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EVALUATION OF HYDROPHOBICITY OF GELS BY USE OF SOME 1-ALKANOLS AS PILOT SOLUTES

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SUMMARY

1-Propanol, -butanol, -pentanol and -hexanol were employed as pilot solutes for evaluating the hydrophobicity of gels. By using both the thermodymamic functions on transfer of the solute from the mobile phase to the gel phase and the specific volume of the gel matrix, the hydrophobicity of gels was evaluated and was found not to be affected adversely by their porosity and particle size. The hydrophobicity of the gels examined increased in the order Bio-Gel P-2 < Sephadex G-15 ⩽ Sephadex G-10 < Toyopearl EW-35 < Sephadex LH-20.

INTRODUCTION

It is well known that many packing materials used in gel chromatography commonly exhibit hydrophobic properties to various extents[1,2]. In general, the hydrophobicity of gels increases with increasing concentration of the gel matrix[2]. The ether-type linkages of dextran gels were formerly regarded as being responsible for hydrophobic adsorption[3–5]. More recently, Janado and co-workers[6–9] ascribed the hydrophobic properties of highly cross-linked gels to the anomalous nature of cooperatively hydrated water in the gel particles. Haglund and Marsden[10] suggested that the cross-links may act cooperatively to orient favourably the non-polar faces of the gel matrices, resulting in the generation of non-polar domains.

The hydrophobic properties of gels have usually been discussed by comparing the elution volumes of a hydrophobic solute or its distribution coefficients on the gels examined. However, no quantitative evaluation of the hydrophobicity of gels has yet been made in which account is taken of both porosity and particle size. The aim of this work was to establish a method for evaluating a parameter representing the hydrophobicity of the gels.

Among hydrophobic solutes, the mechanism of the separation of alkanols on tightly cross-linked gels has been thoroughly studied by thermodynamic and extrathermodynamic approaches[9,11,12]. We have recently elucidated the mechanism of the separation of *n*-alkanols in aqueous dextran gel systems primarily in terms of a hydrophobic interaction, which was substantiated by linear correlations between the

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ΔS^0_{GC} values of the solutes and their ΔS^0_{HY} values, where ΔS^0_{GC} is the standard entropy change on transfer of the solute from the mobile phase outside the gel particles to the gel phase and ΔS^0_{HY} is the entropy of hydration[12].

As one of the important conclusions in that study, it was found that 1-alkanols containing more than two carbon atoms in the molecules behave in a very similar manner, indicating a dominant role of the hydrophobic interaction and a minor participation of hydroxyl groups in the mechanism of their separation. Therefore, 1-propanol, -butanol, -pentanol and -hexanol were employed as pilot solutes for examining the hydrophobic properties of several gels.

The ΔH^0_{GC} and ΔS^0_{GC} values of the 1-alkanols were obtained from the data of the temperature dependence of the K_{av} values on Bio-Gel P-2, Sephadex G-10 and LH-20 and Toyopearl EW-35. ΔH^0_{GC} is the standard enthalpy change on transfer of the solute as for ΔS^0_{GC}. $\Delta(\Delta H^0_{GC})/V_s$ and $\Delta(\Delta S^0_{GC})/V_s$ were employed as parameters for the hydrophobicity of the gels which are not affected adversely by porosity and particle size. $\Delta(\Delta H^0_{GC})$ and $\Delta(\Delta S^0_{GC})$ have usually been interpreted as the increments of ΔH^0_{GC} and ΔS^0_{GC}, respectively, due to the addition of a methylene group to the carbon chains of the alkanols. V_s is the ratio of volume of the gel matrix to that of the gel phase, *i.e.*, the specific volume of the gel matrix, as defined by eqn. 2 under Experimental. An enthalpy–entropy compensation test was also carried out to confirm that the solutes obey a common mechanism of separation on each gel examined.

EXPERIMENTAL

Sample solutions

All reagents used were of guaranteed reagent grade from Wako (Osaka, Japan), unless stated otherwise.

