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RETENTION OF AROMATIC ACIDS 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 chromatographic behaviour of aromatic acids. The energy effect on the retention of aromatic acids was calculated from the result for alkylphenols. This packing was less hydrophobic than octadecyl-bonded silica gels, and the energy effect on the retention of aromatic acids was small compared to the values measured on an octadecyl-bonded silica gel. The correlation coefficient between the energy effects of aromatic acids measured on the octadecyl-bonded vinyl alcohol copolymer and silica gels was 0.972 ($n = 36$). However, this bonded organic packing had more selectivity for the retention of *ortho*-substituted alkylbenzoic acids.

INTRODUCTION

Vinyl alcohol copolymer gels are a useful type of column packing for direct analysis of biological samples, *e.g.*, proteins are eluted as in size-exclusion liquid chromatography, then target compounds are separated by the packed columns¹⁻⁶. However, these packings are too polar for some applications. The surface was, therefore, bonded with octadecyl groups and its selectivity was measured as the difference in the retention of alkanes, alkylbenzenes, polyaromatic hydrocarbons and alcohols⁷, and phenols⁸. The retention of these compounds was weak, but polyaromatic hydrocarbons were selectively retained compared to their retention on octadecyl-bonded silica gels⁷.

In this paper, the selectivity of this octadecyl-bonded vinylalcohol copolymer gel was examined by investigating the retention behaviour of aromatic acids.

EXPERIMENTAL

The instrument used was described previously⁸. The chemicals used are listed in Table I. The octadecyl-bonded vinyl alcohol copolymer gel (BHST602C18; Asahi Chemical, Kawasaki, Japan) was packed into a 10 cm × 6.0 mm I.D. column thermostatted in a water-bath at 30°C. The Van der Waals volumes were obtained from ref. 9. The values of the energy effect for phenols were obtained from ref. 7.

TABLE I

COMPOUNDS STUDIED AND THEIR PHYSICAL PARAMETERS

Experimental conditions: column, BHST602C18, 10 cm × 6.0 mm I.D.; eluent, 0.05 *M* phosphoric acid in acetonitrile–water mixtures; column temperature, 30°C.

Compound	V/WV*	log <i>k'</i> (% acetonitrile)					E**	E***
		20	25	30	35	40		
1 Phenol	53.88	0.6081	0.4351	0.2797	0.1413	0.0123	8.721	7.963
2 2-Methylphenol	65.03	0.8926	0.6984	0.5221	0.4991	0.2014	8.801	8.410
3 4-Methylphenol	65.03	0.8383	0.6280	0.4213	0.2786	0.1212	8.916	8.653
4 2,3-Dimethylphenol	76.18	1.1576	0.9274	0.7075	0.5212	0.3496	8.991	9.197
5 2,4-Dimethylphenol	76.18	1.1251	0.8956	0.6795	0.4976	0.3255	9.017	9.057
6 2,5-Dimethylphenol	76.18	1.1230	0.8928	0.6904	0.4389	0.3114	9.040	9.045
7 2,6-Dimethylphenol	76.18	1.1300	0.9096	0.6990	0.5058	0.3397	9.005	8.984
8 3,4-Dimethylphenol	76.18	1.0309	0.7981	0.5841	0.4148	0.2339	9.112	9.570
9 3,5-Dimethylphenol	76.18	1.0627	0.8289	0.6155	0.4223	0.2517	9.094	9.337
10 2,3,5-Trimethylphenol	87.33	1.3687	1.1150	0.8742	0.6636	0.4632	9.186	9.844
11 2,3,6-Trimethylphenol	87.33	1.3387	1.1089	0.8723	0.6635	0.4764	9.178	9.844
12 2,4,6-Trimethylphenol	87.33	1.3517	1.0992	0.8606	0.6542	0.4693	9.184	9.644
13 2,3,5,6-Tetramethylphenol	98.48	1.5895	1.3044	1.0489	0.8195	0.6127	9.347	10.46
14 2-Ethylphenol	75.26	1.1809	0.9546	0.7230	0.5303	0.3729	8.942	8.627
15 3-Ethylphenol	75.26	1.1117	0.8237	0.6281	0.4315	0.3031	9.026	8.946
16 4-Ethylphenol	75.26	1.0978	0.8511	0.6406	0.4368	0.2642	9.050	8.946
17 Benzoic acid	65.36	0.6239	0.4053	0.2237	0.0701	-0.0645	9.131	10.00
18 2-Methylbenzoic acid	76.51	0.8750	0.6235	0.4143	0.2395	0.0827	9.278	10.76
19 3-Methylbenzoic acid	76.51	0.9052	0.6570	0.4382	0.2480	0.0937	9.264	10.51
20 4-Methylbenzoic acid	76.51	0.8828	0.6367	0.4227	0.2354	0.0827	9.278	10.61
21 2,4-Dimethylbenzoic acid	87.66	1.1520	0.8754	0.6292	0.4297	0.2527	9.423	11.27
22 2,5-Dimethylbenzoic acid	87.66	1.1310	0.8607	0.6217	0.4112	0.2366	9.440	11.43
23 2,6-Dimethylbenzoic acid	87.66	0.6428	0.4445	0.2477	0.0760	-0.0650	9.791	13.00
24 3,4-Dimethylbenzoic acid	87.66	1.1385	0.8413	0.6015	0.3928	0.2185	9.457	11.62

