

CHROM. 2000

CHROMATOGRAPHIC BEHAVIOUR OF ALKYL BENZENES AND ALKYL BENZOATES ON POROUS MICRO-SPHERICAL POLY(STYRENE-DIVINYLBENZENE) GEL

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(Received October 14th, 1975)

SUMMARY

Homologous series of alkylbenzenes and alkyl benzoates were analyzed by high-performance liquid chromatography employing a porous micro-spherical poly(styrene-divinylbenzene) gel as the stationary phase with various proportions of methanol and water as the mobile phase. In each series of alkylbenzenes and alkyl benzoates, the elution order followed the order of increasing alkyl chain length. The capacity factors increased with increasing water content in the eluent, but decreased as the column temperature increased. The logarithm of the capacity factor for each homologous series was directly proportional to the carbon number. The reciprocal of the column temperature was also directly proportional to the logarithm of the capacity factor. The results might be explainable in terms of the theory of liquid-liquid partition chromatography.

INTRODUCTION

Recently, high-efficiency separations by liquid chromatography have been studied extensively by many investigators, because liquid chromatography is now comparable to gas chromatography in terms of convenience, speed and efficiency. Liquid chromatography is a useful means for the direct analysis of high-molecular-weight, thermally unstable, inorganic and non-volatile compounds.

The lipophilic groups of surface-active agents are usually determined by gas chromatography. This method, however, requires their conversion into volatile derivatives before analysis. In a previous paper¹, we reported the separations of homologous series of alkylbenzyltrimethylammonium chlorides and alkylpyridinium halides by high-performance liquid chromatography without pre-treatment, employing a porous micro-spherical poly(styrene-divinylbenzene) gel as a stationary phase with

a methanolic solution of inorganic acids or their salts as the mobile phase. We also postulated that the separation mechanism might be explainable in terms of the theory of liquid-liquid partition chromatography, as the logarithms of the capacity factors were directly proportional to the alkyl chain length of each homologous series. However, for liquid chromatography using a porous micro-spherical poly(styrene-divinylbenzene) gel as the stationary phase, the separation mechanism is usually considered as a kind of hydrophobic adsorption or Van der Waals' interactions². Because the eluent is usually a polar solvent, such as methanol or mixtures of methanol and water, the elution order of solutes is generally the same as the order of solute polarity.

In this paper, a study of the chromatographic behaviour of homologous series of alkylbenzenes and alkyl benzoates on a porous micro-spherical poly(styrene-divinylbenzene) gel is described in order to examine the separation mechanism, and we will show that the behaviour is better interpreted in terms of the partition phenomenon between the mobile phase and the stationary phase.

THEORETICAL

The capacity factor, k' , is defined by

$$k' = KV_s/V_m = V_N/V_m \quad (1)$$

where K is the distribution coefficient, V_s is the stationary phase volume in the column, V_m is the mobile phase interstitial volume and V_N is the net retention volume.

The specific retention volume, V_g , is given by

$$V_g = V_N/W_s \quad (2)$$

where W_s is the weight of the stationary phase. V_m and W_s are both constant and we can therefore derive the equation

$$V_g = k'V_m/W_s = k'C \quad (3)$$

where C is a constant.

Locke and Martire³⁻⁵ considered solute retention in liquid-liquid partition chromatography thermodynamically and found the following relationships. The ratio of the activity coefficient of the solute at infinite dilution in the mobile phase, $\gamma^{m,\infty}$, to that in the stationary phase, $\gamma^{s,\infty}$, is related to the specific retention volume of the solute by the equation

$$V_g = (\gamma^{m,\infty}/\gamma^{s,\infty}) (M_m/M_s \rho_m) \quad (4)$$

where M_m and M_s are the molecular weights of the mobile and stationary phase, respectively, and ρ_m is the density of the eluent at the column temperature, T . The dependence of the column temperature on the specific retention volume was found to be

$$R \left[\frac{d \ln V_g}{d(1/T)} \right] = \Delta h^\circ + RT^2 \alpha_m \quad (5)$$

where Δh^e is the partial molar enthalpy of transfer of the solute from the mobile to the stationary phase and α_m is the coefficient of thermal expansion of the mobile phase. The dependence of the carbon number of the solute of a homologous series of organic compounds, n , on the specific retention volume is given by

$$\ln V_g = a + bn \quad (6)$$

where a and b are constants, determined by the mobile and stationary phases.

