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Mixed Surfactants in Acetonitrile-Water Mixtures for the Separation of Organic Compounds by HPLC

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The effect of the addition of surfactants (50 nM, *i.e.*, above cmc) as organic modifiers into a solution containing 50% acetonitrile (+ 50% water) at neutral pH (7.0) for separating 17 organic compounds has been studied experimentally. The results have shown that the capacity factors, k′, of the organic compounds are sharply dependent on the types and the number of surfactants used. Surfactants (single, binary or ternary) have shown to provide dramatic reduction in the capacity factors of the organic compounds studied. Binary additives have lowered the capacity factor more than single additive. The use of binary mixed surfactants comprised of anionic (dioctylsulfosuccinate) (DOSS) and cationic (cetyltrimethylammonium) (CTAB) surfactants result in greater lowering in the capacity factor more than the use of other binary additives. Ternary additives lowered the capacity factor than the binary additives. A ternary mixture of surfactants from anionic (dicetylsulfosuccinate) (DOSS), cationic (cetyltrimethylammonium) (CTAB) and nonionic (poly oxyethylene) (23) dodecanol) (Brij-35) appeared to be more effective in lowering the capacity factor than the other ternary additives systems.

INTRODUCTION

Reversed phase high-performance liquid chromatography (RP-HPLC) has been the most popular HPLC technique for the separation of a broad range of organic analytes with significantly different hydrophobicity [1]. In the past decade it has been shown that incorporation of additive to the mobile phase can lead to impressive separation in capillary electrophoresis, ion-pair chromatography and micellar liquid chromatography[2–5]. Rodgers *et al.*[6] have reported that a major constraint of the silica bonded phase in HPLC is the limited pH range between 2.5 and 7.5. This is troublesome in the separation of poorly retained strong organic acids and bases whose dissociation constants are either outside or close to the boundaries of this range. In such cases, introduction of electrostatic interaction through the addition of a small amount of moderately hydrophobic surfactant to the eluent provides adequate retention and selectivity.

Because of the lipophilic character of the micelles, usually applied in aqueous solvents, different compounds can be separated, even if they are almost insoluble in water. Micellar liquid chromatography (MLC) is also capable of separating charged and uncharged compounds. In MLC, a longer chain surfactant is used at higher concentration (above the critical micelle concentration, cmc) to ensure the formation of micellar aggregates. Micellar aggregates have found a wide range of use in separation techniques. For example, poly (oxyethylene) (23) dodecanol (Brij-35) has been demonstrated in previous studies to be useful as pseudo-stationary phase in micellar electrokinetic capillary chromatography (MEKC)[7]. Initial studies of this micellar system showed particular promise in altering the selectivity factor of benzene and benzaldehyde which are inseparable in sodium dodecyl sulfate (SDS) but were easily separated using a Brij-35/ SDS mixed micellar systems[8]. Over the years, MEKC has been developed into one of the most successful electrophoretic separation techniques. The addition of a micelle-forming surfactant to the mobile phase has even become routine for the separation of partly ionized compounds to give additional selectivity in the separation. The most commonly used micellar system in MEKC is SDS due to its low cost, availability in high purity, UV absorption characteristics, and intrinsic micellar properties. Several methods have been proposed to expand the application range of MEKC to various organic compounds: the use of surfactants other than anionic SDS such as cationic and nonionic, or the use of mixed surfactants system. Also, a frequently applied procedure is to change the solvent strength of the aqueous phase by addition of organic modifiers such as urea, cyclodextrins, methanol, acetonitrile, or chloric acid and its derivatives[9–13].

To the best of our knowledge, there has not been any study on the use of mixed surfactants in the separation of organic compounds by reversed phase high performance liquid chromatography. The work reported herein concerns the use of mobile phases containing various single, binary and ternary mixed surfactant additives for separating various analyte mixtures containing 17 organic compounds.

EXPERIMENTAL

All chromatographic experiments were carried out on a High-Performance Liquid Chromatography (Perkin-Elmer) consisting of an HPLC pump (Series 200 LC pump, Perkin-Elmer), an automated gradient controller and a variable-wavelength UV detector (Model 785A Programmable Absorbance Detector, Perkin-Elmer) at 254 nm. The column was high-speed cartridge column 5 cm in length (4.6 mm i.d.) C_{18} reversed-phase packing. The column was used at ambient temperature. Sample injector was Rheodyne 7725I (stainless steel) supplied with a 20 μL loop (Perkin-Elmer).

