CHROM. 23 408

Investigation of the causes of reduced efficiency in micellar liquid chromatography

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ABSTRACT

Reduced chromatographic efficiency is a major drawback of micellar liquid chromatography (MLC). The Knox equation $h = Av^{1/3} + B/v + Cv$, was used to determine the individual contribution of the flow anisotropy (A term), molecular band broadening (B term) and mass transfer processes (C term) to the final band broadening. Knox plots of h, the reduced plate height, *versus* v, the reduced linear flow-rate, were determined on the same column (i) with an aqueous–organic mobile phase, (ii) with a micellar mobile phase and (iii) with the same aqueous–organic phase. The changes in the A, B and C terms are discussed. Two stationary phases were used: a classical C_{18} monomer phase and a densely grafted (3.5 μmol/m^2) C_{14} phase. Two micellar solutions were used: a non-ionic micellar solution of Brij 35 and an anionic solution of sodium dodecyl sulfate (SDS). Test solute diffusion coefficients were measured in each mobile phase used. The increase in A is mainly responsible for reduced MLC efficiency. However, the B and C terms also increased significantly with micellar solutions. It is shown that the observed changes in the Knox parameters can be explained by the change in the stationary phase produced by surfactant adsorption; 6% of the adsorbed SDS (0.14 μmol/m^2) was irreversibly adsorbed on the C_{14} phase whose initial efficiency could not be restored. Such a small amount of adsorbed surfactant was able to degrade completely the initial efficiency of the C_{14} stationary phase. A model explaining how that irreversible adsorption may occur with ionic long-chain surfactants and densely grafted stationary phases with long-chain ($>C_8$) bonding moieties is proposed.

INTRODUCTION

Since the inception of micellar liquid chromatography (MLC) by Armstrong and co-workers [1,2], the technique has been extensively studied to determine the advantages and disadvantages associated with the substitution of a surfactant, present at a concentration higher than the critical micellar concentration (CMC), for the typical organic solvent component of a classical LC mobile phase. Unique separation selectivities, enhanced detection modes and practical advantages such as non-toxicity, non-flammability, low cost or simplified waste disposal have been reported in several

recent reviews of MLC [3–6]. A serious disadvantage, common to all MLC systems studied to date, however, has been reduced chromatographic efficiency. The significance of this deficiency is most apparent when viewed in the context of resolution: about 30–50% fewer components can be resolved per unit time with the chromatographic efficiencies typically observed in MLC, when compared to commonly used aqueous–organic mobile phases.

Several workers have addressed this problem. Dorsey *et al.* [7] showed that MLC chromatographic efficiency can be improved by the addition of 3% of *n*-propanol to the micellar mobile phase, which serves to overcome the postulated poor mobile phase wetting of the stationary phase. Yarmchuck *et al.* [8] suggested that reduced MLC efficiency is caused by slow solute exit rate from the micelle and the stationary phase which produces poor mass transfer between the bulk phases., They recommended the use of low mobile phase flow-rates, elevated operating temperatures and minimum surfactant concentrations.

Surfactant adsorption on the stationary phase was also suspected to have a major impact on the MLC efficiency. Surfactants were found to absorb on the stationary phase in amounts approximating that of the bonded hydrocarbon [9,10]. The increase in the film thickness of the stationary phase due to adsorbed surfactant was thought to be responsible for the decreased MLC efficiency [9,11,12]. It was shown that the efficiency improvement induced by addition of a short-chain alcohol was due to surfactant desorption out of the stationary phase [13]. Pentanol was the most efficient additive for efficiency improvement [14].

In this work, MLC efficiency was studied by applying a rate equation to determine the contributions to the final solute band width. The Knox equation has been widely used in liquid chromatography for this purpose [15,16]. It can be expressed as

$$h = Av^{1/3} + \frac{B}{v} + Cv \tag{1}$$

where A, B and C are the constants of the Knox equation, h is the reduced plate height calculated as $h = H/d_p$, where H is the column plate height ($H = L/N$, L being the column length and N the number of theoretical plates), d_p is the stationary phase particle diameter, v is the reduced mobile phase velocity, *i.e.*, $v = \mu d_p/D_m$ (μ being the mobile phase velocity in cm/s and D_m the solute diffusion coefficient in the mobile phase in cm^2/s).

The A, B and C terms of the Knox equation are related to the flow anisotropy, molecular longitudinal diffusion and mass transfer processes, respectively. These three terms were determined for the same column on the same chromatographic system with a reference aqueous–organic mobile phase and with a micellar phase. The variations of the A, B and C terms with micellar phases allow one to highlight changes attributable to a particular effect.

