

CHROMSYMP. 986

## EFFECT OF ENTHALPY ON RETENTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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### SUMMARY

Retention in reversed-phase liquid chromatography has been found to be related to the Van der Waals volume, pi-energy and hydrogen-bonding energy effects. However, higher-molecular-weight compounds were retained more strongly than expected. In order to investigate this effect more fully, the retention times of phenols were measured on an octadecyl-bonded silica gel in acidic acetonitrile–water mixtures at different temperatures. The enthalpies of phenols were then calculated from their  $\log k'$  values. The magnitude of the enthalpy effect increases with increasing molecular size, but the polarity of the molecule is the predominant factor in the enthalpy effect.

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### INTRODUCTION

Octadecyl-bonded silica gels are a most popular packing material in liquid chromatography. In particular, these packings are used in aqueous-phase liquid chromatography for separating a variety of polar and non-polar compounds, but the retention mechanism involved is not well understood. Retention times in reversed-phase liquid chromatography have been discussed in conjunction with physico-chemical parameters, namely the Van der Waals volume, pi-energy and hydrogen-bonding energy effects<sup>1–3</sup>. However, the predicted retention times of higher-molecular-weight compounds were less than the observed retention times. An increase in the number of methylene units in alkylbenzenes did not significantly affect the pi-energy effect on their retention, but the enthalpy effect increased dramatically<sup>4</sup>. This means that an hydrophobic compound can be adsorbed directly onto an octadecyl-bonded silica gel. The value of the enthalpy effect of a methylene unit in alkylbenzenes was calculated to be 500 cal/mol<sup>4</sup>. Enthalpy effects were also measured for the retention of phenols on an octadecyl-bonded silica gel in acidic acetonitrile–water mixtures.

### EXPERIMENTAL

The liquid chromatograph used consisted of an ERC Model 3510 degasser (ERMA Optical Works, Tokyo, Japan), a CCPD pump (Toyo Soda, Tokyo, Japan), a Model 7161 injector (Rheodyne, Cotati, CA, U.S.A.), a Model SE11 refractometer

TABLE I  
 PHYSICAL PARAMETERS AND THE LOGARITHMS OF THE CAPACITY FACTORS FOR PHENOLS  
 For experimental conditions see Fig. 1.

