatographic development. It is due to the fact that in a mixed solvent system a gradient in solvent composition is produced during the movement of the mixed solvent on the adsorbent layer.

The results of the mixed combinations of methanol and 10% aqueous sodium formate in different volume ratios are presented in Table 2. At lower concentration of methanol (10% methanol) in the solvent system $M_5$ (methanol: 10% aqueous sodium formate, 10:90, v/v), the HTAC remains at the point of application while DTAB ($R_F = 0.64$) gives higher mobility as compared to TTAB ($R_F = 0.10$). It is clear from Table 2 that with the increase in concentration of methanol in the system from 20–90% ($M_6$–$M_{13}$), the mobilities of DTAB ($R_F = 0.97$), TTAB ($R_F = 0.96$) and HTAC ($R_F = 0.94$) have increased from the solvent system $M_6$–$M_{13}$, the $M_7$ consisting of methanol:10% aqueous sodium formate (30:70, v/v) provides useful medium for the separation (Fig. 1) of the coexisting DTAB, TTAB, and HTAC. The mobility of DTAB ($R_F = 0.75$), TTAB ($R_F = 0.48$) and HTAC ($R_F = 0.10$) are in correlation with the chain length of the hydrophobic group of these surfactants as DTAB > TTAB > HTAC.

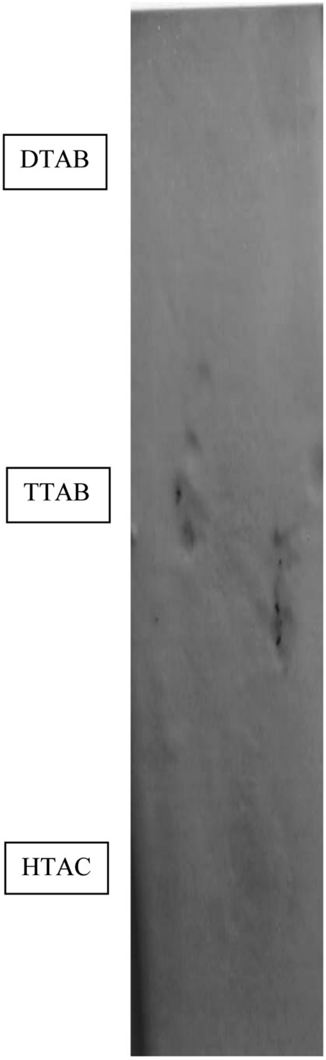

***Figure 1.*** Chromatogram showing resolution of coexisting DTAB, TTAB, and HTAC on kieselguhr developed with $M_7$.

The effect of the nature of alcohols (methanol, ethanol, and n-propanol) on the mobility of DTAB, TTAB, and HTAC was examined and the results are presented in Figs. 2 and 3. From Fig. 2 it is clear that the mobility of all the three surfactants tended to increase with the increase in the carbon chain length of alcohols. Consequently, the capacity factor values were also found to decrease with the increase in the chain length of alcohols (Fig. 3). The lowest capacity factor value of surfactants in n-propanol containing the solvent system $M_{15}$ as compared to ethanol and methanol containing the solvent systems $M_{14}$ and $M_7$ respectively indicates that there is a strong interaction between surfactants and the n-propanol containing the solvent system which results in increased mobility of all the three surfactants. This may be due to increase in the hydrophobic nature in the solvent system.

The effect of the nature of the formate anion of sodium formate substituted by the acetate anion ($CH_3COO^-$) of sodium acetate and benzoate anion ($C_6H_5COO^-$) of sodium benzoate in the solvent system $M_7$ resulting in the systems $M_{16}$ and $M_{17}$ respectively on the mobility of DTAB, TTAB, and HTAC are shown in Figs. 4 and 5. From Fig. 4, it is clear that the $R_F$ value increases in sodium acetate containing the solvent system ($M_{16}$), while in case of sodium benzoate containing the

