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RETENTION BEHAVIOUR OF A POROUS STYRENE-DIVINYLBENZENE COPOLYMER IN THE SEPARATION OF FATTY ACIDS AND RELATED COMPOUNDS

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SUMMARY

Using a porous styrene-divinylbenzene copolymer for the chromatographic separation of straight-chain fatty acids, we found the following properties: (1) a linear relationship between the logarithm of the capacity factor and the number of methylene groups in the alkyl chain, (2) almost identical slopes of the lines for three groups of fatty acid analogues and benzoic acid esters and (3) a linear relationship between the slope and the concentration of methanol in the mobile phase.

INTRODUCTION

Porous silica, either alone or layered on spherical glass beads, has been widely used in high-performance liquid chromatography and these materials, coated or chemically bonded with various liquid phases, are being investigated in order to clarify the operating conditions and the nature of selectivity^{1,2}. However, neither the chemical bond nor the silica matrix is stable under all conditions of chromatographic separations, especially in alkaline media³.

Porous styrene-divinylbenzene copolymers, which have no functional groups attached on the surface, are expected to be stable under a wider range of conditions and have been used in separations that are similar to reversed-phase chromatography⁴. The elution behaviour has been studied with dissociable compounds and neutral benzene derivatives. Reports have been published relating retention volume to the number of carbon atoms in the solute, the effect of the acidity of the mobile phase and the dissociation constants of the solutes^{5,6}.

In this work, the elution behaviour of fatty acids in their undissociated form has been studied and compared with the elution behaviour of benzoic acid esters. Well defined characteristics of the resin were found in the separation of these materials.

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EXPERIMENTAL

Apparatus

A carboxylic acid analyzer^{7,8} (Seishin Seiyaku Co., Tokyo, Japan) was used for the analysis of carboxylic acids. A high-pressure chromatograph was assembled from a reciprocating pump (KHD-26, Kyowa Seimitsu Co., Mitaka, Tokyo, Japan) and a 254-nm UV detector (LC-1, Iatron Co., Tokyo, Japan). Columns (50 cm × 3 mm I.D.) made of thick-walled Pyrex glass tubing and metal packers were obtained from Kyowa Seimitsu Co.

Materials

The packing material used was Hitachi Gel 3011, a spherical porous styrene-divinylbenzene copolymer consisting of beads 10 μm in diameter. Chemicals and solvents were of reagent grade (Tokyo Kasei Kogyo, Tokyo, Japan, and Kanto Chemicals Co., Tokyo, Japan).

Preparation of columns and mobile phases

After equilibrating the gel with the mobile phase for at least 1 day, the columns were filled by the slurry technique using a packer to pump the slurry into the column at a higher flow-rate than would be used in the chromatographic separation. After packing, the columns were stabilized overnight by pumping the mobile phase through the column at 0.31 ml/min (the flow-rate used for the chromatographic separation). The mobile phase consisted of methanol or methanol-water acidified to 0.01 *N* with concentrated hydrochloric acid. The column temperature was maintained at 50 °C.

RESULTS AND DISCUSSION

Retention volume and number of methylene groups in alkyl chains

Fig. 1 shows an example of the chromatographic separation of undissociated straight-chain fatty acids. A column efficiency of several hundred theoretical plates was achieved, which is sufficient to obtain reliable values for the capacity factor (k') for each compound. In the calculation of k' , the elution volume of serine is assumed to be the void volume. Serine, with a carboxyl group, is easily detected by the carboxylic acid analyzer. Because a hydrophobic interaction between the solute and the resin is assumed to be the main mechanism for retention, the charged primary amino groups of serine in the acidic mobile phase are expected to have little affinity for the stationary phase and we can therefore assume negligible retention. This assumption seems to hold, because the elution volume of serine with the same mobile phase gave a coefficient of variation of 1.8% and no steady increase was observed on elution with lower methanol concentrations.

