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Novel additives for the separation of organic compounds by high-performance liquid chromatography

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Abstract

This paper proposes the use of novel surfactant additives at concentrations generally above their critical micelle concentrations in aqueous solution but without micelle formation for the separation of various organic compounds. The presence of these additives in organic–water mixtures greatly improves the separation of alkylbenzenes, polycyclic aromatic hydrocarbons, alkylphenols, and some other aromatic compounds. Compared with separations obtained without additives, shorter retention times and sharper peaks are obtained. The reason for the improvement appears to be a stronger interaction between the analyte molecules and the mobile phase due to the presence of long hydrocarbon chain(s) or polyoxypropylene segments in the additive molecules. The retention times of late-eluting compounds are reduced by a larger percentage than the retention times of earlier peaks. This effect is similar to that obtained with gradient elution but here only isocratic elution with an organic–water eluent containing an appropriate additive is used. Solvent strength and selectivity can be varied by controlling the type and concentration of the additive. Binding constants between solute and surfactant additives were calculated by relating capacity factor to surfactant concentration.

Keywords: Mobile-phase composition; Surfactants; Alkylbenzenes; Hydrocarbons, aromatic; Alkylphenols; Polynuclear aromatic hydrocarbons; Phenols

1. Introduction

The concept of adding an organic modifier to the mobile phase to improve HPLC separations has been studied. Addition of a suitable organic modifier results in additional interactions either between the analytes and the stationary phase, e.g. when a long chain alcohol is used to coat the surface of the stationary phase [1], or between the analytes and the mobile phase, e.g. when cyclodextrins are used for chiral separations [2]. Walbroehl and Jorgenson postulated a dynamic association equilibrium between the tetrahexylammonium ion and neutral organic solutes in acetonitrile–water [3]. They used this association to separate several neutral organic compounds by capillary zone electrophoresis (CZE). However, no one has ever taken advantage of this kind of equilibrium for HPLC separations.

Surfactants, such as sodium dodecylsulfate (SDS) [4] and cetyltrimethylammonium bromide (CTAB) [5], have been used in ion-pair chromatography, generally at concentrations below their critical micelle concentrations. They coat the stationary phase and act as dynamic ion exchangers for ionic analyte species. Charged analytes such as rare earth metals [4] and inorganic anions [5] can be retained and separated using stationary phases such as silica C_{18}. Surfactants have also been used in aqueous solvents at concentrations above their critical micelle con-

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centration to replace the organic solvent component used in conventional hydro-organic chromatography. This is the so-called micellar liquid chromatography (MLC) [6,7]. Micellar liquid chromatography has several advantages over conventional HPLC, but it is often associated with broader peaks and hence lower efficiency compared to conventional HPLC due to slow mass transfer from micelle to stationary phase [6,7].

In the present paper, the effect of surfactants is studied in mobile phases containing 39–70% organic solvent. Under such conditions, the formation of micelles is unlikely. However, a dramatic decrease in retention time was observed with several of the additives studied and sharp peaks were obtained. This is like a "bridge" step between micellar liquid chromatography and conventional HPLC because surfactants are used at concentrations above their critical concentrations in aqueous solution but no micelle formation is required.

2. Experimental

2.1. Chromatographic system

The chromatographic system consisted of several components. A Dionex DXP pump (Dionex, Sunnydale, CA, USA) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered 10-μl samples which were detected with a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ, USA). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instruments, Vienna, Austria). Supelcosil LC-18 columns (150× 4.6 mm I.D.), or in several cases, an Alltech Nucleosil C_{18} column (150×4.6 mm I.D.) were used as separation columns.

2.2. Reagents and chemicals

Methanol and acetonitrile were of HPLC grade and used as obtained from Fisher (Pittsburgh, PA, USA). The mobile-phase additives and analyte chemicals were reagent grade except for phenylacetaldehyde, which was 90%, and were all used as obtained from Aldrich (Milwaukee, WI, USA), J.T.Baker (Phillipsburg, NJ, USA), BASF (Parsippany, NJ, USA) and Sigma (St. Louis, MO, USA). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA) was used to further deionize distilled water for all eluents and sample mixtures.

2.3. Chromatographic procedure

A flow-rate of 1 ml/min was selected for all chromatographic separations. The separation column was equilibrated with mobile phase containing no additives, i.e. 60%, 50% or 39% acetonitrile, or 70% methanol, until the baseline was stabilized. Then the desired eluent was used. The baseline was stable after about 0.5 h. Sample injections were made at this point. The eluted species were detected by a UV–Vis detector at 254 nm with an output range of 0.010 AUFS.

Capacity factors, k', were calculated according to expression: $k'=(t_r-t_o)/t_o$. The system dead time, t_o, used to calculate the capacity factor k', was measured by injecting nitrate solution onto the system. An average of at least three replicates was used to perform all calculations.

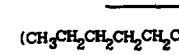
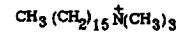
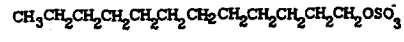
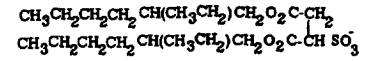
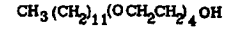
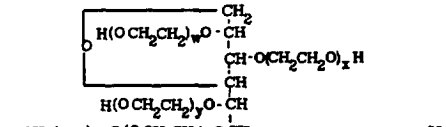
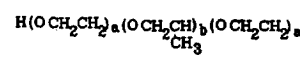
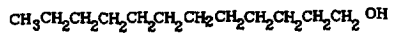
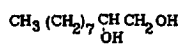
3. Results and discussions

3.1. Effect of additives

Table 1 gives a list of additives that have been used for the separations of the analytes. Pluronic L-31 contains alternating hydrophobic polyoxypropylene and hydrophilic polyoxyethylene segments. The others are all amphiphilic compounds with one or more long alkyl hydrophobic chains and a hydrophilic head group varying in chemical nature. These additives are either ionic or nonionic. The hydrophilic part helps to solubilize them in aqueous–organic solvent while the hydrophobic part helps them to exert their function as mobile-phase modifiers to improve the separation of various organics.

