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# REVERSED-PHASE LIQUID CHROMATOGRAPHY ON RIGID VINYL ALCOHOL COPOLYMER GELS

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## SUMMARY

The selectivity and specificity of rigid vinyl alcohol copolymer gels in reversed-phase liquid chromatography were examined. The results were compared with those obtained on octadecyl-bonded silica gels. The selectivity for polyaromatic hydrocarbons was very weak compared with that of polystyrene gels but was stronger than that of octadecyl-bonded silica gels. The phenomenon was explained as the difference in the energy effects of the retention of some groups of compounds. The HETP of columns packed with the 9-μm gel was less than 0.02 mm.

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## 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 have been used in a variety of research applications. These packing materials 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. The separation time for amino acids in an amino acid analyser with ion-exchange resins is equivalent to that for an amino acid analyser with silica gel-based packings. Saccharides, organic acids and nucleic acids may be analysed on ion-exchange resins. Proteins are purified in columns packed with organic porous polymers.

One widely used organic porous polymer is polystyrene gel. The gel itself is an excellent packing for size-exclusion liquid chromatography, and modified ion-exchange resins are better for some chromatographic purposes than ion exchangers based on silica gel. However, such organic porous polymer gels have limitations, because the column efficiency is lower for compounds with relatively long retention times, owing to the poor pore size distribution and the strong selectivity due to the π-electrons of the aromatic rings[1].

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TABLE I

COMPOUNDS AND THEIR PHYSICAL PARAMETERS

Experimental conditions: column, GS-520M, 10 cm × 7.6 mm I.D.; eluent, aqueous acetonitrile solutions; column temperature, 35°C.

| *No.* | *Compound* | *VWV** | *log P*** | *Elution volume (ml)* | | |
|---|---|---|---|---|---|---|
| | | | | *Acetonitrile–water (55:45)* | *Acetonitrile–water (50:50)* | *Acetonitrile–water (45:55)* |
| 1 | Benzene | 48.36 | 2.280 | 3.925 | 4.380 | 5.075 |
| 2 | Naphthalene | 73.96 | 3.208 | 4.835 | 5.635 | 7.295 |
| 3 | Biphenyl | 90.08 | 3.772 | 4.695 | 5.565 | 7.575 |
| 4 | Fluorene | 93.22 | 3.906 | 5.630 | 6.975 | 9.885 |
| 5 | Phenanthrene | 99.56 | 4.378 | 6.775 | 8.815 | 12.97 |
| 6 | Anthracene | 99.56 | 4.378 | 6.945 | 9.065 | 13.79 |
| 7 | Pyrene | 109.04 | 5.030 | 9.075 | 12.57 | 20.02 |
| 8 | Benzanthracene | 125.16 | 5.545 | 10.42 | 15.64 | – |
| 9 | Chrysene | 125.16 | 5.545 | 10.88 | 16.12 | – |
| 10 | Benzopyrene | 134.64 | 6.200 | 15.72 | – | – |
| 11 | Picene | 150.76 | 6.960 | 18.70 | – | – |
| 12 | Toluene | 59.51 | 2.588 | 3.950 | 4.375 | 5.102 |
| 13 | Ethylbenzene | 69.74 | 3.118 | 3.865 | 4.355 | 5.190 |
| 14 | Propylbenzene | 79.97 | 3.648 | 3.870 | 4.395 | 5.435 |
| 15 | Butylbenzene | 90.20 | 4.178 | 3.905 | 4.480 | 5.750 |
| 16 | Hexylbenzene | 110.66 | 5.238 | 3.905 | 4.710 | 6.495 |
| 17 | Heptylbenzene | 120.89 | 5.768 | 3.975 | 4.845 | 6.970 |
| 18 | Octylbenzene | 131.12 | 6.297 | 4.022 | 4.980 | 7.530 |
| 19 | Nonylbenzene | 141.35 | 6.828 | 4.055 | 5.165 | 8.150 |
| 20 | Decylbenzene | 151.58 | 7.358 | 4.192 | 5.320 | 8.825 |
| 21 | Ethyl alcohol | 31.94 | −0.259 | 3.650 | 3.655 | 3.640 |
| 22 | Propyl alcohol | 42.17 | 0.271 | – | 3.640 | 3.655 |
| 23 | Butyl alcohol | 52.40 | 0.801 | 3.510 | 3.610 | 3.655 |
| 24 | Pentyl alcohol | 62.63 | 1.331 | 3.480 | 3.590 | 3.675 |
| 25 | Hexyl alcohol | 72.86 | 1.861 | 3.445 | 3.585 | 3.700 |
| 26 | Heptyl alcohol | 83.09 | 2.391 | 3.405 | 3.585 | 3.730 |
| 27 | Octyl alcohol | 93.32 | 2.921 | 3.375 | 3.555 | 3.767 |
| 28 | Decyl alcohol | 113.78 | 3.981 | 3.325 | 3.560 | 3.872 |
| 29 | Dodecyl alcohol | 134.24 | 5.041 | – | 3.640 | 4.156 |
| 30 | Pentane | 58.03 | 2.994 | 3.645 (tailing) | – | – |
| 31 | Hexane | 68.26 | 3.524 | 4.010 (tailing) | – | – |
| 32 | Heptane | 78.49 | 4.054 | – | – | – |
| 33 | Octane | 88.72 | 4.584 | – | – | – |
| 34 | Decane | 109.18 | 5.644 | – | – | – |
| 35 | Undecane | 119.41 | 6.174 | – | – | – |
| 36 | Dodecane | 129.64 | 6.704 | – | – | – |
| 37 | Fructose | | | 4.045 | 3.840 | 3.716 |

