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SELECTIVITY OF HYDROPHOBIC COMPOUNDS ON AN OCTADECYL-BONDED VINYL ALCOHOL COPOLYMER GEL

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SUMMARY

The selectivity of an octadecyl-bonded vinyl alcohol copolymer gel was examined by investigating the difference in the enthalpy effect for alkylbenzenes, alkanes, alcohols and polyaromatic hydrocarbons. The retention of these compounds on an octadecyl-bonded silica gel decreased as the column temperature increased. However, their chromatographic behaviour on an octadecyl-bonded vinyl alcohol copolymer gel was not simple. The values of the enthalpy effect for aliphatic compounds were positive above 35°C, negative below 35°C and small at low temperatures, which indicates that the structure of the stationary phase was somehow changed at *ca.* 33°C. Polyaromatic hydrocarbons were selectively retained on this octadecyl-bonded vinyl alcohol copolymer gel.

INTRODUCTION

Octadecyl-bonded silica gels are widely used in liquid chromatography. Their physical strength allows them to be used for a wide range of substances, but their chemical instability has caused some problems, especially in biochemical analyses. On the other hand, porous organic polymer gels, which have also been used in a variety of research applications, are chemically very stable, but physically weaker than silica gel-based packings. The recent development of rigid, porous organic polymer gels permits high-speed separations of many biologically important substances. One widely used organic porous polymer gel is polystyrene-divinylbenzene copolymer. Such gels constitute excellent packings for size-exclusion chromatography, and ion-exchange resins based on polystyrene gels are better than ion exchangers based on silica gels for some chromatographic purposes. However, organic porous polymer gels do have limitations, because the column efficiency is lower for compounds with relatively long retention times. This can be ascribed to the poor pore-size distribution and the strong selectivity due to the π -electrons of the aromatic rings¹. Rigid vinyl alcohol copolymer gels, which are very polar and useful for the analysis of biological substances²⁻⁴, were therefore surface-modified with octadecyl groups and examined for selectivity and column efficiency.

Retention in reversed-phase liquid chromatography is related to the hydrophobicity or the partition coefficient (P) of the solutes between octanol and water.

The hydrophobicity and the partition coefficient can also be related to the solubility of the solutes in water.

The partition coefficient between octanol and water is given as the Hansch π -constant⁵ or as the log P values described by Rekker⁶. The log P values calculated from the fragmental constant are used for the optimization of reversed-phase liquid chromatography. However, this method is not suitable for the optimization of mixtures of different types of compounds⁷⁻¹¹.

The Van der Waals volume of solutes can be related to their hydrophobicity, but their retention times are not related only to their Van der Waals volume. However, the retention of undissociated compounds can be predicted from their Van der