Figs. 1–5 show separations with and without additives in the mobile phase. In Fig. 1 a separation

Table 1
Additives utilized for the separation of organic compounds

Abbreviation	Name	Structure
1. THPA	Tetraheptylammonium	
2. CTAB	Cetyltrimethylammonium	
3. SDS	Sodium dodecylsulfate	
4. DOSS	Dioctylsulfosuccinate	
5. Brij-30	Polyoxyethylene(4) dodecyl ether	
6. Tween-60	Polyoxyethylene(20) sorbitan monostearate	 x+y+z+w=20
7. Pluronic L-31	Polyoxyethylene-polyoxypropylene copolymer	 M.W. = 1172.84 19% EO, w/w
8. 1-dodecanol		
9. 1,2-decanediol		

of alkylphenols is shown with acetonitrile–water (60:40) as the mobile phase. The separation was completed in ca. 22 min. When 50 m*M* Pluronic L-31 was added to the mobile phase, baseline separation was still obtained in only 14 min (Fig. 2). For the separation of the benzene through perylene series, the additive effect is even more evident. As shown in Fig. 3, these six compounds were completely separated with acetonitrile–water (60:40) as the mobile phase. However, it took more than 54 min to elute all the analytes from the column because of the strong interactions between stationary phase and these very hydrophobic analytes. When 40 m*M* Tween 60, which contains one saturated C_{17} hydrocarbon chain in each molecule, was added to the mobile phase, much shorter retention times and much sharper peaks were obtained. The same separation took only about 10 min to finish (Fig. 4). In the case of THPA, which contains four saturated C_7 hydrocarbon chains in each molecule, a similar effect was observed but to a lesser degree due to the weaker hydrophobic interaction between THPA and these PAH molecules (Fig. 5). Similar effects of additives were also observed for alkylbenzene separations.

Table 2 gives the retention times of alkylbenzenes, polycyclic aromatic hydrocarbons and alkylphenols with 50 m*M* SDS, DOSS, Pluronic L-31, THPA, Brij 30, CTAB or 40 m*M* Tween 60 as the additive in acetonitrile–water (60:40) eluent. Retention times of these analytes with acetonitrile–water (60:40) alone as the eluent are also shown for comparison. The different retention times shown in Table 2 using only acetonitrile–water (60:40) as the eluent are caused by the use of two different analytical columns. Table 3 shows the retention times with and without Brij 30 as the additive to a methanol–water (70:30) mobile phase. Brij 30 caused a much smaller change in retention times in methanol than in acetonitrile. From these tables, we can easily see that the retention of all analytes was decreased in the presence of the additives but to different degrees. The retention of larger, more hydrophobic molecules was generally reduced more than that of smaller, less hydrophobic ones. Different additives had different modifying powers when the

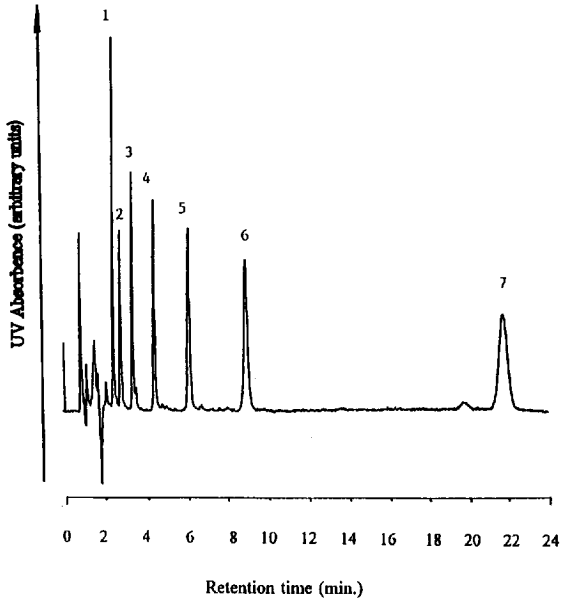


Fig. 1. Chromatographic separation on Supelcosil LC-18 (150× 4.6 mm I.D.) column. Eluent, acetonitrile–water (60:40); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=phenol, 2=*p*-cresol, 3=4-ethylphenol, 4=4-*n*-propylphenol, 5=4-*n*-butylphenol, 6=4-*n*-amylphenol, 7=4-*n*-heptylphenol.

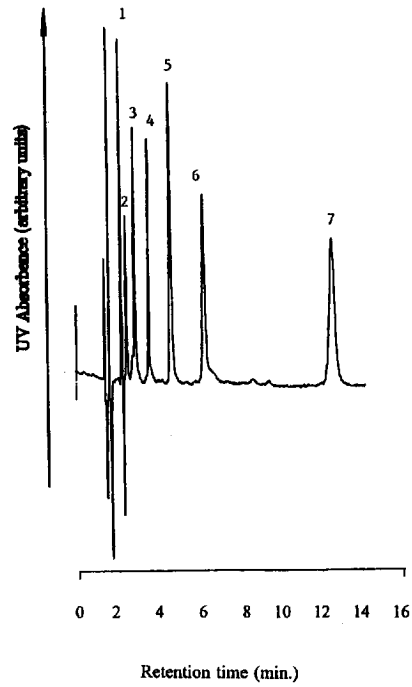


Fig. 2. Conditions as in Fig. 1, except that the eluent is acetonitrile–water (60:40) containing 50 m*M* Pluronic L-31.

same concentration was used. The degree of the reduction in the retention times of the analytes is determined by the hydrophobic chain length and the chemical nature of the additive. In the case of alkylphenols, hydrogen bond formation between the hydroxyl groups in Tween 60 and those in the phenols probably takes place in addition to hydrophobic interactions between the hydrophobic parts of the analytes and Tween 60. Both kinds of interactions help to reduce the retention of phenols and improve their separation.

3.2. Retention mechanism

Micelle formation

To investigate whether micelles play an important role in accomplishing the improved separations as they do in micellar HPLC, a literature study was performed. Acetonitrile and methanol are reported to be micelle-inhibiting solvents and to be able to break all micelles down at concentrations above 15–20% and 10–15%, respectively [8–10]. Thus, it is likely that no micelles exist in our system where at least 39% acetonitrile or 70% methanol was present. In addition, THPA never forms micelles even in pure water because it has a symmetric tetrahedral geometry [9]. It is obvious that micelle formation is not required for our success in improving the separations.

