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HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF SIMPLE COMPOUNDS ON ALKYL-AGAROSES WITH DIFFERENT ALKYL CHAIN LENGTHS AND CHAIN DENSITIES

MECHANISM AND THERMODYNAMICS

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SUMMARY

The retention of homologous *n*-alcohols in dilute phosphate buffer solution was determined on pentyl-, octyl- and dodecyl-agarose as a function of the degree of substitution of the alkyl-agaroses. The occurrence of two retention determining processes, *i.e.*, liquid–liquid distribution and bimolecular association, is revealed by the retention data. The standard thermodynamic functions for the interaction were calculated and are discussed.

INTRODUCTION

In previous papers we have demonstrated for the sorbent Octyl-Sepharose that both the sorption of *n*-alcohols[1] and the chromatographic retention of simple test substances with varying amounts of co-solvents in the eluent[2,3] can be described by a simple partition model. The agarose behaves as an inert support for the layer of octylglycidyl (OG) groups. The activity coefficients of the solutes in this layer are close to unity and they do not vary with the eluent composition. The OG layer has properties closely resembling those of liquid *n*-octanol. The chromatography of simple model compounds on Octyl-Sepharose can therefore be considered as a form of liquid–liquid chromatography (LLC), the retention being governed by an equilibrium much like that for a solute between two liquid phases. In this paper we refer to this mechanism as to LLP (liquid–liquid partition). Thus, the behaviour of the alkyl-agarose (at least, of the commercial product Octyl-Sepharose) is totally different from that of the alkyl-silicas used in reversed-phase high-performance liquid chromatography (HPLC).

At first sight, an LLP mechanism is unexpected as the degree of substitution, *P*, of Octyl-Sepharose is relatively low, *i.e.*, 0.40 mol OG per mol disaccharide[4]. However, if the multi-chain agarose fibres[5,6] remain intact during the synthesis of Octyl-Sepharose[7], the resulting high surface coverage[2] may produce a coherent,

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liquid-like film around the fibres. It is anticipated that the properties of such a layer depend on the chain length and the amount of alkyl groups per unit of fibre surface area (henceforth denoted as "chain density"), and that at very low chain densities the LLP mechanism breaks down. It is the aim of this paper to investigate whether such a dependence exists.

We used home-made pentyl-, octyl- and dodecyl-agaroses with widely varying chain densities as adsorbents and simple compounds as model sorbates. The chromatographic retention data obtained enabled us to calculate the relevant thermodynamic data. Other studies on the influence of the alkyl chain length and chain density[8–12] dealt mainly with protein sorption. The results of these studies are of course important for practical purposes, but are less suitable for interpretation in terms of hydrophobic interaction.

THEORETICAL

From basic chromatographic theory it follows that

$$V_g = K_B V_s \tag{1}$$

where V_g is the specific retention volume (ml g^{-1} of OG), K_B is the molar distribution constant of a test substance B and V_s is the volume of 1 g of OG. With the approximation[13,14] that the specific weights of OG and of other alkylglycidyl (AG) ethers are equal to to 1 g ml^{-1}, it follows that V_g is numerically equal to K_B. With the low P values used in this study, a correction of V_g for the interaction between B and unsubstituted agarose is necessary, yielding a corrected specific retention volume:

$$V_g^* = \frac{(V_e - V^0) - (V_a - V_a^0)}{w_{AG}^0} \tag{2}$$

Here, V_e and V^0 denote the elution volumes, corrected for the out-of-column dead volume, of B and a supposedly unretarded compound on a column containing $(1 + w_{AG}^0)$ g of alkyl-agarose with 1 g of agarose and w_{AG}^0 g of AG groups. V_a and V_a^0 denote the same data for a column containing 1 g of unsubstituted agarose. Note that it is assumed that the AG groups do not hinder the interaction of B with agarose and that eventual cooperative interactions between B, AG and agarose are not taken into account.

Values of K_B (or of V_g) at several temperatures can be used to calculate the partial molar standard thermodynamic data for the partition of B over the eluent and the AG layer. (The uncertainty in the temperature coefficient of the specific weight of this layer introduced errors of only minor importance.) In this paper, the same procedure[2] is applied to V_g^* values obtained on agarose with high P.