EXPERIMENTAL

Chromatographic system

Two systems were used. The first consisted of components from Waters Assoc. (Milford, MA, USA) *viz.* a Model M6000 A pump, Model 441 UV detector and

Model 720 system controller. The second system was constructed in the laboratory with a Shimadzu LC-5A pump, Rheodyne Model 7520 0.5-μl injection valve and Shimadzu SPD-6A UV detector (Touzard et Matignon, Paris, France). Two columns were used. A 10 cm × 5 mm I.D. Radial Pak cartridge (Waters Assoc.) packed with C_{18}, non-end-capped 10-μm particles was used with Brij 35 micellar solutions and an acetonitrile (ACN)–water (30:70, v/v) reference solution. The second column was a 15 cm × 4 mm I.D. column packed with a laboratory-grafted C_{14} phase with a maximum bonding density of the monomer type (particle diameter 5 μm, surface area 210 m^2/g, pore volume 0.45 ml/g, mean pore diameter 9 nm, carbon load 17.9% and C_{14} monomer bonding coverage 3.5 μmol/m^2 [17]). It was used with sodium dodecyl sulfate (SDS) micellar solutions and a methanol–water (70:30, v/v) reference solution.

Chemicals

Table I gives the physico-chemical properties of the two surfactants used. The non-ionic polyoxyethylene 23 dodecyl ether (Brij 35) was obtained from Sigma (St. Louis, MO, USA), SDS and methanol from Merck (Darmstadt, Germany), ACN and benzene from Burdick & Jackson (Muskegon, MI, USA), benzyl alcohol and benzaldehyde from Fisher Scientific (Raleigh, NC, USA) and toluene and ethyl-, propyl- and butylbenzene from Fluka (Buchs, Switzerland). Water was distilled, deionized and filtered with a Barnstead Nanopure system.

Determination of diffusion coefficient

Diffusion coefficients were determined using the Taylor dispersion technique [19], as described in recent papers [11,20]. Five replicate measurements were made in all instances.

Determination of the A, B and C terms of the Knox equation

Chromatograms were obtained with mobile phase flow-rates ranging from 0.1 to 1.6 ml/min with the 15-cm C_{14} column and from 0.4 to 3 ml/min with the 10-cm C_{18} column. All parameters were determined from digitally acquired data after cor-

TABLE I

PHYSICO-CHEMICAL PROPERTIES OF SURFACTANTS AND MICELLES

Data from refs. 4 and 18.

Surfactant	Molecular weight (g/mol)	CMC[a]		Micelle		
		mol/l	ppm	Radius (nm)	Aggregation No.	V^b (l/mol)
SDS ($C_{12}H_{25}SO_3^- Na^+$)	288.4	$8.2 \cdot 10^{-3}$	2360	2.6	62	0.246
Brij 22 [$C_{12}H_{25}(OCH_2CH_2)_{10}OH$]	626	$8 \cdot 10^{-5}$	50	2.8	97	0.6
Brij 35 [$C_{12}H_{25}(OCH_2CH_2)_{25}OH$]	1200	$9 \cdot 10^{-5}$	108	2.6	40	1.07

[a] Critical micellar concentration.

[b] Micellar molar volume.

recting for extra-column contributions as described [20]. The number of theoretical plates, N, was calculated by use of the inflection point method:

$$N_{0.6h} = 4\left(\frac{t_r}{W_{0.6h}}\right)^2 \tag{2}$$

the method derived by Foley and Dorsey [21]

$$N_{0.1h} = 41.7 \cdot \frac{(t_r/W_{0.1h})^2}{(b/a) + 1.25} \tag{3}$$

or by the moment method:

$$N_{moment} = \frac{M_1^2}{M_2} \tag{4}$$

where t_r is the retention time, W_{xh} is the peak width, expressed in time units, and measured at proportion x of the peak height, h, a and b refer to the $0.1h$ peak width and the retention time [21] with $a + b = W_{0.1h}$. M_i is the ith moment: M_0 is the peak area defined as

$$M_0 = \int C(t) \, dt \tag{5}$$

where $C(t)$ is the detector signal at time t,

$$M_1 = \frac{1}{M_0} \int t \, C(t) \, dt \tag{6}$$

is the first reduced moment corresponding to the peak retention time [22] and

$$M_2 = \frac{1}{M_0} \int (t - M_1)^2 \, C(t) \, dt \tag{7}$$

is the second central reduced moment corresponding to the peak variance.

The efficiency measurement method employed may significantly affect the Knox parameters, as pointed out recently [23]. In this work, the moment method was most often employed. Each plate count value, obtained using the moment method, was double-checked using the Foley–Dorsey equation [24]. In one instance (C_{14} column and methanol–water mobile phase, after SDS exposure), the peak tailings were so large that the computer could not find the peak terminations. In that case, the inflection point method was used for efficiency measurements. The A, B and C terms of the Knox equation were determined using a computer fitting method [24]. The uncertainty margin that can be as high as $\pm 50\%$ of a fitted value for tailing peaks is always given in parentheses after every Knox parameter listed.