Sample solutions were prepared by dissolving 1-propanol (1-PrOH), 1-butanol (1-BuOH), 1-pentanol (1-PeOH) and 1-hexanol (1-HexOH) in the eluent. The sample concentration was $1 \cdot 10^{-2}$ *M*, except for 1-HexOH, for which a saturated solution was used because of its poor solubility.

Each sample solution contained Dextran T-2000 (Pharmacia, Uppsala, Sweden) at a concentration of 0.25 wt.-% as a standard material to determine the void volume of the columns. Tritiated water (New England Nuclear, Boston, MA, U.S.A.) was also used to determine the V_s values of the gels, except for Bio-Gel P-2.

Columns

Bio-Gel P-2 (Bio-Rad Labs., Richmond, CA, U.S.A.; wet particle size 75–150 μm), Sephadex LH-20 (Pharmacia; dry particle size 40–120 μm) or Toyopearl EW-35 (Toyo Soda, Tokyo, Japan; 30–60 μm)* was packed into a column (Pharmacia, K16/100) as described in a previous paper[13].

Eluent

The eluent was 0.01 *M* sodium chloride solution. On Bio-Gel P-2 the pH of the eluent was adjusted to 7 with phosphate buffer, because the apparent internal liquid volume of the gel column obtained by using Blue Dextran 2000 and tritiated water varied considerably with the pH of the eluent[14]. The concentration of the buffer was kept at $5 \cdot 10^{-4}$ *M*.

* This gel was kindly offered by the manufacturer and is not commercially available.

Elution

Elution was performed at 10, 15, 20, 25 and 30 ± 0.05°C, as reported previously in detail[12]. The elution volumes of the sample and Dextran T-2000 were determined with a Model R-403 differential refractometer (Waters Assoc., Milford, MA, U.S.A.). The activities of triated water of the effluent collected in each fraction (*ca.* 1 cm^3) were measured with a Model 2660 liquid scintillation spectrometer (Packard, Downers Grove, IL, U.S.A.).

Calculation of the parameters for evaluating the hydrophobicity of gels

The K_{av} value was obtained from the equation[1]

$$K_{av} = (V_e - V_0)/(V_T - V_0) \tag{1}$$

where V_e is the elution volume of the sample and V_T and V_0 are the total bed volume and the void volume of the gel column, respectively. The K_{av} values of the 1-alkanols on Bio-Gel P-2, Sephadex LH-20 and Toyopearl EW-35 are summarized in Table I. Those on Sephadex G-10 were determined by using their K_d values obtained previously[12] and the coefficients F_g (defined by eqn. 5), for transforming K_d into K_{av} values on Sephadex G-10 at 10–35°C[15].

The ΔH^0_{GC} and ΔS^0_{GC} values were calculated by Van 't Hoff analysis of the temperature dependence of the K_{av} values, as described previously[12]. The $\Delta(\Delta H^0_{GC})$ or $\Delta(\Delta S^0_{GC})$ value was obtained from the slope of a linear plot of ΔH^0_{GC} or ΔS^0_{GC} *vs.* the number of carbon atoms in the 1-alkanol molecules. $\Delta(\Delta H^0_{GC})$ and $\Delta(\Delta S^0_{GC})$ on Sephadex G-15 were obtained by the same procedure using the ΔH^0_{GC} and ΔS^0_{GC} values calculated from the temperature dependence of the K_d values[12], because with Sephadex G-10 $\Delta(\Delta H^0_{GC})$ and $\Delta(\Delta S^0_{GC})$ evaluated from both the K_d and K_{av} values were in excellent agreement with each other.

V_s was evaluated from the equation

$$V_s = (V_T - V_t)/(V_T - V_0) \tag{2}$$

where V_t is the total liquid volume of the gel column. When tritiated water is used as a standard, the V_t value is presumably overestimated because of an isotopic exchange reaction of hydrogen atoms between the tritiated water and the hydroxyl, carboxyl, amino and imino groups of the gel matrices. Accordingly, eqn. 3 should be used instead of eqn. 2 for determining the true V_s value.