We can derive eqns. 7 and 8 by combining eqns. 3, 5 and 6:

$$R \left[\frac{d \ln k'}{d(1/T)} \right] = \Delta h^e + RT^2 \alpha_m \quad (7)$$

$$\ln k' = a' + bn \quad (8)$$

where a' is a constant. From the above discussion, we can say that in liquid-liquid partition chromatography, the logarithm of the capacity factor is directly proportional to the reciprocal of the column temperature and the carbon number of the solute.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was constructed from separate components. The detector (Model 1205 UV monitor, Laboratory Data Control, Riviera Beach, Fla., U.S.A.) was operated at 254 nm. The pump (FLC 150 positive-displacement syringe pump, Japan Spectroscopic, Tokyo, Japan) was capable of delivering solvent at a flow-rate of 0-3.0 ml/min at a pressure of up to 150 kg/cm². The column temperature was controlled to within $\pm 0.05^\circ$.

Column packing

Porous micro-spherical particles of a copolymer of styrene and divinylbenzene (Hitachi Gel 3010, average diameter 25 μ m) were used as a column packing material. The column packing material, dispersed in methanol-water (1:1), was packed into the glass column by the slurry packing procedure and the packed column was treated with methanol before use.

Reagents

The eluent was prepared by mixing methanol with water in various proportions. Sample solutions were prepared by dissolving 1 ml of alkylbenzene (benzene, toluene and ethyl-, *n*-propyl- and isopropylbenzene) or alkyl benzoate (methyl, ethyl, *n*-propyl, *n*-butyl, isopropyl, isobutyl and isoamyl benzoate) in 10 ml of methanol.

Analytical procedure

About 5 μ l of a sample solution were injected in the column with a micro-syringe through a septum injector. The capacity factor, k' , was calculated according to eqn. 1. The interstitial volume of the mobile phase was determined by measuring the retention volume of non-retained water.

TABLE I
CAPACITY FACTORS OF ALKYL BENZENES AND ALKYL BENZOATES

| Compound | Eluent | | | | | | | | | | | |
|---------------------------|----------|------|------|------|------|-----------------------|------|------|------|------------------------|------|------|
| | Methanol | | | | | Water-methanol (5:95) | | | | Water-methanol (10:90) | | |
| | 10°C | 20°C | 30°C | 40°C | 50°C | 20°C | 30°C | 40°C | 50°C | 30°C | 40°C | 50°C |
| Benzene | 0.97 | 0.88 | 0.79 | 0.73 | 0.67 | 1.37 | 1.17 | 1.03 | 0.95 | 1.73 | 1.51 | 1.36 |
| Toluene | 1.33 | 1.19 | 1.06 | 0.95 | 0.86 | 1.97 | 1.67 | 1.45 | 1.29 | 2.63 | 2.21 | 1.97 |
| Ethylbenzene | 1.52 | 1.35 | 1.19 | 1.07 | 0.97 | 2.41 | 2.03 | 1.76 | 1.57 | 3.41 | 2.85 | 2.48 |
| <i>n</i> -Propylbenzene | 1.77 | 1.57 | 1.37 | 1.23 | 1.08 | 2.97 | 2.48 | 2.15 | 1.89 | 4.43 | 3.69 | 3.17 |
| Isopropylbenzene | — | — | 1.23 | 1.09 | 1.00 | 2.53 | 2.17 | 1.88 | 1.67 | 3.86 | 3.21 | — |
| Methyl benzoate | 1.65 | 1.41 | 1.23 | 1.09 | 0.95 | 2.02 | 1.75 | 1.48 | 1.30 | 2.48 | 2.07 | — |
| Ethyl benzoate | 1.73 | 1.49 | 1.30 | 1.13 | 0.95 | 2.37 | 2.03 | 1.71 | 1.47 | 3.07 | 2.53 | — |
| <i>n</i> -Propyl benzoate | 2.17 | 1.83 | 1.57 | 1.35 | 1.17 | 3.07 | 2.59 | 2.15 | 1.84 | 4.17 | 3.39 | — |
| <i>n</i> -Butyl benzoate | 2.82 | 2.34 | 1.99 | 1.68 | 1.45 | 4.27 | 3.50 | 2.87 | 2.40 | 6.10 | 4.83 | — |
| Isopropyl benzoate | 1.75 | 1.49 | 1.33 | 1.15 | 0.99 | 2.53 | 2.15 | 1.81 | 1.53 | 3.45 | 2.81 | — |
| Isobutyl benzoate | 2.29 | 1.93 | 1.68 | 1.46 | 1.23 | 3.55 | 2.95 | 2.45 | 2.06 | 5.13 | 4.10 | — |
| Isoamyl benzoate | 3.08 | 2.57 | 2.19 | 1.86 | 1.55 | 5.03 | 4.12 | 3.33 | 2.75 | 7.67 | 5.97 | — |

