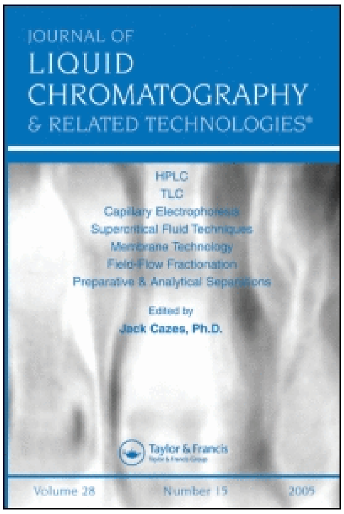

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PREPARATION AND CHARACTERIZATION OF AMINE MODIFIED POROUS POLYMER FOR RPLC

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ABSTRACT

The retention behaviour and selectivity of various aromatic compounds, including halobenzenes and alkylbenzenes, were examined on the stationary phase of chloromethylated polystyrene(CMS) beads, of which the chloride group was substituted by the alkylamines: n-hexyl(C_6), n-octyl(C_8), n-decyl(C_{10}),n-dodecyl(C_{12}), n-tetradecyl(C_{14}) or n-hexadecyl(C_{16}). Changes in the distribution coefficients of benzene derivatives are demonstrated not only by the changes of the eluent compositions but also by the changes of chain length in the stationary phase. In general, a longer alkyl chain in the stationary phase gives longer retention time and different selectivity. The effects of MeOH/H_2O ratios on retention and selectivity was also demonstrated on the C_{14} stationary phase. Good separations of halobenzenes and alkylbenzenes were obtained with C_{14} stationary phase. Moreover, the retention time for samples was shortened by about a half compared to polystyrene stationary phase. These results show the chemical modification of CMS gel with long chain alkyl amines decreases tailing and provides new supports with different selectivities.

INTRODUCTION

The silica-supported stationary phases are widely used in high performance liquid chromatography(HPLC)(1-6), because they do not change size with changes in solvent conponents or eluent

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pressure. On the other hand, the silica-supported stationary phases (e.g. ODS) dissolve in an alkali or decompose in an acidic solution of low pH due to hydrolysis of the siloxane bonds. Generally, silica supports cannot be used routinely above pH 8 or below pH 2(7).

A new stationary phase that may be superior for separating the bioactive materials was our goal. Bioactive materials are generally water-soluble, and the selectivities in HPLC separation are often affected by a change of pH(8). Thus chloromethylated polystyrene(CMS) beads was used as a support stable to a wide pH range. The distribution coefficients of halobenzenes and alkyl benzenes was examined on various amine modified CMS beads as well as the effects of the alkyl chain length of the modified surface and the eluent composition on the distribution coefficients and the retention times of benzene derivatives.

EXPERIMENTAL

Materials and Reagents; Six kinds of n-alkylamines, viz., n-hexyl, n-octyl-, n-decyl-, n- dodecyl-, n-tetradecyl- and n-hexadecylamine, were of reagent grade (Tokyo Kasei, Japan). All the compounds as sample solutes were of reagent grade and purchased from several sources. They were benzene, toluene, p-xylene, m-xylene, mesitylene, chloro-, p-dichloro-, m-dichloro-, 1,2,4-trichloro-, 1,3,5-trichloro-, bromo-, p-dibromo-, m-dibromo-, 1,3,5-tribromo- and iodobenzene. Macroporous (ca. 250Å) chloromethylated polystyrene(CMS) beads (10μm, Nippon Chemical Co., Japan) were compared to 10μm porous polystyrene(PS) gels(#3011, Hitachi, Japan). Gels were packed into 6 mmϕ x 15 cm columns by the high pressure slurry procedure.

