

SILICA GEL THIN-LAYER CHROMATOGRAPHIC SEPARATION OF CETYLPYRIDINIUM CHLORIDE (CPC) FROM POLYOXYETHYLENE (20) SORBITAN MONOLAURATE (TWEEN-20)

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SUMMARY

Thin-layer chromatography of eleven cationic and non-ionic surfactants has been performed on silica gel layers with tetrahydrofuran (THF)-containing aqueous mobile phases. The mobile phase tetrahydrofuran–water 6:4 was identified as best for mutual separation of cetylpyridinium chloride (CPC) and polyoxyethylene (20) sorbitan monolaurate (Tween-20). The effect of the nature of the adsorbent (silica gel, alumina, or kieselguhr) on the mobility of the surfactants was examined and the comparative efficiency of each adsorbent was evaluated. The effect of metal cations as foreign substances on the mutual separation of CPC and Tween-20 was also examined. Limits of detection were determined for CPC and Tween-20 and semi quantitative determination of CPC was also attempted.

INTRODUCTION

Surfactants, which are amphipathic compounds, have many applications, for example in the chemical, pharmaceutical, and food industries, and in hospitals, homes, and other environments, for cleaning, emulsification, solubilization, and moisturization [1]. Surfactant mixtures are used in a variety of industrial processes, for example in synthetic detergents, in drug design, in waste-water purification, and in oil production [2–4]. Cationic and non-ionic surfactants are known to affect biological and chemical processes. For example, cationic surfactants, including quaternary ammonium compounds, have antimicrobial activity [1,5] and are known to disrupt cell membranes [6], interrupt protein function [7], release intracellular K⁺ and other constituents [8,9], and induce cell autolysis [10,11]. Non-ionic surfactants promote penetration of hydrocortisone through skin [12], enhance

the fluorescence of metal chelates [13], prevent adsorption of bacteria by hydrophobic surfaces [14,15], and interact with phospholipids [16]. The biological activity of non-ionic surfactants depends on the physicochemical character of the hydrophobic moiety and on the number of hydrophilic ethylene oxide groups [17,18].

Numerous chemical and physicochemical methods have been developed for qualitative and quantitative analysis of surfactants; the most widespread are use of microbial sensors [19] and amperometric biosensors [20], ion-pair formation with in-situ flow-injection analysis using dynamic surface tension detection [21], use of ion-selective electrodes [22], ion-pair chromatography with suppressed conductivity detection [23], spectrophotometry [24–31], capillary electrophoresis [32,33], solid-phase extraction with surface-assisted laser desorption/ionization spectrometry [34], electrospray ionization mass spectrometry with flow-injection analysis [35], liquid chromatography combined with atmospheric-pressure ionization mass spectrometry [36], and flow-injection analysis combined with atmospheric-pressure ionization-mass spectrometry [37]. All these methods require use of sophisticated instruments and expertise, and development of simple, rapid, and selective methods for identification, separation and determination of surfactants is still a challenge to chemists.

In this paper a simple and rapid thin-layer chromatographic (TLC) method for analysis of cationic and non-ionic surfactants is reported. The method can be used to identify the types of surfactant present in different aqueous systems. We have successfully separated CPC (a cationic surfactant) from non-ionic surfactants, including Tween-20. Mutual separation of CPC and Tween-20 is important because of their wide applicability in many environmentally benign systems [38–41].

EXPERIMENTAL

All experiments were performed at room temperature ($30 \pm 2^\circ\text{C}$).

Chemicals and Reagents

All chemicals were analytical reagent grade. Sodium chloride, methanol, and tetrahydrofuran (THF, 0.89 kg L^{-1}) were from Merck, India.

The surfactants studied were Triton-X (Tx-100), Brij-35 (Bj-35), Tween-20 (Tw-20), Cween-20 (Cw-20), Cween-40 (Cw-40), Cween-60 (Cw-60), cetylpyridinium chloride (CPC), cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), hexadecyl-

trimethylammonium chloride (HDTAC), and dodecyltrimethylammonium bromide (DTAB). Solutions (1%, i.e. 1 g/100 mL) of the surfactants were prepared in methanol.