HPLC grade acetonitrile, alkylbenzenes, polyaromatic hydrocarbons, and alkylphenols were used as obtained from Merck. Nonionic surfactants (polyoxyethylene) (23) dodecanol (Brij-35) and polyoxyethylene tert-octylphenol (Triton X-100), anionic surfactants (sodium dodecyl sulfate) (SDS) and dioctyl sulfosuccinate (DOSS), cationic surfactants (cetyltrimethylammonium) (CTAB), tetraheptylammonium (THPA), and trioctylmethylammonium (TOMA) were purchased from Merck and were used as received.

Separation Condition

Before each experiment, the column was rinsed with mobile phase containing no additives, *i.e.*, 50–60% acetonitrile. The mobile phase was prepared by combination of surfactant solution/acetonitrile/doubly distilled water. All running mobile phases were adjusted to pH 7.0 and were filtered through 0.45 μm membrane prior to use. All measurements were performed at 25°C. A flow rate of 1.0 mL/min was selected for all experiments. Mobile phase delivery was accomplished with a syringe pump at 2000 psi. Injection valve of 20 μL was used. Detection of eluting components was done with UV/Vis absorption detector at 254 nm. Capacity factor, k′, was calculated according to the expression $k' = (t_R - t_M)/ t_M$. The system dead time, t_M, used to calculate capacity factor, k′, was measured by injecting nitrite solution into the system.

RESULTS AND DISCUSSION

Effect of Single Surfactant Additive on the Separation

As with reversed-phase HPLC, solutes can be separated using single surfactant in acetonitrile-water mixture on the basis of the difference in their hydrophobic character. Resolutions were optimal when solutes have relatively small k′ values (typically less than 5). The resolution of hydrophobic molecules is improved due to a reduction in k′ values after addition of almost any single surfactant. The capacity factors, k′, of 17 organic compounds using 50 mM single surfactant in 50% acetonitrile at flow rate of 1.0 mL/min are shown in Table-1. The capacity factor in column 1 demonstrates the analytical separation of the organic compounds without the addition of surfactants. Generally, the effect of single surfactants additive in lowering the k′ values increase in the order SDS < DOSS < CTAB < THPA < TOMA < Triton X-100 < Brij-35.

Figure 1 illustrates a typical 45 min separation of 17 organic compounds using Brij-35, the most effective single surfactant in lowering the capacity factor. The result has shown that in almost all the chromatograms with single surfactant additive peaks 12 and 13 are not resolved and peaks 14, 15, 16 and 17 have very long retention times.

It is interesting to note from Table-1 that the capacity factors of large hydrophobic analytes were reduced by larger percentage than those of smaller analytes. The chemical compositions of the hydrophobic tails and head groups in surfactants significantly influence their interaction with analytes as well as with C_{18} surface of the stationary phase. In other words, the physico-chemical and structural properties of the surfactant molecules have a major influence on the capacity factor, k′. Analytes' interactions with micelles occur through various mechanisms such as surface adsorption, comicellization or partitioning into the hydrophobic core of the micelles. The investigated surfactants concentration range is above the cmc in aqueous solution (50 mM). This suggests that the mechanism of surfactant action involves the virtually complete absorption and migration of the analytes into the micellar interior phase. Although the nucleus of the micelles is strongly hydrophobic, even rather polar compounds are absorbed to some extent and can be separated. On the other hand, ion pair interaction of the surfactant with analytes in the mobile phase is also possible. This is primarily because of the high percentage of acetonitrile in the mobile phase. It has been reported previously that large organic modifier concentration disrupts micellization in capillary electrophoresis[14]. The possibility that the micelles can tolerate higher concentration of organic modifier is also possible and has been reported in literature[10].

The results have shown that Brij-35 was generally the most effective surfactant in lowering the capacity factor. This is caused by the presence of long chain alkyl group that has a repeating chain of ethoxy group terminating in a hydroxyl group. These two groups enhance the ability of Brij-35 to form association complexes in solution and thus lower the capacity factor.