25	3,5-Dimethylbenzoic acid	87.66	1.1857	0.9067	0.6528	0.4365	0.2530	9.416	11.16
26	2,4,6-Trimethylbenzoic acid	98.81	0.9819	0.7152	0.4752	0.2755	0.0995	9.919	13.44
27	4-Ethylbenzoic acid	86.74	1.1782	0.8989	0.6417	0.4280	0.2466	9.396	10.85
28	2-Chlorobenzoic acid	74.84	0.7977	0.5392	0.3509	0.1793	0.0401	9.294	11.09
29	3-Chlorobenzoic acid	74.84	1.2042	0.9291	0.6966	0.4971	0.3327	8.963	9.715
30	4-Chlorobenzoic acid	74.84	1.1888	0.9419	0.7065	0.5055	0.3388	8.961	9.419
31	2,4-Dichlorobenzoic acid	84.32	1.3733	1.0829	0.8331	0.6456	0.4716	9.094	10.30
32	2,5-Dichlorobenzoic acid	84.32	1.3275	1.0716	0.7023	0.5096	0.4324	9.161	10.70
33	2,6-Dichlorobenzoic acid	84.32	0.8012	0.6211	0.4438	0.2712	0.1169	9.499	12.48
34	3,4-Dichlorobenzoic acid	84.32	1.6698	1.3667	1.0862	0.8688	0.6784	9.059	9.223
35	3,5-Dichlorobenzoic acid	84.32	1.7429	1.4493	1.1488	0.9476	0.7756	8.765	8.791
36	2-Bromobenzoic acid	77.96	0.8800	0.6397	0.4425	0.2569	0.1117	9.307	11.35
37	3-Bromobenzoic acid	77.96	1.2982	1.0443	0.7966	0.5980	0.4169	8.961	9.641
38	4-Bromobenzoic acid	77.96	1.3278	1.0558	0.8130	0.6068	0.4310	8.946	9.522
39	Phenylacetic acid	79.55	0.3950	0.1905	0.0186	-0.1496	-0.2704	9.777	12.88
40	2-Tolylacetic acid	86.74	0.5839	0.3631	0.1662	-0.0078	-0.1621	9.858	12.83
41	3-Tolylacetic acid	86.74	0.6461	0.4206	0.2137	0.0247	-0.2680	9.909	12.51
42	2-Chlorophenylacetic acid	85.07	0.7250	0.4831	0.2729	0.0869	-0.0715	9.705	12.34
43	4-Chlorophenylacetic acid	85.07	0.9049	0.6578	0.4338	0.2255	0.0582	9.558	11.57
44	4-Phenyl- <i>n</i> -butyric acid	96.05	0.8911	0.6293	0.3889	0.1786	-0.0039	9.941	13.05
45	1-(+)-Mandelic acid	80.18	-0.1832	-0.3365	-0.4109	-0.5582	-0.6494	10.23	16.03
46	<i>trans</i> -Cinnamic acid	82.32	0.9331	0.6634	0.4371	0.2275	0.0614	9.471	11.56
47	4-Methylcinnamic acid	93.47	1.2326	0.9257	0.6620	0.4262	0.2431	9.592	12.08
48	3-Phenyl- <i>n</i> -propionic acid	86.04	0.6634	0.4334	0.2207	0.0314	-0.1222	9.789	12.49
49	Indol-3-acetic acid	91.65	0.6706	0.4175	0.1914	0.0088	-0.1466	9.979	15.42
50	Indole-3-propionic acid	114.11	0.9424	0.6475	0.3916	0.1692	-0.0067	10.56	18.19
51	Indole-3-butylic acid	121.34	0.9235	0.8095	0.5294	0.2654	0.0789	10.61	18.44
52	Hippuric acid	96.15	-0.1884	-0.4359	-0.5376	-0.7361	-0.8124	10.86	19.31
Void volume (fructose)			1.683	1.671	1.659	1.657	1.675 (1.643)		