The metal ion solutions studied, as their 1.0% aqueous solutions, were the nitrates of Zn^{2+} , Al^{3+} , Pb^{2+} , Tl^{3+} , and UO_2^{2+} , the chlorides of Ni^{2+} , Hg^{2+} , and Co^{2+} , the sulphates of Cu^{2+} and Fe^{2+} , and $K_2Cr_2O_7$.

Chromatography

Thin layer chromatography was performed on plates coated with silica gel G (Merck, India; code S₁), alumina (CDH, India; S₂), and kieselguhr (CDH; S₃).

Preparation of TLC Plates

TLC plates were prepared by mixing the adsorbent with double-distilled water in the ratio 1:3. The resulting slurry was mechanically shaken for 5 min then coated on 20 cm × 3.5 cm glass plates to give layers 0.25 mm thick, by means of a TLC applicator. The plates were first dried in air at room temperature then activated by heating at 100°C for 1 h. After activation, the plates were kept in an air-tight chamber until used.

Procedure

The surfactant solutions (10 µL) were spotted on TLC plates by means of a micropipette and the spots were left to dry at room temperature. Chromatography was performed in 24 cm × 6 cm glass jars, with lids, previously equilibrated with mobile phase for 10 min. The plates were developed to a distance of 10 cm from the origin with the mobile phases listed in Table I. The development time was 25–30 min.

After development, all surfactant spots were detected by use of modified Drangendorff's reagent, which was prepared from two solutions, A and B. Solution A was prepared from two solutions, a solution of bismuth subnitrate ($BiONO_3 \cdot H_2O$, 1.7 g) in acetic acid (20 mL) diluted to 100 mL with water, and a solution of potassium iodide (65 g in 200 mL water). These solutions were transferred to a 1-L volumetric flask, acetic acid (200 mL) was added, and the contents were diluted to one litre with water. Solution B was prepared by dissolving barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$, 290 g) in 1 L water. Solutions A and B were mixed in the volume ratio 2:1 and the resulting mixture was applied to the plates by means of a glass sprayer.

Table I

The mobile phases used

Code	Components ^a	Composition
M ₁	THF	
M ₂	THF–DDW	1 : 9
M ₃	THF–DDW	2 : 8
M ₄	THF–DDW	3 : 7
M ₅	THF–DDW	4 : 6
M ₆	THF–DDW	5 : 5
M ₇	THF–DDW	6 : 4
M ₈	THF–DDW	7 : 3
M ₉	THF–DDW	8 : 2
M ₁₀	THF–DDW	9 : 1

^a THF, tetrahydrofuran; DDW, double distilled water

For separation of mixtures of surfactants, equal volumes of the surfactant solutions were mixed and 10 μ L of the resulting mixture was applied to a TLC plate (S₁). The plate was developed with mobile phase M₇, the spots were detected, and R_F values of the surfactant spots were calculated.

To study of effect of nature of the adsorbent layer (silica gel G, alumina, or kieselguhr) on mobility, the surfactants were chromatographed by use of mobile phase M₇ and the plates were compared.

For separation of microgram quantities of Tween-20 from milligram quantities of CPC, a TLC plate was first spotted several times with 10 μ L Tween-20 solution (100 μ g). After complete drying of the spots CPC standard solutions (10 μ L containing 0.1–0.77 mg) were spotted at the same positions on the plate. Similarly, for separation of microgram quantities of CPC from milligram quantities of Tween-20, a TLC plate was first spotted several times with 10 μ L CPC solution (100 μ g). Tween-20 standard solutions (10 μ L containing 0.1–1.0 mg) were spotted at the same positions on the plate. The spots were dried and the plates were developed with M₇. The separated spots were visualized with Drangendorff's reagent, and the R_F values of the separated surfactants were calculated.