The LLP model predicts that the V_g^* values do not depend on P. However, it should be realized that models based on other mechanisms (adsorption, bimolecular association) also predict constant V_g^* values. Additional information about the sorption mechanism can be obtained from the value of the activity coefficient of B infinitely diluted in the layer of AG, $\gamma_{B,AG}^{\infty}$, which can be calculated from the mole

fraction partition constant, $K_{X,B}$, as described in ref. 3. The expression for $\log \gamma^{\infty}_{B,AG}$ is as follows:

$$\log \gamma^{\infty}_{B,AG} = \log \gamma^{\infty}_{B,aq} - \log K_{X,B} = \log \gamma^{\infty}_{B,aq} - \log 5.55 \cdot 10^{-2} M_{AG} V^{*}_{g} \quad (3)$$

Thus, values of $\log \gamma^{\infty}_{B,AG}$ can be calculated from V^{*}_{g}, the molecular weight of the AG group, M_{AG}, and the (literature) value of the activity coefficient of B in the aqueous eluent, $\gamma^{\infty}_{B,aq}$, which is about equal to that in water. The values obtained in this way can be compared with predictions from solubility theories if the bonded AG groups are considered as mobile molecules.

Constant V^{*}_{g} values at extremely low P values are interpreted in terms of bimolecular association. If we represent the complex formation as

$$AG + B, aq \leftrightarrows AGB$$

we can define:

$$K_{AS} = C_{AGB} C^{-1}_{AG} C^{-1}_{B,aq} \quad (4)$$

Here, $C_{B,aq}$, C_{AG} and C_{AGB} denote the molar concentrations of B, AG and the complex AGB, respectively, in the eluent. In this case, the capacity ratio is equal to the ratio of C_{AGB} and $C_{B,aq}$ and it follows from basic chromatographic theory and from eqn. 2 that:

$$\frac{C_{AGB}}{C_{B,aq}} = \frac{(V_e - V^0) - (V_a - V^0_a)}{V^0} = \frac{V^{*}_{g} w^0_{AG}}{V^0} \quad (5)$$

The standard free energy of association of 1 mol of solute is given by $\Delta G^0_{AS} = -RT \ln K_{AS}$ or

$$\Delta G^0_{AS} = -RT \ln \frac{V^{*}_{g} w^0_{AG}}{V^0 C_{AG}} = -RT \ln 10^{-3} M_{AG} V^{*}_{g} \quad (6)$$

Values of ΔH^0_{AS} and $\Delta C^0_{p,AS}$ can be obtained from a Van 't Hoff plot.

EXPERIMENTAL

Preparation and characterization of alkyl-agarose

Pentyl-, octyl- and dodecyl-agaroses were synthesized from one single batch of Sepharose CL-4B (Pharmacia, Uppsala, Sweden) according to ref. 7. Their alkyl concentration was determined by ^{1}H NMR spectroscopy (90 MHz)[8]. We added a small quantity of trifluoroacetic acid in order to shift the interfering water signal[11] to lower field.

Chromatographic experiments

Column experiments were performed as described before[2,3]. The 30-ml bed volumes contained *ca.* 1 g of dry alkyl-agarose. The sample dose was 0.1–0.5 mg

depending on the retention time. The gels with the lowest alkyl contents were packed in 60-ml beds, and the sample dose was reduced to 10 μg, the smallest dose possible with the differential refractive index detector. Low doses are necessary to avoid overloading of the column, as can be seen as follows. With $P = 0.010$, there are *ca.* 10^{-6} mol of AG groups per ml of gel bed. This value must be sufficiently large with respect to the solute concentration in the column. A 10-μg dose of *n*-nonanol corresponds to *ca.* $3 \cdot 10^{-8}$ mol per ml of gel bed. The fraction of octanol-bonded AG groups is thus less than 3%. So the concentration of free AG groups in eqn. 6 can be taken to be equal to the total concentration of AG groups, C_{AG}. The gel beds were slightly compressed prior to use, to avoid contraction during experiments. The test substances were eluted with a phosphate buffer (ionic strength 0.1, pH = 7.0). The elution peaks were symmetrical.

RESULTS

In Table I, P values are given for the alkyl-agaroses used; also listed are values of w^0_{AG}. The experimental error in the P values is estimated from duplicate determinations to be 4% or 0.004 for low values of P.

The dry specific weight and the hydrophobicity of the alkyl-agarose increase with increasing P. The latter fact makes the highly substituted gels less able to swell in water, *cf.*, ref. 8. Fig. 1 shows the bed volumes, V_{bed}, of $(1 + w^0_{AG})$ g of swollen alkyl-agarose as a function of w^0_{AG}. In the extreme case, the gel did not swell at all.