The elution behaviour of 14 straight-chain fatty acids was examined. The retention volume for the carboxylic acids increased with the length of the alkyl chain and it was not possible to elute all of them separately with a single mobile phase. Various compositions of mobile phases from 40% (v/v) methanol in water to pure methanol were used to obtain reasonable separations of these acids. Plots of $\log k'$ against the number of methylene groups in the alkyl chain were linear for all concentrations of methanol (Fig. 2). This result indicates that the intrinsic affinity of each methylene

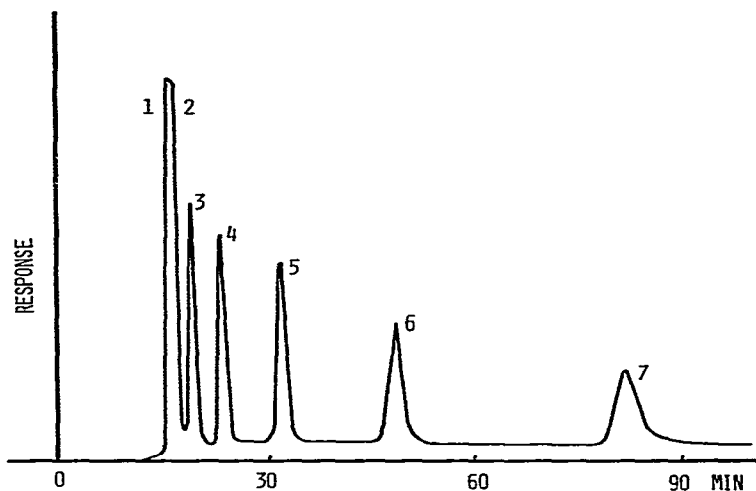


Fig. 1. Separation of straight-chain fatty acids. Mobile phase, 60% methanol; sample size, 0.2 μ mol. Other conditions as in text. Acids: 1 = formic; 2 = acetic; 3 = propionic; 4 = butyric; 5 = valeric; 6 = caproic; 7 = heptanoic acid.

group for the stationary phase is constant for straight-chain fatty acids. However, the slope of the line decreased with an increasing concentration of methanol in the mobile phase.

Linearity was also observed for the separation of dicarboxylic acids and *trans*- α,β -unsaturated fatty acids. The value for acrylic acid did not fall on the line

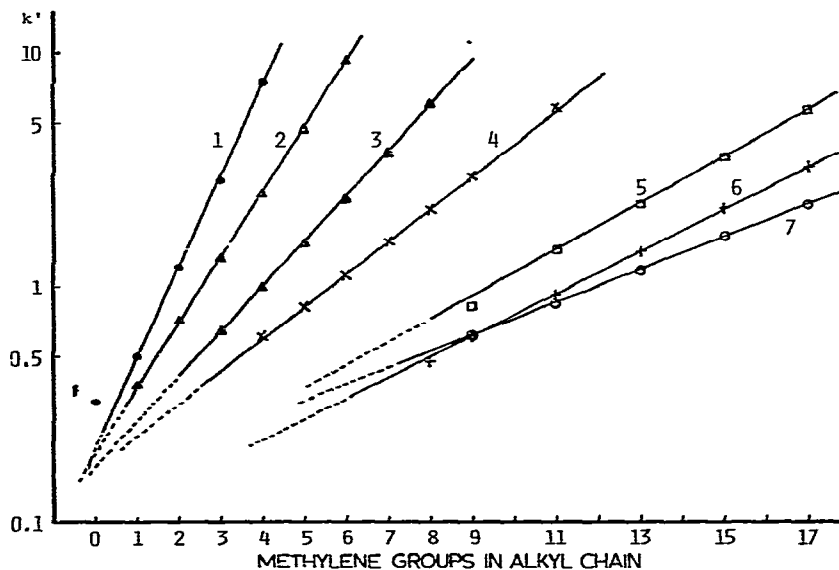


Fig. 2. Capacity factors versus number of methylene groups in the alkyl chain of straight-chain fatty acids. Methanol concentration: 1, 40%; 2, 60%; 3, 75%; 4, 85%; 5, 95%; 6, 97%; 7, 100%. Other conditions as in Fig. 1.