Preparation of packing materials; Porous CMS gel beads (4.86g) was added to 45 mmol of alkylamine (n-hexyl, n-octyl, n-decyl, n-dodecyl, n-tetradecyl, n-hexadecyl) in 160 ml of benzene and reacted with stirring at ca. 250 rpm for 24 hrs at 60 °C. After removing benzene by filtration, the polymer beads were rinsed with 500ml methanol and 500ml benzene with stirring for one hour, respectively. After that the polymer beads were washed with methanol and benzene for 16 hrs each using a Soxhlet extractor, followed by drying in vacuo at 50°C for 18 hrs. Amine content was determined by elemental analyses (Table 1).

Chromatograph The high speed liquid chromatograph(#HLC-803D, TOYO SODA, Japan) equipped with a reflectometer RI-8(TOYO SODA, Japan), and a valve loop injector (100 μl, Rheo dyne, USA) was used at room temperature (ca. 25°C). Eluents were mixtures of methanol and distilled water(v/v), degassed and filtered through the 0.45μm membrane filter before use. Sample solutions (1~2mM) were prepared by dissolving in the eluents. Eluent flow rate was 0.8 ml/min.

RESULTS AND DISCUSSION

Elution behaviors of halobenzenes

Fig. 1 compares the separation of monosubstituted halobenzenes on the C_{14} stationary phase(A) with that on the PS phase(B). The elution order of species was benzene, chlorobenzene, bromobenzene and iodobenzene, and the elution

TABLE 1. The N CONTENTS and the DEGREES of CONVERSION of various ALKYLAMINE-modified CMS BEADS

	Alkylamines					
	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}
N content(%)	3.22	2.72	2.54	2.97	2.34	2.28
conversion(%)	50.16	47.64	49.61	63.87	50.06	58.16

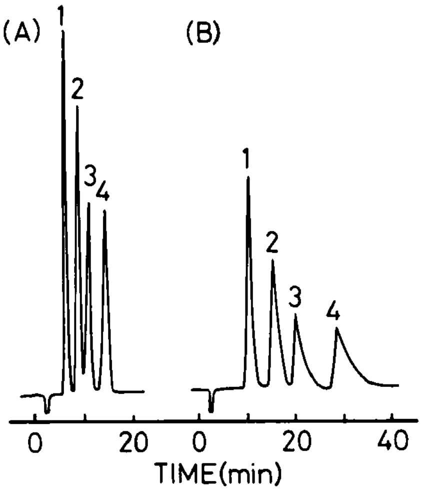

FIGURE 1 Chromatogram of benzene(1), chlorobenzene(2), bromobenzene(3) and iodobenzene(4) on the C_{14} stationary phase(A) and on the PS stationary phase(B). Eluent:87.5% MeOH; flow rate: 0.8ml/min; column: 4.6mm x 15cm; detector:RI-8.

times were within 20 min. Compared to the PS support, the C_{14} substituent decreases retention, increases peak height and decreases peak width. It is clear that the resolution is much enhanced by using the stationary phase with long alkylamino chain.

Fig. 2 shows the separation of chlorobenzene, p-dichlorobenzene, m-dichlorobenzene, 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene on the C_6(A), C_8(B) and C_{14}(C) stationary phases. The retention time increases as the number of halogens giving the order, benzene, chlorobenzene, p-dichlorobenzene, m-dichlorobenzene, 1,2,4-trichlorobenzene and 1,3,5-