In Table II, the retention volumes, $(V_e - V^0)$, of some *n*-alcohols on various alkyl-agaroses (dry weights $1 + w^0_{AG}$ g) are listed. We estimate the precision of these values to be 0.08 ml or 0.8% for large values of $(V_e - V^0)$. The values of V^0 and

TABLE I

DEGREE OF SUBSTITUTION, P, AND MASS OF ALKYLGLYCIDYL GROUPS PER GRAM OF AGAROSE OF ALKYL-AGAROSES

*Gel**	P**	w^0_{AG}***	*Gel*	P	w^0_{AG}
a	0.000	0.000	O6	0.170	0.103
P1	0.050	0.024	O7	0.190	0.115
P2	0.095	0.045	O8	0.22	0.134
P3	0.115	0.054	O9	0.43	0.261
P4	0.212	0.100	D1	0.035	0.028
P5	0.275	0.129	D2	0.048	0.038
P6	0.47	0.221	D3	0.060	0.047
P7	0.71	0.334	D4	0.084	0.066
P8	0.74	0.348	D5	0.167	0.132
O1	0.010	0.006	D6	0.223	0.176
O2	0.025	0.015			
O3	0.060	0.036	Ph	0.40	0.243
O4	0.070	0.043			
O5	0.090	0.055			

* a = Unsubstituted agarose; P = pentyl-agarose; O = octyl-agarose; D = dodecyl-agarose and Ph = Octyl-Sepharose CL-4B.

** Moles of alkyl per mol of disaccharide.

*** Mass of alkylglycidyl groups (g per g of agarose).

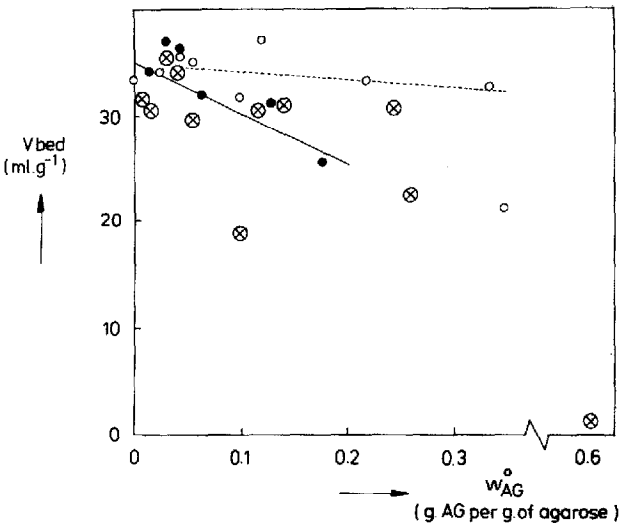


Fig. 1. Bed volumes (ml per g of agarose) of various alkyl-agaroses as a function of w^0_{AG} (g AG per g of agarose). Symbols: ○, ..., pentyl-agarose; ⊗, ——, octyl-agarose; ●, ——, dodecyl-agarose.

TABLE II

RETENTION VOLUMES, $(V_e - V^0)$ (ml/g OF AGAROSE) OF *n*-ALCOHOLS AT 25°C ON ALKYL-AGAROSES

*Gel**	*n***					*Benzene*
	5	*6*	*7*	*8*	*9*	
S		0.00	0.17	0.47	1.37	
P1			0.55	1.18	1.99	
P2		0.41	0.86	1.77	3.47	
P3	0.39	0.63	1.49	2.53	8.54	
P4	0.79	1.93	5.44	14.7	45.0	3.51
P5	1.19	3.76	11.9	36.3		
P6	3.76	11.5	38.1	119.7		22.4
P7	5.96	19.7	72.8	242.9		
P8	6.20	20.9	71.1	248.4		42.3
O1			0.42	0.85	1.80	
O2		0	0.74	1.51	3.62	
O3		0.73	1.83	4.57	12.43	
O4	0.33	0.77	1.54	3.40	7.75	
O5	1.08	3.00	9.04	27.7		
O6	2.16	6.80	22.2	74.4		
O7	2.35	7.58	25.8	86.0	274.8	
O8	2.91	9.75	34.3	104.1		
O9	6.31	22.2	75.4	240.9		
D1		1.73	4.45	11.9	30.4	2.64
D2		2.16	6.93	18.9	61.8	3.37
D3	1.35	4.52	14.0	41.1	133.1	6.79
D4	1.50	5.66	20.2	63.0		
D5	3.41	12.6	42.8	138.8		
D6	4.89	16.9	58.7	195.9		28.5

* See Table I.