Effect of Binary Surfactants Additives on the Separation

Anionic-Cationic Additives Pairs. Addition of binary surfactants at concentration of 50 mM (25 mM from each) to the 50% acetonitrile mobile phase was investigated. In each case the binary additives resulted in lowering the capacity factor more than those observed for single additive. Figure 2 shows a chromatographic separation of 17 organic compounds with the use of 25 mM

DOSS + 25 mM CTAB mixed additives in 50% acetonitrile, the most effective two additives in lowering the k' values. Now, in all the binary additives peaks 12 and 13 are well resolved and the retention time of the peaks 15, 16, and 17 are much shorted. The capacity factors of the 17 organic compounds in several two additives are summarized in Table 2. Figures 3, 4, and 5 show a typical chromatograms of anionic-cationic additives of TOMA + DOSS, THPA + SDS, and THPA + DOSS, respectively. The results has shown that the use of anionic-cationic binary surfactants additives has resulted in very favorable synergistic effect, increase in the order THPA + SDS < TOMA + DOSS < THPA + DOSS < CTAB + DOSS.

TABLE-1

EFFECT OF A SINGLE SURFACTANT ADDITIVE (50 MM EACH) ON CAPACITY FACTORS (k′) OF ORGANIC COMPOUNDS[a]

Compound	No additive	SDS	DOSS	CTAB	THPA	TOMA	Triton X-100	Brij-35
Benzene	2.52	1.99	1.98	1.89	1.80	1.75	1.62	1.53
Toluene	3.73	3.24	3.15	2.84	2.71	2.66	2.44	2.25
Ethylbenzene	5.39	4.25	4.15	4.03	3.83	3.69	3.24	3.09
Propylbenzene	8.45	6.95	6.85	6.27	5.98	5.34	5.08	4.85
Butylbenzene	13.51	10.99	10.89	9.89	9.81	9.04	7.98	7.35
Naphthalene	5.12	4.58	4.29	3.87	3.65	3.46	3.26	3.04
Pyrene	16.34	15.26	15.04	16.34	14.37	14.24	11.89	11.62
Chrysene	24.68	23.45	21.46	16.80	20.09	15.87	12.35	11.64
Perylene	39.07	29.00	24.86	19.78	17.96	17.86	17.31	17.00
Phenol	0.89	0.82	0.78	0.75	0.73	0.70	0.64	0.61
Cresol	1.22	1.18	1.05	1.08	0.98	0.91	0.85	0.82
Ethylphenol	1.53	1.46	1.29	1.38	1.30	1.24	1.17	1.05
Propylphenol	2.31	2.21	2.00	1.92	1.83	1.76	1.65	1.60
Butylphenol	3.21	3.12	2.99	2.81	2.78	2.60	2.46	2.30
Amylphenol	4.95	4.90	4.10	4.06	3.93	3.46	3.45	3.42
Heptylphenol	13.01	12.82	11.45	10.98	10.34	10.06	8.70	7.97

[a] Separation column was Supelcosil LC-18 (50 mm × 4.6 mm). Eluent was 50% acetonitrile-water. Each additive was 50 mM.

The reasons for the observed synergistic effect for the use of anionic-cationic mixed additives are the electrostatic attraction of their head groups and the hydrophobic interaction between their hydrophobic tails. These two types of interactions result in the formation of ion pairs, which result in lowering k''values to a greater extent than either additive alone. On the other hand, the difference in behaviour between different anionic-cationic binary additive systems resulted from their structural differences. For example, CTAB has only one long hydrocarbon chains, whereas TOMA and THPA have three and four long hydrocarbons chains, respectively. As a result, these molecules are much more bulky and can sterically hinder the approaching of other molecules. Thus, SDS or DOSS cannot approach TOMA or THPA effectively and the electrostatic and hydrophobic interactions between them will be less effective than the interactions that contain only one hydrocarbon chain, such as the interactions between SDS and CTAB or DOSS and CTAB

TABLE 2.
EFFECT OF ANIONIC + CATIONIC BINARY ADDITIVE SYSTEMS (25 mM EACH) ON CAPACITY FACTORS (k′) OF ORGANIC COMPOUNDS.