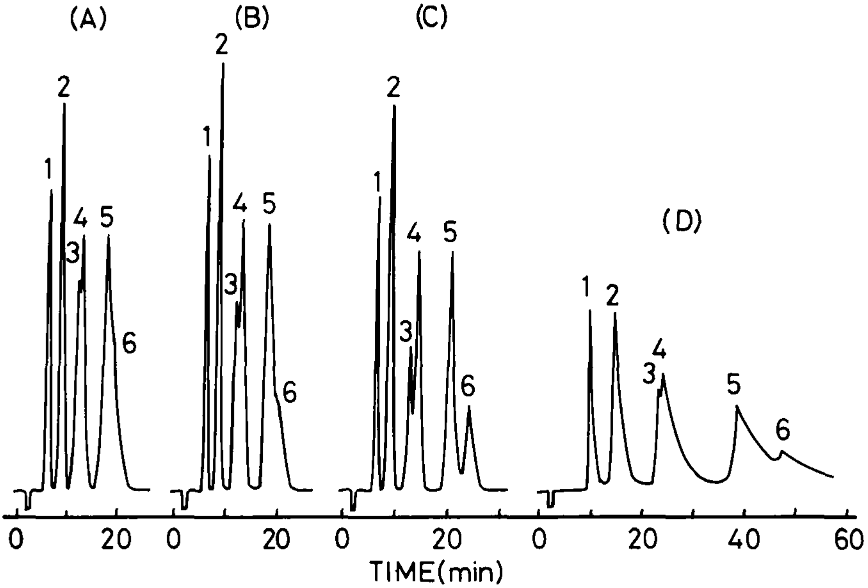

FIGURE 2 Chromatogram of benzene(1), chlorobenzene(2), p-dichlorobenzene(3), m-dichlorobenzene(4), 1,2,4-trichlorobenzene(5) and 1,3,5-trichlorobenzene(6) on C_6 phase(A), on C_8 phase(B), on C_{14} phase(C) and on PS phase(D). Eluent: 87.5% MeOH; flow rate: 0.8 ml/min.

trichlorobenzene, for all three phases. The retention times become slightly longer and the distribution coefficients change a little with an increase in the stationary phase alkyl chain length. The C_6 and C_8 stationary phases do not resolve trichloro isomers, i.e., 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene, but the C_{14} stationary phase resolves completely these isomers. The PS phase showed the retention times about twice those on the C_{14} phase, and more peak-tailing. Fig. 3 shows a satisfactory

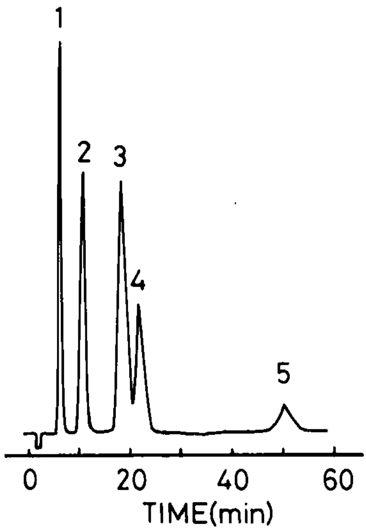

FIGURE 3 Chromatogram of benzene(1), bromobenzene(2), p-dibromobenzene(3), m-dibromobenzene(4) and 1,3,5-tribromobenzene(5) on C_{14} phase. Eluent: 87.5% MeOH; flow rate: 0.8 ml/min.

separation on the same C-14 phase of bromine compounds similar to the chloro compounds.

The effect of the carbon number ,n, of alkylamino group of the stationary phase on the retention was investigated in detail. The result is shown in Figure 4 as the plot of $\log K_{av}$ vs n. The distribution coefficient K_{av} is defined by the following equation, $K_{av} = V_e/V_x$, where V_e and V_x are the elution volume of a given solute and the column volume, respectively(9). The $\log K_{av}$ values increase with an increase in the chain length of alkylamino group on the stationary phase. The elution order remains the same for most samples, but it changes for p-dibromobenzene and 1,2,4-