RESULTS AND DISCUSSION

Solute diffusion in micellar solutions

Molecular diffusion is the predominant plate height contribution at low mobile phase velocities [20,25]. This parameter, D_m, is essential in the calculation of v ($v = \mu d_p/D_m$). It was directly determined in the different micellar phases and in the reference aqueous–organic phases. Table II lists the diffusion coefficients obtained by

TABLE II

DIFFUSION COEFFICIENTS IN VARIOUS MOBILE PHASES AT 24°C

Micellar phases

Solute	Micellar partition coefficient[a]	Diffusion coefficient ($\times 10^6$ cm^2/s)
Brij 22, (5%, w/v; 8·10^{-2} mol/l)		
Benzyl alcohol	1600 ± 200	5.1 ± 0.1
Benzaldehyde	2100 ± 300	4.7 ± 0.1
Benzene	7000 ± 800	2.88 ± 0.01
Micelle	−	0.87[b]
Brij 35, (5%, w/v; 4.2·10^{-2} mol/l)		
Benzyl alcohol	400 ± 40	6.3 ± 0.1
Benzaldehyde	640 ± 50	5.5 ± 0.1
Benzene	1800 ± 200	4.4 ± 0.2
Micelle	−	0.94[b]
SDS, (1.4%, w/v; 5·10^{-2} mol/l)		
Benzene	4800 ± 500	6.8 ± 0.3
Toluene	15 000 ± 1000	3.4 ± 0.2
Ethylbenzene	43 000 ± 3000	1.7 ± 0.1
Propylbenzene	120 000 ± 10 000	0.94 ± 0.08
Butylbenzene	340 000 ± 40 000	0.68 ± 0.07
SDS micelle	−	0.57[c]

Hydro-organic mobile phases

Solute	Diffusion coefficient ($\times 10^6$ cm^2/s)		
	Water[d]	ACN–water (30:70, v/v)	Methanol–water (70:3, v/v)
Benzene	11.2 ± 0.7	11.4 ± 0.1	8.65 ± 0.06
Toluene	10.5 ± 0.7	−	8.0 ± 0.05
Ethylbenzene	9.9 ± 0.7	−	7.4 ± 0.05
Propylbenzene	8.4 ± 0.6	−	6.5 ± 0.05
Butylbenzene	7.0 ± 0.5	−	5.5 ± 0.05
Benzyl alcohol	8.7 ± 0.4	9.6 ± 0.4	−
Benzaldehyde	8.9 ± 0.3	10.08 ± 0.03	−

[a] Data from refs. 9, 12, 14, 26, 27.
[b] Calculated from the Stokes-Einstein equation using the micelle radii given in Table I.
[c] From ref. 11.
[d] Calculated using eqn. 9 in ref. 11.

the Taylor dispersion technique. Measurements were done in SDS, Brij 35 and Brij 22 solutions. The last surfactant was not utilized for efficiency comparison in this work but it will be used in future work.

As demonstrated previously [11], the inclusion of a solute in a micelle produces a significant decrease in its diffusion coefficient, D_m. The equation expressing D_m as a function of D_{mic} and D_{aq}, the diffusion coeffcient of the micelle and the solute diffusion coefficient in water, respectively, is [11]

$$D_m = \frac{D_{mic}}{1 + \Psi} + \frac{D_{aq}}{1 + \dfrac{1}{\psi}} \qquad (8)$$

where

$$\Psi = \frac{N(1 - CV)}{PCV} \qquad (9)$$

N is the micellar aggregation number, C is the surfactant concentration in the micelle, *i.e.*, the total surfactant concentration minus the CMC, and V is the surfactant micellar molar volume. N, CMC and V values are listed in Table I. The diffusion coefficients, D_{aq}, in water without micelles were calculated from the experimental D_m values obtained in the three different micellar solutions. They are in close agreement, within ± 5%. For example, the benzene D_{aq} values calculated from the D_m values in Brij 22, Brij 35 and SDS solutions at 25°C were $1.02 \cdot 10^{-5}$, $1.17 \cdot 10^{-5}$ and $1.18 \cdot 10^{-5}$ cm^2/s, respectively.

A, B and C Knox parameters

Fig. 1 shows the theoretical contributions of each term for a "good" column ($A = 1$, $B = 2$, $C = 0.035$). As already stated, the B term, related to molecular diffusion, is the predominant plate height contribution at low reduced velocities ($v < 2$). The stationary phase mass transfer processes (C term), in contrast, become increasingly
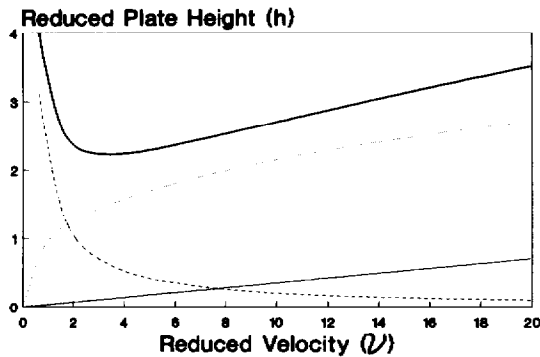