Compound	THAP + SDS	TOMA + DOSS	THPA + DOSS	CTAB + DOSS
Benzene	2.20	1.95	1.78	1.09
Toluene	2.95	2.34	2.02	1.73
Ethylbenzene	3.89	3.24	3.01	2.49
Propylbenzene	5.85	5.24	4.55	3.90
Butylbenzene	8.45	7.65	6.23	5.85
Naphthalene	3.74	3.34	2.97	2.19
Pyrene	11.62	10.12	9.04	7.10
Chrysene	14.64	12.21	10.04	8.48
Perylene	22.00	19.34	17.25	12.79
Phenol	0.65	0.62	0.55	0.45
Cresol	1.15	1.00	0.67	0.65
Ethylphenol	1.35	1.14	1.01	0.76
Propylphenol	2.03	2.08	1.83	1.07
Butylphenol	2.89	2.12	1.87	2.10
Amylphenol	4.22	4.64	3.35	3.08
Heptylphenol	10.97	9.34	7.45	5.00

[a] Separation column was Supelcosil LC-18 (50 mm × 4.6 mm). Eluent was 50% acetonitrile-water containing 25 mM anionic and 25 mM cationic surfactants.

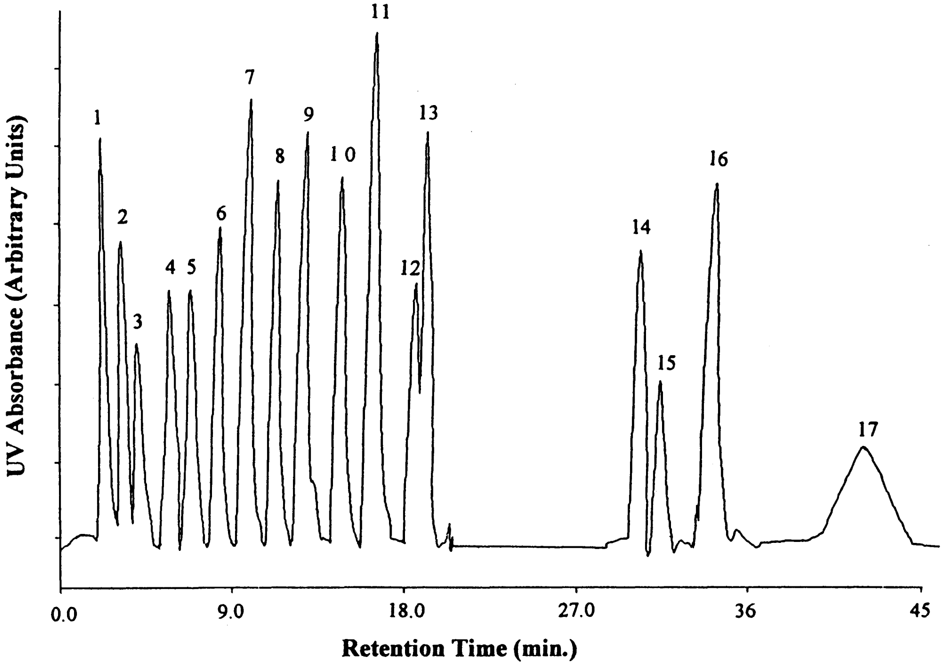

Fig. 1 Chromatographic separation of supelcosil LC-18 (50 mm × 4.6 i.d.) column. Mobile phase 50% acetonitrile-water containing 50 mM Brij-35. Peak identification: (1) phenol, (2) benzene, (3) cresol, (4) toluene, (5) naphthalene, (6) ethyl phenol, (7) propyl phenol, (8) ethyl benzene, (9) butyl phenol, (10) propyl benzene, (11) anthracene, (12) amyl phenol, (13) butyl benzene, (14) pyrene, (15) chysene, (16) heptyl phenol and (17) perylene.

TABLE 2.
EFFECT OF ANIONIC + CATIONIC BINARY ADDITIVE SYSTEMS (25 mM EACH) ON CAPACITY FACTORS (k′) OF ORGANIC COMPOUNDS.

