# Use of Nonionic Poly(Ethylene glycol) *p*-Isooctyl-Phenyl Ether (Triton X-100) Surfactant Mobile Phases in the Thin-Layer Chromatography of Heavy-Metal Cations

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## Abstract

The analytical potential of poly(ethylene glycol) p-isooctyl-phenyl ether (Triton X-100), a nonionic surfactant, is used as a mobile phase in the thin-layer chromatographic separation of heavy-metal cations. The surfactant concentration below its critical micellar concentration (CMC) as well as above the CMC value is used to investigate the migrational behavior of some heavy-metal ions on silica gel layers. The mobility of the metal ions is found to change marginally with the increase of surfactant concentration from 0.001M (below CMC) to 0.1M (above CMC). The influence of the pH of the medium, nonelectrolyte organic (urea and alkanols), and inorganic electrolyte (NaCl) additives in the surfactant containing mobile phase on the mobility of heavy metals on the silica gel layer is examined. For separating metal ions, surfactant must be used in the presence of buffers. Triton X-100 (0.02M) at pH 2.3 is found to be the best mobile phase for the separation of heavy-metal cations. In general, the presence of alcohol in aqueous surfactant solutions results in a decrease in the mobility of metal ions. Besides Cu2+ and Fe<sup>3+</sup>, all of the metal ions show a trend of increasing the retardation factor beyond a minima at 0.1 or 0.3M of added urea or NaCl. The proposed method is successfully applied for the simultaneous detection of Zn2+ and Cd2+ from a spiked human blood sample.

# Introduction

Thin-layer chromatography (TLC) is a very convenient and effective technique for the separation and identification of inorganic ions. It permits selective separations, simple detection, and easy manipulation of the mobile phase. As a result, numerous sorbents and an even greater number of mobile phases have been developed for achieving improved chromatographic performance in terms of selectivity, resolution, rapidity, and reproducibility.

From literature (1–3), the mobile phases used in inorganic TLC have been individual organic solvents; a mixture of organic solvents; mixed aqueous–organic (or hydro-organic)

solvents; or aqueous solutions of acids, bases, and salts. Although organic solvents such as benzene, chloroform, acetone, methanol, acetonitrile, carbon tetrachloride, dioxane, acetic acid, phenols, cyclohexane, and hexane are quickly removed from the sorbent layer after development, most of these are toxic to some extent. However, aqueous micellar solutions of surfactants are almost nontoxic (especially biodegradable surfactants), nonflammable, and odorless. Aqueous micellar systems capable of mimicking certain properties of organic solvents (i.e., solubilizing nonpolar solutes) have been considered as an attractive alternative to organic solvents as the mobile phase in the chromatographic analysis of complex mixtures. The highly selective partitioning of solutes to micelles results in unique separation possibilities for both ionic and nonionic solutes.

Surfactants have long been used as mobile phase components in ion-pair chromatography (IPC) and micellar liquid chromatography (MLC) (4-9). The surfactant concentration in IPC is intentionally maintained below the critical micellar concentration (CMC) in order to avoid the formation of micelles. In this study, the surfactants are used as counter ions in the separation of ionic substances. In MLC the surfactant concentration in the mobile phase is kept well above the CMC value. In this case micelles formed by surfactant molecules are aggregations suspended in the water mobile phase, and the system is microscopically heterogeneous. Since the first report by Armstrong and Henry (10), interest in MLC has grown rapidly and it is now being used as a substitute for traditional liquid chromatography with reversed phases (11–14). The fascinating feature of micellar systems is the presence of three phases (e.g., micellar micropseudo phase, bulk mobile phase, and the stationary phase) that provide sites of multiple interactions, resulting in unique simultaneous separation capabilities of hydrophobic and hydrophilic compounds.