Fig. 1. Theoretical *h versus* v plot for a good column. Dotted line: flow anisotropy contribution, $Av^{1/3}$, with $A = 1$. Dashed line: longitudinal molecular diffusion contribution, B/v, with $B = 2$. Full line: mass transfer contribution, Cv, with $C = 0.035$. Bold line: the Knox plot, sum of the three contributions.
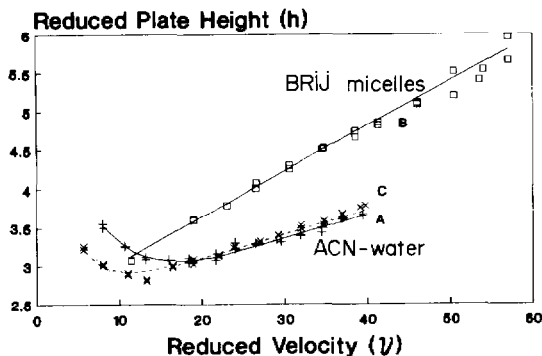
Reduced Plate Height (h)

Fig. 2. Knox plots with a C_{18} column and the solute benzene. (A) +, ACN–water (30:70, v/v) on the new column; $A = 0.65$, $B = 16.4$, $C = 0.026$. (B) □, Brij 35 (5%, w/v) mobile phase; $A = 1.0$, $B = 6$, $C = 0.03$. (C) ×, ACN–water (30:70, v/v) after Brij exposure. Dotted line: $A = 0.75$, $B = 10.2$, $C = 0.025$. Dashed line: $A = 0.91$, $B = 8.6$, $C = 0.10$.

significant as the reduced velocity increases. The flow anisotropy (A term) contributes to *ca.* 50% or more of the plate height at all reduced flow velocities greater than 2.

Fig. 2 shows the reduced plate height *versus* reduced efficiency for the solute benzene on the 10 cm × 5 mm I.D. C_{18} column with (A) ACN–water (30:70, v/v) on the new column, (B) a 5% (w/v) Brij 35 mobile phase and (C) ACN–water (30:70, v/v) again after a column wash with the aqueous–organic mobile phase. Fig. 3 shows the same set of *h versus v* plots on the 15 cm × 4 mm I.D. C_{14} column: (A) with methanol–water (70:30, v/v) on the new column, (B) with 1.4% (w/w) SDS (0.05 *M*), (C) with methanol–water (70:30, v/v) after a column wash with methanol. Table III lists the capacity factors and A, B and C terms of the Knox equation obtained with the different mobile phases using the fitting procedure described in ref. 24. The uncertainty margin, indicated with every fitted term, is illustrated in Fig. 2C. Table III lists the parameters $A = 0.83 \pm 0.08$, $B = 9.4 \pm 0.8$, $C = 0.017 \pm 0.007$. The dotted line in Fig. 2 is the theoretical Knox plot with $A = 0.75$, $B = 10.2$ and $C = 0.025$, which are the

Reduced Plate Height (h)
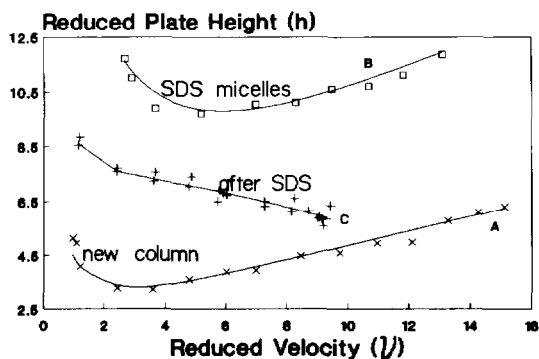

Fig. 3. Knox plots with a C_{14} column and the solute benzene. (A) ×, methanol–water (70:30, v/v) on the new column; $A = 1.1$, $B = 3.1$, $C = 0.22$. (B) □, SDS (1.4%, w/v) mobile phase; $A = 3.2$, $B = 20$, $C = 0.20$. (C) +, methanol–water (70:30, v/v) after SDS exposure; $A = 0.70$, $B = 5.7$, $C = -0.7$.

