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## SEPARATION OF HOMOLOGOUS ALKYL BENZYL DIMETHYLAMMONIUM CHLORIDES AND ALKYL PYRIDINIUM HALIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

Homologous series of alkylbenzyl dimethylammonium chlorides and alkylpyridinium halides with  $C_{10}$ - $C_{18}$  alkyl groups were separated by high-performance liquid chromatography, employing porous micro-spherical poly(styrene-divinylbenzene) gel as the stationary phase. The recommended conditions are as follows: column, 500 mm  $\times$  4 mm I.D.; mobile phase, 0.5 M perchloric acid in methanol; column temperature, 30°. The logarithm of the capacity factor for each homologous series was directly proportional to the alkyl chain length. The reciprocal of the column temperature was also directly proportional to the logarithm of the capacity factor of each constituent. The results might be explainable in terms of liquid-liquid partition chromatography.

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### INTRODUCTION

Alkylbenzyl dimethylammonium chlorides (BzAC) and alkylpyridinium halides (APH), typical cationic surface-active agents, have been widely used as germicides and textile softeners and in many other industrial applications. These commercial products are usually homologous series containing mixtures of  $C_{10}$ - $C_{18}$  alkyl groups.

The separation of homologous series of surface-active agents has been carried out by gas chromatography, but this method requires their conversion into volatile derivatives before analysis. Several studies on BzAC and APH have been reported. Warrington<sup>1</sup> formed the alkyldimethylamines quantitatively from BzAC by catalytic hydrogenation. Metcalfe<sup>2</sup> attempted the determination of certain quaternary ammonium compounds by a Hofmann degradation with direct injection on an alkaline-treated gas chromatographic column. The peaks observed did not correspond to the anticipated long-chain  $\alpha$ -olefins but to the tertiary amines. Uno *et al.*<sup>3</sup> observed nearly

quantitative (about 90%) degradation of BzAC into alkyl dimethylamines and benzyl chloride, and APH<sup>4</sup> were also degraded into alkyl halides and pyridine under the same conditions. Jennings and Mitchner<sup>5</sup> reported that the modified Hofmann degradation of BzAC gave the corresponding alkenes and benzyl dimethylamine. Kojima and Oka<sup>6</sup> reported that BzAC were reduced to alkyl dimethylamines with lithium aluminium hydride and APH to N-alkyl dihydropyridine with sodium borohydride.

Liquid chromatography has made great progress in recent years as a result of developments in detectors, column packings and pumps, and it has become comparable to gas chromatography in terms of convenience, speed and efficiency. High-molecular-weight, non-volatile, thermally unstable and ionic compounds can be analyzed by liquid chromatography with or without prior treatment of the samples and we considered that it would be applicable to the analysis of surface-active agents without the need for any chemical conversion. In a previous paper<sup>7</sup> we briefly reported the separations of homologous series of BzAC and APH by high-performance liquid chromatography (HPLC), employing a porous micro-spherical poly(styrene-divinylbenzene) gel as the stationary phase and a methanolic solution of inorganic acids or their salts as the mobile phase. Recently, it has been possible to use smaller particles of poly(styrene-divinylbenzene) gel, of average diameter 10–15  $\mu\text{m}$ . Using this gel, homologous series of BzAC and APH can be separated within 30 min. This paper describes the procedure and presents results that indicate that this method may be applicable to the analysis of other surface-active agents.

## EXPERIMENTAL

### *Apparatus*

The liquid chromatograph was constructed from separate components. The pump (KHU-45 reciprocating pump, Kyowa Seimitsu, Mitaka, Tokyo, Japan) was capable of delivering solvent at a flow-rate of 0.5–4.5 ml/min at pressures up to 170 kg/cm<sup>2</sup>. The parts of the pump that came into contact with solvent (pump head, plunger, etc.) were made of PTFE. The column (500 mm  $\times$  4 mm I.D.) was made of Pyrex glass, with a water-jacket for control of the column temperature. A spectrophotometer (Model 139, Hitachi Perkin-Elmer, Tokyo, Japan) with a 20 mm  $\times$  2 mm I.D. PTFE flow cell of volume *ca.* 63  $\mu\text{l}$ , was used as the detector. All fittings and tubing (0.5 mm I.D. and 2 mm O.D.) were made of PTFE. The detector was equipped with a recorder (Model U-125M, Nihondenshikagaku, Kyoto, Japan) and an electronic digital integrator (Model 104R, Infotronics, Shannon Airport, Ireland).