* Van der Waals volume calculated by Bondi's method⁹.

** Energy effect obtained from the observed log k' values.

*** Energy effect from ref. 9.

RESULTS AND DISCUSSION

The $\log k'$ values, measured in 20–40% acidic acetonitrile–water mixtures, are listed in Table I together with the Van der Waals volumes and the values of the energy effect. For each group of compounds there was a linear relationship between $\log k'$ and the Van der Waals volume, as seen in Fig. 1.

Chloro-substituted acids were retained more strongly than alkyl-substituted acids, as in the case of chloro- and alkylbenzenes¹⁰. *Ortho*-substituted alkylaromatic acids were retained less strongly than *meta*- and *para*-substituted acids, and *ortho*-substituted alkylphenols were strongly retained more than the *meta*- and *para*-derivatives on octadecyl-bonded silica gels¹⁰. As seen in Fig. 2, *ortho*-substituted benzoic acids (23 and 26) are retained less strongly than *meta*- and *para*-substituted benzoic acids (21, 22, 24 and 25), and *ortho*-substituted phenols (4–7) are retained more strongly than *meta*- and *para*-substituted phenols (8 and 9). In addition, the octadecyl-bonded vinyl alcohol copolymer gel had greater selectivity for *ortho*-substituted compounds than had octadecyl-bonded silica gels.

A possible explanation for the difference between alkyl-substituted phenols and aromatic acids is that there is hydrogen bonding between the hydroxy group of phenols and other entities in the system, but that this hydrogen bonding is sterically hindered by neighbouring methyl groups; in the case of aromatic acids, the carboxyl group is sterically unhindered.