TABLE III

PARAMETERS OF THE KNOX EQUATION WITH DIFFERENT MOBILE PHASES

Mobile phase	Solute	k'	A		B		C	
Acetonitrile–water (30:70, v/v)	Benzyl alcohol	2.2	1.4	± 0.1	2.5	± 0.6	0.029	± 0.009
	Benzaldehyde	6.6	1.0	± 0.04	10	± 0.5	0.027	± 0.004
	Benzene	17.3	0.65	± 0.03	16.4	± 0.6	0.026	± 0.004
Water–Brij 35 (5%, w/v)	Benzyl alcohol	4.5	1.3	± 0.15	10	± 3	0.15	± 0.03
	Benzaldehyde	11.0	1.4	± 0.15	8	± 2.5	0.04	± 0.015
	Benzene	26.1	1.0	± 0.12	6	± 2	0.03	± 0.01
Acetonitrile–water (30:70, v/v) after Brij exposure	Benzyl alcohol	1.9	1.3	± 0.1	11.2	± 1.1	0.08	± 0.02
	Benzaldehyde	5.2	1.0	± 0.1	10.8	± 0.9	0.04	± 0.01
	Benzene	15.2	0.83	± 0.08	9.4	± 0.8	0.017	± 0.007
Methanol–water (70:30, v/v)	Benzene	1.1	1.1	± 0.15	3.1	± 0.2	0.22	± 0.02
	Toluene	2.1	1.05	± 0.15	3.5	± 0.2	0.16	± 0.02
	Ethylbenzene	3.5	1.1	± 0.15	3.8	± 0.4	0.15	± 0.02
	Propylbenzene	6.0	1.3	± 0.15	5.0	± 0.4	0.09	± 0.02
Water–SDS (1.4%, v/v)	Benzene	15.6	3.2	± 0.6	20	± 5	0.2	± 0.1
	Toluene	29.0	3.0	± 0.7	25	± 6	0.5	± 0.2
	Ethylbenzene	45.0	3.1	± 0.7	23	± 6	0.5	± 0.2
	Propylbenzene	60.5	3.0	± 0.7	20	± 6	0.7	± 0.3
Methanol–water (70:30, v/v) after SDS exposure	Benzene	0.70	5.7	± 0.3	4	± 0.4	−0.7	± 0.1
	Toluene	1.47	5.2	± 0.3	4.2	± 0.4	−0.64	± 0.08
	Ethylbenzene	2.54	4.6	± 0.2	4.4	± 0.5	−0.54	± 0.06
	Propylbenzene	4.54	4.0	± 0.2	4.7	± 0.6	−0.42	± 0.05

minimum A value and the maximum B and C values. The dashed line is the theoretical Knox plot with the maximum A value ($A = 0.91$) and the minimum B and C values ($B = 8.6$, $C = 0.01$). Both lines fit the experimental set of points within the error margins. This illustrates that the Knox equation must be used with care to obtain information on band broadening contributions. For the same set of experimental points, the maximum C value ($C = 0.025$) is 2.5 times greater than the minimum C value ($C = 0.01$). As pointed out [24], the uncertainty in the B adjustment can be very high when the h *versus* v plot does not present a minimum. This was often the case with micellar solutions and explains the high error margins in the parameters obtained with micellar mobile phases. As will be discussed later, the validity of the use of the Knox equation with micelle-exposed columns may be questioned [26].

A term. The A term depends on flow anisotropy. The A term for benzene was 0.65 in an ACN–water mobile phase and 1.0 in the Brij 35 micellar mobile phase, *i.e.*, 40% higher. At a reduced velocity $v = 20$, the reduced plate height was $h = 3.10$ with the ACN–water phase and $h = 3.62$ with the Brij 35 micellar phase (Fig. 2). The A contribution, *i.e.*, $Av^{1/3}$, was 1.76 (56% of the whole band broadening) and 2.71 (75%) with the ACN–water and the Brij 35 micellar phase, respectively. The 40% increase in A is the main factor responsible for micellar efficiency loss. A threefold increase in A induced by the SDS micellar phase was observed for the four alkylbenzenes studied. For benzene, at a reduced velocity $v = 10$, the reduced plate height was $h = 4.9$ with the methanol–water phase and $h = 10.9$ with the SDS micellar phase (Fig. 3). The A contribution was 2.36 (48%) and 6.9 (63%) with the methanol–water and the SDS micellar phase, respectively. Again, the increase in A was the main factor responsible for micellar efficiency loss.

In only one case, propylbenzene and C_{14} phase, the micellar efficiency loss was due to both A and C increases. The A value for propylbenzene was 1.3 in methanol–water and 3.0 in SDS. The corresponding C values were 0.09 and 0.7, respectively (Table III). At a reduced velocity $v = 10$, the reduced plate height was $h = 4.2$ with the methanol–water phase and 15.5 with the SDS phase. The A contribution was 2.8 (66%) and 6.5 (only 42%) with the methanol–water phase and the SDS micellar mobile phase, respectively. The C contribution, *i.e.*, Cv, increased from 0.9 (22%) to 7 (45%). The particular case of propylbenzene is discussed further below.

It seems that the presence of micelles and the surfactant-induced stationary phase modifications significantly increase the flow anisotropy. The surfactant adsorbed layer may change the column porosity and permeability [12,26]. This point will be discussed later.