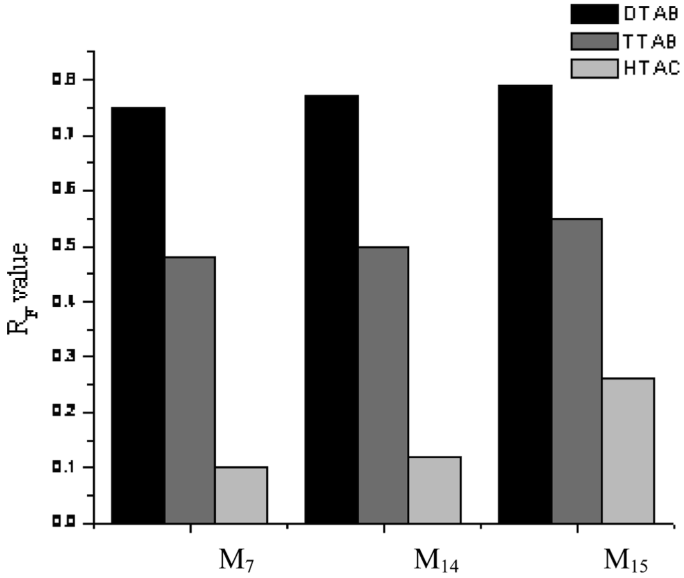

***Figure 2.*** Effect of alcohol chain length on the $R_F$ value of DTAB, TTAB, an HTAC on kieselguhr layer ($S_1$).

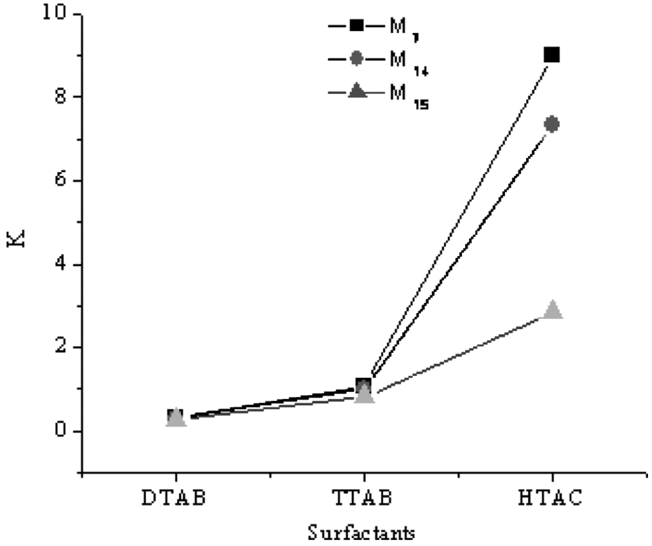

***Figure 3.*** Capacity factor (K) value vs. the alkane chain length of surfactants in $M_7$, $M_{14}$ and $M_{16}$ solvent systems.

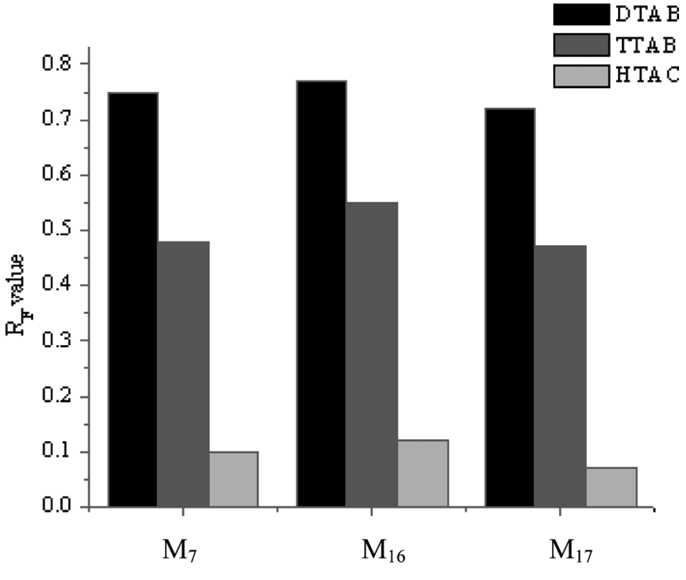

***Figure 4.*** Effect of formate, acetate and benzoate anion on the $R_F$ value of DTAB, TTAB, and HTAC.

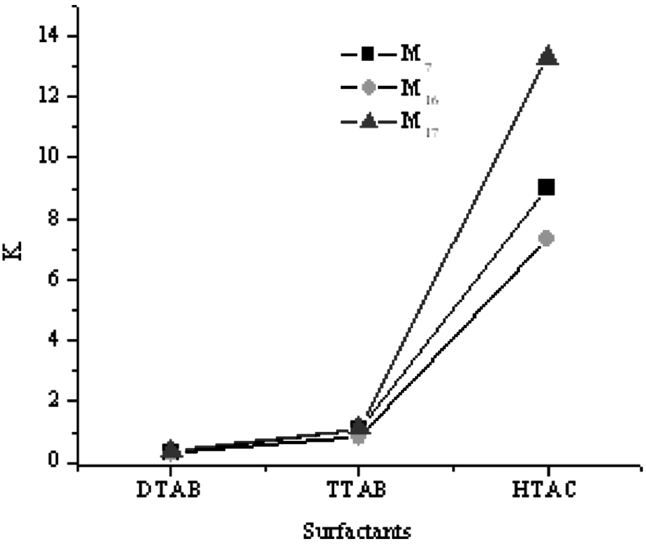

*Figure 5.* Capacity factor (K) value vs. the alkane chain length of surfactants in $M_7$, $M_{16}$ and $M_{17}$ solvent systems.