$$V_s = \frac{K_{d(THO)}(V_T - V_0) - (V_{THO} - V_0)}{K_{d(THO)}(V_T - V_0)} \tag{3}$$

where V_{THO} and $K_{d(THO)}$ are the elution volume and the K_d value, respectively, of tritiated water. The $K_{d(THO)}$ values of 1.091 and 1.075 reported by Marsden[16] were used on Sephadex G-10 and G-15, respectively. As the gel matrix of Sephadex LH-20 has negligibly small amounts of hydroxyl and carboxyl groups[17], $K_{d(THO)} = 1$ was assumed. The manufacturer reported only that the gel matrix of Toyopearl EW-35 consists of an ether-type polymer produced principally with a hydrophilic vinyl mono-

mer and that its exclusion limit, $(MW)_{EL}$, is $5 \cdot 10^3$ (ref. 18). A $K_{d(THO)}$ value of unity was also postulated, assuming that most of the hydrophilic sites of Toyopearl EW-35 are ether-type oxygens of the gel matrix as in Vinylon.

With Bio-Gel P-2 the $V_T - V_{THO}$ values had a variance of around zero, probably owing to the isotopic exchange reaction mentioned above. Therefore, the V_s value of Bio-Gel P-2 was obtained from the equation

$$V_s = \frac{V_T(1 - W_r/V_d) - V_0}{V_T - V_0} \quad (4)$$

where W_r is the water regain (1.5 g of water per gram of dry gel) and V_d the bed volume (3.8 cm^3 of swollen gel per gram of dry gel)[19].

RESULTS AND DISCUSSION

Enthalpy–entropy compensation test

The enthalpy–entropy compensation test based on a linear free-energy relationship has often been used to differentiate intrinsically similar physico-chemical processes from others[20]. This test for the chromatographic behaviour of *n*-alkanols on Sephadex G-10 and G-15 showed that 1-alkanols containing more than two carbon

TABLE I

K_{av} VALUES OF 1-PROPANOL, 1-BUTANOL, 1-PENTANOL AND 1-HEXANOL AT VARIOUS TEMPERATURES

Solute	*On Bio-Gel P-2**		*On Sephadex LH-20***		*On Toyopearl EW-35***	
	Temperature (°C)	K_{av}	*Temperature (°C)*	K_{av}	*Temperature (°C)*	K_{av}
1-Propanol	10.3	0.628	10.4_5	0.735	10.8	0.758
	15.4	0.646	16.2	0.768	15.6	0.792
	20.3	0.668	20.7	0.787	20.5	0.840
	25.8	0.692	25.9_5	0.830	25.5	0.875
	30.3	0.706	30.8	0.858	30.4_5	0.922
1-Butanol	10.7	0.654	10.5_5	0.808	10.7	0.983
	15.4	0.673	16.3	0.865	15.7	1.06
	20.3	0.701	20.6	0.908	20.5	1.15
	25.8	0.726	25.9_5	0.980	25.5	1.24
	30.3	0.744	30.1	1.02	30.3_5	1.34
1-Pentanol	10.2	0.687	10.5_5	0.924	10.7	1.40
	15.3	0.719	15.5	1.00	15.8	1.58
	20.3	0.750	20.6	1.10	20.3_5	1.76
	25.8	0.779	25.9	1.22	25.5	1.96
	30.3	0.798	30.1	1.31	30.3_5	2.15
1-Hexanol	10.2	0.740	10.5_5	1.09	10.7	2.18
	15.4	0.767	16.2	1.24	15.8	2.55
	20.3	0.804	20.6	1.39	20.6	2.94
	25.8	0.835	25.9	1.61	25.5	3.36
	30.4	0.864	30.1	1.79	30.4	3.86

* With 0.01 *M* HCl at pH 7.
** With 0.01 *M* NaCl.