Retention characteristics

The chromatographic behavior of alkyl homolog series is useful for the investigation of retention mechanisms and for calibration of the retention. According to Guiochon and co-workers [11,12], a linear relationship is generally observed between $\log k'$ and the number of carbons in a homolog series in conventional hydro-organic HPLC. This regular increase of retention due to addition of a methylene group is recognized as a measure of hydrophobic interaction in a given reversed-phase HPLC system. In contrast to this typical relationship, linearity is found between k', not $\log k'$, and the number of carbons of a homogolous series in micellar LC when either a purely aqueous micellar mobile phase or a

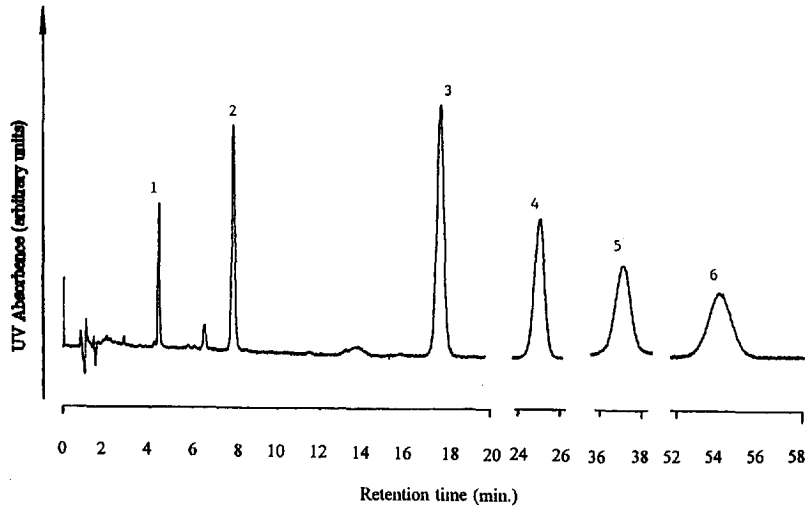


Fig. 3. Chromatographic separation on Supelcosil LC-18 (150×4.6 mm I.D.) column. Eluent, acetonitrile–water (60:40); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=benzene, 2=naphthalene, 3=anthracene, 4=pyrene, 5=chrysene, 6=perylene.

hybrid mobile phase (micellar mobile phase containing organic solvent) is used [13]. In the present system, a linear relationship was found between log k' of alkylbenzenes and carbon number on their side chains with correlation coefficients between 0.998 and 0.999. For alkylphenols, correlation coefficients between log k' and carbon number on their side chains were between 0.997 and 1.000. This was true for all of the additives listed in Table 1 used at different concentrations. This relationship again suggests that the retention mechanism of our system

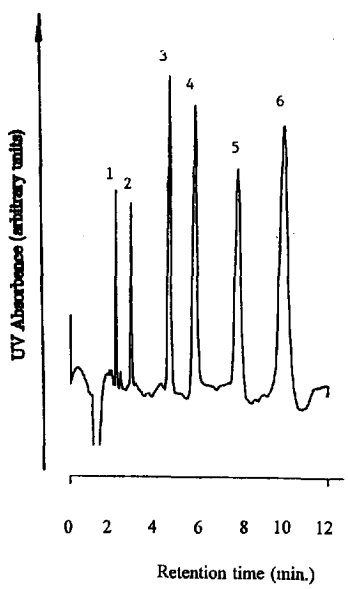


Fig. 4. Conditions as in Fig. 3, except that the eluent is acetonitrile–water (60:40) containing 40 mM Tween 60.

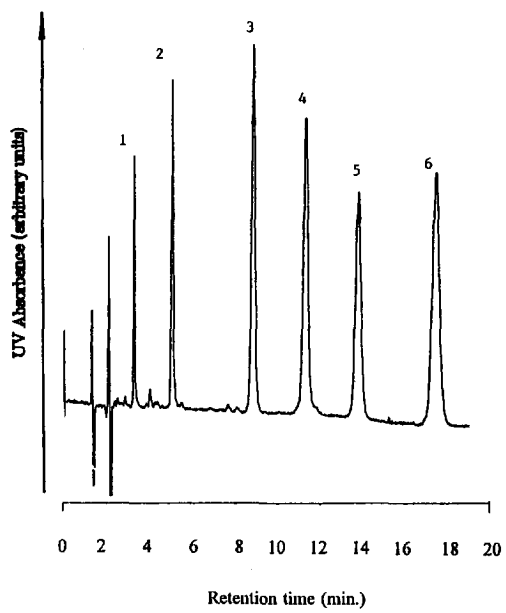


Fig. 5. Conditions as in Fig. 3, except that the eluent is acetonitrile–water (60:40) containing 50 mM tetraheptylammonium bromide.

Table 2
Retention times (min) of various compounds on Supelcosil LC-18 (150×4.6 mm I.D.) column

Compound	60% Acetonitrile +					
	None	50 m*M* SDS	50 m*M* DOSS	50 m*M* Pluronics L-31	50 m*M* THPA	40 m*M* Tween 60
Benzene	4.20	3.09	3.22	2.52	3.28	2.08
Toluene	5.58	4.16	4.23	3.00	4.30	2.36
Ethylbenzene	7.78	5.43	5.57	3.62	5.57	2.64
Propylbenzene	11.78	7.86	8.11	4.80	7.76	3.22
Butylbenzene	18.24	11.65	12.06	6.56	10.98	3.95
Benzene	4.20	3.09	3.22	2.52	3.28	2.08
Naphthalene	7.44	5.46	5.03	3.73	5.04	2.82
Anthracene	16.22	11.96	9.76	7.07	8.81	4.63
Pyrene	22.88	17.98	13.60	9.71	11.34	5.86
Chrysene	33.88	25.27	17.70	13.83	13.80	7.91
Perylene	49.92	39.37	25.66	19.94	17.46	10.06
Phenol	2.56	2.02	1.64	1.85	2.08	1.75
Cresol	2.78	2.24	1.86	1.98	2.34	1.78
Ethylphenol	3.38	2.62	2.08	2.15	2.72	1.92
Propylphenol	4.38	3.34	2.40	2.44	3.40	2.08
Butylphenol	6.02	4.40	3.86	2.94	4.40	2.29
Amylphenol	8.68	6.10	5.24	3.70	5.98	2.63
Heptylphenol	20.84	13.40	11.30	6.96	12.00	3.78

