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COMPARATIVE STUDY OF ALKYL AND FLUOROALKYL N-(2-HYDROXY-ETHOXYETHYL)-AMIDES AS REVERSED-PHASE LIQUID CHROMATOGRAPHIC MODIFIERS

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SUMMARY

The use of the non-ionic surfactant, N-(2-hydroxy-ethoxyethyl)-2,2,3,3,4,4,4-heptafluorobutanamide, as a liquid chromatographic mobile phase modifier has been studied. Comparisons between this fluorinated compound and two similar hydrocarbon surfactants, N-(2-hydroxyethoxyethyl)-hexanamide and N-(2-hydroxyethoxyethyl)-heptanamide, have been made. Although surface tension data were similar for all three surfactants, the fluoroalkyl compound was found to have a larger influence on retention. Likewise, the positional isomers of cresol and toluidine were resolvable using the fluorinated surfactant and were not with the equivalent alkyl surfactant with a similar hydrophilic–lipophilic balance.

INTRODUCTION

Although ionic surfactants have found wide-spread acceptance as mobile phase modifiers in reversed-phase liquid chromatography (RPLC), non-ionic surfactants have not been utilized to the same extent[1–3]. Typically, when non-ionic compounds have been used, they have been either alkylpolyoxyethylene ethers, esters, or amides. In general, except for a few fluorinated alcohols[4,5], fluorine-containing compounds have not been employed as modifiers in RPLC.

The potential of fluoroalkyl surfactants to effect solute retention may be inferred from the chromatographic properties of fluoroalkyl modified silica[6–8]. Under equivalent reversed-phase conditions these surfaces have been found to retain solutes to a lesser degree than the corresponding alkyl modified materials. This coupled with the unique physical properties of fluorine-containing compounds make fluorosurfactants potentially interesting as reversed-phase modifiers.

A major source of interest in non-ionic fluorinated surfactants arise from their potential use in the preparation of blood substitutes[9–11]. In conjunction with this bioengineering work, various physical properties of these compounds such as their critical micelle concentration (CMC), phase inversion temperature, and wetting characteristics have been compared to corresponding alkyl surfactants[10,11]. For a given head group the surface active properties of fluoroalkyl surfactants are

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equivalent to those of structurally similar alkyl surfactant having 1.5 times more carbon in hydrophobic end of the molecule.

In the current study, the properties of N-(2-hydroxy-ethoxyethyl)-2,2,3,3,4,4,4-heptafluorobutanamide (I) as a mobile phase modifier have been investigated. The ability of I to affect chromatographic selectivity has been compared to that of two similar hydrocarbon surfactants, N-(2-hydroxyethoxyethyl)-hexanamide (II), and N-(2-hydroxyethoxyethyl)-heptanamide (III), which has a hydrophilic–lipophilic balance similar to that of I. Significant differences in chromatographic selectivity in the presence of the two classes of surfactants have been observed.

EXPERIMENTAL

Apparatus

Chromatographic experiments were carried out with an IBM Instruments (Danbury, CT, U.S.A.) Model LC/9533 ternary gradient liquid chromatograph equipped with UV and refractive index detectors. Retention data were recorded and processed on an IBM Instrument Model 9000 data system. The octadecyl column was (15 cm × 4.6 mm I.D.) also from IBM Instruments. Surface tension measurements were made using a DuNouy interfacial tensiometer (Central Scientific, Chicago, IL, U.S.A.). All experiments were performed at ambient temperature.

Reagents and procedures

The mobile phases were prepared from high-performance liquid chromatographic (HPLC)-grade methanol (Fisher Scientific, Pittsburgh, PA, U.S.A.) and deionized water which was purified using a Milli-Q reagent water system (Bedford, MA, U.S.A.). Hexanoic acid, heptanoic acid, and methyl heptafluorobutyrate were obtained from Matheson (Houston, TX, U.S.A.), Eastman Kodak (Rochester, NY, U.S.A.), and Aldrich (Milwaukee, WI, U.S.A.), respectively.

Hexanoic acid and heptanoic acid were converted to their methyl esters by treatment with methanol in the presence of concentrated sulfuric acid. Subsequently, these esters as well as methyl heptafluorobutyrate were reacted with excess 2-(2-amino-ethoxy)ethanol in the presence of zinc oxide to yield the corresponding surfactants[12]. The final products were vacuum distilled and characterized by nuclear magnetic resonance and infrared spectrometry.

Prior to use the chromatographic column was rinsed with about 100 ml each of water, methanol, and water and then it was conditioned with a minimum of 100 ml of the mobile phase. This same procedure was used with each new mobile phase. The column void volume was determined using ^2H_2O. All retention and surface tension measurements were made at least twice and in most cases in triplicate.