To study the effect of the presence of metal cations, as impurities, on the separation of the surfactants, standard solutions of CPC and Tween-20 (10 μ L) were spotted several times on the same TLC plate (S₁) followed by spotting, at the same positions, of 10 μ L of the cation being consi-

dered as impurity. The plates were developed with M₇, the spots were detected, and the R_F values of the separated surfactants were calculated.

Limits of detection for CPC and Tween-20 were determined by spotting different amounts of the surfactants on the TLC plates, developing the plates, and detecting the spots. The method was repeated with successive reduction of the amounts of CPC and Tween-20 applied until no spot was detected. The minimum amount of surfactant detectable on the TLC plate was taken as the limit of detection.

For semi-quantitative analysis by measurement of spot-area, a series of standard solutions (1–5%, 10 μ L) of CPC was spotted on silica gel layers. The plates were developed with M₇. After detection, the spots were copied on to tracing paper from the plates and the area of each spot was calculated.

RESULTS AND DISCUSSION

Chromatography of the surfactants was tested with ten mobile phases. With 100% THF almost all the surfactants migrated as badly tailing spots ($R_L - R_T \geq 0.3$, where R_L is the R_F value of the leading edge of the spot and R_T is the R_F value of the trailing edge of the spot). HDTAC was an exception; a well formed compact spot was obtained near the point of application ($R_F = 0.11$). With mobile phases prepared from THF–water mix-

Table II

R_F values of surfactants on silica gel layers developed with different mobile phases

Surfactant	Mobile phase									
	M ₁	M ₂	M ₃	M ₄	M ₅	M ₆	M ₇	M ₈	M ₉	M ₁₀
Triton-X 100	0.45T	0.15T	0.42	0.58	0.72	0.76	0.87	0.87	0.88	0.84
Brij-35	0.43T	0.36T	0.61T	0.57T	0.77	0.80	0.81	0.85T	0.68T	0.30T
Tween-20	0.45T	0.12	0.40T	0.65T	0.76	0.80	0.86	0.82	0.75T	0.32T
Cween-20	0.50T	0.32T	0.40T	0.40T	0.57T	0.67T	0.77T	0.79T	0.77T	0.50T
Cween-40	0.45T	0.31T	0.40T	0.41T	0.42T	0.60T	0.77T	0.68T	0.65T	0.50T
Cween-60	0.23T	0.12	0.37T	0.60T	0.72	0.81	0.81	0.81	0.81	0.30
CPC	0.20T	0.00	0.00	0.03	0.05	0.06	0.07	0.10	0.12	0.07
CTAB	0.45T	0.00	0.00	0.03	0.05	0.08	0.07	0.12	0.14	0.08
TTAB	0.12T	0.00	0.00	0.05	0.05	0.07	0.12	0.10	0.11	0.08
HDTAC	0.11	0.00	0.00	0.05	0.05	0.12	0.11	0.17	0.16	0.10
DTAB	0.25T	0.07	0.07	0.12	0.06	0.08	0.08	0.12	0.12	0.10

T indicates a tailing spot for which $R_L - R_T \geq 0.3$, where R_L is the R_F value of the leading edge of the spot and R_T is the R_F value of the trailing edge of the spot

tures in different volume ratios (M_2 – M_{10}) differential migration of the surfactants was observed. The results are presented in Table II.

From the data presented in Table II it is evident that for low concentrations of THF (up to 30%) almost all the cationic surfactants except DTAB remained at the point of application and all the non-ionic surfactants except Triton-X 100 migrated as tailing spots ($R_L - R_T \geq 0.3$). When mobile phases containing 40–70% THF were used (i.e. M_7 – M_{10}) most of the surfactants (cationic and non-ionic) migrated as compact spots; this range of concentrations of THF is therefore useful for separation of cationic from non-ionic surfactants.