Flow-rate: 1 ml/min. Detection, UV absorbance at 254 nm. SDS=sodium dodecylsulfate; DOSS=sodium dioctylsulfosuccinate; Pluronics L-31=polyoxyethylene–polyoxypropylene copolymer; THPA=tetraheptylammonium bromide; Tween 60=polyoxyethylene(20) sorbitan monostearate

Table 3
Retention times (min) of various compounds on Alltech Nucleosil C_{18} (150×4.6 mm I.D.) column

Compound	70% Methanol +	
	None	50 m*M* Brij 30
Benzene	4.94	5.06
Toluene	7.38	7.38
Ethylbenzene	10.46	10.08
Propylbenzene	16.62	14.88
Butylbenzene	27.43	22.54
Benzene	4.98	4.80
Naphthalene	11.03	10.32
Anthracene	32.88	25.24
Pyrene	52.74	35.64
Chrysene	93.88	50.58
Perylene	162.84	68.64
Phenol	2.42	2.70
Cresol	2.98	3.16
Ethylphenol	3.83	3.94
Propylphenol	5.40	5.28
Butylphenol	8.08	7.46
Amylphenol	12.74	10.78
Heptylphenol	35.18	23.60

Other conditions are the same as in Table 2.

agrees with that found in conventional hydro-organic HPLC rather than in micellar LC.

Interestingly, when $\log k'$ of benzene, naphthalene, anthracene and chrysene was plotted against the number of fused benzene rings, a linear relationship was also obtained with correlation coefficients between 0.994 and 0.999. However, upon adding perylene to the series or replacing chrysene with pyrene, the linearity deteriorated. This is not surprising, considering the effect of molecular shape on the retention as discussed by Sander and Wise [14]. According to their study, the length-to-width ratio of a PAH molecule is among the most important parameters affecting its retention. For PAH molecules with the same number of benzene rings, the higher the ratio, the greater its retention will be. Our findings seem to perfectly agree with those results.

Separation efficiency

Compared to conventional hydro-organic HPLC, MLC suffers from much lower separation efficiency than conventional hydro-organic HPLC. Generally, broad peaks are observed in MLC. For example,

Borgerding et al. [7] reported separations of several organic compounds using methanol–water (50:50) and 0.285 *M* SDS in water as the eluent. Similar retention times were obtained for benzene in the two cases. However, a 75% lower efficiency was obtained in the latter case ($N=1530$ compared to $N=6010$). This effect was even more pronounced for more hydrophobic solutes such as 2-ethylanthraquinone. This is thought to be the result of slow mass transfer due to (i) adsorption of surfactant molecules in the pores of the stationary phase, (ii) poor wettability of the stationary phase by the aqueous mobile phase used in MLC, and (iii) slow exit rate of analyte molecules from micelles to the bulk aqueous mobile phase.

To study the separation efficiency of our system, 50 m*M* Brij 30 in acetonitrile–water (60:40) was selected as an example with acetonitrile–water (60:40) alone as a comparison. With fast chart speed (6 cm/min), each sample peak and dead-time marker peak was recorded. Peak width at half maximum, $w_{1/2}$, and half peak-width at 10% peak height, *A* and *B*, were measured carefully. Peak broadening σ^2 was calculated according to formula $w_{1/2}=2.35\sigma$ and the classical plate number, *N*, was calculated according to: $N=t_0^2(k'+1)^2/\sigma^2$, for each individual compound. By plotting $(1+k')^2$ against σ^2 and taking the slope $[(k'+1)^2/\sigma^2]$, the average classical plate number was calculated from t_0^2/slope. Finally, peak asymmetry *B/A* of each compound was calculated from measured *B* and *A*. The calculated results are shown in Table 4 and Table 5. Comparison of the results with and without 50 m*M* Brij 30 indicates that they offer very similar separation efficiencies.

Table 4
Separation efficiency with ACN–H_2O (60:40) as eluent on Supelcosil LC-18 (150×4.6 mm I.D.) column

Compound	k'	σ^2 (min^2)	*B/A*	*N*
Benzene	1.81	0.00322	1.70	4822
Toluene	2.77	0.00503	1.25	5542
Ethylbenzene	4.14	0.00876	1.40	5915
Propylbenzene	6.71	0.0193	1.30	6036
Butylbenzene	10.78	0.0438	1.20	6220
Naphthalene	3.84	0.00749	1.58	6140
Anthracene	9.33	0.0351	1.27	5964
Chrysene	19.98	0.141	1.16	6132

Average classical plate number $N=6154$.

Table 5
Separation efficiency with ACN–H_2O (60:40) containing 50 m*M* Brij 30 as eluent on Supelcosil LC-18 (150×4.6 mm I.D.) column

Compound	k'	σ^2 (min^2)	*B/A*	*N*
Benzene	1.09	0.00170	1.06	4633
Toluene	1.58	0.00246	1.26	4857
Ethylbenzene	2.24	0.00372	1.26	5063
Propylbenzene	3.55	0.00654	1.11	5692
Butylbenzene	5.43	0.0132	1.15	5629
Naphthalene	2.16	0.00306	1.48	5875
Anthracene	4.67	0.0107	1.23	5387
Chrysene	8.60	0.0304	1.20	5433

Average classical plate number $N=5475$.