In terms of $\log k'$ values, the correlation coefficient between the bonded co-

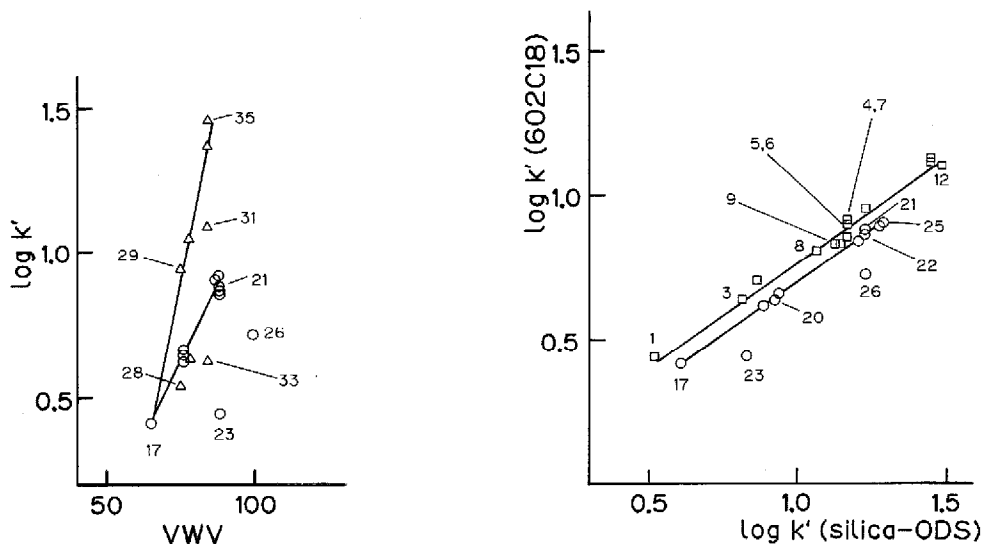


Fig. 1. Relationship between the Van der Waals (VWV) volumes and $\log k'$ (k' = capacity factor) values of benzoic acids on an octadecyl-bonded vinyl alcohol copolymer gel column, BHST602C18, packed in a 10 cm \times 6.0 mm I.D. stainless-steel tube. Eluent: 25% aqueous acetonitrile containing 0.05 *M* phosphoric acid. Column temperature: 30°C. Compound numbers as in Table I; \circ = alkylbenzoic acids; \triangle = halogenated benzoic acids.

Fig. 2. Comparison of the retention of alkylbenzoic acids and phenols on the octadecyl-bonded vinyl alcohol copolymer and silica gels. Experimental conditions as in Fig. 1; $\log k'$ values for phenols from ref. 9. \circ = Alkylbenzoic acids; \square = phenols.

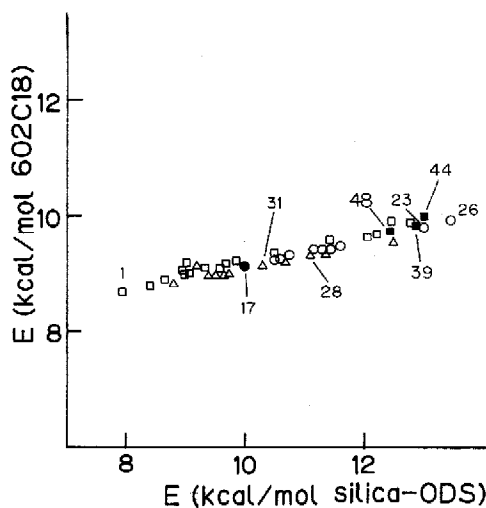


Fig. 3. Comparison of the energy effects of aromatic acids, measured on octadecyl-bonded vinyl alcohol copolymer and silica gels. Experimental conditions as in Fig. 1. \circ = Alkylbenzoic acids; \triangle = halogenated benzoic acids; \square = others.

polymer gel and the octadecyl-bonded silica gel was 0.489 for alkylphenols ($n = 10$), and 0.776 for alkylbenzoic acids ($n = 11$). These low values are also an indication of the higher selectivity of the bonded copolymer gel compared to the octadecyl-bonded silica gel.

The selectivity was examined further by studying the energy effect of aromatic acids. However, it was impossible to measure the capacity factors of alkanes in this system, because the retention times of alkanes are too long compared to those of the acids. The energy effect of aromatic acids was, therefore, calculated from the energy effect of phenols previously obtained⁷. The values are listed in Table I. The hydrogen-bonding energy effect of the carboxyl group of benzoic acid was about 2 units greater than that of a phenolic hydroxy group on an octadecyl-bonded silica gel⁹, and about the same as that of a phenolic hydroxy group on the octadecyl-bonded vinyl alcohol copolymer gel. The difference between the energy effects of benzoic and phenylacetic acids was about 2 units on an octadecyl-bonded silica gel⁹, but only 0.6 on the vinyl alcohol copolymer gel. There was no clear difference between the energy effects of phenylacetic, 3-phenylpropionic and 4-phenylbutyric acids (indicated by filled squares in Fig. 3), such as was found on an octadecyl-bonded silica gel. A similar result was obtained for indo-3-acetic, indole-3-propionic and indole-3-butyric acids.

There was a good linear relationship between the energy effects measured on both the octadecyl-bonded vinyl alcohol copolymer and on silica gel, as seen in Fig. 3. The correlation coefficient between the energy effects measured on these gels was 0.977 ($n = 52$, including phenols) or 0.972 ($n = 36$, only acids). The energy effect was large on a hydrophobic packing, small on a less hydrophobic packing like the octadecyl-bonded vinyl alcohol copolymer gel.

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