TABLE I
PHYSICAL PARAMETERS AND LOGARITHM OF CAPACITY FACTORS OF STANDARD SOLUTES

No.	Solute	VWV*	Log k' at**				
			Temperature $10^5/T$ (K^{-1})				
			309.6	314.5	319.5	324.7	330.0
1	Benzene	48.36	-0.142	-0.133	-0.119	-0.110	-0.125
2	Naphthalene	73.96	0.006	0.022	0.045	0.062	0.057
3	Fluorene	93.22	0.149	0.169	0.195	0.216	0.213
4	Phenanthrene	99.56	0.263	0.291	0.314	0.340	0.342
5	Pyrene	109.04	0.463	0.495	0.531	0.564	0.572
6	Chrysene	125.16	0.553	0.591	0.637	0.674	0.697
7	Benzopyrene	134.64	0.788	0.831	0.883	0.936	0.979
8	Picene	150.76	0.867	0.913	0.988	1.049	1.131
9	Toluene	59.51	-0.164	-0.172	-0.182	-0.183	-0.196
10	Ethylbenzene	69.74	-0.086	-0.090	-0.096	-0.101	-0.123
11	Propylbenzene	79.97	0.018	0.018	0.017	0.015	-0.008
12	Butylbenzene	90.20	0.126	0.132	0.135	0.137	0.118
13	Hexylbenzene	110.66	0.376	0.390	0.402	0.413	0.394
14	Heptylbenzene	120.89	0.512	0.530	0.547	0.563	0.546
15	Octylbenzene	131.12	0.651	0.674	0.695	0.715	0.700
16	Nonylbenzene	141.35	0.794	0.821	0.846	0.870	0.859
17	Decylbenzene	151.58	0.939	0.969	0.999	1.027	1.020
18	Pentane	58.03	0.221	0.242	0.274	0.290	0.288
19	Hexane	68.26	0.351	0.372	0.405	0.424	0.428
20	Heptane	78.49	0.489	0.513	0.552	0.570	0.580
21	Octane	88.72	0.640	0.653	0.694	0.719	0.730
22	Decane	109.18	0.940	0.962	1.013	1.047	1.073
23	Undecane	119.41	1.093	1.130	1.174	1.217	1.247
24	Dodecane	129.64	1.243	1.291	1.336	1.387	1.418

* Van der Waals volume (cm^3/mol), from ref. 12.

** Eluent: acetonitrile-water (70:30).

*** Enthalpy values obtained using acetonitrile-water as the mobile phase, at two different concentrations. The concentration of acetonitrile in the mixture is indicated.

§ Column temperature: 35, 40, 45, 50°C.

§§ Column temperature: 20, 25, 30°C (alkylbenzenes: 15, 20, 25, 30°C).

§§§ From ref. 16.

Waals volumes, π -energy and hydrogen-bonding energy effects¹²⁻¹⁴ on octadecyl-bonded silica gels. We have found that the retention times of highly hydrophobic compounds were longer than predicted and that the difference between the experimental and predicted capacity ratios could be related to enthalpy¹⁵.

There are some differences between octadecyl-bonded silica gels and octadecyl-bonded vinyl alcohol copolymer gels so far as the retention of aromatic compounds is concerned¹⁶. The enthalpies of alkylbenzenes, alkanes, alcohols and polyaromatic hydrocarbons were therefore measured on an octadecyl-bonded vinyl alcohol copolymer gel, and the values were compared with those obtained on an octadecyl-bonded silica gel.

			$-\Delta H$ (kcal/mol) ^{***}				Energy effect ^{§§§}
			High temperature [§]		Low temperature ^{§§}		
335.6	341.3	347.2	80%	70%	80%	70%	
-0.128	-0.134	-	0.666	1.000	-1.011	-0.365	1.895
0.062	0.067	-	1.472	1.738	-0.079	0.405	2.376
0.223	0.232	-	1.883	2.065	0.284	0.769	3.134
0.359	0.373	-	2.329	2.310	0.649	1.255	2.831
0.597	0.618	-	2.757	3.084	1.297	1.862	2.235
0.738	0.785	-	3.329	3.720	3.198	3.564	3.071
1.034	1.099	-	4.251	4.515	4.819	4.860	1.814
1.229	1.342	-	4.952	5.653	9.070	8.552	1.221
-0.209	-0.215	-0.227	0.931	-0.563	-0.850	-0.790	2.320
-0.145	-0.151	-0.165	1.004	-0.464	0.201	-1.052	2.700
-0.033	-0.042	-0.055	1.158	-0.135	-0.768	-1.195	2.958
0.092	0.078	0.067	1.347	0.224	-1.376	-1.330	3.130
0.367	0.352	0.340	1.967	1.118	-1.862	-1.410	3.334
0.519	0.507	0.498	2.367	1.546	-1.415	-1.242	3.367
0.674	0.663	0.657	2.794	1.937	-1.335	-1.114	3.394
0.835	0.828	0.862	3.185	2.301	-1.011	-0.842	3.353
0.999	0.996	0.999	3.549	2.674	-0.727	-0.522	3.327
0.261	0.241	-	1.561	2.172	-1.456	-1.902	0
0.403	0.387	-	2.283	2.291	-1.456	-1.658	0
0.555	0.535	-	2.483	2.563	-1.295	-1.822	0
0.706	0.689	-	2.866	2.531	-1.336	-1.659	0
1.046	1.027	-	3.622	3.387	-1.131	-1.861	0
1.231	1.215	-	4.013	3.785	-0.645	-1.295	0
1.411	1.399	-	4.283	4.340	0.449	-0.771	0