Surface adsorption

Since micelle formation is unlikely, the observed additive effect could arise in two ways. First, the additive molecules might be adsorbed on the surface of the stationary phase with the long alkyl or polyoxypropylene chains interacting with the C_{18} chains on the stationary phase and the hydrophilic head groups sticking out as described by Morris and Fritz [1], and by Montgomery and Wirth [15]. This would give a more hydrophilic surface and thus reduce the retention times of the analytes. Second, the discrete additive molecules in the mobile phase might interact with analyte molecules by hydrophobic interactions between the analyte molecules and the polyoxypropylene or long alkyl chains of the additives. Hydrogen bonding may play a role when both the analyte and the additive contain potential hydrogen bond formation centers as in alkylphenol separations with Brij 30 or Tween 60 as the additive. To evaluate the first aspect, several experiments were performed.

First, if surface adsorption of the additives exists, the additives should be retained by the stationary phase. However, when a solution of SDS, DOSS, CTAB or THPA was injected onto the system with 60% acetonitrile as the eluent, no apparent retention of any of these compounds was observed. Instead, they eluted near the system dead time, which was indicated by the elution time of sodium nitrate. These compounds were detected with conductivity detection.

Second, 150 ml of 5 m*M* CTAB in acetonitrile–water (60:40) was shaken with 2 g of the stationary phase. The mixture was allowed to stand overnight

in a tightly closed container, and was then filtered through a 0.45-μm Nylon-66 filter. The conductivity of both the clear filtrate and the original 5 m*M* CTAB solution was measured by pumping the solution through a Dionex CDM-3 conductivity detector. No decrease of conductivity was observed. This indicated that no CTAB had been lost from the solution due to adsorption onto the stationary phase.

As a further check, we determined the retention times of the analyte molecules using acetonitrile–water (60:40) as the mobile phase before and after continuously running with mobile phase containing the additives. No apparent change in the retention times of the analytes was observed.

When switching the mobile phase to one containing an additive after running without additives, no column re-equilibration time was needed to obtain a stable baseline except for a time period of about 0.5 h to completely replace the previous eluent left in the system. All of the above suggests that no adsorption of the additive molecules occurs on the surface of the stationary phase.

Thus, the second mechanism is probably correct. In other words, the observed effect of these additives on the retention and separations of the analytes is a result of the interactions between the analyte and discrete additive molecules in the mobile phase. This has also been reported by Walbroehl and Jorgenson [3] and Shi and Fritz [16] in CZE.

3.3. Solvent strength and selectivity

Several reports have shown that in micellar LC both solvent strength and selectivity can be controlled by varying the surfactant concentration in the mobile phase [6,7]. This is also true in our system. The capacity factors of various analytes were determined as a function of additive concentration in acetonitrile–water (60:40) for Brij 30, THPA, DOSS and Tween 60. Typical plots are shown in Fig. 6 for PAH analytes with Brij 30 as the additive. Increasing concentrations of Brij 30 result in progressively lower k' values for all of the analytes, but the magnitude of the decrease in k' varies from one analyte to another. The amount by which k' is decreased for any given analyte depends on the type of additive as well as the concentration of the

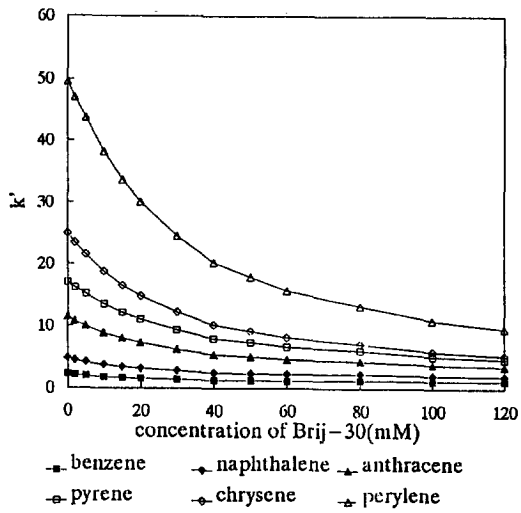


Fig. 6. Effect of Brij 30 on the retention of polycyclic aromatic hydrocarbons. Experimental conditions as in Fig. 1.

additive. Numerical values for the ratio of capacity factors with and without additive are shown in Table 6. The percentage decrease in k' is generally significantly greater for analytes with larger k' values. Thus, the effect of additives is akin to gradient elution in HPLC, both in percent and in absolute magnitude. Furthermore, gradient elution by varying the additive concentration should be possible. Since no surface adsorption is taking place, gradient elution can be very fast because no re-equilibration of the column is required.

The selectivity of such a system for analytes with different functionalities was also studied. Example separations are shown in Fig. 7 and Fig. 8. In Fig. 7, a group of ten compounds with different hydrophobicities and functionalities was separated with acetonitrile–water (60:40) as the eluent. When 50 m*M* DOSS was added to the eluent, the k' values of all the analytes were reduced but to different extents. Most noticeable are peaks 2, 8, 9 and 10. While peaks 2 and 8 move much closer to peaks 1 and 7, and further away from peaks 3 and 9 respectively (Fig. 8), a reversed order of elution resulted for peaks 9 and 10 (Fig. 8). This was confirmed by injections of each individual compound.

In short, by choice of type and/or amount of additive, the solvent strength and selectivity of our

Table 6
Ratio of capacity factors in eluent without additives to those with additives

Compounds	Acetonitrile–water (60:40)						Acetonitrile–water (39:61)	
	40 m*M* Tween 60	50 m*M* Brij 30	50 m*M* Pluronics L-31	50 m*M* THPA	50 m*M* SDS	50 m*M* DOSS	40 m*M* Tween 60	50 m*M* DOSS
Benzene	3.44	1.81	2.28	1.44	1.59	1.49	3.52	1.54
Toluene	3.80	1.88	2.43	1.41	1.48	1.45	4.89	1.66
Ethylbenzene	4.59	1.97	2.73	1.51	1.56	1.51	6.91	1.82
Propylbenzene	5.26	2.00	2.94	1.61	1.59	1.53	10.63	2.06
Butylbenzene	6.22	2.09	3.18	1.74	1.63	1.57	17.54	2.36
Benzene	3.44	1.81	2.28	1.44	1.59	1.49	3.49	1.54
Naphthalene	3.87	1.89	2.47	1.63	1.46	1.63	6.88	3.91
Anthracene	4.39	2.11	2.56	1.98	1.40	1.76	16.26	7.94
Pyrene	4.66	2.09	2.55	2.14	1.29	1.75	NA	NA
Chrysene	4.80	2.44	2.55	2.56	1.34	1.95	NA	NA
Perylene	5.50	2.57	2.60	3.00	1.28	1.99	NA	NA
Phenol	2.50	1.59	2.11	1.55	1.67	1.75	1.84	1.50
Cresol	2.73	1.50	2.02	1.39	1.50	1.65	2.37	1.69
Ethylphenol	3.06	1.62	2.31	1.44	1.54	1.78	3.22	1.78
Propylphenol	3.64	1.60	2.58	1.45	1.46	1.79	4.74	1.97
Butylphenol	4.45	1.66	2.78	1.51	1.51	1.82	7.41	2.21
Amylphenol	5.26	1.71	3.00	1.57	1.51	1.85	11.92	2.52
Heptylphenol	7.62	1.85	3.41	1.82	1.58	1.94	NA	NA