| Compound                     | $VWV^*$ | $\log k'$ at $1/T \cdot 10^{-5}$ | 309.6   | 314.5   | 319.5   | 324.7   | 330.0   | 335.6   | 341.3  | Energy**<br>effect<br>(kcal/<br>mol) | $-\Delta H$<br>(kcal/<br>mol) |
|------------------------------|---------|----------------------------------|---------|---------|---------|---------|---------|---------|--------|--------------------------------------|-------------------------------|
| 1 Phenol                     | 53.88   | -0.2752                          | -0.2591 | -0.2384 | -0.2059 | -0.1903 | -0.1617 | -0.1272 | 7.883  | 1.980                                |                               |
| 2 2-Methylphenol             | 65.03   | -0.1327                          | -0.1140 | -0.0892 | -0.0661 | -0.0424 | -0.0208 | 8.259   | 1.683  |                                      |                               |
| 3 4-Methylphenol             | 65.03   | -0.1718                          | -0.1538 | -0.1312 | -0.1054 | -0.0820 | -0.0627 | 8.704   | 1.910  |                                      |                               |
| 4 2,3-Dimethylphenol         | 76.18   | -0.0313                          | -0.0130 | 0.0125  | 0.0346  | 0.0581  | 0.0829  | 0.1130  | 9.116  | 1.926                                |                               |
| 5 2,4-Dimethylphenol         | 76.18   | -0.0204                          | 0.0058  | 0.0263  | 0.0456  | 0.0708  | 0.0933  | 0.1241  | —      | 1.872                                |                               |
| 6 2,5-Dimethylphenol         | 76.18   | -0.0191                          | 0.0003  | 0.0191  | 0.0489  | 0.0674  | 0.0922  | 0.1220  | 9.013  | 1.880                                |                               |
| 7 2,6-Dimethylphenol         | 76.18   | 0.0183                           | 0.0427  | 0.0624  | 0.0866  | 0.1078  | 0.1440  | 0.1607  | 8.524  | 1.941                                |                               |
| 8 3,4-Dimethylphenol         | 76.18   | -0.0868                          | -0.0681 | -0.0436 | -0.0176 | 0.0025  | 0.0265  | 0.0561  | 9.762  | 1.906                                |                               |
| 9 3,5-Dimethylphenol         | 76.18   | -0.0650                          | -0.0373 | -0.0172 | 0.0052  | 0.0260  | 0.0660  | 0.0820  | 9.489  | 1.889                                |                               |
| 10 2,3,5-Trimethylphenol     | 87.33   | 0.0842                           | 0.1035  | 0.1244  | 0.1547  | 0.1797  | 0.1968  | 0.2294  | 9.851  | 1.947                                |                               |
| 11 2,3,6-Trimethylphenol     | 87.33   | 0.1266                           | 0.1463  | 0.1693  | 0.1962  | 0.2171  | 0.2416  | 0.2721  | 9.360  | 1.940                                |                               |
| 12 2,4,6-Trimethylphenol     | 87.33   | 0.1422                           | 0.1606  | 0.1819  | 0.2113  | 0.2553  | 0.2525  | 0.2837  | 9.175  | 1.898                                |                               |
| 13 2,3,5,6-Tetramethylphenol | 98.48   | 0.2937                           | 0.3140  | 0.3356  | 0.3655  | 0.3888  | 0.4081  | 0.4404  | 10.126 | 1.960                                |                               |
| 14 2-Ethylphenol             | 75.26   | -0.0006                          | 0.0230  | 0.0462  | 0.0659  | 0.0900  | 0.1108  | 0.1444  | 9.192  | 1.877                                |                               |
| 15 3-Ethylphenol             | 75.26   | -0.0561                          | -0.0315 | -0.0087 | 0.0098  | 0.0341  | 0.0541  | 0.0866  | —      | 1.838                                |                               |
| 16 4-Ethylphenol             | 75.26   | -0.0553                          | -0.0281 | -0.0094 | 0.0152  | 0.0386  | 0.0620  | 0.0880  | 9.122  | 1.886                                |                               |
| 17 2-Chlorophenol            | 63.03   | -0.1191                          | -0.0990 | -0.0757 | -0.0559 | -0.0345 | -0.0080 | 0.0111  | 8.017  | 1.899                                |                               |
| 18 3-Chlorophenol            | 63.03   | -0.0915                          | -0.0686 | -0.0435 | -0.0183 | 0.0050  | 0.0315  | 0.0538  | 7.647  | 2.118                                |                               |
| 19 4-Chlorophenol            | 63.03   | -0.1035                          | -0.0853 | -0.0626 | -0.0391 | -0.0044 | 0.0145  | 0.0352  | 7.884  | 2.085                                |                               |
| 20 2,3-Dichlorophenol        | 72.51   | 0.0144                           | 0.0366  | 0.0610  | 0.0816  | 0.1045  | 0.1428  | 0.1512  | 8.225  | 2.061                                |                               |