The use of micellar solutions as the mobile phase in TLC was first reported in 1979 by Armstrong and Terrill (15–16). Micellar chromatography, being a relatively new tool, has expanded the scope of separations by normal-phase TLC. Despite distinct advantages, micellar systems have not been extensively used in TLC compared with their use in reversedphase high-performance liquid chromatography (17–20) and micellar electrokinetic capillary chromatography (21–23). Interesting separations of pesticides, nucleosides, biphenyls, polynuclear aromatic hydrocarbons, phenols, amino acids, aromatic amine alkaloids, drugs, and dyes have been realized using micellar mobile phase systems (24–30). Reports concerning efficiency and the unique chromatographic advantages of surfactant mediated mobile phases in the separation of inorganics (31–36) have demonstrated that the micellar systems are excellent eluents. Recently, T. Okada has admirably reviewed the work on the micellar chromatography of inorganic compounds (37).

In regard to metal ion separation by TLC, some studies reported the use of micellar mobile phases (38–40). TLC separations of metal 1,3-diketonates on silufol plazmachrom plates with sodium dodecyl sulfate (SDS) (41); heavy metal cations on cellulose layers with Brij-35 micellar mobile phases (38); Co(III)-1-(2-pyridylazo)-2-naphthyl complexes on polyamide

layers using a mixture of SDS, Triton X-100 (TX-100), and an acetic acid-sodium acetate buffer (pH 4) (39); and metal piperidinedithiocarbamate complexes on surfactant impregnated silica gel layers using mixed organic solvent systems as the mobile phase (40) have been performed recently.

The work with MLC to date appears to have involved mostly charged cationic or anionic micellar mobile phase systems, and the use of uncharged nonionic surfactants seems to have been generally neglected. The aim of this work was to explore the possibility of using the unique properties of TX-100 (a nonionic surfactant) in the separation of heavy-metal cations on silica gel layers. The roles of various factors such as surfactant concentration, pH of the medium, addition of organic modifiers (alkanols), concentration of nonelectrolyte (urea), and the addition of electrolyte (NaCl) on the retention behavior of metal ions were examined. These additives were selected because of their capabilities of bringing about changes in the microenvironment of micelles in order to influence the selectivity pattern of solutes (31,42–44).

# **Experimental**

#### Reagents

TX-100 was obtained from Loba (Mumbai, India) and used as received. Silica gel G, methanol, and ethanol (Glaxo, New Delhi, India); butanol and pentanol (CDH, Mumbai, India); urea (G.S. Chemical Testing, Mumbai, India); and NaCl (S.d. fine Chemicals, Mumbai, India) were also used. All chemicals used were of analytical reagent grade.

## **Test solution**

Solutions (1%) of nitrates of Pb<sup>2+</sup>, Hg<sup>2+</sup>, Tl<sup>+</sup>, Cd<sup>2+</sup>, and  $UO_2^{2+}$ ; chlorides of Ni<sup>2+</sup>, CO<sup>2+</sup>, and Zn<sup>2+</sup>; and sulfates of Cu<sup>2+</sup> and VO<sup>2+</sup> were prepared in double-distilled water. Ferric chloride and aluminum nitrate solutions were prepared in 0.1M solutions of the corresponding acids. A bismuth chloride solution was prepared in 3.7M HCl.

#### **Detection reagents**

A 1% aqueous solution of potassium ferrocyanide for  $Cu^{2+}$  and  $Fe^{3+}$ , alcoholic dimethylglyoxime for  $Ni^{2+}$  and  $CO^{2+}$ , and a 0.5% solution of dithizone in carbon tetrachloride for  $Cd^{2+}$ ,  $Zn^{2+}$ ,  $Tl^+$ , and  $Bi^{3+}$  were used as detection reagents.