### *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 an average particle diameter of 10–15  $\mu\text{m}$ .

BzAC with C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub> and C<sub>18</sub> alkyl groups were prepared from the corresponding alkyl dimethylamines and benzyl chloride, and APH from the alkyl chlorides or bromides and pyridine. These samples were purified by recrystallization from acetone.

All other reagents were of analytical-reagent grade.

### Procedure

The packing material, dispersed in methanol-water (8:2, v/v), was packed into the glass column under high pressure (*ca.* 130 kg/cm<sup>2</sup>) by the slurry packing procedure. The packed column was treated with methanol before use.

A mixture of each homologue was dissolved in methanol and the solution injected into the column with a microsyringe through a septum injector. BzAC in the effluent was detected at 220 nm and APH at 260 nm.

### RESULTS AND DISCUSSION

In liquid chromatography with poly(styrene-divinylbenzene) gel as the stationary phase, a polar solvent, such as methanol or mixtures of methanol and water, is usually used as the mobile phase. These chromatographic systems, however, are not suitable for the separation of homologous series of BzAC and APH, because methanol and methanol-water (9:1, v/v) elute BzAC and APH with C<sub>10</sub>-C<sub>18</sub> alkyl groups at the solvent front.

Capacity factors of homologous series of BzAC and APH were determined at various column temperatures using methanolic solutions of inorganic acids or their salts as the mobile phase. The results are shown in Table I.

In each series of BzAC and APH, the elution order follows the order of increasing alkyl chain length. With 0.05 M perchloric acid in methanol as the mobile phase, adequate separation of decyl- and dodecylbenzyltrimethylammonium chloride (C<sub>10</sub>- and C<sub>12</sub>-BzAC) was not obtained, but C<sub>16</sub>-BzAC was separated completely from C<sub>18</sub>-BzAC. Using 0.5 M perchloric acid in methanol, the capacity factors of BzAC were higher than those obtained by using 0.05 M perchloric acid and all components of BzAC were well resolved, as shown in Fig. 1. Similar chromatographic behaviour was shown by APH (Fig. 2).

The capacity factors of BzAC and APH increased and the resolutions were improved with increasing perchloric acid content in the mobile phase. As the capacity factors increased, the time required for the analysis of homologous series of BzAC and APH increased several-fold. Therefore, the perchloric acid content in the mobile phase must be controlled by the compositions of the homologous series of BzAC and APH to be analyzed.

Alkylpyridinium bromides with C<sub>10</sub>-C<sub>18</sub> alkyl groups were eluted with retention times identical with those of alkylpyridinium chlorides of the same alkyl chain length. In order to clarify this result, the chromatographic behaviour of a methyl orange-hexadecylpyridinium complex on porous micro-spherical poly(styrene-divinylbenzene) gel was investigated. This complex was extracted with chloroform from an aqueous solution of hexadecylpyridinium chloride and excess of methyl orange and the extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in methanol and the solution injected into the column. Separate solutions of methyl orange and hexadecylpyridinium chloride in methanol were also injected into the column and the column effluents were monitored at 260 nm for methyl orange and hexadecylpyridinium salts and at 510 nm for methyl orange. The results are shown in Fig. 3.

The methyl orange-hexadecylpyridinium complex gave two separate peaks, one being identified as methyl orange and the other as hexadecylpyridinium per-

TABLE I  
CAPACITY FACTORS OF ALKYL BENZYL DIMETHYLAMMONIUM CHLORIDES AND ALKYL PYRIDINIUM CHLORIDES

Type of compound	Compound	Capacity factor						
		Mobile phase*						
		0.05 M HClO <sub>4</sub> (30°)	0.1 M HClO <sub>4</sub> (30°)	0.5 M HClO <sub>4</sub>		0.5 M NaClO <sub>4</sub> (30°)	0.5 M HCl (30°)	
		30°	40°	50°				
Alkylbenzyl dimethylammonium chloride	C <sub>10</sub>	0.44	0.46	0.81	0.63	0.49	0.79	0.57
	C <sub>12</sub>	0.60	0.66	1.21	0.94	0.74	1.18	0.79
	C <sub>14</sub>	0.86	0.96	1.88	1.41	1.10	1.79	1.14
	C <sub>16</sub>	1.23	1.42	2.97	2.16	1.66	2.78	1.67
	C <sub>18</sub>	1.81	2.13	4.81	3.36	2.56	4.44	2.51
Alkylpyridinium chloride	C <sub>10</sub>	0.33	0.39	0.61	0.49	0.38	0.58	0.42
	C <sub>12</sub>	0.45	0.55	0.92	0.73	0.57	0.86	0.60
	C <sub>14</sub>	0.66	0.78	1.44	1.12	0.87	1.32	0.87
	C <sub>16</sub>	0.96	1.16	2.30	1.74	1.34	2.12	1.30
	C <sub>18</sub>	1.42	1.73	3.75	2.74	2.07	3.40	1.96

\* In methanol.