|    |                           |        |         |         |         |        |        |        |        |       |       |
|----|---------------------------|--------|---------|---------|---------|--------|--------|--------|--------|-------|-------|
| 21 | 2,4-Dichlorophenol        | 72.51  | 0.0625  | 0.0851  | 0.1074  | 0.1455 | 0.1555 | 0.1807 | 0.2016 | 7.673 | 2.029 |
| 22 | 2,5-Dichlorophenol        | 72.51  | 0.0551  | 0.0725  | 0.0960  | 0.1208 | 0.1416 | 0.1699 | 0.1936 | 8.004 | 2.031 |
| 23 | 2,6-Dichlorophenol        | 72.51  | 0.0447  | 0.0643  | 0.0869  | 0.1101 | 0.1313 | 0.1683 | 0.1794 | 7.829 | 2.032 |
| 24 | 3,4-Dichlorophenol        | 72.51  | 0.0525  | 0.0715  | 0.0976  | 0.1220 | 0.1464 | 0.1748 | 0.1971 | 7.830 | 2.133 |
| 25 | 3,5-Dichlorophenol        | 72.51  | 0.1331  | 0.1586  | 0.1859  | 0.2093 | 0.2346 | 0.2717 | 0.2797 | 7.204 | 2.209 |
| 26 | 2,3,4-Trichlorophenol     | 81.99  | 0.1803  | 0.1993  | 0.2235  | 0.2494 | 0.2731 | 0.2992 | 0.3215 | 7.943 | 2.083 |
| 27 | 2,3,5-Trichlorophenol     | 81.99  | 0.3663  | 0.3932  | 0.4215  | 0.4477 | 0.4743 | 0.5077 | 0.5282 | 8.226 | 2.374 |
| 28 | 2,3,6-Trichlorophenol     | 81.99  | 0.1924  | 0.2125  | 0.2372  | 0.2613 | 0.2853 | 0.3129 | 0.3349 | 7.989 | 2.093 |
| 29 | 2,4,5-Trichlorophenol     | 81.99  | 0.2271  | 0.2474  | 0.2735  | 0.2993 | 0.3224 | 0.3505 | 0.3744 | 8.083 | 2.157 |
| 30 | 3,4,5-Trichlorophenol     | 81.99  | 0.2418  | 0.2663  | 0.2931  | 0.3207 | 0.3457 | 0.3816 | 0.3990 | 7.411 | 2.335 |
| 31 | 2,3,4,5-Tetrachlorophenol | 91.47  | 0.5336  | 0.5627  | 0.5929  | 0.6195 | 0.6481 | 0.6813 | 0.7024 | 7.489 | 2.470 |
| 32 | 2,3,5,6-Tetrachlorophenol | 91.47  | 0.3936  | 0.4156  | 0.4428  | 0.4682 | 0.4947 | 0.5233 | 0.5470 | 7.335 | 2.251 |
| 33 | Pentachlorophenol         | 100.95 | 0.5349  | 0.5626  | 0.5922  | 0.6205 | 0.6484 | 0.6776 | 0.7024 | 7.434 | 2.439 |
| 34 | 3-Bromophenol             | 66.48  | -0.0484 | -0.0303 | -0.0062 | 0.0190 | 0.0376 | 0.0679 | 0.0909 | 7.966 | 2.039 |
| 35 | 4-Bromophenol             | 66.48  | -0.0611 | -0.0434 | -0.0186 | 0.0050 | 0.0261 | 0.0541 | 0.0769 | 8.199 | 2.024 |
| 36 | 2,4-Dibromophenol         | 79.08  | 0.1478  | 0.1659  | 0.1901  | 0.2150 | 0.2357 | 0.2631 | 0.2854 | 7.854 | 2.021 |
|    | Void volume (ml)          |        | 1.488   | 1.485   | 1.481   | 1.470  | 1.463  | 1.443  | 1.432  |       |       |

\* Van der Waals volume calculated by Bondi's method<sup>5</sup>.

\*\* Energy effect from ref. 3.

(Shodex, Tokyo, Japan), an ERC Model 8710 ultraviolet detector (ERMA Optical Works) and a Model CR3A integrator (Shimadzu, Kyoto, Japan). An IBM PC-5510 computer was used for the calculations. Deionized water was purified further in a Model Pureline still (Yamato Sci. Co., Tokyo, Japan).

Acetonitrile was of high-performance liquid chromatography (HPLC) grade (Kishida Kagaku Co., Tokyo, Japan). The other reagents were mainly supplied by Tokyo Chem. Ind. Co. (Tokyo, Japan), and are listed in Table I with their physical parameters. The octadecyl-bonded silica gel column was an ERC 1000 (10 cm × 6 mm I.D.) from ERMA Optical Works, packed with Hypersil ODS, and it was thermostatted in a water-bath. The eluent was acetonitrile–0.05 *M* aqueous phosphoric acid (pH 2.016).

## RESULTS AND DISCUSSION

The capacity factors measured in 70% aqueous acetonitrile with 0.05 *M* phosphoric acid at different temperatures are listed in Table I. The isomer effect on the retention of alkylphenols is weak, but it is not negligible in the case of chlorophenols. Halogenated phenols were retained more strongly than alkylphenols, and halogenated benzenes more than alkylbenzenes. The relationship between the  $\log k'$  values, measured at 30°C, and the Van der Waals volumes is shown in Fig. 1.

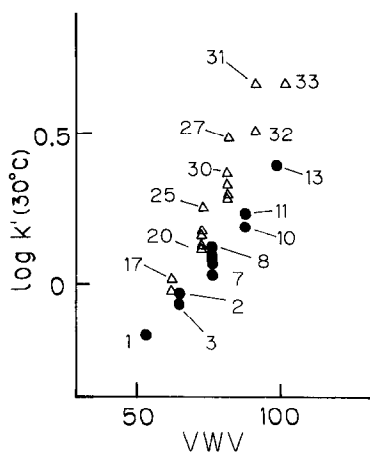


Fig. 1. Relationship between the Van der Waals volumes and  $\log k'$  for phenols in reversed-phase liquid chromatography. Column: ERC 1000, packed in a 10 cm × 6.0 mm I.D. stainless-steel tube. Eluent: 70% aqueous acetonitrile, containing 0.05 *M* phosphoric acid. Column temperature: 30°C. Numbers beside symbols are the same as in Table I. ●, Methylphenols; Δ, chlorophenols.