Table I. The Various Solvent Systems Used	
No.	Composition
Aqueous surfact	tant solutions
M <sub>1</sub>	0.001, 0.002, 0.005, 0.01, 0.02, 0.05, and 0.1M aqueous TX-100
Buffer solutions	
M <sub>2</sub>	buffer solutions of pH 2.3, 3.4, 5.7, and 11.9
Micellar buffere	d solutions
M <sub>3</sub>	0.02M TX-100 in buffer solutions of pH 2.3, 3.4, 5.7, and 11.9
$M_4$	0.10M TX-100 in buffer solutions of pH 2.3, 3.4, 5.7, and 11.9
Micellar buffere	d solutions with organic additives*
M <sub>5</sub>	0.1M TX-100 buffered at pH 2.3 + methanol (95:5, v/v)
M <sub>6</sub>	0.1M TX-100 buffered at pH 2.3 + methanol (90:10, v/v)
M <sub>7</sub>	0.1M TX-100 buffered at pH 2.3 + methanol (80:20, v/v)
M <sub>8</sub>	0.1M TX-100 buffered at pH 2.3 + ethanol (95:5, v/v)
M <sub>9</sub>	0.1M TX-100 buffered at pH 2.3 + propanol (95:5, v/v)
M <sub>10</sub>	0.1M TX-100 buffered at pH 2.3 + butanol (95:5, v/v)
M <sub>11</sub>	0.1M TX-100 buffered at pH 2.3 + pentanol (95:5, $v/v$ )
M <sub>12</sub>	1M methanol in aqueous 0.1M TX-100 (pH 2.3)
M <sub>13</sub>	1M ethanol in aqueous 0.1M TX-100 (pH 2.3)
M <sub>14</sub>	1M propanol in aqueous 0.1M TX-100 (pH 2.3)
M <sub>15</sub>	1M butanol in aqueous 0.1M TX-100 (pH 2.3)
M <sub>16</sub>	1M pentanol in aqueous 0.1M TX-100 (pH 2.3)
M <sub>17</sub>	0.1M TX-100 buffered at pH 2.3 + 0.01 moles of urea
M <sub>18</sub>	0.1M TX-100 buffered at pH 2.3 + 0.1 moles of urea
M <sub>19</sub>	0.1M TX-100 buffered at pH 2.3 + 0.3 moles of urea
M <sub>20</sub>	0.1M TX-100 buffered at pH 2.3 + 0.5 moles of urea
Micellar buffere	d solutions with inorganic additives
M <sub>21</sub>	0.1M TX-100 buffered at pH 2.3 + 0.01 moles of NaCl
M <sub>22</sub>	0.1M TX-100 buffered at pH 2.3 + 0.05 moles of NaCl
M <sub>23</sub>	0.1M TX-100 buffered at pH 2.3 + 0.03 moles of NaCl
M <sub>24</sub>	0.1M TX-100 buffered at pH 2.3 + 0.5 moles of NaCl
* The volume frac order to protect	ction of the organic modifier that was added to the micellar buffered solution was limited to 5% in the integrity of micelles.

#### **Buffer solution**

Buffer solutions at pH 2.3, 3.4, 5.7, and 11.9 were prepared by adding 0, 8, 10, and 60 mL of 0.24M NaOH, respectively, into a 100-mL mixture consisting of equal volumes of boric acid (0.04M) and phosphoric acids (0.04M).

# Mobile phases

The various solvent systems used are found in Table I.

# Chromatography

# Preparation of TLC plates

A homogeneous slurry of the stationary phase (i.e., silica gel G (20 g) and double-distilled water (60 mL)) was prepared by constant shaking for 5 min. The slurry was coated on  $20 \times 3$ -cm plates as a 0.25-min layer by means of a Toshniwal (Mumbai, India) TLC applicator. Plates were dried at room temperature and activated at  $100^{\circ}C \pm 2^{\circ}C$  for 1 h in an electrically controlled oven. After activation, the plates were cooled at room temperature and stored in a closed chamber until use. No additional binder was added in the preparation of the plates.

# Procedure

Chromatography was performed in 24-  $\times$  6-  $\times$  6-cm glass jars. A sample solution (10 µL) was applied to a TLC plate on a line marked at 3 cm from the lower edge using a lambda pipette and left to dry in air. Ascending development in various mobile phases was performed to a distance of 10 cm from the point of application. After development, the plates were air dried at room temperature and the position of the metal ions on the plates was identified by detecting the spots using appropriate chromogenic reagents. Retardation factor (R<sub>F</sub>) values of the metal ions were determined by their respective R<sub>L</sub> (R<sub>F</sub> of leading front) and R<sub>T</sub> (R<sub>F</sub> of trailing front) values.