The low mobility of cationic surfactants may be attributed to their strong electrostatic attraction to negative silanol groups on the silica gel surface whereas the non-ionic surfactants are adsorbed on the silica surface by hydrogen bonding [42,43]. A plot of R_F against volume fraction of THF in the mobile phase (Fig. 1) shows that the best separation of CPC from Tween-20 is achieved by use of the mobile phase containing 0.06 mole fraction of THF (0.94 mole fraction of water). Mobile phase M_7 (THF–DDW, 6:4) was therefore selected for detailed study.

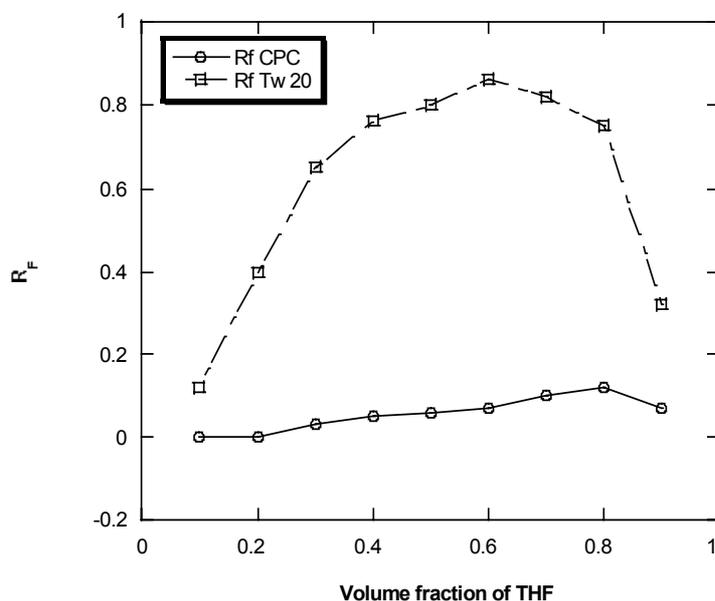


Fig. 1

Plot of R_F against volume fraction of THF for mutual separation of CPC from Tween-20

The effect of the nature of different adsorbents (silica gel, alumina, and kieselguhr) on the mobility of the surfactants was examined using M_7 as mobile phase. From the results presented in Fig. 2 three trends are apparent:

1. The mobility of the surfactants decreased in the order alumina > kieselguhr > silica gel
2. The best separation of each cationic surfactant from all the non-ionic surfactants was obtained on silica layers. Because silica is a weakly acidic cation exchanger ($\text{Si-O-H} \rightleftharpoons \text{Si-O}^- \text{H}^+$) exchange of the cationic surfactants on its surface is possible and they are strongly retained near the point of application. The uncharged non-ionic surfactants, however, migrate with the solvent front. This enables separation of the cationic surfactants from the non-ionic compounds.
3. Separation efficiency decreases in the order: silica gel > kieselguhr > alumina. As expected, worse separations were obtained on both alumina and kieselguhr because they are not cation exchangers.

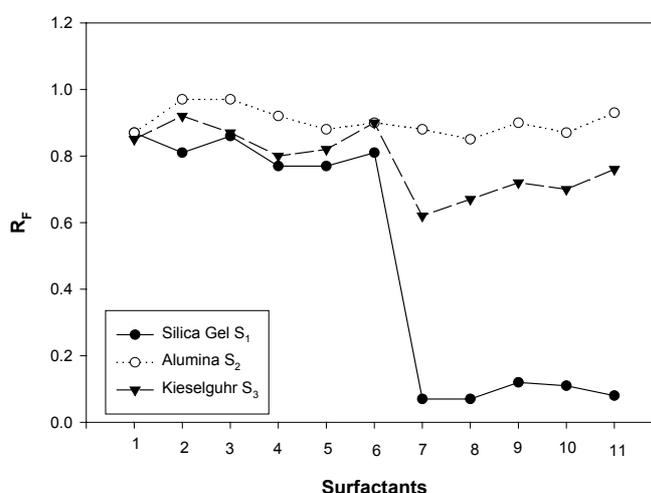


Fig. 2

Effect of the nature of the stationary phase on the mobility of surfactants using M_7 as mobile phase. 1. Triton-X100, 2. Brij-35, 3. Tween-20, 4. Cween-20, 5. Cween-40, 6. Cween-60 (non-ionic surfactants), 7. CPC, 8. CTAB, 9. TTAB, 10. HDTAC, 11. DTAB (cationic surfactants)

It was observed that on silica gel 100 μg Tween-20 could easily be separated from 0.7 mg CPC and that 100 μg CPC could easily be separated from 1.0 mg Tween-20, i.e. this TLC system is capable of separating

microgram quantities of cationic surfactants from milligram quantities of non-ionic surfactants and vice versa.

