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SEPARATION OF HOMOLOGOUS FATTY ACID ALKANOLAMIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Homologous series of fatty acid mono- and diethanolamides with C_{10}–C_{18} alkyl groups were separated by high-performance liquid chromatography, employing a porous micro-spherical poly(styrene–divinylbenzene) gel as the stationary phase. The recommended conditions for the analysis were as follows: column, 500 mm × 4 mm I.D.; mobile phase, water–methanol (3:97); and column temperature, 30°. The logarithm of the capacity factors for each homologous series was directly proportional to their alkyl chain lengths. The reciprocal of the column temperature was also proportional to the capacity factor of each component.

INTRODUCTION

Fatty acid alkanolamides, which are typical non-ionic surface-active agents, are used as foam boosters and foam stabilizers in liquid detergent and cosmetic products. The most widely used material is lauric acid diethanolamide. However, diethanolamides from other fatty acids and the fatty amides of monoisopropanolamine and monoethanolamine are available commercially.

The separation of homologous series of fatty acid alkanolamides has been carried out by gas chromatography. This method, however, requires their conversion into volatile derivatives before analysis. Fatty acid alkanolamides were hydrolysed with strong acid and the fatty acids obtained were identified by determination of the acid number, hydroxyl number, and/or iodine number and by gas chromatography[1]. Lee and Puttnam[2] formed the fatty acid methyl esters from fatty acid ethanolamides by transesterification in a sealed tube at high temperature. Gross and Jones[3] reported that fatty acid alkanolamides were converted into the amines and the methyl esters of fatty acids by refluxing for 4 h with hydrogen chloride in methanol. Oka and Kojima[4] formed trimethylsilyl derivatives of fatty acid alkanolamides with N,O-bis(trimethylsilyl)acetamide (BSA) and the products were analysed by gas chromato-

TABLE I

CAPACITY FACTORS OF FATTY ACID ALKANOLAMIDES

Temperature 30° except where indicated otherwise.

Compound	Mobile phase												
	CH_3OH	H_2O-CH_3OH (3:97)				H_2O-CH_3OH (5:95)	0.05 M $HClO_4$*	0.1 M $HClO_4$*	0.5 M $HClO_4$*	0.5 M $NaClO_4$*	0.5 M HCl*	0.5 M $LiCl$*	0.1 M NH_4Cl*
		30°	40°	50°	60°								
Fatty acid monoethanolamides													
C_{10}	0.46	0.60	0.50	0.44	0.35	0.69	0.36	0.36	0.47	0.61	0.44	0.68	0.57
C_{12}	0.64	0.89	0.74	0.65	0.52	1.09	0.52	0.51	0.70	0.92	0.64	0.95	0.78
C_{14}	0.90	1.36	1.11	0.95	0.75	1.76	0.76	0.75	1.07	1.42	0.95	1.42	1.10
C_{16}	1.30	2.11	1.69	1.40	1.12	2.93	1.11	1.11	1.66	2.23	1.42	2.11	1.58
C_{18}	1.87	3.31	2.59	2.08	1.65	4.90	1.63	1.65	2.64	3.51	2.14	3.23	2.30
Fatty acid diethanolamides													
C_{10}	0.57	0.61	0.51	0.44	0.36	0.71	0.40	0.41	0.50	0.63	0.51	0.71	0.58
C_{12}	0.75	0.92	0.75	0.65	0.53	1.12	0.57	0.58	0.75	0.92	0.74	1.01	0.81
C_{14}	1.02	1.41	1.14	0.96	0.79	1.84	0.84	0.86	1.16	1.42	1.09	1.49	1.15
C_{16}	1.46	2.23	1.75	1.43	1.16	3.08	1.25	1.29	1.85	2.24	1.66	2.25	1.66
C_{18}	2.10	3.56	2.73	2.16	1.72	5.27	1.88	1.94	2.78	3.57	2.56	3.51	2.46

* In methanol.

graphy. O'Connell[5] also reported that the trimethylsilyl derivatives of coconut diethanolamide formed with a silylation reagent consisting of BSA and trimethylchlorosilane were separated by gas chromatography. In previous papers[6,7], we reported the separation of homologous alkylbenzyldimethylammonium chlorides, alkylpyridinium halides and alkylbenzenesulphonates by high-performance liquid chromatography (HPLC) without pre-treatment of the samples and also showed that this method may be applicable to the analysis of other surface-active agents. This paper is concerned with the application of HPLC to the separation of homologous fatty acid alkanolamides.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was as described previously[6].

Reagents and samples

The column packing material was Hitachi Gel 3011, consisting of porous micro-spherical particles of a copolymer of styrene and divinylbenzene with average diameter 10–15 μm.

Fatty acid alkanolamides with C_{10}–C_{18} alkyl groups were prepared from the appropriate amines and fatty acid chlorides as described by Trowbridge et al.[8]. Fatty acid monoethanolamides (FME) were purified by repeated recrystallization from ethanol and fatty acid diethanolamides (FDE) from acetone.

All other reagents were of analytical-reagent grade.