EXPERIMENTAL

The liquid chromatograph used consisted of an ERMA ERC-3310 degasser from ERMA Optical Works (Tokyo, Japan), a Jasco Trirotar-V pump (Jasco, Tokyo, Japan), a Rheodyne 7125 injector, a Jasco Uvidec 100VI ultraviolet detector, an ERMA ERC-7510 refractive index detector and a Shimadzu CR3A recorder (Shimadzu, Kyoto, Japan). An octadecyl-bonded vinyl alcohol copolymer gel, BHST 602 C18, was obtained from Asahi Chemical (Kawasaki, Japan). The column was 10 cm \times 6.0 mm I.D., thermostated at 15–50°C.

Analytical-reagent grade chemicals, supplied by Tokyo Chemical Industry and Kishidakagaku (Tokyo, Japan), were used without further purification. Liquid chromatography-grade acetonitrile from Kishidakagaku and water treated by passage through Pureline WL-2P from Yamato Scientific (Tokyo, Japan) were used for the eluent.

The retention times of alkylbenzenes were measured in 70 and 80% aqueous acetonitrile on the column at 15–50°C, and the values of the enthalpy effect were obtained from these measurements.

RESULTS AND DISCUSSION

The process occurring inside a liquid chromatographic column cannot be characterized simply by a summation of the Van der Waals volume (VWV), π -energy (Π) and hydrogen-bonding energy (HB) effects for hydrophobic compounds. The influence of temperature on retention was therefore measured in order to investigate the enthalpy effect ($-\Delta H$), as described in previous reports^{15,17}. The logarithms of the capacity ratios ($\log k'$) of the alkylbenzenes, alkanes and polyaromatic hydrocarbons investigated are given in Table I. However, because the retention of alcohols was too weak to measure the temperature effect, the value have been omitted from Table I. The elution volume of water was used as the void volume of the column.

The retention behaviour of these compounds changed at around 30 and 35°C, as can be seen in Fig. 1.

The slope between 35 and 50°C was positive whereas that between 20 and 30°C was negative, except for polyaromatic hydrocarbons. Values for the enthalpy effect were therefore calculated separately, these values being given in Table I as $-\Delta H$ (high temperature) and $-\Delta H$ (low temperature). Such a phenomenon was not observed on octadecyl-bonded silica gels.

Because the values of the enthalpy effect obtained above 35°C were larger than those obtained below 30°C, for alkylbenzenes and alkanes, the values of the enthalpy effect above 35°C were first used for further discussion. For alkylbenzenes, the relationship between $-\Delta H$ (high temperature) values and $\log k'$ values and between $-\Delta H$ (high temperature) values and Van der Waals volumes were linear. The correlation coefficients (r) were 0.997 and 0.996 ($n = 8$), respectively, omitting the data for toluene from the calculation (Fig. 2). The enthalpy effect increased as a function of the Van der Waals volume on both the octadecyl-bonded silica gel and the vinyl alcohol copolymer gel.