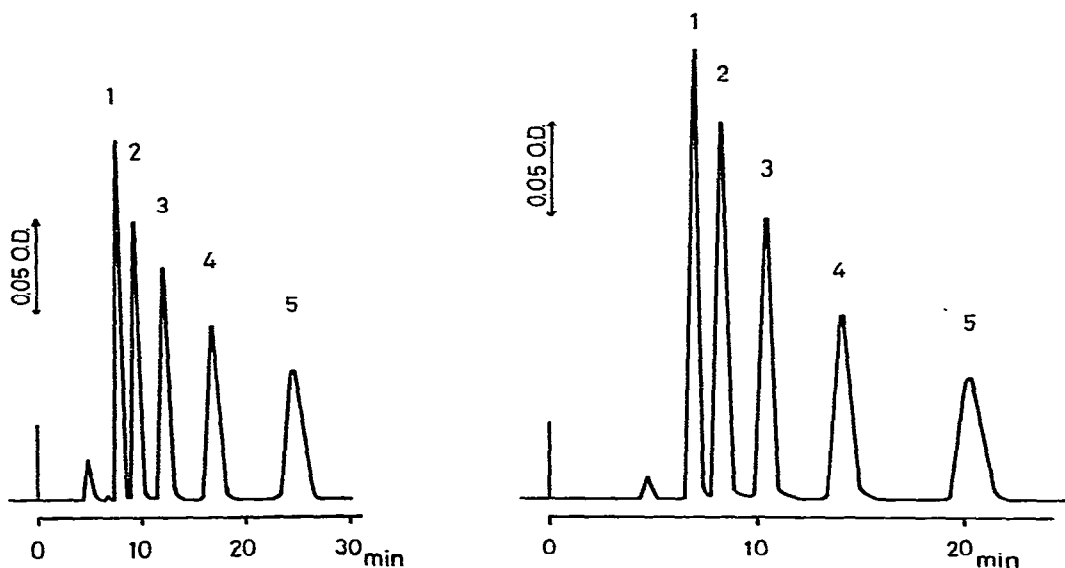


Fig. 1. Chromatogram of alkylbenzyltrimethylammonium chlorides. Column: Hitachi Gel 3011, 500 mm  $\times$  4 mm I.D. Mobile phase: 0.5 M perchloric acid in methanol. Flow-rate: 1.1 ml/min. Pressure: 70 kg/cm<sup>2</sup>. Column temperature: 30°. Detection: 220 nm. Peaks: 1 = decyl-; 2 = dodecyl-; 3 = tetradecyl-; 4 = hexadecyl-; 5 = octadecylbenzyltrimethylammonium chloride.

Fig. 2. Chromatogram of alkylpyridinium chlorides. Conditions as in Fig. 1 except for detection at 260 nm. Peaks: 1 = decyl-; 2 = dodecyl-; 3 = tetradecyl-; 4 = hexadecyl-; 5 = octadecylpyridinium chloride.

chlorate. These results suggest that the counter ion of surface-active agents is exchangeable to a great extent with the counter ion in the mobile phase.

In a previous paper<sup>8</sup>, we indicated that the column temperature is an important factor in chromatographic analyses using poly(styrene-divinylbenzene) gel. In the present study, the effects of column temperature on the separations of each homologous series of BzAC and APH were investigated and the results are shown in Table I. As the column temperature was increased from 30° to 50°, the capacity factor of C<sub>18</sub>-BzAC decreased from 4.81 to 2.56 and the resolution of C<sub>10</sub>- and C<sub>12</sub>-BzAC also deteriorated; similar behaviour was shown by APH. Accordingly, the recommended column temperature for the separation of BzAC and APH with C<sub>10</sub>-C<sub>18</sub> alkyl groups is 30°.

With 0.5 M sodium perchlorate in methanol instead of perchloric acid as the mobile phase, the members of the homologous series of BzAC and APH were eluted in the order of increasing alkyl chain length, and all components of BzAC and of APH were resolved completely. The use of hydrochloric or sulphuric acid also gave satisfactory separations. When using salts dissolved in methanol, such as lithium, ammonium and calcium chloride, similar results were obtained but when acetic acid in methanol was used as the mobile phase, neither BzAC nor APH were separated.