Compound	THAP + SDS	TOMA + DOSS	THPA + DOSS	CTAB + DOSS
Benzene	2.20	1.95	1.78	1.09
Toluene	2.95	2.34	2.02	1.73
Ethylbenzene	3.89	3.24	3.01	2.49
Propylbenzene	5.85	5.24	4.55	3.90
Butylbenzene	8.45	7.65	6.23	5.85
Naphthalene	3.74	3.34	2.97	2.19
Pyrene	11.62	10.12	9.04	7.10
Chrysene	14.64	12.21	10.04	8.48
Perylene	22.00	19.34	17.25	12.79
Phenol	0.65	0.62	0.55	0.45
Cresol	1.15	1.00	0.67	0.65
Ethylphenol	1.35	1.14	1.01	0.76
Propylphenol	2.03	2.08	1.83	1.07
Butylphenol	2.89	2.12	1.87	2.10
Amylphenol	4.22	4.64	3.35	3.08
Heptylphenol	10.97	9.34	7.45	5.00

[a] Separation column was Supelcosil LC-18 (50 mm × 4.6 mm). Eluent was 50% acetonitrile-water containing 25 mM anionic and 25 mM cationic surfactants.

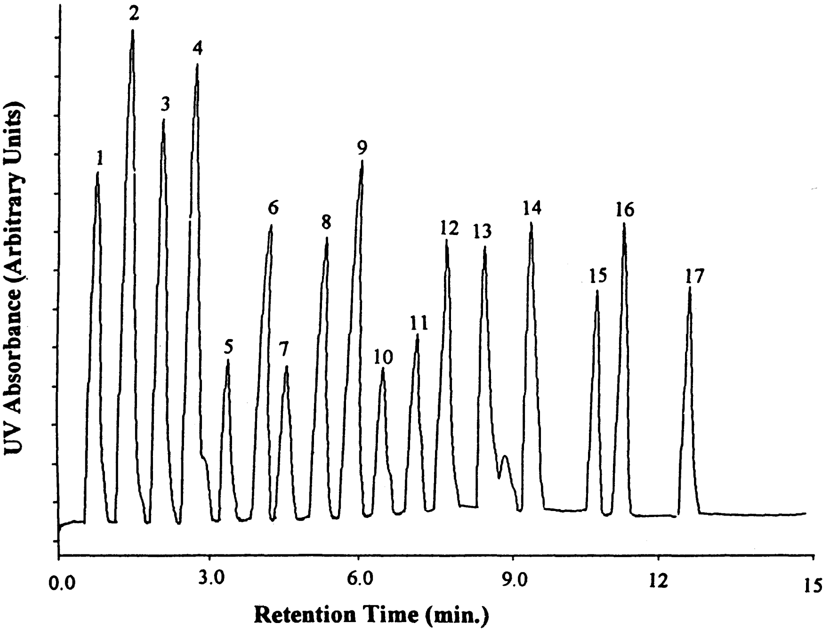

Fig. 2. Chromatographic separation of supelcosil LC–18 (50 mm × 4.6 i.d.) column. Mobile phase 50% acetonitrile-water containing 25 mM DOSS and 25 mM CTAB. Flow rate: 1 mL/min. Detector: UV at 254 nm. Peak identification: (1) phenol, (2) benzene, (3) cresol, (4) toluene, (5) naphthalene, (6) ethyl phenol, (7) propyl phenol, (8) ethyl benzene, (9) butyl phenol, (10) propyl benzene, (11) anthracene, (12) amyl phenol, (13) butyl benzene, (14) pyrene, (15) chysene, (16) heptyl phenol and (17) perylene.