RESULTS AND DISCUSSION

The capacity factors of homologous series of alkylbenzenes and alkyl benzoates were determined at various column temperatures using various eluents. The results are shown in Table I.

In each series of alkylbenzenes and alkyl benzoates, the elution order followed the order of increasing alkyl chain length. As the column temperature was increased, the capacity factors decreased and also the resolution deteriorated. Branched alkylbenzenes and branched alkyl benzoates were eluted earlier than the corresponding straight-chain compounds. Typical chromatograms of *n*-alkylbenzenes, *n*-alkyl benzoates and isoalkyl benzoates with pure and 90% methanol as eluents are shown in Figs. 1, 2 and 3.

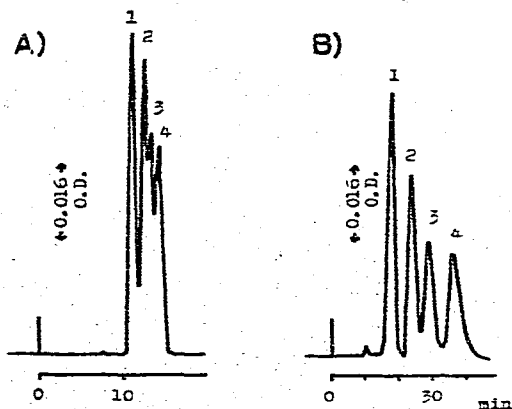


Fig. 1. Chromatograms of *n*-alkylbenzenes. Column: Hitachi Gel 3010, 500 mm × 5 mm I.D. Flow-rate: 1.50 ml/min. Pressure: (A) 10 kg/cm²; (B) 30 kg/cm². Column temperature: 30°. Eluent: (A) methanol; (B) water-methanol (10:90). Samples: 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = *n*-propylbenzene.

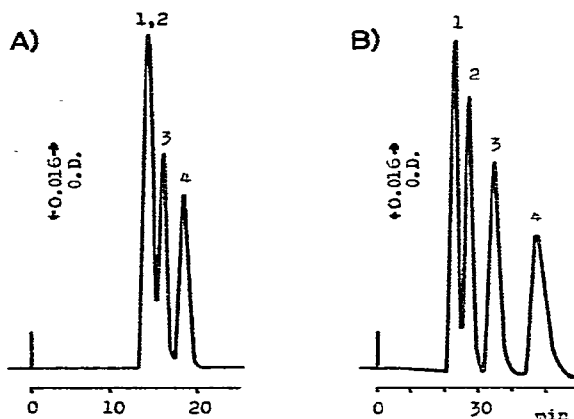


Fig. 2. Chromatograms of *n*-alkyl benzoates. Conditions as in Fig. 1. Samples: 1 = methyl benzoate; 2 = ethyl benzoate; 3 = *n*-propyl benzoate; 4 = *n*-butyl benzoate.

The latter eluent gave a better separation than the former, but the analysis time was several times longer. If water content in the eluent is increased, the elution time for each homologous series is increased.

As expected from eqn. 8, the logarithm of the capacity factor of alkylbenzenes is directly proportional to the carbon number, except for benzene and isopropylbenzene, as shown in Fig. 4. The slope (*b*) increased with increasing water content in the eluent. Therefore, the homologous series of alkylbenzenes can be easily separated by choosing an appropriate eluent composition, as shown in Fig. 1. Similar linear relationships were observed for the isoalkyl benzoate homologous series, as shown in Fig. 5. For the *n*-alkyl benzoate homologues, the linearity did not hold strictly, as shown in Fig. 6, but it can be considered to be approximately linear except for methyl benzoate.