The reported  $R_F$  values are the average of three replicate determinations. They all have a standard deviation of  $\pm~0.05$  from their individual  $R_F$  values.

Table II. Experimentally Achieved Separations on Silica Gel G

## Identification of Zn and Cd in a spiked blood sample

A blood sample was treated with a 10% NaOH solution and 10% sodium tungstate solution. This mixture was acidified by adding a small amount of  $H_2SO_4$  to destroy the precipitate and then it was filtered. The filtrate was spiked with equal amounts of Zn<sup>2+</sup> and Cd<sup>2+</sup>. Approximately 10 µL of the spiked blood sample was spotted on a TLC plate developed with 0.02M TX-100 (pH 11.9), dried, and the separated spots of Zn<sup>2+</sup> and Cd<sup>2+</sup> were identified.

# **Results and Discussion**

The results of this study have been summarized in Table II and Figures 1–4. The mobility of thirteen heavy-metal cations was examined on a silica gel layer using aqueous solutions of TX-100. In order to optimize the experimental conditions, the effect of various factors such as the concentration of the surfactant, the acidity and basicity of the medium, the nature of alkanols in the surfactant mobile phase, and the presence of urea and NaCl in the micellar mobile phase on the mobility of cations was examined.

# Effect of the concentration of TX-100

In order to understand the effect of surfactant concentration, the mobility of an individual metal ion on a silica gel layer was determined using a wide concentration range (0.001-0.10M) of TX-100.

The following trends were noticed.  $UO_2^{2+}$ ,  $VO^{2+}$ , and  $AI^{3+}$  were strongly retained by silica gel and remained at the point of application ( $R_F = 0$ ) over the entire concentration range of the surfactant. Conversely,  $Ni^{2+}$  and  $Co^{2+}$  show maximum mobility ( $R_F = 0.90$ ) at all surfactant concentration levels. In all of the mobile phases containing different TX-100 concentrations,  $Cd^{2+}$  had an  $R_F$  value that was lower than those of  $Ni^{2+}$  or  $Co^{2+}$  except at 0.02M TX-100, in which it was approximately the same. Fe<sup>3+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, and Bi<sup>3+</sup> showed little mobility and moved only slightly from the point of application. All of