B term. According to Giddings [25], the B term can be written as

$$B = 2\left[\gamma_m + \gamma_s\left(\frac{D_s}{D_m}\right)k' \right] \tag{10}$$

where γ_m and γ_s are the obstruction factors for diffusion through granular and/or porous materials. Subscripts m and s refer to the mobile and stationary phase, respectively. The D terms are the solute diffusion coefficient in the mobile (m) and stationary (s) phase, and k' is the solute capacity factor.

B is related to band broadening due to molecular diffusion. The lowering of the

solute diffusion coefficient produced by micellar inclusion (Table II) should cause an increase in the B term. This was the case for all solutes studied, except for the benzene and Brij 35 system, where a decrease in the B term was observed. As shown by Khaledi [28], the relative eluent strength of micellar mobile phase is much lower than that of aqueous–organic phases. In this study, the capacity factors obtained with micellar phases were always higher than the corresponding values obtained with aqueous–organic phases and the same stationary phase. The k' value for benzene was 17.3 with the ACN–water mobile phase and 26.1 with the Brij 35 mobile phase. As the B term decreased from 16.4 to 6, this means that the D_s/D_m ratio was drastically reduced (eqn. 10). The B value obtained with SDS solutions were *ca.* 20 (Table III) with no increase as the capacity factors increased from 15.6 (benzene) to 60.5 (propylbenzene). Given the D_m values listed in Table II, it can be estimated than the D_s values became lower as the hydrophobic character of the solute increased. This observation supports the solubility limit theory proposed by Borgerding and co-workers [27,29]. Very hydrophobic solutes are directly transferred from the micelle interior to the stationary phase organic layer. They do not and cannot go into the aqueous phase because their water solubility is too low. The solute diffusion in the surfactant-modified stationary phase is very restricted.

C term. The C term of the Knox equation represents the mass transfer contribution to solute band broadening. It was written as [25]

$$C = q\left(\frac{k' + \varphi}{1 + k'}\right)^2 \left(\frac{D_m}{\gamma_{sm}\varphi D_m + k'y_s D_s}\right) \tag{11}$$

where the γ terms are obstruction factors, the subscripts s, m and sm represent the mobile, stationary and pore stagnant mobile phase, respectively, φ is the stagnant mobile phase fraction and q is a geometrical factor dependent on porosity [25].

The micellar mobile phase induced an increase in the C term that was as high as 700%, as already noted (propylbenzene and SDS phase). The reduced mass transfer induced by micellar phases was attributed to poor wetting of the stationary phase [5,7] or to surfactant adsorption [9–11,13,26,27] on the stationary phase. This latter point is the most important and warrants further discussion.

Surfactant adsorption

The decrease in efficiency caused by micellar mobile phases is revealed by increases in the three terms of the Knox equation. Micellar mobile phases seem to increase the flow anisotropy, which increases the A term, and to decrease drastically the solute diffusion coefficients in both the stationary and mobile phases, which increases the B and C terms. Both effects can be explained by surfactant adsorption. It was demonstrated by Berthod *et al.* [30] that the important differences between a C_1, C_8, C_{18} or CN bonded stationary phase were reduced in micellar chromatography. All of the surfactant-covered phases behaved similarly.

The surfactant adsorption was *ca.* 70 mg of Brij 35 per gram of C_{18} stationary phase and 140 mg of SDS per gram of C_{14} phase or 2.3 μmol/m^2. As described by Borgerding *et al.* [12,26], the volumes occupied by the surfactants were 63 and 120 mm^3, respectively, producing a decrease in the stationary phase pore volume of 22%

and 28%, respectively. The surfactant tends to clog the smallest pores, with diameters smaller than 7 nm, dramatically decreasing the stationary phase surface area [12,26]. Such changes in the stationary phase physico-chemical characteristics may modify the mobile phase circulation, thus explaining the A term increases.

The surfactant adsorption increases the stationary phase organic layer thickness and decreases the mass transfer rate and D_s, the solute diffusion coefficient in the stationary phase [9,13,26,27,29]. This adsorption may explain the increases in the B (eqn. 10) and C terms (eqn. 11). The significant improvement in efficiency obtained with addition of small amounts of a short-chain organic modifier (propanol [7], pentanol or tetrahydrofuran [13]) is primarily due to the displacement of adsorbed surfactant. Pentanol, which best desorbs ionic surfactants, is the best organic additive for micellar efficiency improvement [14].