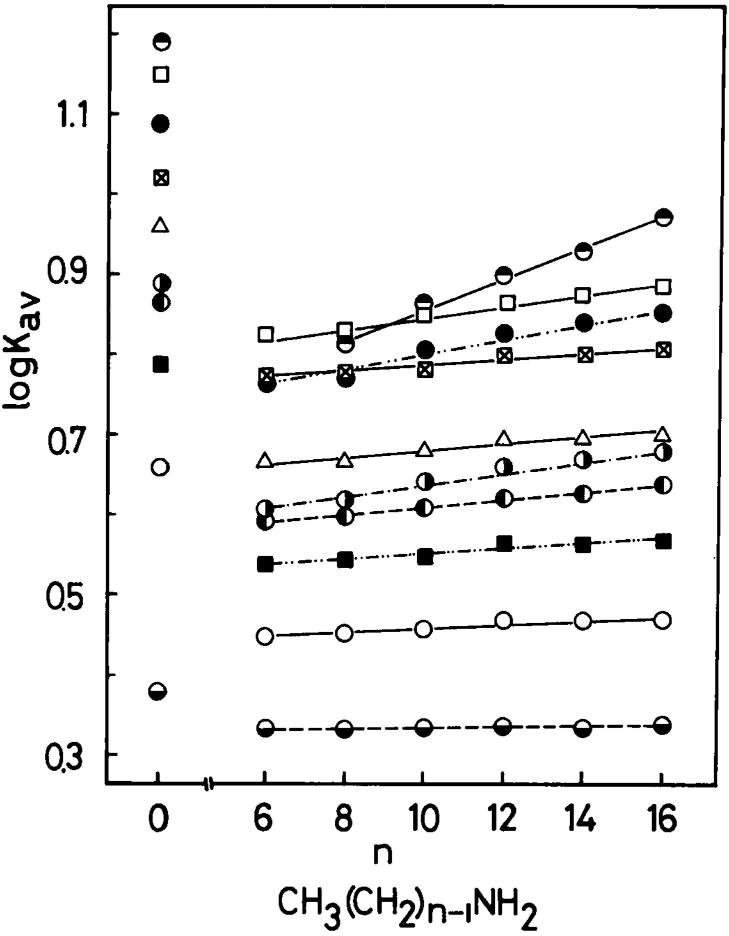

FIGURE 4 Plots of logK_{av} vs chain length n.
Eluent: 87.5% MeOH. --◒--:benzene,
——○——:chlorobenzene, ·–■·–:bromobenzene, --◐--:
p-dichlorobenzene, ·—◑·—:m-dichlorobenzene, ——△——:
iodobenzene, ——⊠——:p-dibromobenzene, ··—●··—:
1,2,4-trichlorobenzene, ——□——:m-dibromobenzene,
——◓——:1,3,5-trichlorobenzene.

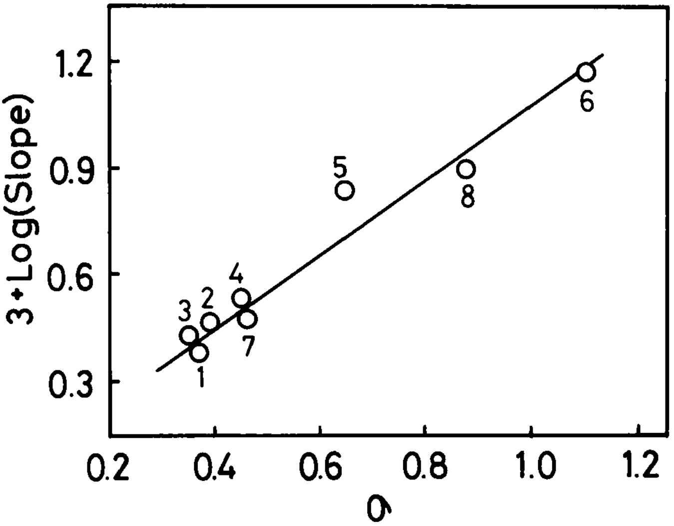

FIGURE 5 Relationship between Hammett's constants(σ) and the slopes of $\log K_{av}$ vs n curves in the Fig.4. Samples are chlorobenzene(1),bromobenzene(2), iodobenzene(3), p-dichlorobenzene(4), m-dichlorobenzene(5), 1,3,5-tri-chlorobenzene(6), p-dibromobenzene(7), m-dibromobenzene(8).

trichlorobenzene, and that of m-dibromobenzene and 1,3,5-trichlorobenzene reverse between the C_8 and C_{10} phase. The effect of the carbon number of the stationary phase is greatest for the most retained sample, 1,3,5-trichlorobenzene.