NA: Data are not available because the retention times of these analytes are very long in acetonitrile–water (39:61) alone.
Experimental conditions as in Fig. 1.

chromatographic system can be varied according to actual needs.

3.4. Determination of solute–surfactant binding constant

In micellar LC, a three-phase equilibrium model relating capacity factor to micellar mobile-phase concentration has been proposed and equations have been derived, which allow calculation of the binding constants between the solute and the micellar aggregates [6,17]. A similar approach was found to be valid for the present study. Two equilibria were considered: that of solute in the mobile phase (E_m) combining with stationary-phase sites (L_s) and that of solute combining with additive (A_m) to form an association complex.

$$E_m + L_s \overset{K_1}{\rightleftharpoons} EL_s \quad (1)$$

$$E_m + A_m \overset{K_2}{\rightleftharpoons} EA_m \quad (2)$$

The equation derived [18] was as follows:

$$\frac{1}{k'} = \frac{[A_m]K_2}{\phi[L_s]K_1} + \frac{1}{\phi[L_s]K_1} \quad (3)$$

where k' is the capacity factor of the solute and ϕ is the phase ratio. A plot of $1/k'$ against the concentration of the additive in the mobile phase should be linear. The desired binding constant, K_2, is obtained by dividing the slope by the intercept when $[A_m]$ is zero.

It should be pointed out that this treatment assumes a 1:1 association between solute and additive. The linear range tends to be limited to rather low concentrations of additive. This may stem from the fact that any distribution of EA between the stationary and mobile phase is ignored.

Table 7 lists the binding constants (K) calculated for several additives. The correlation coefficients for linear regression are reasonably good, and the calculated constants follow the expected trend with the bulkier analytes having larger constants. The values of the binding constants are of course affected by the

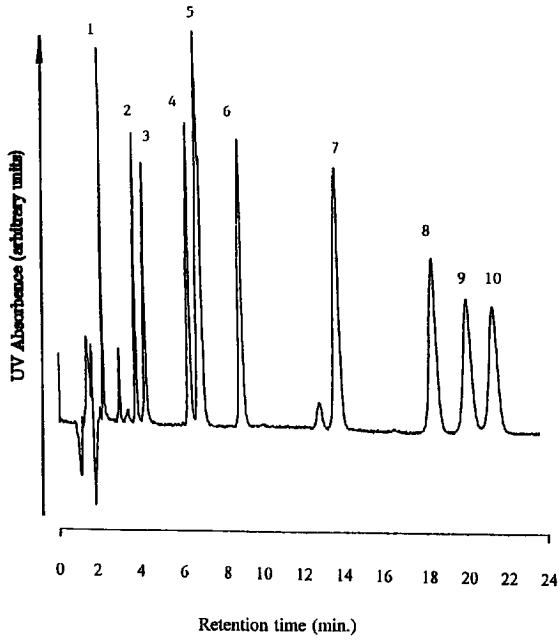


Fig. 7. Chromatographic separation on Supelcosil LC-18 (150× 4.6 mm I.D.) column. Eluent, acetonitrile–water (60:40); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1= benzylbromide, 2=benzylacetate, 3=propylphenol, 4=toluene, 5=bromobenzene, 6=ethylbenzene, 7=propylbenzene, 8= anthracene, 9=butylbenzene, 10=heptylphenol.

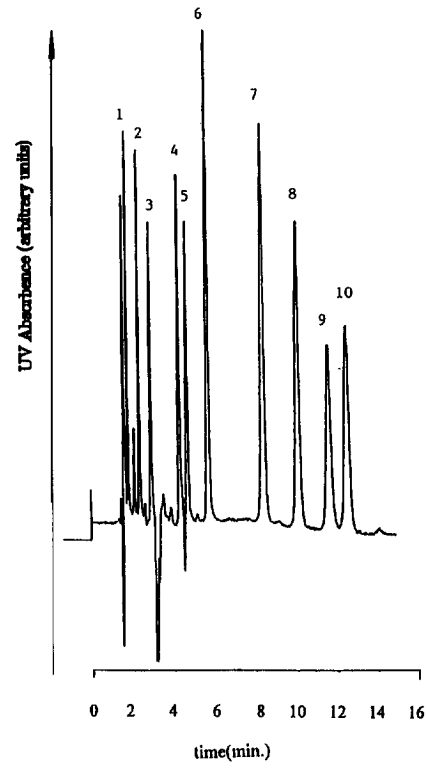


Fig. 8. Chromatographic separation on Supelcosil LC-18 (150× 4.6 mm I.D.) column. Eluent, acetonitrile–water (60:40) containing 50 m*M* DOSS; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=benzylbromide, 2=benzylacetate, 3=propylphenol, 4=toluene, 5=bromobenzene, 6=ethylbenzene, 7= propylbenzene, 8=anthracene, 9=heptylphenol, 10= butylbenzene.

percentage of acetonitrile in the mobile phase. A decrease in the percentage of acetonitrile should decrease the strength of acetonitrile solvation and lead to higher binding constants for the EA complexes.

3.5. Gradient elution

Gradient elution has been commonly used to separate relatively complicated analyte mixtures. In conventional HPLC, a gradient in the percentage of organic solvent is generally applied since an increase in the organic solvent concentration would result in a stronger eluent. It appeared that the use of mobile-phase additives provides similar benefits to conventional solvent gradient elution with a mobile phase of fixed concentration. The additives complex later-eluting analytes more strongly and thereby reduce their retention times more than those of the earlier peaks.