** Number of carbon atoms in the solute molecule.

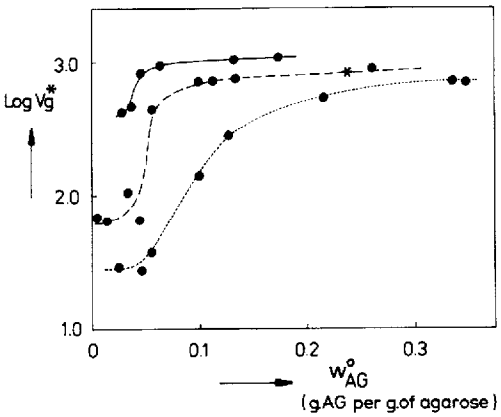


Fig. 2. Specific retention volumes of *n*-octanol as a function of w^0_{AG} (g AG per g of agarose). The asterisk denotes the Pharmacia gel. Curves: ....., pentyl-; ——, octyl-; and ——, dodecyl-agarose.

V^0_a were determined with ethanol and potassium bromide, and are in close agreement with the calculated amount of eluent in the column. For comparison, some values for a non-polar solute, *i.e.*, benzene, are also given.

Values of log V^*_g for *n*-octanol are plotted as a function of w^0_{AG} in Fig. 2. The correction for retention on agarose is significant only for the gels O1-4, P1-3.

Fig. 3 shows values of log $\gamma^\infty_{B,AG}$, calculated from the data in Tables I and II with eqn. 3, as a function of $P^{-\frac{1}{2}}$. Values of log $\gamma^\infty_{B,aq}$ in the eluent were obtained from a data compilation in ref. 15 as the logarithm of the reciprocal of the mole fraction solubility in water. Vertical bars denote the errors caused by the uncertainty in the retention volumes. Tilted bars show the impact of the uncertainty in P on both $P^{-\frac{1}{2}}$ and log $\gamma^\infty_{B,AG}$ values.

With some alkyl-agaroses, *i.e.*, P8, D6, O2, O3 and O5, and with unsubstituted

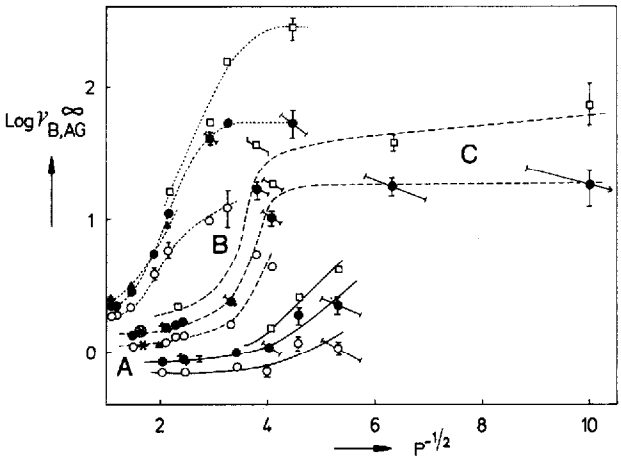


Fig. 3. Activity coefficients of *n*-hexanol (○), *n*-octanol (●), *n*-nonanol (□) and benzene (▲) in the sorbed state as a function of $P^{-\frac{1}{2}}$. Curves: ....., pentyl-; ——, octyl- and ——, dodecyl-agarose.

TABLE III

RETENTION VOLUMES, $(V_e - V^0)$ (ml/g OF AGAROSE) OF *n*-ALCOHOLS AT DIFFERENT TEMPERATURES ON ALKYL-AGAROSES

Gel	*T* (°C)	*n*				
		5	*6*	*7*	*8*	*9*
a	2.5			0.00	0.10	0.21
	11.5			0.02	0.29	0.80
	20.5			0.18	0.42	1.30
	32.0			0.22	0.59	1.39
	39.0			0.41	0.60	1.60
	45.0			0.50	0.82	1.51
P8	3.9	4.38	15.0	51.5	182	
	10.5	5.08	17.3	59.5	207	
	17.7	5.91	20.0	67.9	234	
	25.5	6.33	21.4	72.9	254	
	32.6	7.10	23.4	77.5	271	
	40.5	7.38	24.1	80.8	275	
O2	3.6				0.75	1.70
	12.5				0.86	2.73
	19.0				1.23	3.33
	27.0				1.61	3.92
	39.2				2.14	4.35
	47.0				2.46	4.66
O4	2.5			0.73	1.51	3.78
	13.6			1.11	2.18	5.56
	18.2			1.22	2.89	6.84
	27.6			1.61	3.23	8.23
	34.8			1.95	4.18	9.53
	43.2			1.84	4.09	9.81
D6	3.9	3.41	12.7	44.4	155	
	10.4	4.00	14.0	50.5	171	
	18.3	4.59	16.0	54.8	186	
	24.8	4.80	16.9	57.2	194	
	28.8	5.03	17.3	58.3	195	
	33.5	4.96	17.2	58.9	195	
	40.4	5.09	17.8	59.2	198	

agarose, measurements at different temperatures were performed. The values of $(V_e - V^0)$ and of $(V_a - V_a^0)$ are listed in Table III; V^0 and V_a^0 were determined with ethanol, and are independent of the temperature.