solvent system ($M_{17}$), the mobility decreases as compared in $M_7$. This may be due to the presence of the benzene ring in sodium benzoate which may interact weakly with these surfactants due to the steric hindrance and hence decreases the mobility of these surfactants. The results presented in Fig. 5 show that the capacity factor values are higher for the hydrophobic chain of surfactants containing a greater number of carbon atoms i.e. the capacity factor values are in the order HTAC > TTAB > DTAB. This indicates that the larger chain length of the hydrophobic group have high affinity with the adsorbent and weak interaction with the solvent. It is also clear from Fig. 5 that in a sodium benzoate containing solvent system ($M_{17}$) surfactants have the highest capacity factor as compared to sodium formate ($M_7$) and sodium acetate ($M_{16}$) containing solvent systems. This clearly indicates that sodium benzoate ($M_{17}$) containing solvent system interacts weakly with these surfactants.

The results showing the effect of the nature of adsorbents (kieselguhr, silica gel, and alumina) on the mobility of DTAB, TTAB, and HTAC using $M_7$ (methanol:10% aqueous sodium formate, 30:70, v/v) as eluent are summarized in Figs. 6 and 7. As evident from Fig. 6 the mobility of these surfactants is increased on alumina ($S_3$) and decreased on silica ($S_3$) as compared to their mobilities on kieselguhr ($S_1$). This may be due to the fact that in silica ($S_2$), the negative silanol groups act as active centers for adsorption which have the highest affinity for the positively charged

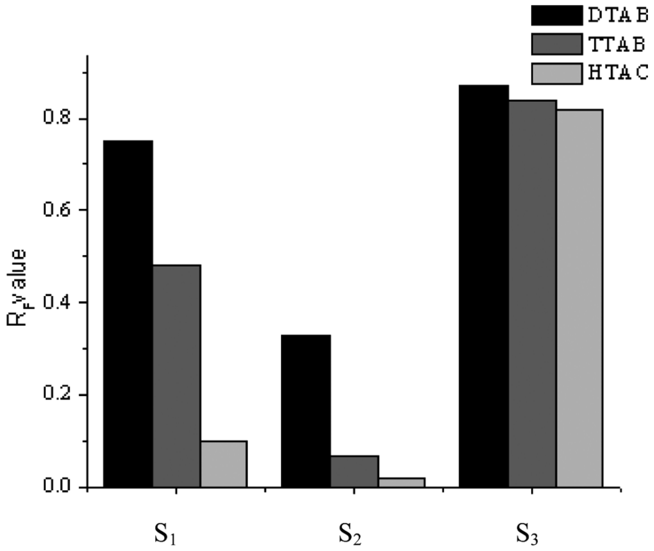

***Figure 6.*** Effect of different adsorbents on the $R_F$ value of DTAB, TTAB, and HTAC in the solvent system $M_7$.

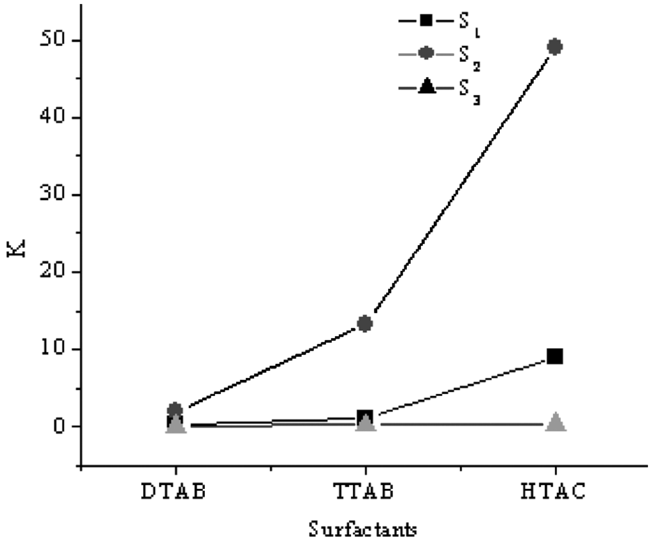

***Figure 7.*** Capacity factor (K) value vs. the alkane chain length of surfactants on different adsorbents in the solvent system $M_7$.

hydrophilic groups (cationic). As compared to alumina ($S_3$), where the hydroxyl groups and oxide ($O^{2-}$) ions act as active centers for the adsorption which have the least affinity for the cationic hydrophilic groups, while in the case of kieselguhr ($S_1$), it is the mixture of various materials containing 90% material of $SiO_2$ and the remaining 10% consists of $Al_2O_3$, $Fe_2O_3$, MgO, $Na_2O$, $K_2O$, CaO, and $TiO_2$ in different proportions (13). This mixed combination of various materials in kieselguhr ($S_1$) provides effective adsorption of these surfactants in the solvent system ($M_7$) for their mutual separation. From Fig. 7 it is clear that the capacity factor value of these surfactants on these adsorbents is in the order silica ($S_2$) > Kieselguhr ($S_1$) > alumina ($S_3$). The highest capacity factor on silica ($S_2$) indicates that these surfactants have strong interaction with the silica ($S_2$) as compared to kieselguhr ($S_1$) and alumina ($S_3$). This moderate affinity of kiselguhr ($S_1$) with the surfactants in the solvent system $M_7$ (Methanol:10% aqueous sodium formate, 30:70, v/v) provides a novel chromatographic system for the resolution of the coexisting mixture of DTAB, TTAB, and HTAC.