In this study, we expected that the percentage of the peak area of each constituent on a chromatogram would be equal to its molar percentage present in the mixture, because a spectrophotometric detector was used for the detection of BzAC

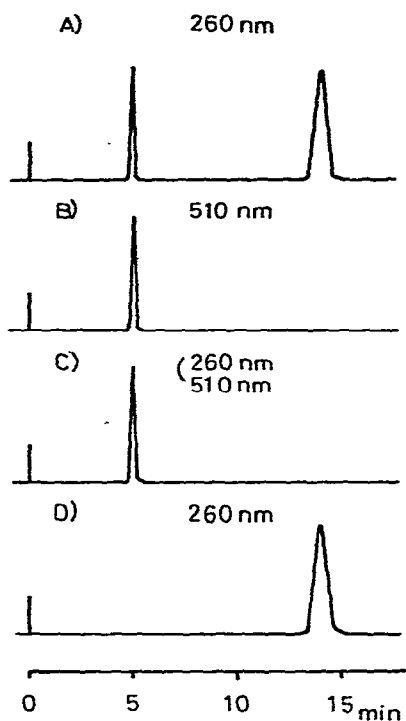


Fig. 3. Chromatograms of hexadecylpyridinium-methyl orange complex, methyl orange and hexadecylpyridinium chloride. Conditions as in Fig. 1. A and B, hexadecylpyridinium-methyl orange complex detected at 260 nm and 510 nm, respectively; C, methyl orange detected at 260 nm and 510 nm; D, hexadecylpyridinium chloride detected at 260 nm.

TABLE II

ANALYSIS OF SYNTHETIC MIXTURES OF ALKYL BENZYL DIMETHYL AMMONIUM CHLORIDES AND ALKYL PYRIDINIUM CHLORIDES

Type of compound	Compound	Present (mol-%)	Found* (peak area, %)	Coefficient of variation
Alkylbenzyl dimethyl ammonium chloride	C <sub>10</sub>	22.04	21.35	0.48
	C <sub>12</sub>	17.53	18.73	1.14
	C <sub>14</sub>	20.97	20.27	1.03
	C <sub>16</sub>	20.22	20.03	1.16
	C <sub>18</sub>	19.24	19.62	1.19
Alkylpyridinium chloride	C <sub>10</sub>	22.57	21.88	0.95
	C <sub>12</sub>	21.40	22.05	0.32
	C <sub>14</sub>	20.12	20.40	0.40
	C <sub>16</sub>	18.07	17.53	0.84
	C <sub>18</sub>	17.85	18.14	1.03

\* Mean of 5 replicate analyses.

and APH in the effluent and the molar absorptivities of each constituent in a homologous series were identical. Synthetic mixtures were prepared from samples of C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub> and C<sub>18</sub>-BzAC and -APH and analyzed and the results are shown in

Table II. Reasonably good agreement between the molar percentage of each component in the synthetic mixtures and the peak-area percentages as measured by HPLC were obtained. The reproducibility of the method was also good, small relative standard deviations for each element being obtained in five replicate analyses.

In a previous paper<sup>8</sup>, we showed that the chromatography of alkylbenzenes and alkyl benzoates using poly(styrene-divinylbenzene) gel as the stationary phase could be interpreted in terms of the theory of partition chromatography and the following two relationships applied:

$$\ln k' = a' + bn \quad (1)$$

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

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

where  $\Delta h^e$  is the partial molar enthalpy of the solute from the mobile to the stationary phase and  $\alpha_m$  is the coefficient of thermal expansion of the mobile phase. In this study, the logarithm of the capacity factors of BzAC and APH was directly proportional to the alkyl chain length, as shown in Figs. 4 and 5. The slope ( $b$ ) in eqn. 1 increase with increasing perchloric acid content in the mobile phase and decreasing column temperature. Using these linear relationships, the elution peaks for both homologous series can be identified and the alkyl chain lengths can be estimated.

On the other hand, the plots of the logarithm of the capacity factors of BzAC and APH versus the reciprocal of the column temperature were straight lines, as shown in Figs. 6 and 7. These results indicate the constancy of the right-hand side of eqn. 2.