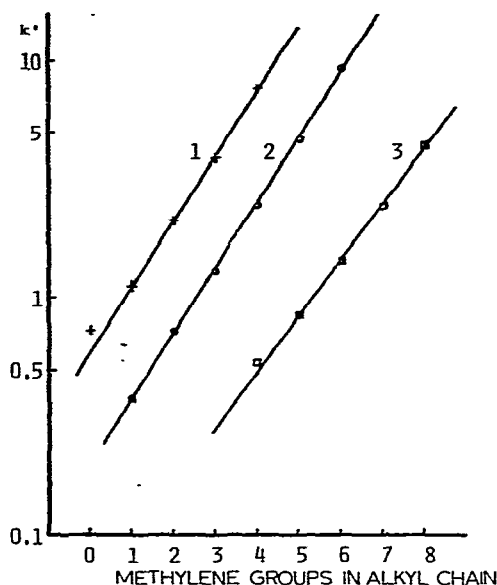


Fig. 3. Capacity factors and number of methylene groups in the alkyl chain of fatty acids, *trans*- α,β -unsaturated fatty acids and dicarboxylic acids. Conditions as in Fig. 1. 1, α,β -Unsaturated acids; 2, fatty acids; 3, dicarboxylic acids.

but such a discrepancy is common for the first member of a homologous series (Fig. 3). The slopes of the lines seem to be almost identical.

A linear relationship between the number of carbon atoms and $\log k'$ has previously been observed for benzoic acid esters by Nakae and Muto⁶. The slope of the line for fatty acids was compared with that of benzoic acid esters by using 95% methanol (Fig. 4). The slopes of both lines were almost identical, although the k' values were much larger for benzoic acid esters. These observations imply that the intrinsic affinity of a methylene group for the stationary phase is approximately equal in these carboxylic acids and esters. In partition chromatography on paper⁹, a similar relationship for the methylene group has been known for many years.

For this polystyrene resin, it is possible to adjust the $\log k'$ value for one methylene group to fall in the range 0.07–0.39 by varying the methanol concentration in the mobile phase from 100% to 40%. The separation of any two homologous compounds that differ by one methylene group can be achieved with almost the same ease, even though their molecular weights exceed several hundred. A similar observation has recently been made by Shimada *et al.*¹⁰, who separated estrogen derivatives with a column of μ Bondapak. For this column, the mechanism of retention is primarily partition.

The distance between parallel lines in Figs. 3 and 4 is a measure of the difference in affinity for various functional groups. The carboxyl group reduces the capacity factor while the introduction of benzoate group or a double bond increases it.

Equations

Two types of retention mechanism have been suggested for chromatographic

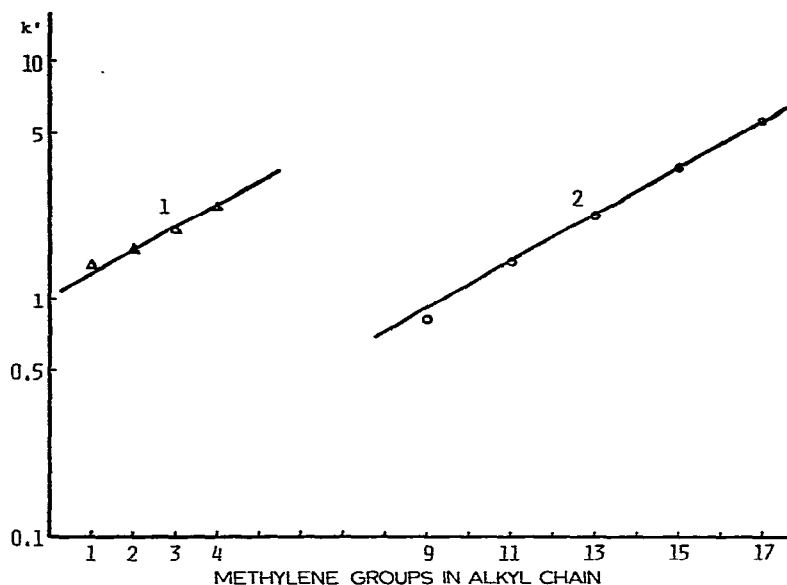


Fig. 4. Capacity factors versus number of methylene groups in the alkyl chain in fatty acids and benzoic acid esters. Mobile phase, 95% methanol. Other conditions as in Fig. 1. 1, Benzoic acid esters; 2, fatty acids.