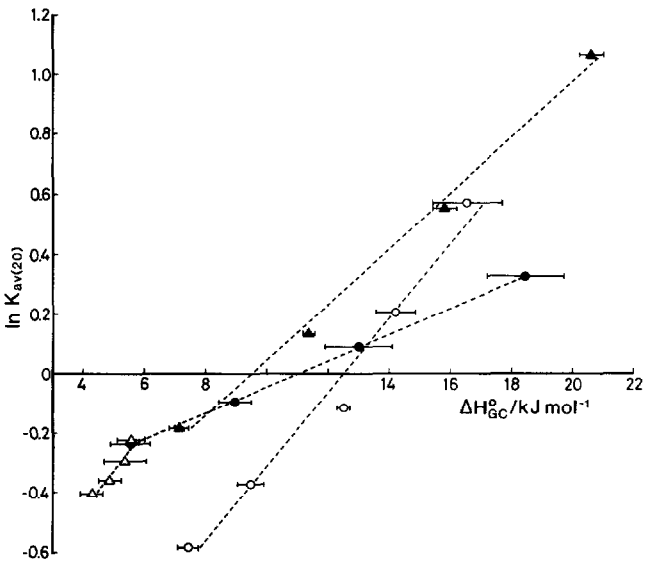

Fig. 1. Plots for enthalpy–entropy compensation test. ○, Sephadex G-10; △, Bio-Gel P-2; ●, Sephadex LH-20; ▲, Toyopearl EW-35. The horizontal lines through the symbols indicate the $P = 0.95$ confidence intervals of the thermodynamic functions.

atoms in the molecules obey common mechanisms of separation on the respective gels[12]. Therefore, the compensation behaviour of 1-PrOH, 1-BuOH, 1-PeOH and 1-HexOH on Bio-Gel P-2, Sephadex LH-20 and Toyopearl EW-35 was examined by the method of Krug *et al.*[21] in order to establish whether the separation of the alkanols on each gel proceeds in an intrinsically similar mechanism. Fig. 1 also includes a plot for the compensation test on Sephadex G-10 in addition to those on the gels stated above.

The linear regression analysis for the plots in Fig. 1 showed that the coefficients of determination were 0.918 with Bio-Gel P-2, 1.00 with Sephadex LH-20, 0.995 with Toyopearl EW-35 and 0.975 with Sephadex G-10, and that each straight line is significant at the $P = 0.05$ level. It was also found that the linear regression lines are distinguishable from one another at the $P = 0.1$ level.

In conclusion, the 1-alkanols employed as pilot solutes show an intrinsically similar mechanism of separation on each gel examined, but their mechanisms of separation differ considerably on the individual gels.

Evaluation of the hydrophobicity of gels

The chromatographic behaviour of *n*-alkanols on tightly cross-linked gels is governed primarily by a hydrophobic interaction, the degree of which is reflected well in the positive values of both ΔH^{0}_{GC} and ΔS^{0}_{GC}[9,11,12]. With 1-alkanols higher than ethanol, we found linear relationships between the ΔH^{0}_{GC} or ΔS^{0}_{GC} values and the number of carbon atoms in the molecules on Sephadex G-10 and G-15[12]. This indicates that the interaction between the terminal hydroxyl groups of the 1-alkanols and the gel matrices contributes to the separation mechanisms of the solutes presumably to the same and small extent on each gel. The slopes of the linear plots of

ΔH^0_{GC} and ΔS^0_{GC} against the number of carbon atoms, $\Delta(\Delta H^0_{GC})$ and $\Delta(\Delta S^0_{GC})$, have usually been interpreted as the increments in ΔH^0_{GC} and ΔS^0_{GC}, respectively, due to the addition of a methylene group to the carbon chains.

Fig. 2 shows plots of ΔH^0_{GC} and ΔS^0_{GC} *vs.* the number of carbon atoms in the 1-alkanol molecules on Bio-Gel P-2, Sephadex G-10, LH-20 and Toyopearl EW-35, which yielded excellent linear correlations with the individual gels. The $\Delta(\Delta H^0_{GC})$ and $\Delta(\Delta S^0_{GC})$ values, obtained from the slopes of the straight lines, are summarized in Table II, including those on Sephadex G-15, which were calculated from the K_d values at various temperatures[1,2] for the reason mentioned under Experimental.