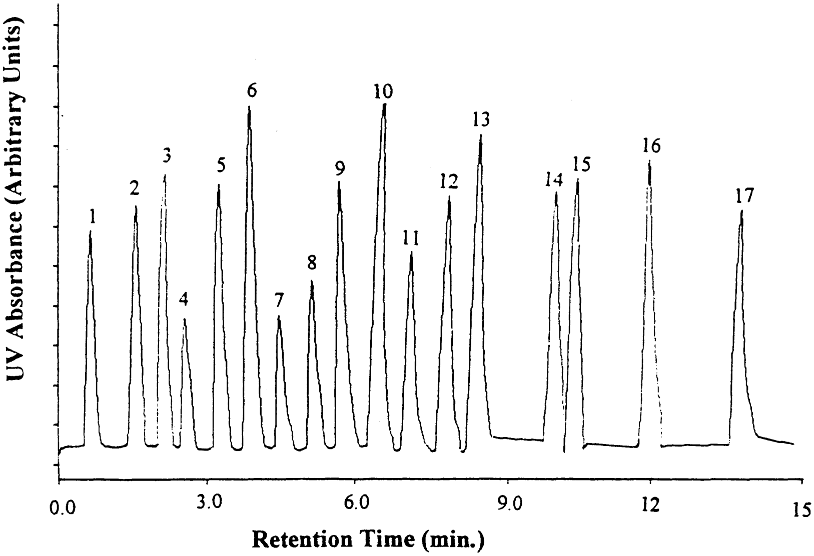

Fig. 3. Chromatographic separation of supelcosil LC-18 (50 mm × 4.6 i.d.) column. Mobile phase 50% acetonitrile – water containing 25 mM TOMA and 25 mM DOSS. Flow rate: 1 mL/min. Detector: UV at 254 nm. Peak identification: (1) phenol, (2) benzene, (3) cresol, (4) toluene, (5) naphthalene, (6) ethyl phenol, (7) propyl phenol, (8) ethyl benzene, (9) butyl phenol, (10) propyl benzene, (11) anthracene, (12) amyl phenol, (13) butyl benzene, (14) pyrene, (15) chysene, (16) heptyl phenol, and (17) perylene.

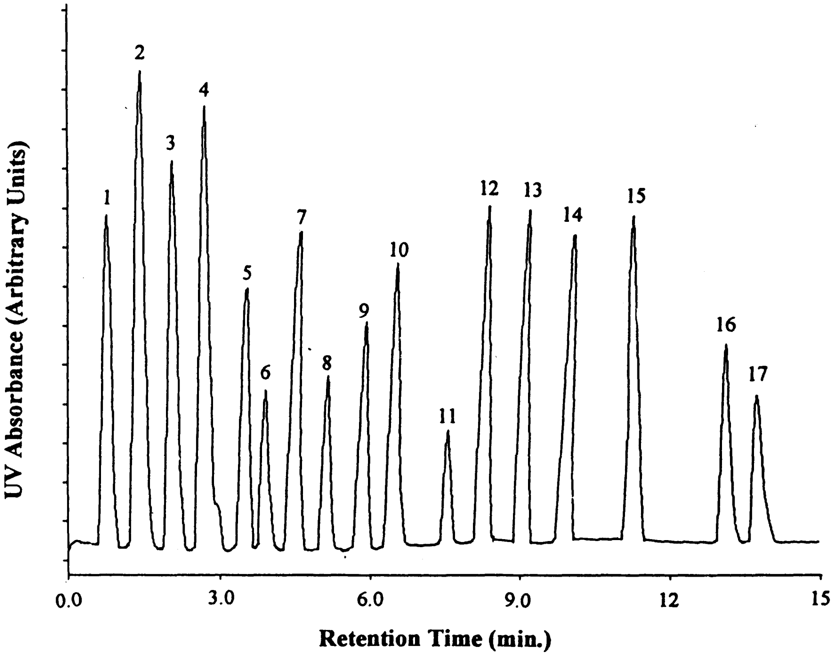

Fig. 4. All the conditions are the same as Fig. 3, except that the eluent was 50% acetonitrile-water containing 25 mM THPA and 25 mM SDS.