In alkylbenzenes, when the alkyl chains are longer than six carbon atoms, their enthalpy effect dramatically increases, but their energy effect becomes fairly constant

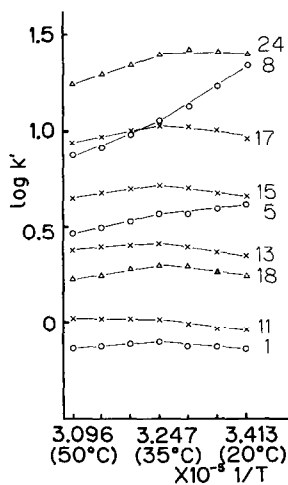


Fig. 1. Relationship between $\log k'$ values and the reciprocal of absolute temperature for (\times) alkylbenzenes, (Δ) alkanes and (\circ) polyaromatic hydrocarbons on an octadecyl-bonded vinyl alcohol copolymer gel with 70% aqueous acetonitrile as the eluent. Numbers adjacent to lines are the same as those in Table I.

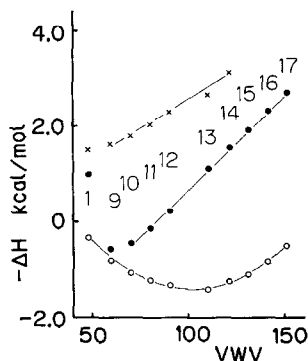


Fig. 2. Relationship between enthalpy effect and Van der Waals volume of alkylbenzenes on octadecyl-bonded vinyl alcohol copolymer gel and octadecyl-bonded silica gel measured in 70% aqueous acetonitrile. Numbers adjacent to symbols are the same as those in Table I. \times , Measured on an octadecyl-bonded silica gel, from ref. 15; \circ [$-\Delta H$ (low temperature)] and \bullet [$-\Delta H$ (high temperature)], measured on an octadecyl-bonded vinyl alcohol copolymer gel. For details, see text.

at *ca.* 3.3, as given in Table I. The energy effect was obtained from the difference in the $\log k'$ values between alkanes and polyaromatic hydrocarbons, and the $\Delta \log k'$ values were related to the delocalization energy of polyaromatic hydrocarbons¹². A similar result was observed on an octadecyl-bonded silica gel¹⁵. Further, the hydrogen-bonding effect of alkanols also became constant at *ca.* 7.1 on an octadecyl-bonded vinyl alcohol copolymer gel¹⁶ and at *ca.* 8.6 on an octadecyl-bonded silica gel¹⁵. This indicates that when their Van der Waals volumes are larger than about 110 cm^3/mol , the direct adsorption of molecules on the surface of the packing will be the predominant force in their retention on octadecyl-bonded vinyl alcohol copolymer gels.

The enthalpy effects of alkylbenzenes on octadecyl-bonded vinyl alcohol copolymer gels are smaller than those on octadecyl-bonded silica gels¹⁵. This may be due to the lower hydrophobicity of a modified vinyl alcohol copolymer gel compared with that of an octadecyl-bonded silica gel, owing to the existence of hydroxyl groups on the surface of the former.

The $\Delta \Delta H$ value of a methylene unit of alkylbenzenes on an octadecyl-bonded vinyl alcohol copolymer gel, except for toluene, is a fairly constant 370 cal/mol calculated from the data in Table I, whereas on an octadecyl-bonded silica gel, $\Delta \Delta H$ for a methylene unit is not constant and is greater for larger molecules¹⁵. However, the $\Delta \Delta H$ value of a methylene unit of alkanols on an octadecyl-bonded silica gel is fairly constant at *ca.* 300 cal/mol ¹⁵. Such a difference can be seen in Fig. 2 as the different slopes.