Results showing the effect of different metal cations as impurities on the separation efficiency of CPC from Tween-20 are presented in Fig. 3.

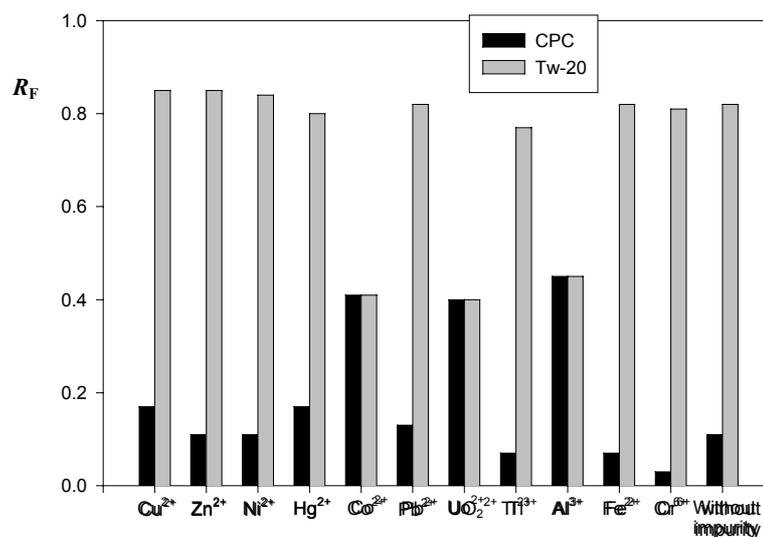


Fig. 3

Separation of CPC from Tween-20 on silica layers, with M_7 as mobile phase, in the presence of metal cations as impurities

It is clear from this figure that separation of CPC from Tween-20 is hampered by the presence of Co^{2+} , UO_2^{2+} , and Al^{3+} , because of co-migration of the surfactants in the presence of these cations. Conversely, separation of CPC from Tween-20 is improved by the presence of Cr^{6+} , Fe^{2+} , and Tl^{3+} , because of reduction of the mobility of CPC. In the presence of the other metal cations (Cu^{2+} , Zn^{2+} , Ni^{2+} , Hg^{2+} , and Pb^{2+}), separation of CPC from Tween-20 was always possible. Thus some metallic impurities in the surfactants have a detrimental effect on chromatographic performance.

The lowest concentration detected after successive dilution was $3 \mu\text{g}/10 \mu\text{L}$ for both CPC and Tween-20. This amount was taken as the detection limit, and indicates the method is a highly sensitive means of detection of cationic and non-ionic surfactants.

In addition to qualitative analysis, quantitative analysis of surfactants is often required. We attempted semi-quantitative determination of the surfactants by measurement of spot area. A linear relationship was obtained between amount of CPC spotted (in the range 100–500 μg) and the area of the spot. The correlation coefficient, r , was 0.980, indicative of high positive correlation. At higher concentrations, a negative deviation from linearity was observed.

Application

To widen the applicability of the method, separation of CPC and Tween-20 from a variety of water samples was investigated. The results presented in Table III show that CPC and Tween-20 can be easily identified in a variety of water samples after separation on S_1 with M_7 as mobile phase.

Table III

Identification and separation of CPC and Tween-20 in water samples

Sample	Separation	
	CPC	Tween-20
DDW	0.14	0.89
Tap water	0.20	0.88
Saline water	0.21	0.81
River water	0.15	0.87

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