* Van der Waals volume (ml/mol), calculated by Bondi's method[5].

** Partition coefficient between octanol and water, calculated by Rekker's method[6]. The volume between UV and RI detectores was 197 μl.

Therefore, rigid vinyl alcohol copolymer gels, which are very polar and useful for the analysis of biological substances[2–4], were surface-modified with octadecyl groups and examined for selectivity and column efficiency. The results were compared with those obtained on octadecyl-bonded silica gels.

## EXPERIMENTAL

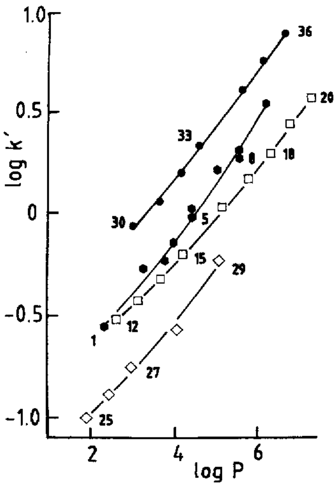
The chromatograph was assembled from several instruments: the degassers were an ERC Model 3310 and an ERC Model 3510 (ERMA Optical Works, Tokyo, Japan); the pumps were a Model Tri Rotar V (Jasco, Tokyo, Japan) and a Model 2150 (LKB Japan, Tokyo, Japan); the injector was a Reodyne Model 7125; the detectors were an ERC Model 7510 refractometer and a Shodex Model SE11 refractometer; the ultraviolet detectors were a Jasco Uvidec 100-VI and an ERC Model 8710; and the recorders were a Model CRA-1 (Shimadzu, Kyoto, Japan) and a SIC Model Chromatocorder 11 (Gasukuro Kogyo, Tokyo, Japan). An IBM PC-5501 computer was used for calculations.

Deionized water was further purified in a Pureline apparatus (Yamato Scientific, Tokyo, Japan). Acetonitrile was of HPLC grade (Kishida Kagaku, Tokyo, Japan). The chemicals were supplied mainly by Tokyo Chemical Industries (Tokyo, Japan) and are listed in Table I with their physical parameters.

The unmodified vinyl alcohol copolymer gel was GS-520M and the octadecyl-modified gels were BHST601C18 and BHST602C18 (Asahi Chemical, Kawasaki, Japan). Their particle size was 9 μm.

## RESULTS AND DISCUSSION

The elution volumes of the standard compounds were measured in 45–55% acetonitrile in water mixtures on an unmodified gel. The results are given in Table



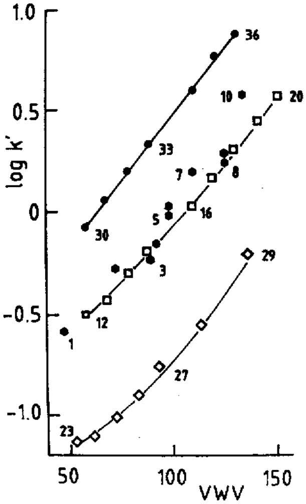


Fig. 1. Relationship between log P and log $k'$ values. Experimental conditions: column, BHST602C18, 10 cm × 6.0 mm I.D.; eluent, acetonitrile–water (80:20); column temperature, 35°C. Each symbol indicates a series of compounds. The numbers refer to those in Table I.

Fig. 2. Relationship between Van der Waals volumes and log $k'$ values. Experimental conditions as in Fig. 1.