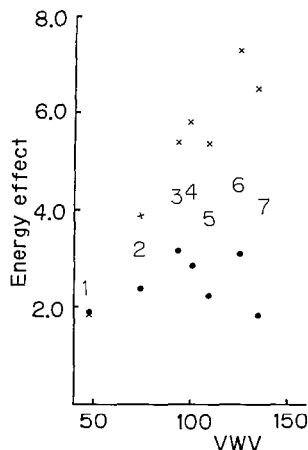
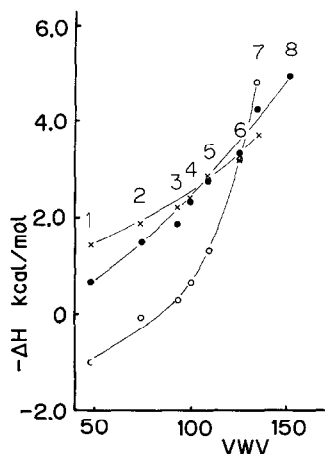


Fig. 3. Relationship between enthalpy effect and Van der Waals volume of polyaromatic hydrocarbons. Numbers adjacent to symbols are the same as those in Table I. Symbols as in Fig. 2. \times , Measured on an octadecyl-bonded silica gel, from ref. 14; \circ [$-\Delta H$ (low temperature)] and \bullet [$-\Delta H$ (high temperature)], measured on the vinyl alcohol copolymer gel in 80% aqueous acetonitrile. For details, see text.

Fig. 4. Relationship between energy effect and Van der Waals volume of polyaromatic hydrocarbons. Numbers adjacent to symbols are the same as those in Table I. \times , Measured on an octadecyl-bonded silica gel, from ref. 14; \bullet , measured on the vinyl alcohol copolymer gel.

The enthalpy effect of alkylbenzenes on octadecyl-bonded vinyl alcohol copolymer gels in 70% aqueous acetonitrile is smaller than that in 80% acetonitrile, as seen in Table I. From Fig. 2, the minimum value of $-\Delta H$ (high temperature) of alkylbenzenes was obtained at 60/VWV and that of $-\Delta H$ (lower temperature) at 100/VWV. This may depend on the degree of solvation of the packing. However, there is no clear explanation for the difference between $-\Delta H$ (high temperature) and $-\Delta H$ (low temperature) at present.

The chromatographic behaviour of alkanes was similar to that of alkylbenzenes, and their retention behaviour changed at *ca.* 33°C. The $\Delta\Delta H$ value of a methylene unit in alkanes was *ca.* 350 cal/mol on both octadecyl-bonded silica and vinyl alcohol copolymer gels. The chromatographic behaviour of polyaromatic hydrocarbons differed from that of other compounds, even though their retention behaviour changed at *ca.* 33°C. The value of the enthalpy effect increased with increasing Van der Waals volume, while the values of $-\Delta H$ (high temperature) on an octadecyl-bonded vinyl alcohol copolymer gel were close to those obtained on an octadecyl-bonded silica gel. However, the values of $-\Delta H$ (low temperature) were dramatically increased, as shown in Fig. 3.

There is no direct way of comparing the values of the enthalpy and the energy effects at present. However, the energy effect obtained on an octadecyl-bonded vinyl alcohol copolymer gel (Fig. 4) was less than the enthalpy effect (*ca.* 2.4) and there was little difference within polyaromatic hydrocarbons. This phenomenon differed from that observed on an octadecyl-bonded silica gel, where the energy effect was greater than the enthalpy effect.

The enthalpy effect may therefore depend on the type of packing used and on

the water content of the eluent. The values of this effect for alkylbenzenes and alkanes on octadecyl-bonded vinyl alcohol copolymer gels, measured below 35°C, were negative. This means that alkylbenzenes and alkanes may not be directly adsorbed on the surface of the packing below 35°C, the retention mechanism appearing to be a partition of some kind. However, polyaromatic hydrocarbons were directly adsorbed on both the octadecyl-bonded vinyl alcohol copolymer gel and the octadecyl-bonded silica gel. Apart from this, they were retained more selectively than aliphatic compounds on the octadecyl-bonded vinyl alcohol copolymer gel. The reasons for the negative enthalpy effect at low temperature are not simple, and further study is required before the liquid chromatographic behaviour on octadecyl-bonded vinyl alcohol copolymer gels can be characterized.

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