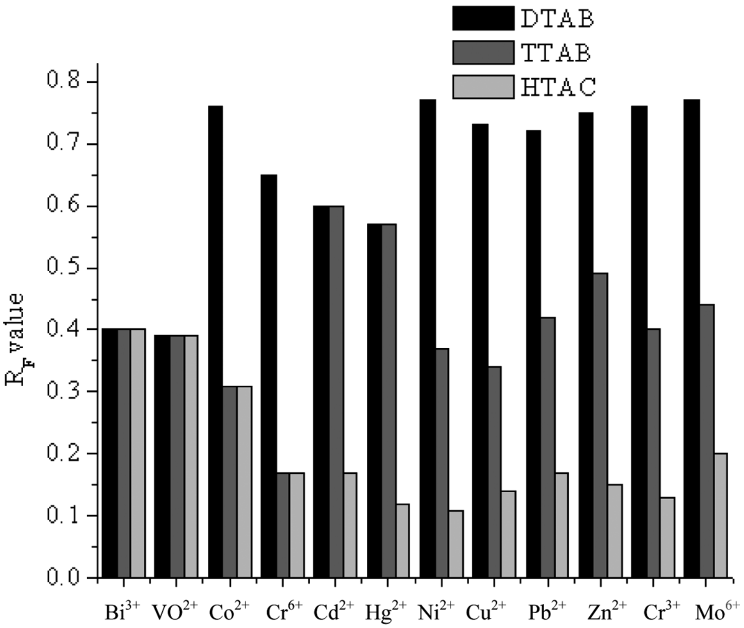

***Figure 8.*** Effect of metal cations as impurities on the mutual separation of coexisting DTAB, TTAB, and HTAC on kieselguhr layers developed with methanol: 10% aqueous sodium formate $M_7$ (30:70, v/v).

The results showing the effect of various metal cations as impurities on the mutual separation of coexisting DTAB, TTAB, and HTAC are presented in Fig. 8. It is clear from this figure that separation is hampered by the presence of $Bi^{3+}$, $VO^{2+}$, $Co^{2+}$, $Cr^{6+}$, $Cd^{2+}$, and $Hg^{2+}$. All the three surfactants DTAB, TTAB, and HTAC co-migrate in the presence of $Bi^{2+}$ and $VO^{2+}$, whereas, in the presence of $Co^{2+}$, $Cr^{6+}$, TTAB, and HTAC and in the presence of $Cd^{2+}$ and $Hg^{2+}$, DTAB, and TTAB co-migrate. In the presence of other metal cations ($Ni^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Cr^{3+}$, and $Mo^{6+}$) mutual separation of coexisting DTAB, TTAB, and HTAC is always possible. Thus, certain metallic impurities in the surfactants have a detrimental effect on their chromatographic performance. This may be due to the complex formation of metal ions with the cationic surfactants.

The low values of standard deviation (S.D.) for DTAB (S.D = 0.017), TTAB (S.D = 0.026) and HTAC (S.D = 0.040) obtained after resolving the mixture (DTAB, TTAB, and HTAC) in six replicate $R_F$ value measurements indicated the robustness of the developed TLC method.

The lowest possible detectable amount of DTAB, TTAB, and HTAC on Kieselguhr plates developed with $M_7$ was 3.3, 3.1, and 2.8 μg/zone, respectively. This indicates that the proposed TLC method is highly sensitive for the detection of surfactants.

## APPLICATION

To widen the applicability of the proposed method water samples are spiked with DTAB, TTAB, and HTAC. The results presented in Table 3 shows that the DTAB, TTAB, and HTAC can be separated from the spiked water samples (double distilled water, tape water, river water, and saline water) on kieselguhr plates developed with $M_7$.

**Table 3.** $R_F$ value of HTAC, TTAB, and DTAB, after their preliminary separation from the mixture spiked in different water samples

| Water samples | HTAC | TTAB | DTAB |
|---|---|---|---|
| Double distilled water | 0.16 | 0.47 | 0.47 |
| Tape water | 0.16 | 0.51 | 0.77 |
| River water | 0.17 | 0.48 | 0.74 |
| Saline water | 0.19 | 0.49 | 0.75 |

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