RESULTS AND DISCUSSION

Shown in Fig. 1 are plots of surface tension *vs.* ln concentration for the three surfactants studied. The linear dependencies of these plots are consistent with trends reported for other non-ionic surfactants[13,14] and indicate that the concentration range studied was below the CMC for I, II and III. The slopes of the plots in Fig. 1 are related to the surface excess concentration of the surfactants according to the Gibbs

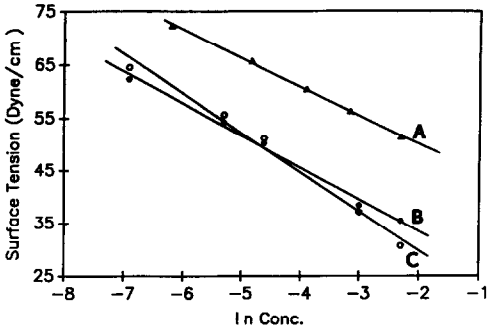

Fig. 1. Surface tension *vs.* ln surfactant concentration. Surfactants: (A) II, (B) III and (C) I.

equation[14]. Similar values were obtained for the two hydrocarbon surfactants and only a slightly larger slope was observed for I. Additionally, the data for I and III (Fig. 1, curves B and C) are nearly superimposable. The above results indicate that there is a relatively small difference between the three surfactants based on surface tension measurements and that I and III have a similar hydrophilic–lipophilic balance.

The ln–ln plots of the capacity factors for *o*-nitroaniline, phenol, and resorcinol *vs.* surfactant concentration are shown in Fig. 2. Linearity in such plots (*i.e.*, ln k' *vs.* ln additive concentration) also have been reported for other reversed-phase systems[15,16]. For a given modifier, simple hydrophobic theory predicts straight line plots of similar slope but different intercepts for solutes which have nearly the same properties[15].

The slopes of the linear fits to the data in Fig. 2 are summarized in Table I. Similar values were observed for both alkyl modifiers for a given solute. However, an approximately three to five-fold increase was noted with the fluoroalkyl modifier. The above trends demonstrate that I has a more pronounced effect on retention than the corresponding hydrocarbon analogue, III. The reductions in solute retention are likely due to unfavorable interactions between solute and surfactant sorbed into the bonded layer with all or part of its fluoroalkyl end exposed. The proposed sorption model is consistent with published chromatographic data obtained on bonded fluoroalkyl phases[6–8]. In this latter instance, reduced retentions have also been explained in terms of unfavorable interactions between the immobilized fluorocarbon chains and the solutes.

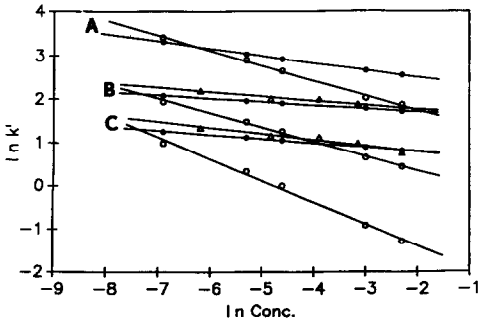

Fig. 2. ln k' *vs.* ln surfactant concentration. Solutes: (A) *o*-nitroaniline, (B) phenol, (C) resorcinol. Surfactants: (○) I, (△) II, and (●) III.

TABLE I

SLOPES OF ln k' *vs.* ln SURFACTANT CONCENTRATION

Solutes	*Surfactant*		
	I	*II*	*III*
Resorcinol	0.54	0.14	0.10
Phenol	0.50	0.10	0.08
o-Nitroaniline	0.41	–	0.17

In order to further evaluate the properties of I, relative changes in k' were measured as a function of increasing carbons for a homologous series of ethyl alkanoate esters (*i.e.*, acetate, propionate, butyrate and valerate). Measurements were made as a function of concentration for the aqueous mobile phase prepared from either I or III. The methylene selectivity for a given mobile phase was determined from a plot of ln k' *vs.* carbon number (Fig. 3). The data from this set of experiments are summarized in Table II. Likewise, for comparative purposes, methylene selectivity was also determined for binary combinations of methanol and water which did not contain surfactant (Fig. 3 and Table II). The methylene selectivities obtained in methanol and water are comparable to previously reported values obtained under similar condi-