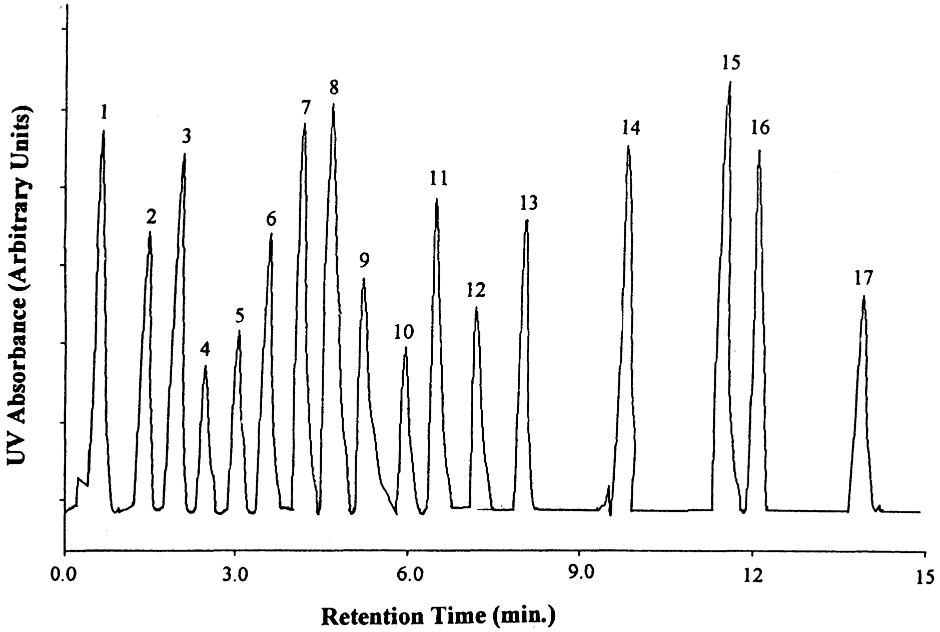

Fig. 5 All the conditions are the same as Figure 3, except that the eluent was 50% acetonitrile – water containing 25 mM THPA and 25 mM DOSS.

TABLE 3.
EFFECT OF ANIONIC + NONIONIC, ANIONIC + ANIONIC, AND NONIONIC + NONIONIC BINARY ADDITIVE SYSTEMS (25 MM EACH SURFACTANT) ON CAPACITY FACTORS (K′) OF ORGANIC COMPOUNDS[A]

Compound	DOSS + SDS	Brij-35 + DOSS	Brij-35 + SDS	Triton X-100 + Brij-35
Benzene	2.7 0	2.23	1.97	1.54
Toluene	3.45	2.54	2.42	2.33
Ethylbenzene	4.89	4.24	4.01	3.09
Propylbenzen	7.91	5.86	4.55	3.90
Butylbenzene	11.13	9.05	7.73	6.88
Naphthalene	4.99	4.84	3.73	3.14
Pyrene	14.62	15.12	14.79	11.10
Chrysene	18.84	14.21	15.14	10.98
Perylene	28.00	17.34	17.25	17.52
Phenol	0.85	0.83	0.82	0.76
Cresol	1.25	1.14	1.07	0.85
Ethylphenol	1.68	1.52	1.23	0.86
Propylpheno	2.67	2.77	2.93	1.85
Butylphenol	3.89	3.12	2.87	2.10
Amylphenol	4.42	3.64	3.35	3.18
Heptylphenol	13.99	10.34	10.45	8.00

[a] Experimental conditions are the same as in Table-2.

(b) *Effect of Other Binary Additive Pairs:*. In addition to the systems containing anionic and cationic additives, those containing anionic-nonionic, anionic-anionic, and nonionic-nonionic additives were also studied. All calculated capacity factors for these systems are listed in Table 3.

Unfortunately, no significant synergistic effect was observed for any of these systems. As shown in Table 3, the resulting capacity factors for these systems are close to the system containing only the stronger one of the two additives or intermediate between them. This result can be explained by taking the potential interactions between these systems. When anionic and nonionic surfactants are mixed and incorporated into the mobile phase, hydrophobic interactions will exist between their hydrophobic tails, ion-dipole interaction between their head groups. The ion-dipole interaction is much weaker than the electrostatic interaction observed for anionic and cationic mixed additives. For anionic-anionic or nonionic-nonionic mixed binary systems, there could be three types of interactions: hydrophobic interaction, hydrophilic-hydrophilic interaction and electrostatic repulsion. The net interaction for these systems is relatively repulsive or zero because of the order of magnitude of these three interactions.

TABLE 4.
EFFECT OF TERNARY ADDITIVE SYSTEMS (16.7 mM EACH) ON CAPACITY FACTORS (k′) OF ORGANIC COMPOUNDS[a]

Compound	SDS + CTAB + Triton X-100	SDS + CTAB + Brij-35	DOSS + CTAB + Triton X-100	DOSS + CTAB + Brij-35
Benzene	1.20	1.55	1.22	0.95
Toluene	2.04	2.00	1.65	1.40
Ethylbenzene	2.89	2.44	2.03	2.30
Propylbenzene	3.45	3.01	2.95	2.80
Butylbenzene	4.93	4.65	4.23	3.85
Naphthalene	2.45	2.34	2.03	1.89
Pyrene	4.62	3.52	3.00	2.10
Chrysene	9.64	8.98	7.54	6.15
Perylene	19.80	17.47	15.25	14.95
Phenol	0.59	0.60	0.52	0.44
Cresol	0.86	0.80	0.72	0.55
Ethylphenol	0.99	0.84	0.79	0.76
Propylphenol	1.32	1.28	1.13	0.99
Butylphenol	1.99	1.62	1.47	1.12
Amylphenol	3.02	2.44	2.35	2.18
Heptylphenol	6.23	5.67	5.40	5.12

[a] Experimental conditions are the same as in Table-2, except that the eluent was 50% acetonitrile-water containing anionic, cationic and nonionic surfactants at 16.7 mM each.