DISCUSSION AND CONCLUSIONS

Fig. 1 shows that the bed volume in water decreases gradually with increasing w_{AG}^0, *i.e.*, with increasing hydrophobicity of the gel. The decrease is stronger with octyl- and dodecyl-agarose than with pentyl-agarose. If it is assumed that the fibre

structure of the agarose remains intact during the synthesis of the alkyl derivative, then the surface area of 1 g of agarose is independent of *P*. Hence, $P^{-\frac{1}{2}}$ (Fig. 3) is a measure of the mean distance between the points of attachment of the AG groups on the agarose fibres. The use of this unit greatly improves the legibility of Fig. 3 at low P values.

Retention on unsubstituted Sepharose

It is seen in Tables II and III that *n*-alcohols are little retained on unsubstituted agarose. Nevertheless, the very low AG-group concentrations of some of our adsorbents give rise to retention volumes of comparable magnitude and in these cases the contribution of the interaction with agarose cannot be neglected. The interaction with agarose shows features of hydrophobic interaction: (i) the retention volumes increase with the number of carbon atoms, *n*, in the alcohols [Table II; in addition $n = 10$ yields $(V_a - V_a^0) = 3.0$ ml g^{-1}] and (ii) retention increases with temperature (Table III).

The methylene increment in the log $(V_a - V_a^0)$ values, *i.e.*, 0.40, is smaller than the corresponding increment for Octyl-Sepharose, *i.e.*, 0.60.

Retention on alkyl-agarose

Fig. 2 shows the log V_g^* values of *n*-octanol on alkyl-agarose. They depend strongly on the chain density of the AG groups. At large values of w_{AG}^0 (for dodecyl-, octyl- and pentyl-agarose: w_{AG}^0 greater than, say, 0.05, 0.10 and 0.25, respectively), log V_g^* is approximately constant and the retention is proportional to the mass of the AG groups in the column. This is in agreement with, but not a proof of an LLP mechanism.

LLP mechanism. In Fig. 3, values of log $\gamma_{B,AG}^{\infty}$ in the LLP region appear in the lower left corner, at relatively short distances between the bonded alkyl chains, in the region denoted by A. It appears that the values of log $\gamma_{B,AG}^{\infty}$ range from -0.2 to 0.4, which corresponds to $0.7 < \gamma_{B,AG}^{\infty} < 2$. Such values are commonly encountered for solutes in solvents that resemble the pure solute. If we consider the bonded AG groups as mobile molecules, these features can be compared with the predictions of

TABLE IV

COMPARISON OF CALCULATED AND EXPERIMENTAL ACTIVITY COEFFICIENTS IN THE LAYER OF ALKYLGLYCIDYL GROUPS AT HIGH *P* (REGION A IN FIG. 3)

Solute	$\bar{V}_B$ *(ml)*	δ_B*	log $\gamma_{B,AG}^{\infty}$					
			Adsorbent P8		*Adsorbent O9*		*Adsorbent D6*	
			*Calc.***	*Exptl.*	*Calc.*	*Exptl.*	*Calc.*	*Exptl.*
n-Hexanol	126	21.7	0.16	0.26	0.00	0.05	−0.10	−0.16
n-Octanol	157	20.9	0.36	0.34	0.11	0.14	−0.05	−0.08
Benzene	89	18.8	0.45	0.41			−0.08	+0.04

* $\delta_B^2 = (\Delta H_{vap}^0 - RT)/\bar{V}_B$; ΔH_{vap}^0 from ref. 17.

** $\log \gamma_{B,AG}^{\infty} = \log \frac{\bar{V}_B}{\bar{V}_{AG}} + \frac{1}{2.30}\left(1 - \frac{\bar{V}_B}{\bar{V}_{AG}}\right) + \frac{\bar{V}_B}{2.30\,RT}(\delta_B - \delta_{AG})^2$, from ref. 16. $\delta_{AG} = 22$, 23 and 24.5 for dodecyl-, octyl- and pentylglycidyl groups, respectively.

solubility theories. We used the Flory–Huggins–Hildebrand–Scott equation, already successfully adopted before by Tewari *et al.*[16] and by the present authors[1]. Values for the molar volumes, $\bar{V}$, of the AG groups were calculated as the ratio of the molecular weights and the densities of the corresponding alkylglycidyl ethers[14]. The best results were obtained with adopted values of the solubility parameter for the AG groups, δ_{AG}, equal to 22, 23 and 24.5 $J^{\frac{1}{2}}$ $mol^{-\frac{1}{2}}$ m for dodecyl-, octyl- and pentyl-agarose, respectively. These values correspond to slightly more polar liquids than the related *n*-alcohols with $n = 12$, 8 and 5, respectively. Results for the solutes *n*-hexanol, *n*-octanol and benzene are given in Table IV. It is seen that the agreement between calculated and experimental values of log $\gamma^{\infty}_{B,AG}$ is good. The mean deviation is 0.06. We conclude that the retention of simple compounds can be described with the LLP model in region A.