Fig. 4 shows that the distribution coefficients decrease by introducing the alkylamino groups on CMS gels and increase with increasing carbon number, n, of the alkylamino residues. It was generally observed that sample with large retention showed a greater change in its retention as the alkyl chain length was increased. The slopes of $\log K_{av}$ vs n curves were plotted against the Hammett constants σ of the corresponding samples in Fig. 5. A good linearity is found: it implies that the charge distribution in a solute molecule a primary factor in determining the elution behaviour on the present stationary phase.

When the length of alkyl chains of the stationary phase increases, the characteristics as a reverse phase support become dominant. The reverse phase effect is weakened with a decrease in the alkyl chain length because of the electrostatic interaction caused by the amino group on the stationary phase. In summary, Fig. 4 shows that different retentions and some major differences in the distribution coefficient could be provided by the differences in alkyl chain length of the stationary phase (10-14).

The effect of the ratio of MeOH to H_2O in the eluents on retention was also examined for the C_{14} support (Fig. 6). A decrease in K_{av} with an increase in MeOH agrees with the common characteristics of reversed phase liquid chromatography(15).

Elution behaviours of alkylbenzenes

Figure 7 illustrates the chromatogram of the alkyl derivatives of benzene: toluene, p-xylene, m-xylene and mesitylene. Also in the separation of alkylbenzenes, the selectivity is improved and the retention time is shortened on the C_{14} stationary phase compared to those on the PS stationary phase. Figures 8 and 9 show dependences of K_{av} on the carbon number n and the MeOH concentration in the mobile phase, respectively. A larger number of n on the stationary phase and a higher percentage of water in the mobile phase give a better selectivity, although the retention time become longer.

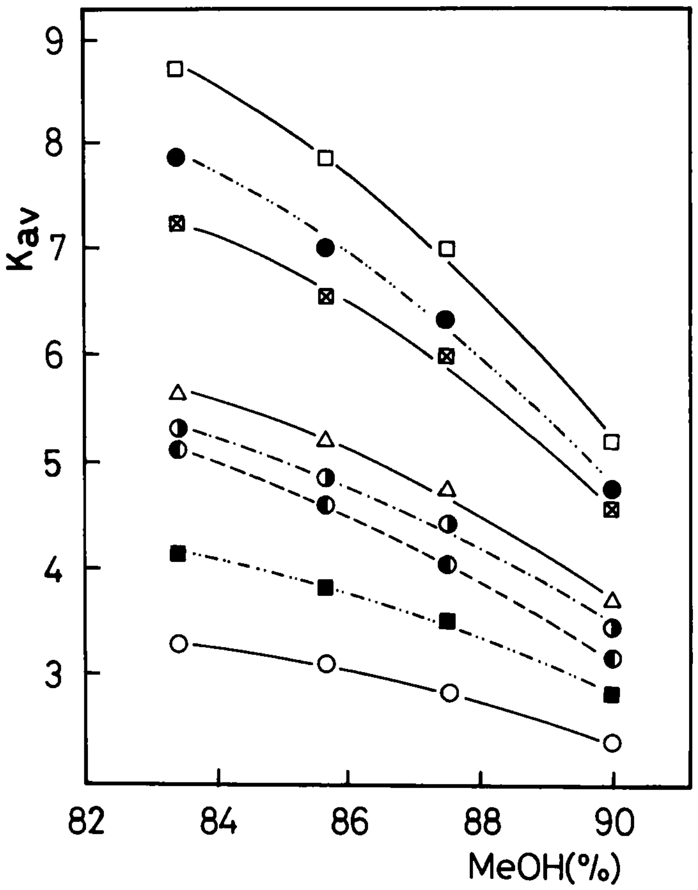

FIGURE 6 Plots of K_{av} vs MeOH concentration in the mobile phase on the C_{14} phase.

—○—:chlorobenzene, ··—■··—:bromobenzene,

——◐——:p-dichlorobenzene, ·—·◑—·:m-dichlorobenzene,

—△— :iodobenzene, —⊠—:p-dibromobenzene, ··—●··—:

1,2,4-trichlorobenzene, —□—:m-dibromobenzene.