Both of the $\Delta(\Delta H^0_{GC})$ and the $\Delta(\Delta S^0_{GC})$ values increase in the order Bio-Gel P-2 < Sephadex G-15 < Sephadex G-10 < Sephadex LH-20 < Toyopearl EW-35. This order, however, may not reflect that of the true hydrophobicity of the gel matrices, because both the porosity and the particle size differ among the gels. Accordingly, the $\Delta(\Delta H^0_{GC})/V_s$ and $\Delta(\Delta S^0_{GC})/V_s$ values were employed as parameters representing the true hydrophobicity of the gels in this work. As V_s is the proportion of the volume occupied by the gel skelton to that of the gel phase, $\Delta(\Delta H^0_{GC})/V_s$ and $\Delta(\Delta S^0_{GC})/V_s$ imply the increments in ΔH^0_{GC} and ΔS^0_{GC}, respectively, when each gel phase theoretically consists solely of the gel matrix.

In the last two columns in Table II are given the $\Delta(\Delta H^0_{GC})/V_s$ and $\Delta(\Delta S^0_{GC})/V_s$ values thus obtained on the five gels, which lead to a revised order of the hydrophobicity of the gels, especially with Sephadex LH-20 and Toyopearl EW-35. The difference in the hydrophobicity between Sephadex G-10 and G-15 is uncertain. Therefore, it is concluded that the hydrophobicity of the gels examined increases in the order Bio-Gel P-2 < Sephadex G-15 ⩽ Sephadex G-10 < Toyopearl EW-35 < Sephadex LH-20. Both the $\Delta(\Delta H^0_{GC})/V_s$ and $\Delta(\Delta S^0_{GC})/V_s$ values resulted in the same order of hydrophobicity because a linear relationship based on enthalpy–en-

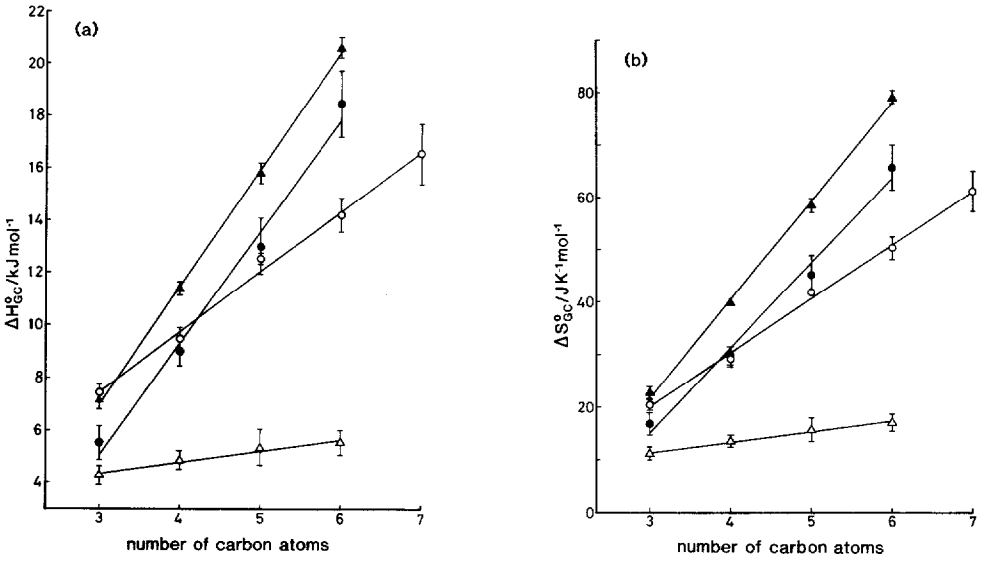

Fig. 2. Plots of ΔH^0_{GC} and ΔS^0_{GC} against number of carbon atoms. Symbols and the vertical lines through them as in Fig. 1.

TABLE II

PARAMETERS FOR EVALUATING THE HYDROPHOBICITY OF GELS

Temperature, 25°C.