separation on a column of porous styrene-divinylbenzene copolymer: partition⁶ and adsorption⁵. For partition chromatography on paper, Martin's equation has been successfully applied⁹:

$$RT \ln K = n\Delta\mu_x + m\Delta\mu_y + \dots \quad (1)$$

where K is the partition coefficient, $\Delta\mu_x, \Delta\mu_y, \dots$ are the chemical potentials of groups x, y, \dots and n, m, \dots are the numbers of groups x, y, \dots in a molecule. When eqn. 1 is applied to homologues of fatty acids, it is simplified to the following equation:

$$\log k' = a + bn \quad (2)$$

where k' is the capacity factor, n is the number of methylene groups and a and b are constants. A similar equation is also derived for adsorption chromatography from the assumption that each group in the molecule has an intrinsic affinity for the adsorbent. However, when this equation is applied to the separation of homologues on a polar adsorbent such as silica or alumina, it holds only for compounds with $n \leq 6$, and any increase in the second term in eqn. 2 levels off at higher values of n (ref. 11). On the other hand, for the adsorption of fatty acids on to active charcoal from ethanolic solution, Claesson¹² reported a case in which the linear relationship extended to 20 carbon atoms. Recently, Colin *et al.*¹³ also reported a similar observation on graphitized thermal carbon black. Consequently, eqn. 2 can be applied to express chromatographic separation, assuming either a partition or an adsorption mechanism for retention.

Methanol concentration in mobile phase

Applying eqn. 2 to our results, b was evaluated as a function of the concentration of methanol in the mobile phase. A linear relationship was observed (Fig. 5). Recently, Karch *et al.*¹⁴ reported a linear relationship between $\log k'$ and methanol concentration (0–60%) for the elution of lower alcohols from a column of Permaphase ODS; the present results seem to be consistent with their observations. The dependence of slope on the methanol concentration and the linear relationship between $\log k'$ and the number of methylene groups make it possible to estimate a preferred methanol concentration in the mobile phase for all members of a homologous series. Thus, the separation obtained between homologues can be estimated if the number of theoretical plates in the column is known for a given set of conditions.

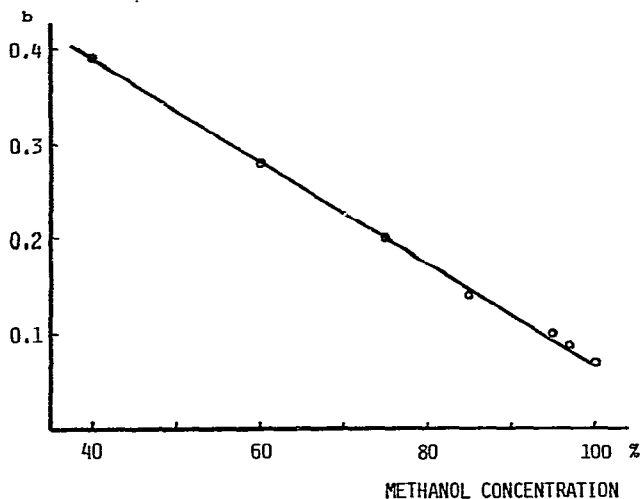


Fig. 5. Effect of methanol concentration on the value of b in eqn. 2.

CONCLUSION

This clarification of retention behaviour is of great practical value. The results obtained here for the porous styrene-divinylbenzene copolymer combined with the variation of retention characteristics with pH for dissociable molecules⁵ will further increase the versatility of this packing material for high-performance liquid chromatography. One drawback to the resin is that skewed peaks are occasionally obtained when the k' values are greater than 10, so that the practical range of k' is limited. However, compounds eluting as skewed peaks under one set of conditions give symmetrical peaks on elution with stronger solvents.

From the results, it is not clear whether or not the linearity of b versus methanol concentration can be extended beyond 40% methanol to more dilute solutions. The results obtained by Chu and Pietrzyk¹⁵ for the adsorption of aromatic compounds indicate that the logarithm of the distribution coefficient between XAD resin and ethanol solution seem to be linear at lower ethanol concentrations.

The extrapolation of the lines in Fig. 2 suggests the presence of a point to which all lines converge. If this observation is correct, then a linear relationship exists between the first term in eqn. 2 and the concentration of methanol in the mobile phase. Because $\log k'$ is defined by only two terms on the right-hand side of eqn. 2, the linear relationship between $\log k'$ and the concentration of methanol in the mobile phase might extend to other types of compounds. It will require more precise experiments to determine whether or not the lines in Fig. 2 converge to a single point. However, it can be assumed they cross in at least a narrow area and the degree of the convergence will be a measure of the validity of the above discussion.

The presence of similar relationships for other types of compounds is being studied.

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