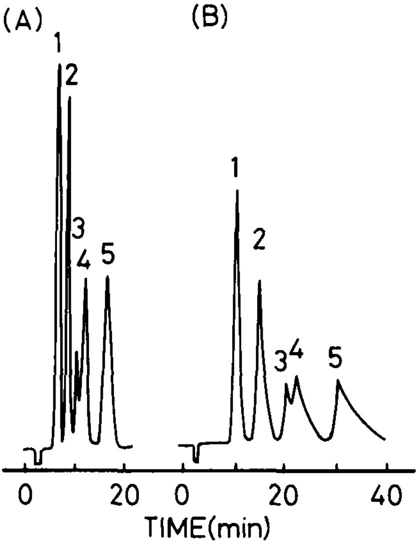

FIGURE 7 Chromatogram of benzene(1), toluene(2), p-xylene(3), m-xylene(4) and mesitylene(5) on C_{14} phase(A) and on PS phase(B). Eluent:87.5% MeOH; flow rate:0.8 ml/min.

Figure 10 shows the plot of logK_{av} on the C_6 stationary phase against logK_{av} on the C_{16} stationary phase. A good linearity is obtained, suggesting that the separation mechanism is the same on the C_6 and the C_{16} stationary phase in this liquid chromatographic process.

CONCLUSION

These results of the present investigations lead the following conclusions. The CMS beads modified with long chain n-alkylamines are promising as non-silicic stationary phase packings in RPLC. The alkyl chain length on stationary phase beads and the solvent composition in the mobile phase are also important factors on the selectivity and retention behaviors.

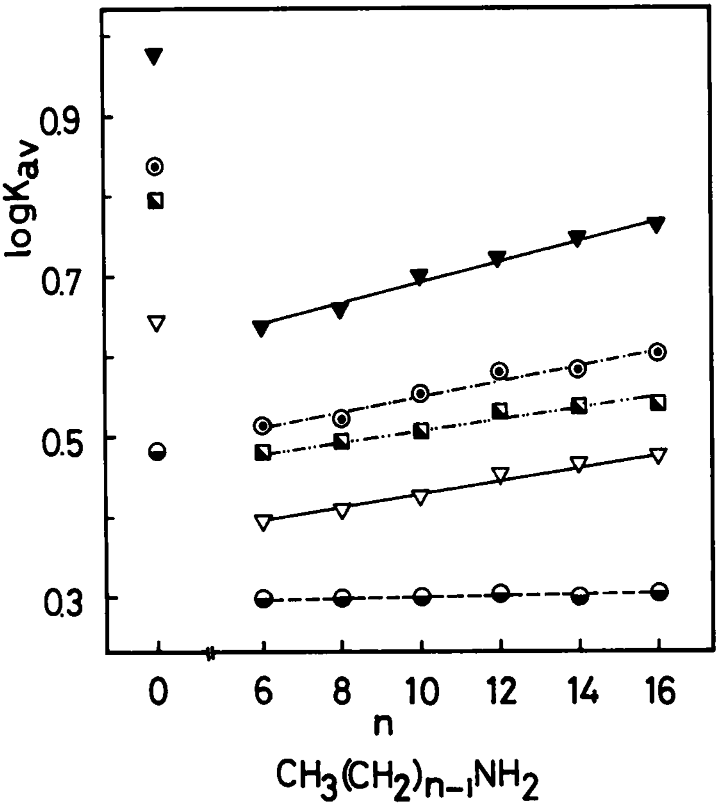

FIGURE 8 Plots of logK$_{av}$ vs n on 87.5% MeOH.

—◒- -:benzene, —▽—:toluene, ··—⧅··—:p-xylene,

·—·◉—·:m-xylene, —▼—:mesitylene.