Mobile phase	Separations* (R <sub>F</sub> )
0.001M TX-100	Ni <sup>2+</sup> (0.91)–Pb <sup>2+</sup> (0.00),UO <sub>2</sub> <sup>2+</sup> (0.00)–Cd <sup>2+</sup> (0.67),Co <sup>2+</sup> (0.91)–Fe <sup>3+</sup> (0.05)
0.002M TX-100	Ni <sup>2+</sup> (0.85)–Bi <sup>3+</sup> (0.20),Co <sup>2+</sup> (0.85)–Bi <sup>3+</sup> (0.20),Ni <sup>2+</sup> (0.90)–Al <sup>3+</sup> (0.00),
	Cd <sup>2+</sup> (0.70)–Al <sup>3+</sup> (0.00),Ni <sup>2+</sup> (0.85)–VO <sup>2+</sup> (0.10)
0.005M TX-100	Tl+(0.75)-Ni <sup>2+</sup> (0.90),Zn <sup>2+</sup> (0.10)-Co <sup>2+</sup> (0.80)
0.01M TX-100	Cu <sup>2+</sup> (0.10)–Cd <sup>2+</sup> (0.70),Ni <sup>2+</sup> (0.85)–Al <sup>3+</sup> (0.00)
0.02M TX-100	Cd <sup>2+</sup> (0.87)–Pb <sup>2+</sup> (0.00),Ni <sup>2+</sup> (0.92)–UO <sub>2</sub> <sup>2+</sup> (0.00)
0.05M TX-100	Ni <sup>2+</sup> (0.85)–Pb <sup>2+</sup> (0.00),UO <sub>2</sub> <sup>2+</sup> (0.00)–Cd <sup>2+</sup> (0.70),Ni <sup>2+</sup> (0.85)–Zn <sup>2+</sup> (0.10)
0.1M TX-100	Tl+(0.15)-Ni <sup>2+</sup> (0.90),Pb <sup>2+</sup> (0.00)-Cd <sup>2+</sup> (0.67),Pb <sup>2+</sup> (0.00)-Co <sup>2+</sup> (0.85)
0.02M TX-100 in buffer of pH 2.3	Pb <sup>2+</sup> (0.00)–Tl+(0.60),Tl+(0.60)–Fe <sup>3+</sup> (0.00),
	Hg <sup>2+</sup> (0.70)–Pb <sup>2+</sup> (0.00),Pb <sup>2+</sup> (0.00)–Co <sup>2+</sup> (0.80),
	$VO^{2+}(0.35), UO_2^{2+}(0.00) - Cu^{2+}(0.40) - Ni^{2+}(0.80),$
	Pb <sup>2+</sup> (0.00)–Zn <sup>2+</sup> (0.45)–Ni <sup>2+</sup> (0.90)
0.02M TX-100 in buffer of pH 11.9	$Zn^{2+}(0.00)-Cd^{2+}(0.65), Hg^{2+}(0.50)-Co^{2+}(0.85), Ni^{2+}(0.90)-Cd^{2+}(0.62), Ni^{2+}(0.90)-Cd^{2+}(0.67)-Zn^{2+}(0.00), Ni^{2+}(0.90)-Cd^{2+}(0.$



Figure 1. Mobility of metal ions on silica gel layers developed in buffer (▲), buffered 0.02M aqueous surfactant (■), and buffered 0.1M aqueous micellar solutions (♦).



**Figure 2.** Migration behavior of metal ions on silica gel layers developed in buffered 0.1M TX-100 (pH 2.3) with 5% added alcohol: (C0) no added alcohol, (C1) methanol, (C2) ethanol, (C3) propanol, (C4) butanol, and (C5) pentanol.

these metal ions can be separated from Ni<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup>, which migrate with the mobile phase irrespective of the surfactant concentrations. The behavior of Tl<sup>+</sup> was peculiar. It showed higher mobility ( $R_F = 0.75$ ) with mobile phases containing a surfactant concentration up to 0.02M, and above this concentration (i.e., 0.05 or 0.10) the mobility of Tl<sup>+</sup> suddenly decreased ( $R_F = 0.15$ ). Certain metal ions (e.g., Cu<sup>2+</sup>, Bi<sup>3+</sup>, Hg<sup>2+</sup>, and Zn<sup>2+</sup>) showed occasional tailing ( $R_L$ – $R_T > 0.3$ ).

The increase in concentration of TX-100 from 0.001 to 0.1M resulted in minor changes in the mobility of metal ions, which indicated that the various types of concentration-dependent structures formed by surfactant molecules with concomitant change in their aggregates (45) do not effectively influence the mobility of metal ions. At a 0.001–0.02M TX-100 concentration, which is below its CMC value (0.028 M), the mobile phase mainly consisted of monomer surfactant (e.g., no micelles present), whereas at a 0.1M concentration (well above the CMC) the mobile phase was composed of surfactant molecules in the form of monomers and aggregates (or micelles). Below CMC, the amount of adsorbed surfactant onto the stationary phase was considered to increase with the increase in surfactant concentration in the mobile phase, whereas above CMC the activity of the surfactant molecules remained more or less constant and thus the adsorption of surfactant may be considered independent of the surfactant concentration in the mobile phase. However, these different (46,47) characteristics of chromatographic systems could not provide better separation possibilities of metal ions.