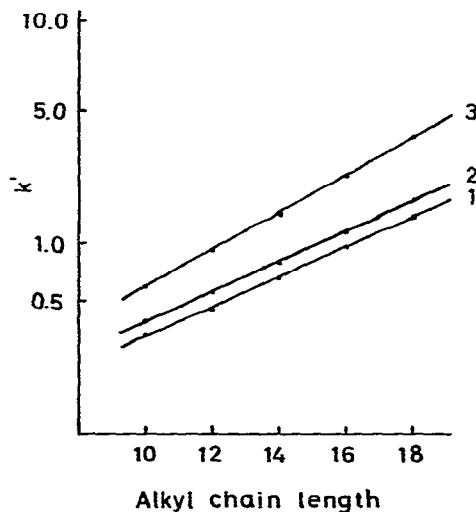
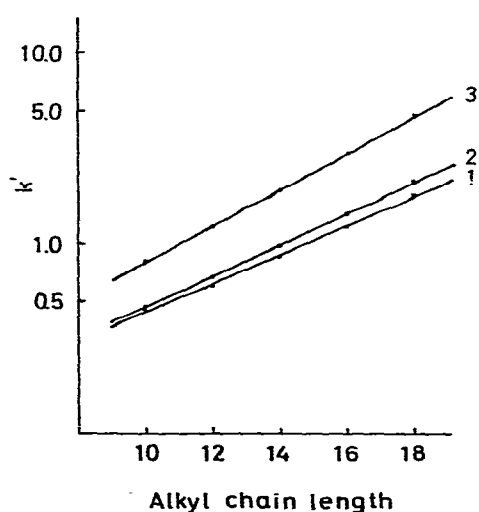


Fig. 4. Relationships between the capacity factors and alkyl chain lengths of alkylbenzyltrimethylammonium chlorides. Mobile phase, perchloric acid in methanol: 1 = 0.05 M; 2 = 0.1 M; 3 = 0.5 M.

Fig. 5. Relationships between the capacity factors and alkyl chain lengths of alkylpyridinium chlorides. Mobile phase, perchloric acid in methanol: 1 = 0.05 M; 2 = 0.1 M; 3 = 0.5 M.

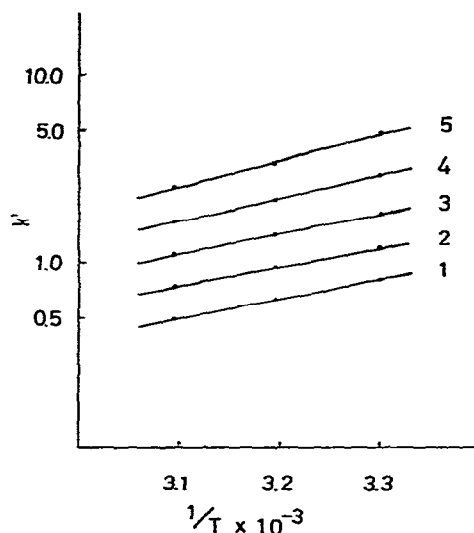
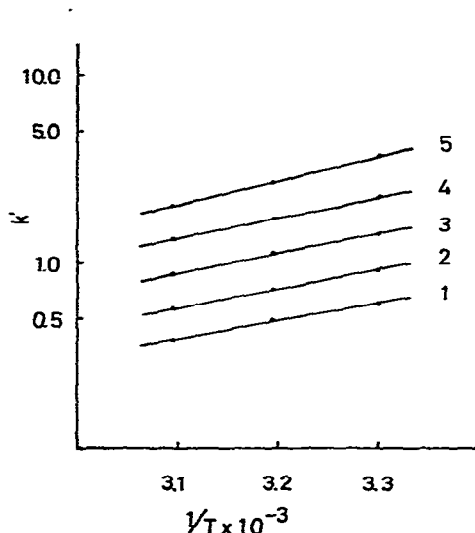


Fig. 6. Relationships between the capacity factors of alkylbenzyltrimethylammonium chlorides and column temperature. 1 = Decyl-; 2 = dodecyl-; 3 = tetradecyl-; 4 = hexadecyl-; 5 = octadecylbenzyltrimethylammonium chloride.

Fig. 7. Relationships between the capacity factors of alkylpyridinium chlorides and column temperature. 1 = Decyl-; 2 = dodecyl-; 3 = tetradecyl-; 4 = hexadecyl-; 5 = octadecylpyridinium chloride.

As shown in the above discussion, the results of the chromatography of homologous series of BzAC and APH can also be explained in terms of the theory of partition chromatography and the hydrophilic groups of BzAC and APH appear to have little influence on the separations. Therefore, these chromatographic systems might be applicable to the separation of homologous series of other surface-active agents such as alkylbenzenesulphonates. Detailed studies of these homologous series are now in progress.

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