Gel	$\Delta(\Delta H^0_{GC})$ (kJ mol^{-1})	r^{2*}	$\Delta(\Delta S^0_{GC})$ (J °K^{-1} mol^{-1})	r^{2*}	V_s	$\Delta(\Delta H^0_{GC})/V_s$ (kJ mol^{-1})	$\Delta(\Delta S^0_{GC})/V_s$ (J °K^{-1} mol^{-1})
Bio-Gel P-2	0.43	0.960	2.0	0.983	0.35_5	1.2	5.6
Sephadex G-10	2.29	0.994	10.2	0.997	0.425	5.4	24
Sephadex LH-20	4.28	0.989	16.2	0.989	0.265	16	61
Toyopearl EW-35	4.48	0.999	18.7	0.998	0.375	12	50
Sephadex G-15	1.70	0.989	7.1	0.990	0.34_6**	4.9	20

* r^2 = Coefficient of determination.
** Value at 20°C.

tropy compensation between ΔH^0_{GC} and ΔS^0_{GC} exists on each gel, as mentioned in the preceding section.

We have recently examined the temperature dependence of the coefficients, F_g, for transforming the K_d into K_{av} values on Bio-Gel P-2, Sephadex G-10, LH-20 and Toyopearl EW-35[15], where F_g is defined by the equation*

$$F_g = (V_{THO} - V_0)/(V_T - V_0) \quad (5)$$

The F_g values decreased linearly with increasing temperature on Sephadex G-10, LH-20 and Toyopearl EW-35, although those on Bio-Gel P-2 remained constant regardless of temperature. The temperature dependence of the F_g values increased in the order Bio-Gel P-2 < Sephadex G-10 < Toyopearl EW-35 ≈ Sephadex LH-20, which corresponds approximately to that of the hydrophobicity of the gels obtained in this work. This suggests that the temperature-dependent dehydration of the gels possibly gives an alternative measure of the hydrophobicity of gels, although it may be less accurate than the measure proposed in this paper.

The hydrophobicity of the gels obtained in this work is reasonable, taking account of the structures of the gel matrices. Bio-Gel P-2, with the smallest hydrophobicity, is a polyacrylamide gel cross-linked with N,N'-methylenebisacrylamide and its $(MW)_{EL}$ is 1800[19]. The hydrophilic sites of the gel are amino, imino, cabonyl and carboxy groups, the density in the gel matrix of which should be relatively high compared with the hydrophobic sites.

Sephadex G-10 and G-15 consist of dextrans chiefly with 1,6-α- and 1,3-α-glucoside bonds cross-linked with epichlorhydrin and their $(MW)_{EL}$ values are 700 and 1500, respectively[22]. The higher the degree of cross-linking, the larger will be the hydrophobicity, because the cross-links may promote non-polar domains, as suggested Haglund and Marsden[10]. Accordingly, the hydrophobicity of Sephadex G-10 may be larger than that of Sephadex G-15.

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* The K_d values in our studies have been calculated according to the equation $K_d = (V_e - V_0)/(V_{THO} - V_0)$. Therefore, an apparent F_g value, F'_g, was defined as in eqn. 5. The F_g value is represented by $F_g = (V_{THO} - V_0)/(V_T - V_0)K_{d(THO)}$ for the true K_d value.

Sephadex LH-20 is a hydroxypropyl derivative of Sephadex G-25 with an $(MW)_{EL}$ value of 4000[1], and Toyopearl EW-35 consists of a ether-type polymer produced principally with a hydrophilic vinyl monomer[18]. The hydrophilic sites of both gels are fundamentally ether-type oxygens, although other hydrophilic groups contained in raw materials may partly remain. This probably confess much higher hydrophobicity on these gels.

We propose the use of some 1-alkanols containing more than two carbon atoms in their molecules as pilot solutes for evaluating the hydrophobicity of gel packings, which is not affected by porosity and particle size. The ΔH^0_{GC} or ΔS^0_{GC} values of the 1-alkanols can be obtained from the temperature dependence of the K_{av} values, which are determined by refractometric detection. An approximate $\Delta(\Delta H^0_{GC})$ or $\Delta(\Delta S^0_{GC})$ value on a gel can be obtained even from the K_{av} values of two 1-alkanols at two different temperatures. In addition, the V_s value of the gel can easily be evaluated according to eqn. 3 or 4. When measuring the W_r and V_d values, if necessary, the gel is not necessarily dried completely, because the effect of incomplete drying is actually cancelled in the term W_r/V_d in eqn. 4.

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