#### Effect of acidity and basicity of the medium

Thin-layer chromatography of metal ions was performed using 0.02 and 0.1M TX-100 solutions prepared in phosphateborate buffer solutions of different pH values (pH 2.3–11.9). The results obtained with buffered surfactant solutions (0.02 and 0.1M) and buffered solutions (without surfactant) were compared in Figure 1. It was apparent from Figure 1 that the surfactant in buffered solutions plays an important role in influencing the mobility of metal ions. In general, the mobility (or  $R_F$  value) of metal ions was in the order of: 0.02M buffered surfactant monomers > buffer solutions > 0.1M buffered micellar solution. This trend shows that the mobility of metal ions was simultaneously controlled by the pH of the medium, surfactant monomers, and the micelles of the surfactant. However, better separation possibilities were at pH 2.3, but certain separations such as Zn<sup>2+</sup>–Cd<sup>2+</sup>, Ni<sup>2+</sup>–Cd<sup>2+</sup>, and Ni<sup>2+</sup>–Cd<sup>2+</sup>–Zn<sup>2+</sup> were only possible at pH 11.9. The important separations realized experimentally using different mobile phases are summarized in Table II.

#### Effect of alkanols

The effect of an alkanol chain length on the mobility of metal ions was examined using mobile phases consisting of 0.1M TX-100 (pH 2.3) and various alkanols (methanol, ethanol, propanol, butanol, or pentanol) in a 95:5 ratio (v/v). The results shown in Figure 2 indicate that the mobility of metal ions was minimum with propanol. The organic solvents (i.e., alcohols) modified the retention mechanism and controlled the relative mobility of metal ions. The added alcohols can affect the CMC, the aggregation number, and the actual strength of the micelle assembly. There was a general trend of decrease in mobility, giving minimum value with propanol.

Upon increasing the added methanol concentration from 5% to 20%, the following trends in the mobility of metal ions were observed. A sharp decrease in the  $R_{\rm F}$  values of  $Ni^{2+}$  and  $Co^{2+}$  from 0.8 to 0.5 and a sharp increase in  $R_{\rm F}$  values of  $Cu^{2+}$  and  $Zn^{2+}$  from 0.1 to 0.4 were observed on increasing the concentration of added methanol from 5% to 10%. Upon further increasing the alcohol from 10% to 20%, no change in the  $R_{\rm F}$  value of all the four cations was noticed. The mobility of Tl<sup>+</sup> ( $R_{\rm F}$  = 0.4),  $Bi^{3+}$  ( $R_{\rm F}$  = 0.2), and Fe<sup>3+</sup> ( $R_{\rm F}$  = 0.1) remained unaltered irrespective of the concentration of added methanol (5–20%).

The effect of the equimolar concentration of different alcohols in aqueous 0.1M TX-100 at pH 2.3 was also examined on the mobility of metal ions in order to determine whether the variation in  $R_F$  at 5% added alcohols was a result of the variable concentrations of alcohols or was the result of different polarities of alcohols. When metal ions were chromatographed using 1.0M alcohols in aqueous 0.1M TX-100 (pH 2.3) (such as methanol, ethanol, propanol, butanol, and pentanol) all the metal ions showed unaltered mobility irrespective of the type of alcohol present in the mobile phase. Thus, all five alcohols had the same effect at 1.0M, but the  $R_F$  values in alcohol were different than those in  $H_2O$ .

#### Effect of urea and electrolyte additive

The effect of urea (organic nonelectrolyte) and NaCl



(inorganic electrolyte) at different concentration levels in fixed surfactant molarity (0.1M TX-100, pH 2.3) on the mobility of metal ions was examined. The results summarized in Figure 3 show that the mobility of Fe remained almost unaffected at all concentration levels of urea and NaCl, whereas the mobility of Cu<sup>2+</sup> successively decreased with the increase in NaCl concentration, thus showing a salting-out effect as observed by earlier workers during the TLC separation of metal complexes with nonmicellar mobile phases (48). However, the mobility of  $Cu^{2+}$  in the presence of urea remains almost unchanged. With NaCl, the mobility of Tl<sup>+</sup> showed a sharp increase at 0.5M NaCl after maintaining an R<sub>F</sub> value constancy in the concentration range of 0.01 to 0.3M. With other metal ions, the mobility (or  $R_{\rm F}$ ) decreased with an increase in the concentration of urea or NaCl in the mobile phase passing through a minima at the 0.1 or 0.3M concentration level followed by an increase in mobility at a higher concentration of added NaCl–urea.