Effect of Ternary Surfactant Additives on the Separation: In this investigation four ternary mixed surfactants additives were tested. The first containing DOSS, CTAB, and Brij-35, the second containing SDS, CTAB, and Brij-35, the third containing DOSS, CTAB and Triton X-100, the fourth containing SDS, CTAB and Triton X-100. Table 4 displays the capacity factors of 17 aromatic compounds in systems containing 16.7 mM from each of the three additives in 50% acetonitrile mobile phase. We have found that ternary additives containing anionic, cationic and nonionic surfactants have more synergistic effect than binary additive systems. The synergistic effects of the ternary surfactant additives increase in the order SDS, CTAB, and Triton X-100 < SDS, CTAB, and Brij-35 < DOSS, CTAB, and Triton X-100 < DOSS, CTAB, and Brij-35.

The interesting point in the use of ternary additive systems is that in these systems the separation of the 17 organic compounds, as depicted in Figure 6, takes about 8 min without any peak overlapping, with all of them completely resolved. It is believed that when a 50% acetonitrile mobile phase containing three mixed surfactants; anionic, cationic and nonionic, ternary micelles will be formed as a result of hydrophobic interaction between hydrophobic tails, electrostatic interaction between the hydrophilic groups, ion-dipole interactions between their head groups. These interactions account for the formation of mixed micelles. The mixed micelles are believed to be responsible for lowering the capacity factor. We do not yet have an explanation as to why these ternary additives prepared from anionic, cationic and nonionic mixed surfactants are different in their ability to lower the capacity factor.

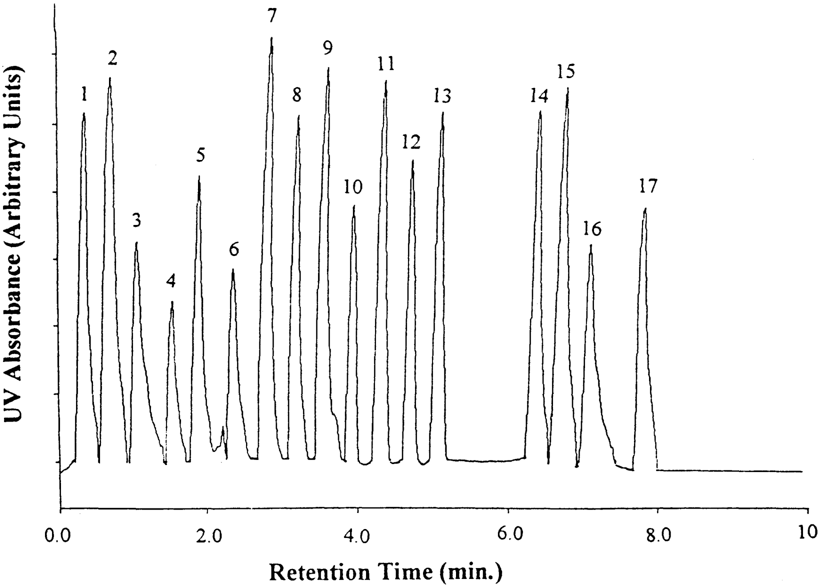

Fig. 6 . Chromatographic separation of supelcosil LC-18 (50 mm × 4.6 i.d.) column. Mobile phase 50% acetonitrile-water containing DOSS, CTAB and Brij-35 at 16.7 mM each. Flow rate: 1 mL/min. Detector: UV at 254 nm. Peak identification: (1) phenol, (2) benzene, (3) cresol, (4) toluene, (5) naphthalene, (6) ethyl phenol, (7) propyl phenol, (8) ethyl benzene, (9) butyl phenol, (10) propyl benzene, (11) anthracene, (12) amyl phenol, (13) butyl benzene, (14) pyrene, (15) chysene, (16) heptyl phenol, and (17) perylene.

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