TABLE II

COMPARISON OF HYDROPHOBICALLY MODIFIED POLYVINYL ALCOHOL GEL AND SILICA GEL

Experimental conditions: column, PV-ODS (601), 10 cm × 6.0 mm I.D.; eluent, aqueous acetonitrile solutions; column temperature, 35°C.

| *Compound** | *log k'* | | | | *Energy effect (cal/mol)* | *log k' on ODS (ref. 7)* | | *Energy effect (cal/mol) refs. 7 and 8)* |
|---|---|---|---|---|---|---|---|---|
| | *Acetonitrile–water (90:10)* | *Acetonitrile–water (80:20)* | *Acetonitrile–water (70:30)* | *Acetonitrile–water (60:40)* | | *Acetonitrile–water (90:10)* | *Acetonitrile–water (80:20)* | |
| 1 | −0.6960 | −0.4449 | −0.2432 | −0.0398 | 1.895 | −0.2112 | 0.0109 | 1.948 |
| 2 | −0.4583 | −0.1888 | −0.0524 | 0.3145 | 2.376 | −0.0108 | 0.2371 | 3.874 |
| 3 | −0.5104 | −0.1965 | −0.0729 | 0.3707 | 3.754 | 0.0591 | 0.3424 | 5.625 |
| 4 | −0.3617 | −0.0495 | 0.2178 | 0.5164 | 3.134 | 0.1376 | 0.4216 | 5.285 |
| 5 | −0.2033 | 0.0800 | 0.3440 | 0.6471 | 2.831 | 0.1960 | 0.4800 | 5.708 |
| 6 | −0.1787 | 0.1142 | 0.3838 | 0.6944 | 2.623 | 0.3186 | 0.6133 | 4.513 |
| 7 | 0.0097 | 0.3116 | 0.5860 | 0.8962 | 2.235 | 0.3767 | 0.6678 | 5.414 |
| 8 | 0.0794 | 0.3780 | 0.7096 | 1.038 | 3.013 | 0.4445 | 0.7677 | 7.413 |
| 9 | 0.0562 | 0.3966 | 0.6933 | 1.027 | 3.071 | — | — | — |
| 10 | 0.3463 | 0.6641 | 0.9852 | — | 1.814 | 0.6587 | 0.9918 | 6.226 |
| 11 | 0.4052 | 0.7545 | 1.108 | — | 1.221 | — | — | — |
| 12 | −0.6585 | −0.3609 | −0.1372 | 0.1068 | 2.320 | −0.0804 | 0.1536 | 2.322 |
| 13 | −0.5878 | −0.2934 | −0.0476 | 0.2034 | 2.700 | 0.0229 | 0.2808 | 2.755 |
| 14 | −0.6143 | −0.2124 | 0.0709 | 0.3518 | 2.958 | 0.1512 | 0.4325 | 2.900 |
| 15 | −0.4199 | −0.1065 | 0.1969 | 0.4973 | 3.130 | 0.2770 | 0.5844 | 3.061 |
| 16 | −0.2177 | 0.1332 | 0.4740 | 0.8207 | 3.334 | 0.5394 | 0.8971 | 3.312 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17 | −0.1067 | 0.2634 | 0.6267 | 0.9946 | 3.367 | 0.6758 | 1.060 | 3.365 |
| 18 | 0.0061 | 0.4007 | 0.7705 | 1.175 | 3.394 | 0.8132 | 1.223 | 3.489 |
| 19 | 0.1235 | 0.5408 | 0.9264 | – | 3.353 | – | – | – |
| 20 | 0.2408 | 0.6796 | 1.084 | – | 3.327 | 1.091 | – | – |
| 21 | −1.587 | −1.292 | −1.140 | −1.126 | 6.024 | – | – | – |
| 22 | −1.425 | −1.203 | −1.032 | −1.009 | 6.142 | – | – | – |
| 23 | −1.349 | −1.147 | −0.9498 | −0.8794 | 6.508 | −0.5852 | −0.5200 | 7.493 |
| 24 | −1.265 | −1.050 | −0.8412 | −0.7075 | 6.713 | −0.4855 | −0.3640 | 7.867 |
| 25 | −1.162 | −0.9399 | −0.7355 | −0.5551 | 6.889 | −0.3413 | −0.2210 | 8.174 |
| 26 | −1.044 | −0.8136 | −0.6216 | −0.4317 | 7.025 | −0.2060 | −0.0630 | 8.211 |
| 27 | −0.9248 | −0.6902 | −0.4868 | −0.2771 | 7.116 | −0.0729 | 0.0961 | 8.407 |
| 28 | −0.6704 | −0.4315 | −0.1859 | 0.0559 | 7.121 | 0.2000 | 0.4179 | 8.509 |
| 29 | −0.4280 | −0.1586 | 0.1006 | 0.4129 | 7.152 | 0.4852 | 0.7511 | 8.558 |
| 30 | −0.2669 | 0.0434 | 0.3050 | 0.5361 | 0 | 0.3070 | 0.5787 | 0 |
| 31 | −0.1723 | 0.1330 | 0.4330 | 0.7299 | 0 | 0.4483 | 0.7424 | 0 |
| 32 | −0.0564 | 0.3095 | 0.5712 | .9202 | 0 | 0.5876 | 0.9088 | 0 |
| 33 | 0.0503 | 0.4167 | 0.7474 | 1.115 | 0 | 0.7302 | 1.076 | 0 |
| 34 | 0.2728 | 0.7311 | 1.053 | – | 0 | 1.015 | 1.415 | 0 |
| 35 | 0.4041 | 0.8287 | 1.220 | – | 0 | – | – | 0 |
| 36 | 0.5248 | 0.9679 | – | – | 0 | 1.306 | – | 0 |
| 37** | 2.033 | 1.878 | 3.769 | 5.272 | | 1.455 | 1.430 | |
| 38*** | 1.967 | 1.905 | 1.875 | 1.867 | | | | |