Procedure

The column was packed by using the slurry packing procedure described previously[6].

FME and FDE were dissolved in methanol and the sample solution was injected into the column with a microsyringe through a septum injector. The detector was operated at 215 nm.

RESULTS AND DISCUSSION

Poly(styrene–divinylbenzene) gel is well suited as a stationary phase in reversed-phase partition chromatography. Methanol or mixtures of methanol and water are usually used as the mobile phase. In this study, these chromatographic systems were applied to the separation of homologous FME and FDE. The results are given in Table I.

In each series of FME and FDE, the elution order follows the order of increasing alkyl chain length. The capacity factors of FME and FDE increased and the resolutions were improved with increasing water content in the mobile phase. As the capacity factors increased, the time required for the separation of the homologous series increased several-fold. On the other hand, the capacity factors decreased and the resolution deteriorated on increasing the column temperature. Adequate separations of homologous FME and FDE with C_{10}–C_{18} alkyl groups were obtained with water–methanol (3:97) as the mobile phase at a column temperature

of 30°, as shown in Figs. 1 and 2. For the separation of homologous ionic surface-active agents by HPLC, it is necessary to add strong acids or their salts to the mobile phase[6,7]. For the separation of homologous FME and FDE, however, water in the mobile phase was more effective than strong acids or their salts. It may be that the difference between the chromatographic behaviour of ionic surface-active agents and that of fatty acid alkanolamides is based on the distinction between ionic and non-ionic surface-active agents.
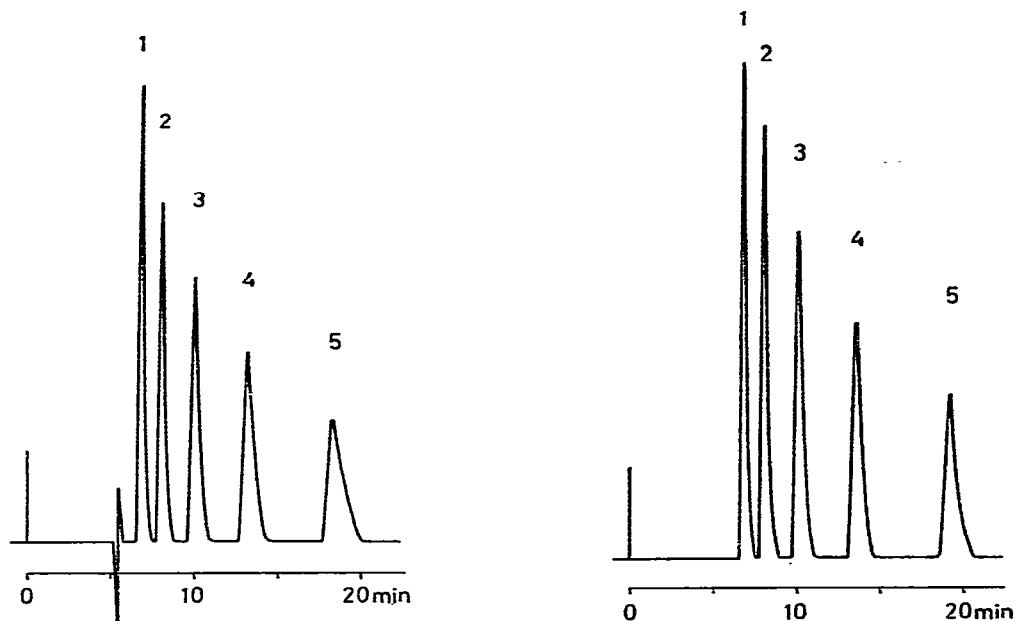
Fig. 1. Chromatogram of fatty acid monoethanolamides. Column: Hitachi Gel 3011, 500 × 4 mm I.D. Mobile phase: water–methanol (3:97). Flow-rate: 1.1 ml/min. Pressure: 40 kg/cm². Column temperature: 30°. Detector: UV (215 nm), 0.5 a.u.f.s. Peaks: 1 = capric acid monoethanolamide; 2 = lauric acid monoethanolamide; 3 = myristic acid monoethanolamide; 4 = palmitic acid monoethanolamide; 5 = stearic acid monoethanolamide.

Fig. 2. Chromatogram of fatty acid diethanolamides. Conditions as in Fig. 1. Peaks: 1 = capric acid diethanolamide; 2 = lauric acid diethanolamide; 3 = myristic acid diethanolamide; 4 = palmitic acid diethanolamide; 5 = stearic acid diethanolamide.