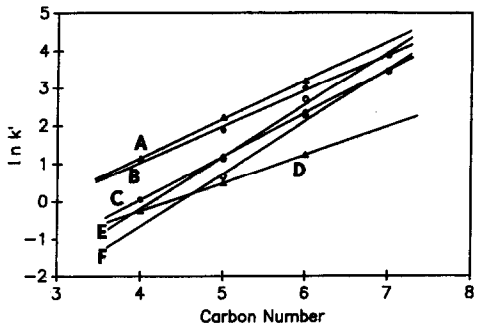

Fig. 3. ln k' *vs.* carbon number for ethyl alkanoate esters. Mobile phase: (A) methanol–water (20:80), (B) 0.02 *M* III in water, (C) 0.10 *M* III in water , (D) methanol–water (50:50), (E) 0.02 *M* I in water, (F) 0.08 *M* I in water.

TABLE II

SLOPES OF ln k' *vs.* CARBON NUMBER ETHYL ALKANOATE ESTERS

Concentration	*Slope*		
	I	*III*	*Methanol–water*
0.02	1.39	0.95	1.08 (20% Methanol)
0.05	1.40	1.07	1.00 (30% Methanol)
0.08	1.38	–	0.85 (40% Methanol)
0.10	–	1.15	0.75 (50% Methanol)

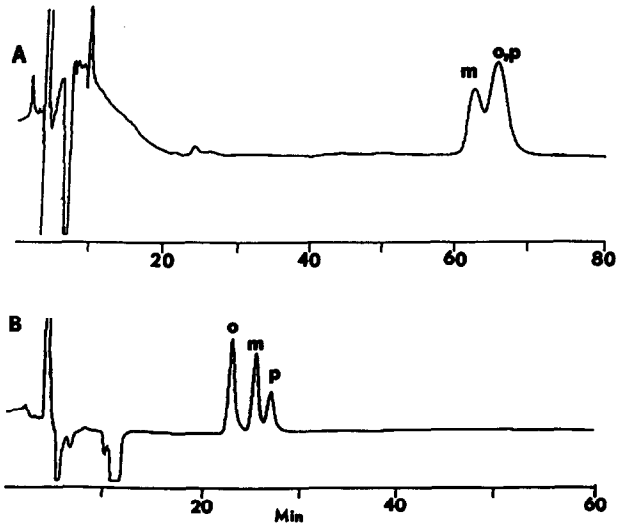

Fig. 4. Chromatograms of *o*-, *m*- and *p*-cresol. Mobile phase: (A) 0.10 *M* III and (B) 0.05 *M* I.

tions[17–19]. Of particular significance in the current study is the 30–50% higher methylene selectivity with the fluoroalkyl surfactant compared to either the corresponding hydrocarbon surfactant, III, or binary combinations of methanol and water.

Fig. 4 and 5 show differences in the separation of positional isomers of cresol and toluidine using I and III as mobile phase additives. Resolution was significantly enhanced for both sets of isomers with the fluoroalkyl surfactant added to the mobile phase. The overall retention times of the total chromatogram were equal or less than that obtained with the alkyl surfactant using only half the concentration of I compared to III. Also, a significant improvement in peak shape was observed for the toluidine isomers when the fluorinated surfactant was used. This is also further evidence for the

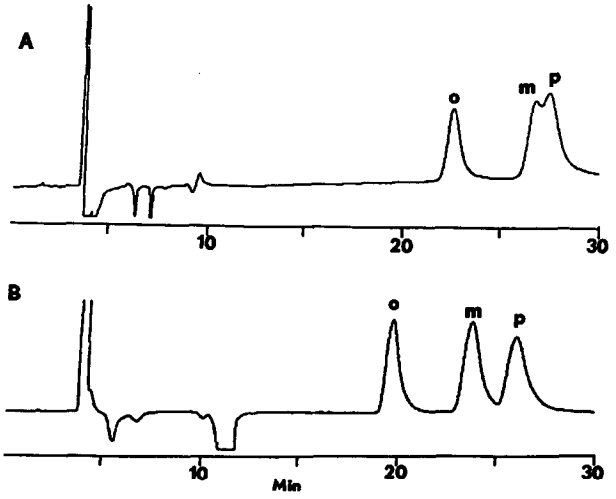

Fig. 5. Chromatograms of *o*, *m* and *p*-toluidine. Mobile phase: (A) 0.10 *M* III and (B) 0.05 *M* I.

presence of sorbed surfactant which blocks residual silanols. This behavior is similar to that obtained with nitrogen containing organic modifiers used to reduce peak tailing in reversed-phase chromatography.

The current results demonstrate the potential usefulness of non-ionic fluorinated surfactants in liquid chromatography. Similar studies, with other fluorinated surfactants are now in progress.

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