Surfactant adsorption reversiblity

An important point has not been well addressed in the literature, namely the question of whether it is possible to desorb completely the surfactant adsorbed on a bonded stationary phase. Fig. 2 shows that the Knox plot obtained with a new C_{18} column roughly corresponds to the plot obtained with the same solute and column after surfactant exposure. The column was washed with ACN–water (30:70, v/v) solution; a column wash with pure methanol or pure isopropanol would have completely eliminated any trace of adsorbed Brij 35, restoring the initial column efficiency. However, this was not observed with the C_{14} column. After SDS exposure, the C_{14} column was washed overnight with pure methanol as recommended by Berthod and Roussel [13]. Fig. 3C shows the unusual h *versus* v plot obtained with the surfactant-exposed C_{14} column. After looking at the results, the column was washed one more time with pure isopropanol. Another identical (within experimental error) h *versus* v plot was obtained (Fig. 3C). The peak tailings were so large that the computer software could not find the peak terminations. The inflection point method was used for plate count computation, which may explain the unusual decrease in the h *versus* v plot observed in Fig. 3C.

It was not possible to restore the initial efficiency of this column. Irreversible adsorption of SDS was suspected. The column was opened and drained, pushing with methanol. The stationary phase was collected, dried and sent for elemental analysis. Trace amounts of sulfur (0.09 $\pm$ 0.03%, w/w) were found. This corresponds to about 9 mg of SDS irreversibly adsorbed on the C_{14} phase; 9 mg of SDS represents only 32 μmol, or 0.14 μmol/m^2, which is only 6% of the SDS initially adsorbed on the C_{14} phase. This small amount of irreversibly adsorbed SDS is enough to modify critically the organic bonded C_{14} layer, producing very low chromatographic efficiency. It is not possible to discuss the Knox parameters obtained with such a modified stationary phase because the peak efficiencies may not have been accurately evaluated and the Knox equation may not correctly depict the efficiency evolution *versus* flow-rate. Theoretically (eqn. 11), is not possible to obtain negative C values. If the C values are not correct, the corresponding A and B terms have no meaning.

Such an irreversible SDS adsorption was observed by Knox and Hartwick [31] on a monomeric C_{18} bonded phase. However, we observed fully reversible SDS adsorption on the same kind of C_{18} phases [10,13]. We have never observed irreversible adsorption on short-chain bonded phases [10,13,30]. Further, irreversible ad-
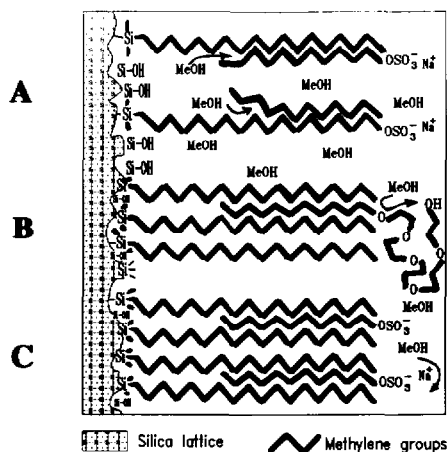
Fig. 4. Surfactant adsorption–desorption. (A) On a medium-density bonded stationary phase, methanol can go inside the bonded layer to desorb the surfactant molecules. (B) On a densely grafted stationary phase, the hydrophobic chain of an nonionic surfactant is inserted in the bonded organic layer, but its bulky hydrophilic part is an easy grasp to extract the molecule. (C) On a densely grafted stationary phase, the long hydrophobic chain of an ionic surfactant is tightly inserted in the bonded organic layer. Organic solvents cannot grap the small polar head, the surfactant adsorption is irreversible. The high efficiency of the column is irreversibly lost after surfactant exposure. MeOH = methanol.

sorption was not observed with short-chain surfactants [31]. It seems that irreversible surfactant adsorption is more likely with densely grafted monomer stationary phases and small polar head group surfactants, with both the surfactant and bonding moiety having hydrophobic chains longer than C_8. Fig. 4 illustrates this mechanism. With a low bonding density (<2.5 $\mu mol/m^2$) stationary phase (Fig. 4A), the hydrocarbon tail of the surfactant molecule is not tightly inserted in the bonded hydrocarbon layer. The surfactant molecules can be washed out by methanol. The long polar chain of a nonionic surfactant provides an easy "handle" with which to extract the inserted part of the molecule (Fig. 4B). With a long-chain ionic surfactant and a densely grafted stationary phase (Fig. 4C), it is difficult to get a grip on the surfactant molecule and to extract it from the bonded layer. The inserted SDS molecules may induce some local rigid crystallinity which restrains solute–stationary phase exchanges. Fig. 4C shows also that the surfactant ionic groups face the mobile phase. The ion-exchange properties of such surfactant-exposed stationary phases have been shown to be useful for ion analysis [31–33]. The polarity increase of the C_{14}–SDS surfactant-exposed stationary phase was evidenced by the decrease in the capacity factor of the hydrophobic solutes after exposure to the surfactant [compare the data for methanol–water (70:30, v/v) before and after SDS exposure, Table III].