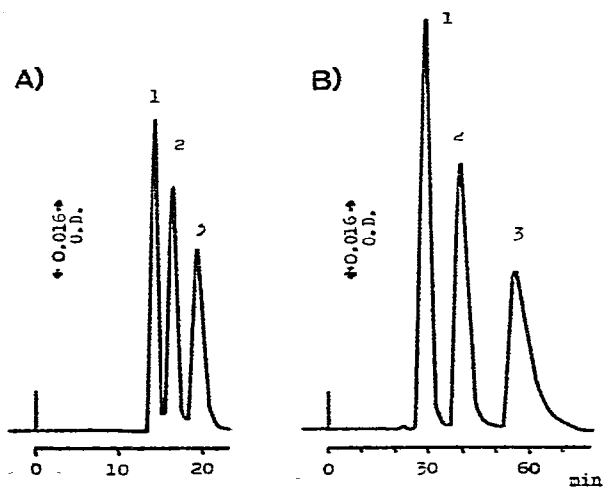


Fig. 3. Chromatograms of isoalkyl benzoates. Conditions as in Fig. 1. Samples: 1 = isopropyl benzoate; 2 = isobutyl benzoate; 3 = isoamyl benzoate.

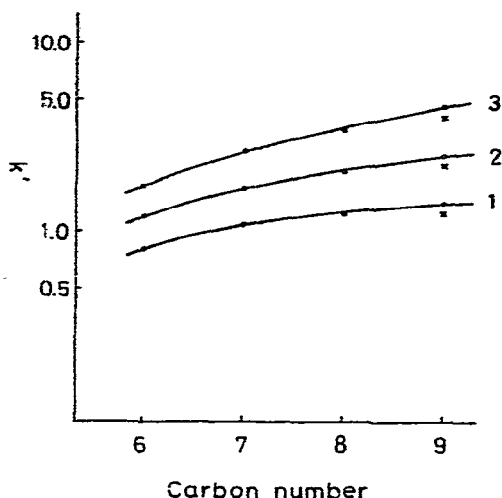


Fig. 4. Relationships between capacity factor and carbon number of alkylbenzenes at 30°. \times , Isopropylbenzene. Eluent: 1 = methanol; 2 = water-methanol (5:95), 3 = water-methanol (10:90).

The plots of logarithm of capacity factor for any solute *versus* the reciprocal of the column temperature were straight lines, as shown for ethylbenzene in Fig. 7 as an example.

As predicted by eqn. 7, the results indicate the constancy of Δh^e over this temperature range, because the second term on the right-hand side is smaller than Δh^e . The slope increased with increasing water content in the eluent. The partial molar enthalpies of transfer of solute from the mobile to the stationary phase at 20° were calculated from the slope obtained by using the least-squares method and eqn. 7. The

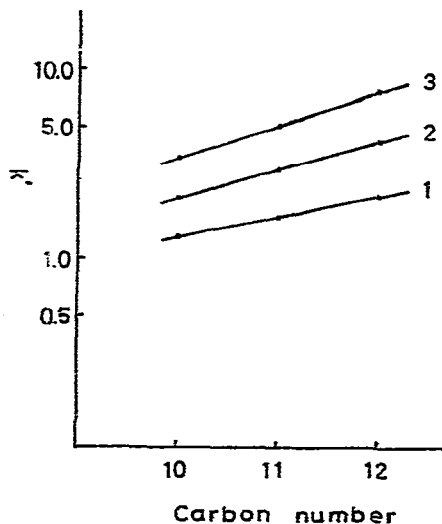


Fig. 5. Relationships between capacity factor and carbon number of isoalkyl benzoates at 30°. Eluent: 1 = methanol; 2 = water-methanol (5:95); 3 = water-methanol (10:90).