* See Table I.

** Fructose (ml).

*** Water (ml).

I. The pore size of the gel was calculated from the exclusion limit of polyethylene glycol in gel filtration chromatography to be about 300 Å.

The elution volume did not increase linearly with increasing size of molecules. The retention of polyaromatic hydrocarbons was stronger on vinyl alcohol copolymers than on octadecyl-bonded silica gels. Infrared spectroscopy showed no trace of double bond compounds on the gel, suggesting that polyaromatic hydrocarbons may be trapped in water or acetonitrile adsorbed on the surface. Because this organic gel was less hydrophobic than octadecyl-bonded silica gels it seemed that it was rich in hydroxy groups on the surface. A size-exclusion effect was observed with aliphatic alcohols and alkylbenzenes in acetonitrile–water (55:45). Fructose, used for the measurement of the void volume of octadecyl-modified silica gel columns, could not be used in this system, because the elution volume of fructose was greater than that of several alcohols.

The capacity ratios of standard compounds were measured on the octadecyl-modified vinyl alcohol copolymer gels in acetonitrile–water mixtures. One example of a relationship between log capacity ratio ($k'$) and log $P$ values, measured on BHST602C18 packing, is shown in Fig. 1, and the relationship between log $k'$ and Van der Waals volume is shown in Fig. 2. The linearity between log $k'$ and log $P$ or Van der Waals volume was not good. The pore size of these modified gels was over 500 Å, calculated from the size-exclusion limit of polyethylene glycol.

Polyaromatic hydrocarbons were more retained than alkylbenzenes. Similar results were obtained with BHST601C18 packing. The capacity ratios of standard compounds are listed in Table I with their energy effect and the results obtained on octadecyl-bonded silica gel. The energy effect of alkylbenzenes, obtained on an octadecyl-bonded silica gel[7], was used as a standard, and the energy effect of the retention of these compounds on octadecyl-modified vinyl alcohol copolymer gels was calculated from their capacity ratios. The values are listed in Table II.

The log $k'$ values of polyaromatic hydrocarbons, alkylbenzenes and alcohols were all linearly related to their log $P$ values on octadecyl-bonded silica gels. However, this relationship did not apply for different groups of compounds on the octadecyl-modified vinyl alcohol copolymer gels. Polyaromatic hydrocarbons were

Fig. 3. Chromatogram of a mixture of alkylbenzenes. Experimental conditions: column, BHST601C18, 10 cm × 6.0 mm I.D.; eluent, acetonitrile–water (80:20); column temperature, ambient; flow-rate, 1 ml/min. Compounds: 1 = butylbenzene; 2 = octylbenzene; 3 = decylbenzene.

more retained than alkylbenzenes. The same results were observed when their capacity ratios were compared with their Van der Waals volumes (see Fig. 2).

The energy effect, obtained from the difference between the capacity ratios of *n*-alkanes and other compounds, clearly indicated the selectivity of the packings. When the energy effect of alkylbenzenes was used as the standard, the energy effect of polyaromatic hydrocarbons and alkyl alcohols obtained on octadecyl-modified vinyl alcohol copolymer gels was less than that obtained on an octadecyl-modified silica gel. The hydrogen-bonding energy effect of these alcohols was about 1 kcal less, and the $\pi$-energy effect of polyaromatic hydrocarbons was about half of that obtained on octadecyl-bonded silica gels. The hydrophobicity of the vinyl alcohol copolymer gels was weak; about 20% less acetonitrile was required to obtain similar elution volumes to those given in Table II. A chromatogram of a mixture of alkylbenzenes is shown in Fig. 3. The height equivalent to a theoretical plate (HETP) of the modified vinyl alcohol copolymer gels was less than 0.02 mm.

The hydrophobicity and the selectivity of octadecyl-modified vinyl alcohol copolymer gels may be useful in the analysis of biological samples such as those obtained from columns of unmodified vinyl alcohol gels[3,4].

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