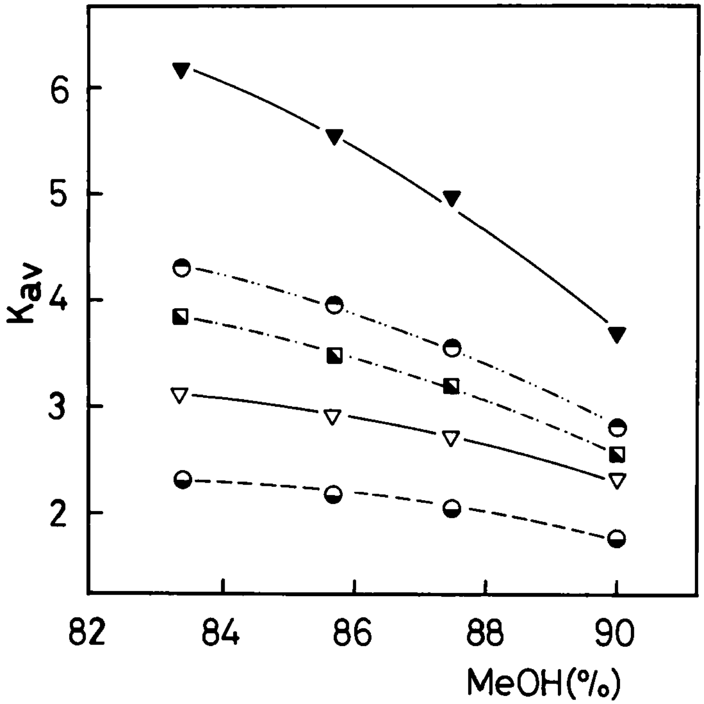

FIGURE 9 Plots of K_{av} vs different MeOH composion in the mobile phase on C_{14} phase. ——◒——:benzene, ——▽——: toluene, ··—◪··—:p-xylene, ·—· ◉—·:m-xylene, ——▼——:mesitylene.

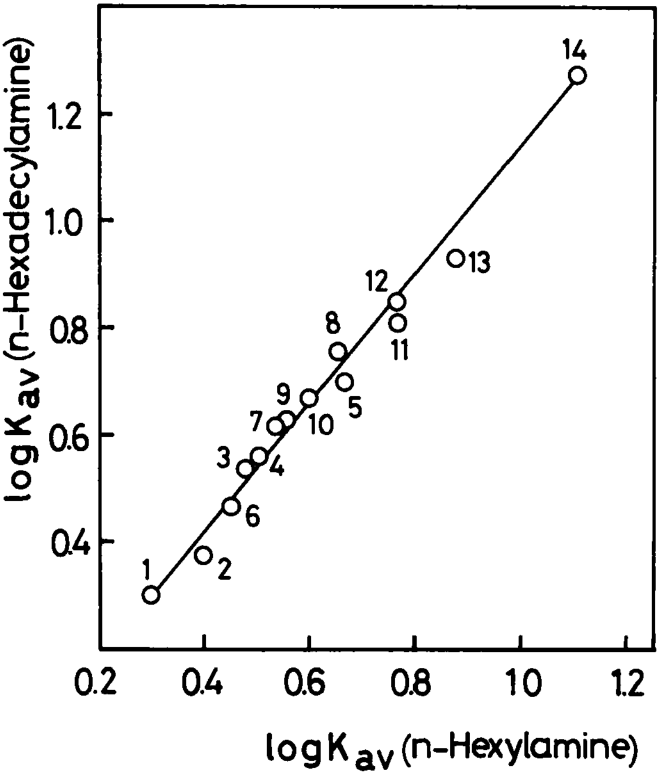

FIGURE 10 Plot of $\log K_{av}$ on the C_6 phase vs $\log K_{av}$ on the C_{16} phase using 87.5 % MeOH as a mobile phase. Samples are benzene(1), toluene(2), p-xylene(3), m-xylene(4), mesitylene(5), chlorobenzene(6), bromobenzene(7), iodobenzene(8), p-dichlorobenzene(9), m-dichlorobenzene(10) p-dibromobenzene(11), 1,2,4-tribromobenzene (12), m-dibromobenzene(13), 1,3,5-tribromobenzene(14).

The longer alkyl chains on the stationary phase beads provide much better selectivities for halobenzenes and alkylbenzenes.

Further work on characterizing these stationary phases is in progress.

ACKNOWLEDGEMENT

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