Reduction of analyte retention times was compared using a higher concentration of acetonitrile and by the use of mobile-phase additives. First of all, a separation of eighteen aromatic compounds was obtained using 80% acetonitrile in water as the eluent (Fig. 9). The separation took 40 min. While the later peaks are quite far from each other, the early peaks are very crowded, especially, peaks 1, 2 and 3, peaks 4 and 5, and peaks 13 and 14. Decreasing the acetonitrile concentration to 70% resulted in a much better separation for the middle peaks. However, it took more than two hours to elute all eighteen compounds while peaks 2 and 3, 4 and 5 were still not baseline resolved. Besides, benz[*e*]acephenathrylene and perylene are co-eluted

Table 7
Calculated solute–additive association constants with associated statistical analysis for various aromatic solute compounds

Analyte	DOSS/39% ACN		Tween 60/60% ACN		Brij 30/60% ACN		THPA/60% ACN	
	K	Corr. coeff.	*K*	Corr. coeff.	*K*	Corr. coeff.	*K*	Corr. coeff.
Benzene	15.44	0.99	241.82	0.99	18.64	0.98	13.22	0.99
Toluene	16.85	0.99	311.46	0.9994	19.11	0.985	12.65	0.99
Ethylbenzene	19.55	0.992	376.84	0.9997	21.38	0.99	13.71	0.97
Propylbenzene	22.49	0.993	422.42	0.9996	23.33	0.994	14.85	0.96
Butylbenzene	27	0	488.00	0.999	25.85	0.997	16.98	0.96
Benzene	15.44	0.99	241.82	0.99	18.64	0.98	13.22	0.99
Naphthalene	22.52	0	255.50	0.995	22.89	0.996	15.24	0.98
Anthracene	33.13	0.99	242.59	0.999	28.67	0.9997	20.57	0.982
Pyrene	36.46	0.98	291.52	0.96	29.27	0.998	–[a]	–[a]
Chrysene	NA	NA	168.79	0.99	35.26	0.999	33.58	0.994
Perylene	NA	NA	169.71	0.98	43.64	0.999	–[a]	–[a]
Phenol	20.45	0.94	134.86	0.96	12.54	0.996	–[a]	–[a]
Cresol	20.93	0.96	162.49	0.98	13.27	0.992	–[a]	–[a]
Ethylphenol	22.64	0.96	194.72	0.983	15.41	0.994	–[a]	–[a]
Propylphenol	24.21	0.96	227.76	0.98	17.13	0.999	–[a]	–[a]
Butylphenol	26.58	0.95	271.27	0.98	19.68	0.998	–[a]	–[a]
Amylphenol	30.62	0.94	316.67	0.981	20.97	0.992	–[a]	–[a]
Heptylphenol	38.95	0.94	398.38	0.992	27.29	0.996	–[a]	–[a]

K=the binding constant between the solute and the additive molecules; L/mol.
NA: Data are not available because the retention times of chrysene and perylene were too long.
[a] Not detected.

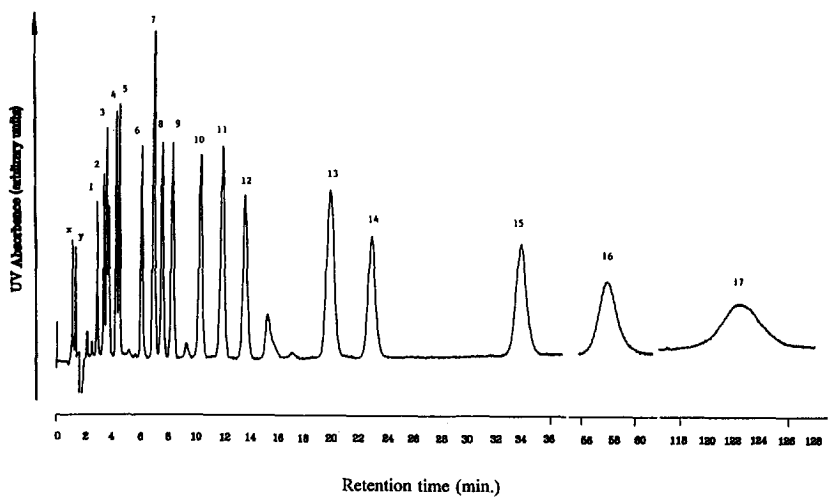


Fig. 9. Chromatographic separation on Supelcosil LC-18 (150×4.6 mm I.D.) column. Mobile phase, acetonitrile–water (80:20); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=benzophenone, 2=benzene, 3=toluene, 4=naphthalene, 5=ethylbenzene, 6=fluorene, 7=phenanthracene, 8=anthracene, 9=butylbenzene, 10=pyrene, 11=2,3-benzofluorene, 12=chrysene, 13=benz[*e*]acephenathrylene, 14=perylene, 15=benzo[*a*]pyrene, 16=benzo[*ghi*]perylene, 17=rubrene, 18=3,4,9,10-dibenzopyrene. All other peaks are impurity peaks.