These interesting observations reflect the impact of added electrolyte and nonelectrolyte solutes in controlling the retention behavior of metal ions. The added electrolytes modify the





solute-micelle or solute-stationary phase interactions by bringing about a change in the microenvironment of the micellar system and thus influencing the retention behavior of metal ions.

In order to bring out a clearer picture regarding the effect of urea and NaCl on the selectivity of silica gel layers towards metal ions, the results obtained in the presence of these additives were compared with those obtained in their absence. The R<sub>F</sub> values  $[(R_F \text{ on silica layer developed with } 0.1M \text{ TX-100 (pH 2.3)} - R_F \text{ on}]$ silica layer developed with 0.1M TX-100 (pH 2.3) + 0.3M urea or NaCl (1:1, v/w) were plotted in Figure 4, which demonstrates how the selectivity of silica was altered by the presence of additives in the micellar mobile phase. Positive R<sub>F</sub> values show that the silica layer was more selective for Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, and Tl+ when a urea containing micellar mobile phase was used. Conversely, the silica layer was less selective (negative  $R_F$  value) for Bi<sup>2+</sup>, Fe<sup>3+</sup>, and Cu<sup>2+</sup>. When a NaCl containing micellar mobile phase was used, the silica layer showed greater selectivity towards Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Tl<sup>+</sup>, and Bi<sup>3+</sup> (positive  $R_F$  value) but poor selectivity towards Ni<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup> as indicative of negative R<sub>F</sub> values. The reverse trend in the selectivity of the

> silica layer towards metal ions in urea and NaCl containing micellar mobile phases may be attributed to the nature of the added compounds. Urea (an organic molecular substance) was assumed to be present entirely in the aqueous phase (49), whereas NaCl (an electrolyte) was capable of moderating the attractive interactions resulting in the modified selectivity (44). The effectiveness of NaCl in extracting water from the homogeneous phase in microemulsion systems has been well-documented (50–51).

## Application

The proposed method was applied to detect Zn<sup>2+</sup> and Cd<sup>2+</sup> from their mixture in a spiked sample of human blood with preliminary separation on a silica gel layer. The amount of both Zn<sup>2+</sup> and Cd<sup>2+</sup> as low as 3.3 µg can be clearly detected on TLC plates after separation from their mixture. The  $R_F$  values for  $Zn^{2+}$  and  $Cd^{2+}$  were 0.10 and 0.4, respectively. The separation of  $Cd^{2+}$  in the presence of  $Zn^{2+}$  is biologically important because in some metalloenzymes Zn<sup>2+</sup> is substituted by Cd<sup>2+</sup> thus leading to cadmium toxicity. Similarly, the detection of Cd<sup>2+</sup> assumes significance because of its tendency to react with sulfhydryl groups of essential enzymes.

# Conclusion

The usefulness of TX-100 nonionic surfactant solutions as mobile phases in the normal-phase TLC of heavy-metal cations has been examined. The surfactant concentration from 0.001 to 0.1M resulted in minor changes in the mobility of individual metal ions. In order to achieve effective separations, the surfactant must be used in buffer solutions. The optimum experimental conditions for separating various metal ions include the use of a 0.02M buffered surfactant solution (pH 2.3 and 11.9) as the mobile phase and silica gel G as the stationary phase. TX-100 at a concentration level of 0.02M, which was below its CMC value (0.028M), appeared to be coated on the stationary phase. The modification of the 0.1M TX-100 buffered solution (pH 2.3) with added alcohols, urea, or NaCl resulted in minor changes in mobility for most of the metal ions.

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