The enthalpy of a solute can be calculated from the following equations and the capacity factors measured at different temperatures:

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = -RT \ln K$$

$$k' = \phi K$$

Therefore,  $\ln k' = -\Delta H/RT + \Delta S/R + \ln \phi$ , where  $G$  is the free energy,  $H$  is the enthalpy,  $T$  is the absolute temperature,  $S$  is the entropy,  $R$  is the gas constant,  $K$  is the partition coefficient and  $k'$  is the capacity factor. The phase ratio,  $\ln \phi$ , is approximately zero in this system, therefore, this equation can be rewritten as:

$$\ln k' = -\Delta H/RT + \Delta S/R$$

The slope of a plot  $\log k'$  vs.  $1/T$  of gives the calculated enthalpy for each compound, which is listed in Table I. The correlation coefficient is 0.996 ( $n = 37$ ), the enthalpy effect of methylphenols being about 1.910 kcal/mol. No large difference in the magnitude of the effect was found among the isomers of this group, as shown in Fig. 2.

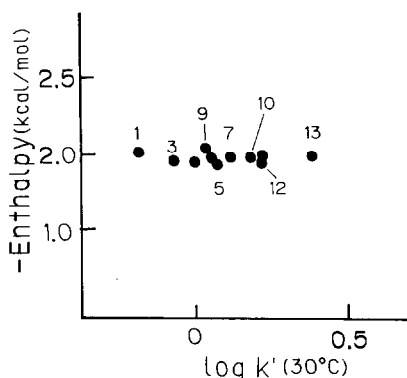


Fig. 2. Relationship between the enthalpy,  $-\Delta H$ , and  $\log k'$  values for methylphenols on an ERC 1000 column, eluted with acetonitrile-0.05  $M$  aqueous phosphoric acid. Numbers beside symbols are the same as in Table I.

The difference in the enthalpy effect between toluene and monomethyl-substituted phenols is about 200 cal/mol, and that between ethylbenzenes and monoethyl-substituted phenols is about 100 cal/mol. The energy effect for the retention of alkylphenols, however, varies from 7.883 kcal/mol for phenol to 10.126 kcal/mol for 2,3,5,6-tetramethylphenol. This means that the retention of alkylphenols is mainly due to pi- and hydrogen-bonding energy effects and not to the enthalpy effect.

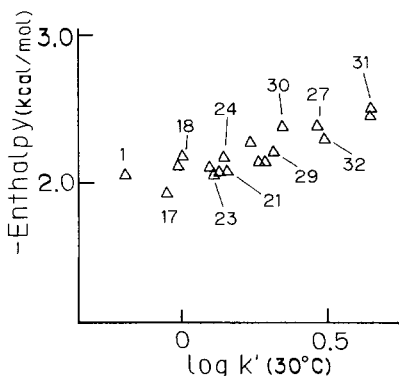


Fig. 3. Relationship between the enthalpy,  $-\Delta H$ , and  $\log k'$  values for chlorophenols. Details as in Fig.

The difference in the enthalpy effect between chlorobenzene and monochlorophenols is the same as in the case of alkylphenols, but the effect increases slightly for polychloro-substituted phenols. Differences among isomers were observed, as shown in Fig. 3.

The energy effect of *ortho*-substituted halogenated phenols is less than that of the corresponding *meta* and *para* compounds, but it is still greater than their enthalpy effect. This result indicates that the predominant factors in the retention of phenols in this system are the pi- and hydrogen-bonding energy effects, not the enthalpy effect. Thus, polar compounds may not be directly adsorbed onto the surface of an octadecyl-bonded silica gel under the above experimental conditions, and the retention of phenols can be described by the following equation

$$\log k' = \log k' (\text{VWV}) - \log k' (\text{pi}) - \log k' (\text{OH})$$

where VWV is the Van der Waals volume of the solute, pi is the pi-energy effect of the phenyl group in phenols and OH is the hydrogen-bonding energy effect of the hydroxy group in phenols<sup>6</sup>.

#### REFERENCES

- 1 T. Hanai and J. Hubert, *J. Chromatogr.*, 290 (1984) 197.
- 2 T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
- 3 T. Hanai and J. Hubert, *J. Chromatogr.*, 302 (1984) 89.
- 4 T. Hanai, A. Jukurogi and J. Hubert, *Chromatographia*, 19 (1984) 266.
- 5 A. Biondi, *J. Phys. Chem.*, 68 (1964) 441.
- 6 T. Hanai, *J. Chromatogr.*, 332 (1985) 189.