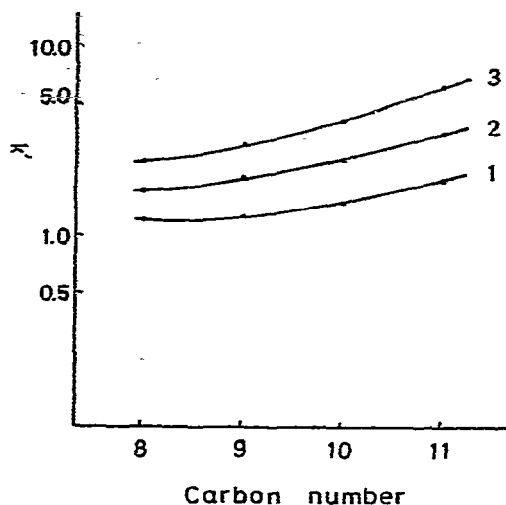


Fig. 6. Relationships between capacity factor and carbon number of *n*-alkyl benzoates at 30°. Eluent: 1 = methanol; 2 = water-methanol (5:95); 3 = water-methanol (10:90).

results are given in Table II. The coefficients of thermal expansion of the mobile phase, α_m , were calculated from the densities⁶.

The partial molar enthalpies of alkylbenzenes obtained in our chromatographic system were greater than those obtained by Locke⁵ for the squalene-acetonitrile system, *i.e.*, our system was more susceptible to the column temperature than the squalene-acetonitrile system for the separation of alkylbenzenes. This necessitates adequate control of the column temperature in order to achieve reproducible analyses.

As shown by the above discussion, the present chromatographic system can

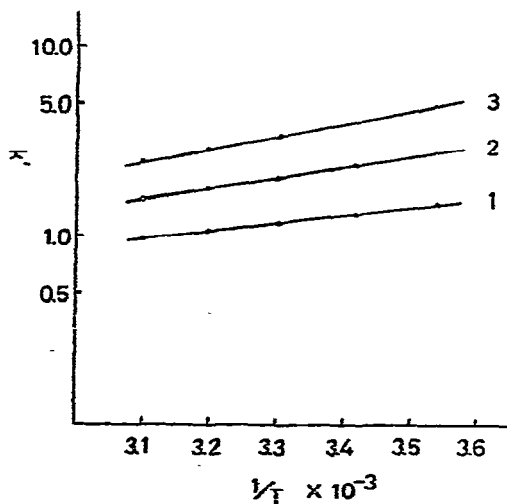


Fig. 7. Relationships between capacity factor of ethylbenzene and column temperature. Eluent: 1 = methanol; 2 = water-methanol (5:95); 3 = water-methanol (10:90).

TABLE II

PARTIAL MOLAR ENTHALPIES OF ALKYL BENZENES AND ALKYL BENZOATES

Enthalpies are given in kcal/mole.

| Compound | Eluent | | |
|---------------------------|----------|--------------------------|---------------------------|
| | Methanol | Water-methanol (5:95) | Water-methanol (10:90) |
| Benzene | 1.48 | 2.13 | 2.19 |
| Toluene | 1.79 | 2.47 | 2.63 |
| Ethylbenzene | 1.85 | 2.50 | 2.92 |
| <i>n</i> -Propylbenzene | 2.01 | 2.63 | 3.07 |
| Isopropylbenzene | 1.82 | 2.43 | 3.30 |
| Methyl benzoate | 2.27 | 2.62 | 3.23 |
| Ethyl benzoate | 2.47 | 2.83 | 3.48 |
| <i>n</i> -Propyl benzoate | 2.57 | 3.05 | 3.74 |
| <i>n</i> -Butyl benzoate | 2.82 | 3.43 | 4.24 |
| Isopropyl benzoate | 2.34 | 2.97 | 3.66 |
| Isobutyl benzoate | 2.56 | 3.23 | 4.06 |
| Isoamyl benzoate | 2.88 | 3.62 | 4.56 |

be interpreted in terms of the theory of partition chromatography and, moreover, we have given the theoretical basis for the analysis of homologous series of surface-active agents. Detailed studies of these homologous series are in progress.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Takeo Yamabe, Dr. Shinsaku Shiraishi and Dr. Nobuharu Takai of the Institute of Industrial Science, University of Tokyo, for valuable suggestions and discussions.

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