This conclusion enabled us to calculate the values of the thermodynamic standard data of LLP for *n*-alcohols over the aqueous eluent and the layer of AG. This was performed for the adsorbents P8, D6 and Octyl-Sepharose CL-4B (ref. 13). Standard free energy values were calculated as $\Delta G^0_{AG} = -RT \ln K_B = -RT \ln V^*_g$ from data in Table III. Results for ΔG^0_{AG} and ΔH^0_{AG} at 25°C, obtained with Van 't Hoff plots, are presented in Fig. 4 as a function of *n*. They will be discussed together with the thermodynamic data for bimolecular association.

Bimolecular association. The increase in log $\gamma^{\infty}_{B,AG}$ with increasing $P^{-\frac{1}{2}}$ in region B in Fig. 3 reflects a decreasing cooperation between the AG chains interacting with a solute. In region C in Fig. 3, log γ again becomes approximately constant. Here, the AG groups may be so far apart that the solute can interact with only one bonded alkyl chain.

Thermodynamic standard data for bimolecular association were calculated

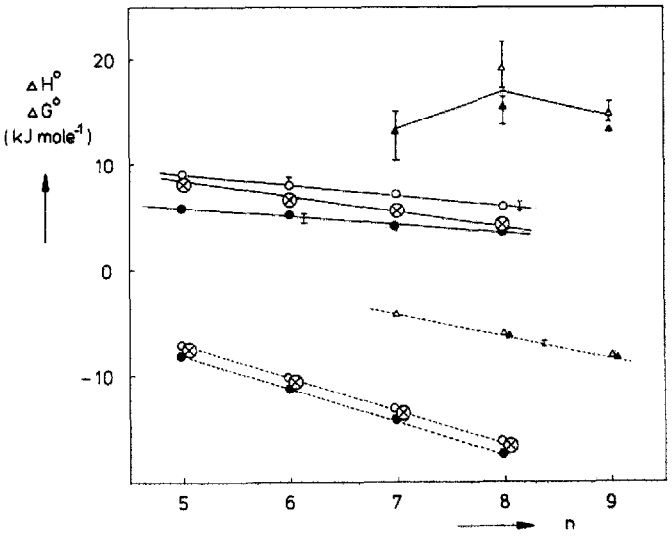


Fig. 4. Standard free energies and enthalpies for the interaction of *n*-alcohols with alkyl-agarose as a function of the number of carbon atoms, *n*, of the *n*-alcohol. Symbols: ○, P8; ⊗, Octyl-Sepharose CL-4B (ref. 13); △, O2; ▲, O4 and ●, D6. The full lines refer to ΔH^0 values, dotted lines to ΔG^0 values. Models: partition (○, ⊗, ●); bimolecular association (△, ▲).

from the data for absorbents O2 and O4 in Table III, with eqn. 5 and a Van 't Hoff plot. The correction for interaction with agarose was performed with values obtained from a least-squares parabola through the data points (Table III) for Sepharose as a function of temperature. Values of ΔG^0_{As} and ΔH^0_{As} are shown in Fig. 4.

The free energy values for adsorbents O2 and O4 are identical within 0.2 kJ mol^{-1}, which is consistent with identical retention mechanisms. The same conclusion holds for the densely substituted gels P8, Octyl-Sepharose and D6. The methylene increment of the ΔG^0_{As} values from $n = 7$ to $n = 9$ is -2.0 ± 0.2 kJ mol^{-1}, and is thus considerably less negative than the corresponding value for LLP, *i.e.*, -3.2 kJ mol^{-1}. Such a difference between the methylene increments for bimolecular association on the one hand and for multimolecular association or partition on the other hand is in agreement with data in the literature. For multimolecular associations, values close to -3.3 kJ mol^{-1} are the rule[2], whilst for bimolecular association between alkyl chains the following values have been found: -1.5 kJ mol^{-1} for the association of alkyltrimethylammonium ions with alkyl carboxylates[18]; -1.3 kJ mol^{-1} for the dimerization of carboxylic acids in water[19]; -2.8 kJ mol^{-1} for the association of alkyl sulphate and S-alkylisothiuronium ions[20].