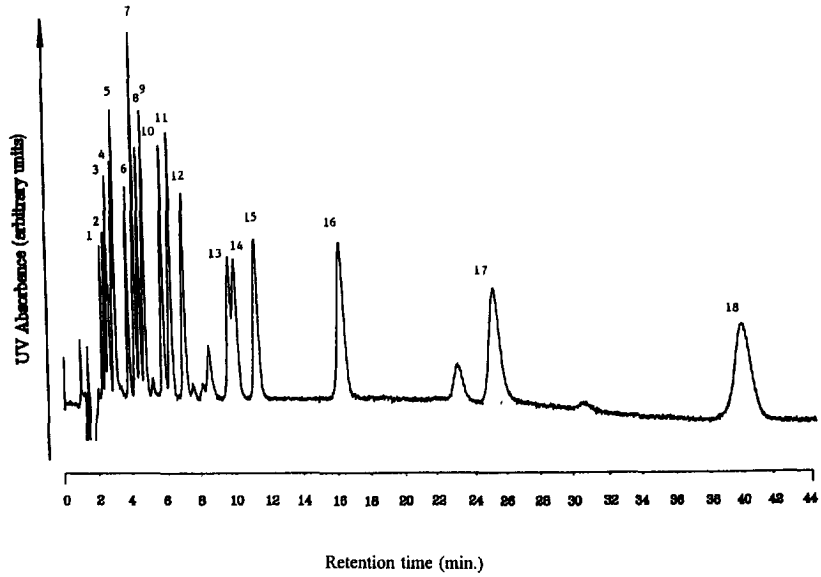


Fig. 10. Chromatographic separation on Supelcosil LC-18 (150×4.6 mm I.D.) column. Mobile phase, acetonitrile–water (70:30); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=benzophenone, 2=benzene, 3=toluene, 4=naphthalene, 5=ethylbenzene, 6=fluorene, 7=phenanthracene, 8=anthracene, 9=butylbenzene, 10=pyrene, 11=2,3-benzofluorene, 12=chrysene, 13=benz[*e*]acephenathrylene and perylene, 14=benzo[*a*]pyrene, 15=benzo[*ghi*]perylene, 16=rubrene, 17=3,4,9,10-dibenzopyrene. Peaks x and y are the injection peaks and all other peaks are impurity peaks.

(Fig. 10). With an eluent of 70 m*M* Brij 30 in acetonitrile–water (50:50), the separation took only ca. 31 min (Fig. 11). Also, the sample peaks were rather evenly distributed within this time period. This is a noticeable gradient elution feature although no gradient elution was actually used.

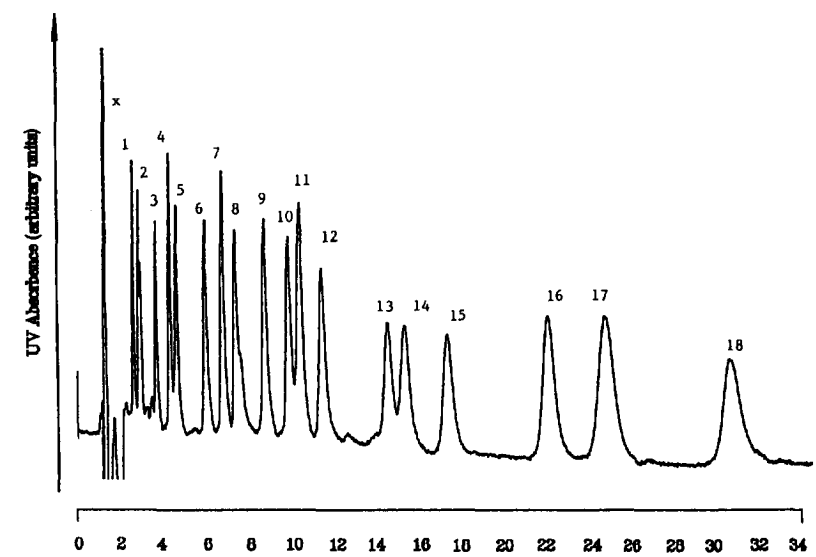


Fig. 11. Conditions as in Fig. 9, except that the eluent is acetonitrile–water (50:50) containing 70 m*M* Brij 30. Peak x is the injection peak and all other peaks are impurity peaks.

4. Conclusions

In the present study, a novel type of additive for chromatographic separations of organic compounds is described. This system acts as a bridge between conventional and micellar HPLC in that it uses surfactants as additives as in micellar LC but does not depend on the presence of micelles to accomplish separations. It offers a better separation window than conventional hydro-organic mobile phases and superior separation efficiency compared to micellar LC. Interaction between the analytes and additives in solution is the basis of the improved separations. Control of solvent strength and selectivity is possible by careful choice of the type of surfactant and concentration. Rapid gradient elution may be possible because no column re-equilibration is required. Binding constants between solute and surfactant molecules in the mobile phase can be calculated from capacity factor and surfactant concentration.

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References

[1] J. Morris and J.S. Fritz, Anal. Chem., 66 (1994) 2390.
[2] T. Takeuchi, H. Asai and D. Ishii, J. Chromatogr., 357 (1986) 409.
[3] Y. Walbroehl and J. Jorgenson, Anal. Chem., 58 (1986) 479.
[4] D.J. Barkley, M. Blanchette, R.M. Cassidy and S. Elchuk, Anal. Chem., 58 (1986) 2222.
[5] M. Wu, V. Pacakova, K. Stulik and G.A. Sacchetto, J. Chromatogr., 439 (1988) 363.
[6] D.W. Armstrong, Sep. Purif. Methods, 14 (1985) 213
[7] M.F. Borgerding, R.L. Williams, Jr., W.L. Hinze and F.H. Quina, J. Liq. Chromatogr., 12 (1989) 1367.
[8] M.S. Bakshi, J. Chem. Soc. Faraday Trans., 89 (1993) 4323.
[9] L. Magid, in K.L. Mittal (Editor), Solution Chemistry of Surfactants, Plenum Press, New york, NY, 1979, p. 427.
[10] L.G. Ionescu, L.S. Romanesco and F. Nome, in K.L. Mittal and B. Lindman (Editors), Surfactants in Solution, Plenum Press, New York, NY, 1984, p. 789.
[11] H. Colin and G. Guiochon, J. Chromatogr. Sci., 18 (1980) 54.
[12] E. Grushika, H. Colin and G. Guiochon, J. Chromatogr., 248 (1982) 325.
[13] M.G. Khaledi, E. Peuler and J. Ngeh-Ngwainbi, Anal. Chem., 59 (1987) 2738.
[14] L.C. Sander and S.A. Wise, Adv. Chromatogr., 25 (1986) 139.
[15] M.E. Montgomery, Jr. and M.J. Wirth, Anal. Chem., 66 (1994) 680.
[16] Y. Shi and J.S. Fritz, J. High Resolut. Chromatogr., 17 (1994) 1.
[17] M. Arunyanart and L.J. Cline Love, Anal. Chem., 56 (1984) 1557.