CONCLUSIONS

As far as the Knox equation can be significantly used to study the efficiency changes in micellar liquid chromatography, the efficiency loss produced by a micellar mobile phase in mainly due to an increase in the A term. The B and C terms also

increased, contributing to the decrease in efficiency. These changes can be explained by the significant surfactant adsorption on the stationary phase that occurs with aqueous micellar solutions. The adsorbed surfactant decreases the column porosity, permeability and tortuosity (A increase); it also decreases the solute diffusion in the stationary phase, and the solute mass transfer (C increase). The surfactant adsorbed on the stationary phase can be washed out by a pure organic solvent. However, the adsorption can be partially irreversible, especially when the stationary phase is a high-density "brush-type" monomer phase and the surfactant is an ionic, small polar head group molecule.

REFERENCES

1 D. W. Armstrong and R. Q. Terrill, *Anal. Chem.*, 51 (1979) 2160.
2 D. W. Armstrong and F. Nome, *Anal. Chem.*, 53 (1981) 1662.
3 D. W. Armstrong, *Sep. Purif. Methods*, 14 (1985) 212.
4 W. L. Hinze, in W. L. Hinze and D. W. Armstrong (Editors), *Ordered Media in Chemical Separations*, (*ACS Symposium Series*, Vol. 342), American Chemical Society, Washington, DC, 1987, Ch. 1, pp. 2–82.
5 J. G. Dorsey, *Adv. Chromatogr.*, 27 (1987) 167.
6 A. Berthod and J. G. Dorsey, *Analusis*, 16 (1988) 75.
7 J. G. Dorsey, M. T. DeEchegaray and J. S. Landy, *Anal. Chem.*, 55 (1983) 924.
8 P. Yarmchuck, R. Weinberger, R. F. Hirsch and L. J. Cline-Love, *J. Chromatogr.*, 283 (1984) 47.
9 M. F. Borgerding and W. L. Hinze, *Anal. Chem.*, 57 (1985) 2183.
10 A. Berthod, I. Girard and C. Gonnet, *Anal. Chem.*, 58 (1986) 1356.
11 D. W. Armstrong, T. J. Ward and A. Berthod, *Anal. Chem.*, 58 (1986) 579.
12 M. F. Borgerding, W. L. Jinze, L. D. Stafford, G. W. Fulp and W. C. Hamlin, *Anal. Chem.*, 61 (1989) 1353.
13 A. Berthod and A. Roussel, *J. Chromatogr.*, 449 (1988) 349.
14 W. L. Hinze, Z. S. Fu, R. W. Williams, F. S. Sadek and A. Berthod, *J. Chromatogr.*, submitted for publication.
15 J. H. Knox and J. N. Done, *J. Chromatogr. Sci.*, 10 (1972) 606.
16 J. H. Knox, *J. Chromatogr. Sci.*, 15 (1977) 352.
17 A. Chartier, C. Gonnet, D. Morel, J. L. Rocca and J. Serpinet, *J. Chromatogr.*, 438 (1988) 263.
18 K. L. Mittal and B. Lindman (Editor), *Surfactants in Solution*, Plenum Press, New York, 1984.
19 G. I. Taylor, *Proc. R. Soc. London, Ser. A*, 223 (1954) 446.
20 A. Berthod, F. Chartier and J. L. Rocca, *J. Chromatogr.*, 469 (1989) 53.
21 J. P. Foley and J. G. Dorsey, *Anal. Chem.*, 55 (1983) 730.
22 B. A. Bidlingmeyer and F. V. Warren, *Anal. Chem.*, 56 (1984) 1583A.
23 A. Berthod, *J. Liq. Chromatogr.*, 12 (1989) 1187.
24 A. Berthod, *J. Liq. Chromatogr.*, 12 (1989) 1169.
25 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
26 M. F. Borgerding, *Ph. D. Dissertation*, Wake Forest University, Winston-Salem, NC, 1988.
27 M. F. Borgerding, F. H. Quina, W. L. Hinze, J. Bowermaster and H. McNair, *Anal. Chem.*, 60 (1988) 2520.
28 M. G. Khaledi, *Anal. Chem.*, 60 (1988) 876.
29 M. F. Borgerding, R. L. Williams, W. L. Hinze and F. H. Quina, *J. Liq. Chromatogr.*, 12 (1989) 1367.
30 A. Berthod, I. Girard and C. Gonnet, in W. L. Hinze and D. W. Armstrong (Editors), *Ordered Media in Chemical Separations*, (*ACS Symposium Series*, Vol. 342), American Chemical Society, Washington, DC, 1987, Ch. 5, pp. 130–141.
31 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
32 A. Berthod, I. Girard and C. Gonnet, *Anal. Chem.*, 58 (1986) 1359.
33 F. G. P. Mullins, in W. L. Hinze and D. W. Armstrong (Editors), *Ordered Media in Chemical Separations*, (*ACS Symposium Series*, Vol. 342, American Chemical Society, Washington, DC, 1987, Ch. 4, pp. 115–129.