To the best of our knowledge, no information exists about the free energies of bimolecular association of *n*-alcohols with neutral alkyl chains. However, we may compare our results with those for the association of cyclohexanol and cyclohexane[21,22] in water. The former compound is approximately as lipophilic as *n*-pentanol. With our standard state, ΔG^0_{As} is equal to 0.2 kJ mol^{-1}. Extrapolation of our ΔG^0_{As} values to $n = 5$ (Fig. 4) yields a similar value.

The enthalpy values for adsorbents O2 and O4 do not differ significantly. They are strongly endothermic, as found before[19,22,23] for the association of non-polar (parts of) molecules in water. The value for the bimolecular association of cyclohexanol with cyclohexane in water[21,22] has the same magnitude, *i.e.*, 14.4 kJ mol^{-1}. The process of bimolecular association is in our case much more endothermic than LLP, where negative methylene increments of ΔH^0 have been found (*cf.* Fig. 4, refs. 2, 13 and references therein). In the case of *n*-octanol we find a difference of *ca.* 13 kJ mol^{-1} between ΔH^0 for bimolecular association and that for partition. The dimerization of benzene and the interaction of cyclohexane with cyclohexanol are also found to be more endothermic than related partition processes, *i.e.*, by 19 and 15 kJ mol^{-1}, respectively[21].

The heat capacities for bimolecular association (not shown) were arbitrarily set equal to zero for the lowest member of the series. Results for *n*-nonanol are -0.3 and -0.4 kJ mol^{-1} K^{-1}, and for *n*-octanol, -0.5 kJ mol^{-1} K^{-1}. These strongly negative values are in agreement with literature data[22,23]. For LLP, the heat capacity change is about -0.3 kJ mol^{-1}, as found before[2,13]. Thus, also in our case, it holds that the interaction between *n*-alcohols and single OG groups becomes exothermic at temperatures near 60–70°C, exactly as found previously[22] (if ΔC^0_p is assumed to be constant up to this temperature).

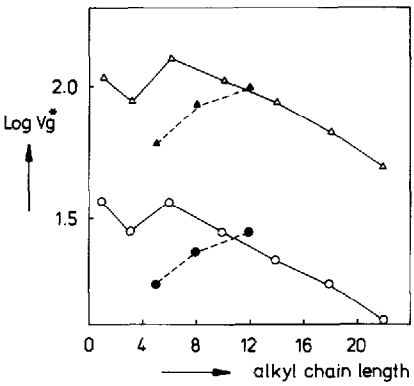
We conclude that the thermodynamics of the interaction of *n*-alcohols with octylglycidyl groups at low values of P show all the features of a bimolecular association of hydrophobic, mobile moieties in water. The strength of this strongly endothermic interaction is much lower than of cooperative interactions between several OG chains and the solute.

Mixed mechanisms govern the retention in region B in Fig. 3, where log $\gamma^{\infty}_{B,AG}$ strongly depends on $P^{-\frac{1}{2}}$ and on the length of the bonded chains. The latter dependence is much stronger than can be accounted for with the simple model of a homogeneous distribution of bonded chains on the surface of the agarose fibres. In that case, upon increasing the mean distance between the points of attachment by lowering P, water is expected to penetrate the layer of AG independently of the chain length, and the change from LLP to bimolecular association should take place at about the same values of P. This is clearly not observed in Fig. 3: the same increase in log $\gamma^{\infty}_{B,AG}$ for *n*-octanol of, say, 0.40 (with respect to values in region A) is brought about by taking $P^{-\frac{1}{2}} = 2$, 3.5 and 5 for pentyl-, octyl and dodecyl-agarose, respectively. Approximately the same ratio of $P^{-\frac{1}{2}}$ values —nearly equal to the corresponding ratio of the lengths of the alkyl chains— is found for other solutes. Apparently, upon increasing $P^{-\frac{1}{2}}$, the layer of AG breaks up into clusters of a few mutually interacting chains and cooperative interaction with a solute is much stronger than bimolecular association. This is especially so for dodecyl-agarose: at $P^{-\frac{1}{2}} = 3$ an LLP model is still valid, whereas for pentyl-agarose a bimolecular association model would be more approximate. It is caused by the much stronger association of the longer dodecyl chains.

Comparison with alkyl-silicas

Alkyl-silicas are frequently used in reversed-phase HPLC. Several studies on the influence of the chain length and chain density of the bonded alkyl groups have revealed a complex retention mechanism (see, *e.g.*, refs. 25–28 and references therein).