The relationships between the capacity factors of each homologous series and their alkyl chain lengths are shown in Figs. 3 and 4. The logarithm of the capacity factors was directly proportional to the alkyl chain lengths and the slope increased with increasing water content in the mobile phase. Using these linear relationships, the elution peaks for both homologous series can be identified and the alkyl chain lengths can be estimated. The reciprocal of the column temperature was also proportional to the logarithm of the capacity factor of each component, as shown in Figs. 5 and 6. In a previous paper[9], we demonstrated that the chromatography of alkylbenzenes and alkyl benzoates with poly(styrene–divinylbenzene) gel as the sta-
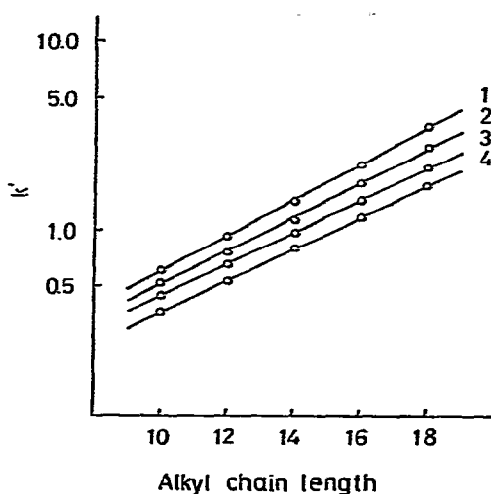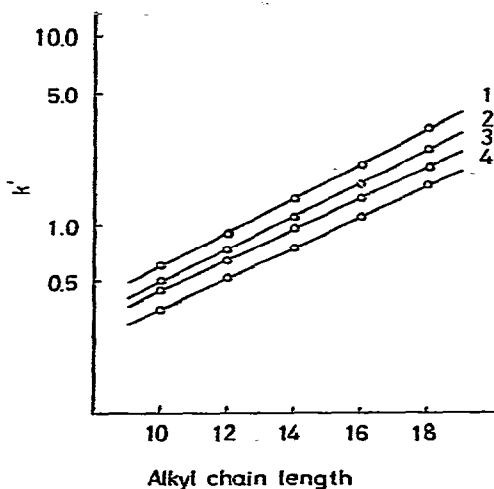
Fig. 3. Relationships between the capacity factors and alkyl chain lengths of fatty acid monoethanol-amides. Mobile phase: water–methanol (3:97). Column temperature: $1 = 30°$; $2 = 40°$; $3 = 50°$; $4 = 60°$.

Fig. 4. Relationships between the capacity factors and alkyl chain lengths of fatty acid diethanol-amides. Mobile phase: water–methanol (3:97). Column temperature: $1 = 30°$; $2 = 40°$; $3 = 50°$; $4 = 60°$.
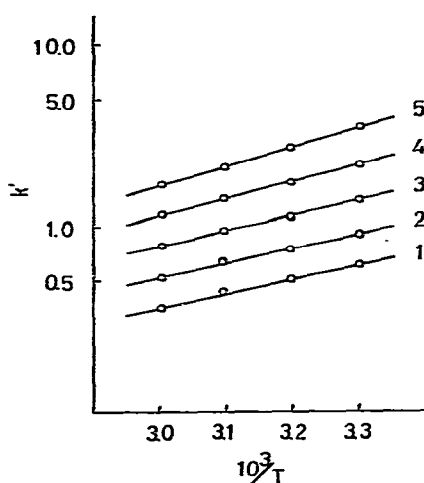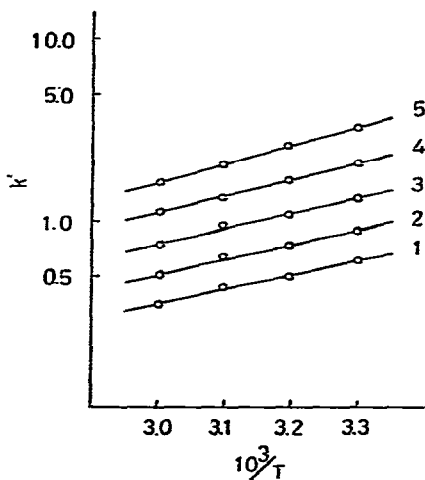

Fig. 5. Relationship between the capacity factors of fatty acid monoethanolamides and column temperature. $1 =$ Capric acid monoethanolamide; $2 =$ lauric acid monoethanolamide; $3 =$ myristic acid monoethanolamide; $4 =$ palmitic acid monoethanolamide; $5 =$ stearic acid monoethanolamide.

Fig. 6. Relationships between the capacity factors of fatty acid diethanolamides and column temperature. $1 =$ Capric acid diethanolamide; $2 =$ lauric acid diethanolamide; $3 =$ myristic acid diethanolamide; $4 =$ palmitic acid diethanolamide; $5 =$ stearic acid diethanolamide.

tionary phase could be interpreted in terms of the theory of partition chromatography and the following two relationships applied:

$$\ln k' = a' + bn \tag{1}$$

where a' and b are constants and n is the number of carbon atoms in the solute of homologous compounds, and

$$R \cdot \frac{d\ln k'}{d(1/T)} = \Delta h^e + RT^2 \alpha_m \tag{2}$$

where Δh^e is the partial molar enthalpy of transfer of the solute from the mobile phase to the stationary phase and α_m is the coefficient of thermal expansion of the mobile phase. Therefore, the results obtained in the chromatography of homologous FME and FDE, as well as alkylbenzyldimethylammonium chlorides, alkylpyridinium halides and alkylbenzenesulphonates, can be explained in terms of the theory of partition chromatography.

ACKNOWLEDGEMENTS

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