Berendsen and De Galan[26] studied the effect of the chain length at maximum surface coverage. We calculated log V_g^* values from their capacity ratios for *n*-propanol and *n*-butanol with water as the eluent using the characteristics of their bonded phases described in ref. 25. The results are plotted as a function of the bonded chain length in Fig. 5. Also shown are our log V_g^* values for *n*-pentanol and *n*-hexanol in



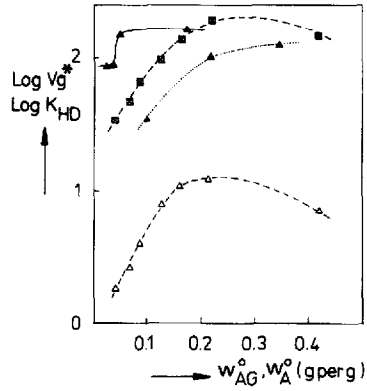


Fig. 5. Comparison of alkyl-agaroses and alkyl-silicas with different chain lengths. Symbols: ●—●, *n*-pentanol; ▲—▲, *n*-hexanol; both on alkyl-agarose in the partition region; ○—○, *n*-propanol; △—△, *n*-butanol; both on alkyl-silicas with maximum surface coverage and water as the eluent[26].

Fig. 6. Comparison of alkyl-agaroses and alkyl-silica with different chain densities. Symbols: ▲, benzene on pentyl-agarose (.....) and on dodecyl-agarose (——), with water as eluent; △, benzene; ⊠, pyrene; both on octadecyl-agarose, with methanol–water (70:30, v/v) as eluent[27,28].

the partition region. First, there exists a large discrepancy between the log V_g^* values for, *e.g.*, *n*-butanol on both types of sorbents. (This follows from extrapolation of our data to $n = 4$.) We observed and discussed this in previous work[1]. Secondly, it is seen in Fig. 5 that extension of the bonded alkyl chains has the opposite effect on the log V_g^* values. Recalculated log V_g^* values for all the other model substances (also for methanol–water eluents) in ref. 26 also decrease with increasing chain length of the bonded alkyl groups. According to Berendsen and De Galan, a solute molecule interacts only with the outer parts of the alkyl chains, keeping its most polar part (if present) in the eluent[26]. They named this mechanism "compulsory absorption". Clearly, this mechanism is not operative on alkyl-agarose, where steric factors favouring such a mechanism are absent.

Hennion *et al.*[27] investigated octadecyl-silicas of lower surface coverage, using methanol–water (70:30) as eluent. In such an eluent, silanophilic interactions can contribute to the retention, especially at low surface coverages. Nikolov[28] eliminated these contributions from the data of Hennion *et al.* in his "partial partition coefficient", K_{HD}. Values of log K_{HD}, which are comparable with our values of log V_g^*, are plotted in Fig. 6 as a function of the weight of the octadecyl chains per g of silica. The solutes are benzene and pyrene (other solutes give the same picture). For comparison, our data for benzene are shown.

It is seen that the influence of the chain density at w^0 values less than 0.25 is qualitatively the same on pentyl-agarose in water and on octadecyl-silica in 70% methanol. We explain this as follows. In the 70% methanol eluent, the association between the octadecyl chains that occurs in water is virtually absent[28]: the chains are solvated. This is also the case with pentylglycidyl chains in water (except at very high w^0); these short chains mutually interact weakly and are solvated by water. Hence, the solute retention on pentyl-agarose and on octadecyl-silica is governed by comparable mechanisms in this region; *i.e.*, the mixed mechanism discussed before. This is in contrast to the interaction with dodecyl-agarose in water. Although these alkyl groups are much shorter than octadecyl groups, the horizontal curve for this sorbent up to low alkyl densities clearly shows the cooperative interactions between the dodecyl groups. The difference in behaviour between dodecyl-agarose and octadecyl-silica is probably caused by the difference in solvating power of the two eluents for alkyl chains. At high values of w_A^0, steric effects[25,26,29] apparently cause a reduction in the retention on octadecyl-silica. The solute is (partially) squeezed out of the alkyl layer and a transformation to the "compulsory absorption" mechanism takes place. In this respect it is noteworthy that values of log V_g^* for benzene on alkyl-silicas with 50% surface coverage (corresponding to $w_A^0 = ca.\ 0.2$ for octadecyl-silica; see Fig. 6) in methanol–water eluents are linearly related to the alkyl chain length ($n = 6$–18) in the same way as on alkyl-agarose in the LLP region, contrary to the maximally substituted alkyl-silicas (calculated from data in ref. 27).

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