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INFLUENCE OF ADDITIVES TO THE ELUENT ON HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF SIMPLE COMPOUNDS

I. SORPTION ISOTHERMS OF *n*-ALCOHOLS ON OCTYL-AGAROSE

P. M. BRANDTS*, W. J. GELSEMA and C. L. DE LIGNY
Analytical Chemistry Laboratory, State University of Utrecht, Croesestraat 77A, 3522 AD Utrecht (The Netherlands)
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SUMMARY

Sorption isotherms of some *n*-alcohols on octyl-agarose were determined with a static batch method, based on a highly accurate concentration determination. The extent of sorption is compared to that on octyl-silica. Using solution theories and a partition model, the extent of sorption on octyl-agarose and on octyl-silica was predicted and compared to the experimental values. The mechanism of sorption is discussed.

INTRODUCTION

Retention of proteins and other biopolymers on hydrophobic gels can be influenced by adding other substances to the aqueous eluent. On a neutral adsorbent, adsorption is generally promoted especially by the so-called structure-making electrolytes. Desorption is brought about in a medium of low ionic strength, eventually containing organic substances such as glycol, urea or propanol. Many separations of complex mixtures have been performed in this way.

The influence of organic substances on the retention is probably caused by the modification of the properties both of the solvent and of the hydrophobic adsorbent, as a result of adsorption of the organic co-solvent. In this paper the adsorption isotherms of some *n*-alcohols, *viz.*, ,methanol, propanol, butanol and pentanol, on octyl-agarose are discussed. The influence of these and some other organic co-solvents and of electrolytes on the chromatographic retention of simple compounds will be discussed in forthcoming papers. The chromatographic retention of these compounds with an aqueous eluent was described previously[1].

THEORETICAL

Adsorption and partition

Sorption isotherms on octyl-silica are commonly interpreted in terms of mono-

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or multi-layer adsorption. With octyl-agarose, interpretation in terms of adsorption is difficult. In our previous paper[1] the nature of the octyl surface was discussed and found to be ill-defined. In addition, the agarose gels shrink in an alcohol-rich medium and this probably affects their surface areas.

A partition model may also be used to interpret sorption phenomena. In this approach the additive is considered to be extracted from water by an organic phase composed of octylglycidyl groups. The extent of agreement between experimental and predicted sorption data enables the applicability of the model to be judged. In our previous paper such a model was found to be realistic for the chromatographic retention of simple compounds.

The sorbed amount of an additive can be determined from the partition model as follows. Consider a system comprised of a mixed aqueous solution and the sorbent octyl-agarose, containing w_{H_2O} grams of water and w_A grams of additive A. The sorbed amount of water is expected to be very small, for the following reasons. First, we found octylglycidyl ether to dissolve only 0.6 wt. % of water. Secondly, from solubility data[2,3], mixtures of two substances of different polarities, *e.g.*, alcohols and hydrocarbons, dissolve much less water than the pure constituents before mixing. Therefore, we suppose exclusive extraction of the additive. In that case, at equilibrium, the aqueous solution comprises w_{H_2O} grams of water and w'_A grams of A, the difference, $w_A - w'_A = W_{s,A}$, being sorbed. The experimentally accessible equilibrium concentration, C'_A, is given by:

$$C'_A = \frac{w'_A}{w'_A + w_{H_2O}} = \frac{w_A - W_{s,A}}{w_{H_2O} + w_A - W_{s,A}} \tag{1}$$

Rearrangement and division by the amount of the octylglycidyl groups, w_0, leads to the expression for the sorbed amount per gram octylglycidyl groups, $W^0_{s,A}$:

$$W^0_{s,A} = \frac{w_A - (w_{H_2O} + w_A)\, C'_A}{w_0\,(1 - C'_A)} \tag{2}$$

A similar result is obtained from an adsorption model, assuming exclusive adsorption of the additive[4]. In calculations on partition, the partition constant on a mole fraction basis is often used. It is given by

$$K_{X,A} = \frac{X_{A,or}}{X_{A,aq}} = \left(\frac{W^0_{s,A} M_A^{-1}}{W^0_{s,A} M_A^{-1} + M_0^{-1}}\right) X^{-1}_{A,aq} \tag{3}$$

where $X_{A,or}$ and $X_{A,aq}$ are the mole fractions of A in the organic and aqueous phase, respectively, and M_A and M_0 the molecular weights of the additive and the octylglycidyl groups, respectively. Note that attached groups are treated as free molecules. Also, it is tacitly assumed that the supporting matrix, *i.e.*, agarose, is inert.

Partition and chromatography

It is difficult to evaluate $K_{X,A}$ at low concentrations from batch experiments. However, if the chromatographic conditions are equal to those in a static batch

experiment, the limit of $K_{X,A}$, extrapolated to zero concentration, is related in a simple way to the chromatographically determined capacity ratio, k'_A, with pure water as the eluent. This is derived as follows. The capacity ratio is given by:

$$k'_A = \frac{n_{A,or}}{n_{A,aq}} = \frac{V_e - V^0}{V^0} \tag{4}$$

Here, $n_{A,or}$ and $n_{A,aq}$ are the numbers of moles of A in the organic and aqueous phase, respectively, in the column and V_e and V^0 are the elution volumes of A and a supposedly unretarded compound. $K^0_{X,A}$, the limit of $K_{X,A}$ at infinite dilution, is given by

$$K^0_{X,A} = \frac{X_{A,or}}{X_{A,aq}} = \frac{n_{A,or}}{n_{O,or}} \cdot \left(\frac{n_{A,aq}}{n_{H_2O,aq}}\right)^{-1} \tag{5}$$

where $n_{O,or}$ is the number of moles of octylglycidyl groups in the column and $n_{H_2O,aq}$ the number of moles of water in the column. It follows that:

$$K^0_{X,A} = k'_A \cdot \frac{n_{H_2O,aq}}{n_{O,or}} \tag{6}$$

EXPERIMENTAL

Determination of concentration of additive, C'_A

The calculation of $W^0_{s,A}$ using eqn. 2 requires the measurement of C'_A. The relatively large ratio of the mobile phase volume to the estimated volume of octylglycidyl groups, *i.e.*, about 150, strongly reduces the effect of sorption on C'_A and, as a consequence, a high-precision determination of the concentration had to be used. (Common methods such as column stripping or breakthrough could not be used.) We determined C'_A with a differential refractive index (RI) detector (R 403; Waters, Milford, MA, U.S.A.).

The method is based on the observed linearity of the detector signal with respect to the concentration difference, ΔC, of the solutes between its sample and reference channels. Both outlets were connected to a peristaltic pump (MINIPULS 2; Gilson, Villiers-le-Bel, France) and pulse dampeners. The detector was thermostatted by air at 20°C. Through the reference channel, a standard solution of concentration $C_{A,ref.}$ was drawn at a flow-rate of about 40 ml h^{-1}. The baseline signal, S^0, was obtained by passing the same standard through the sample channel. A measurement was performed by placing the sample channel inlet into a solution, equilibrated with the gel in a batch experiment (henceforth denoted as "sample solution"), or into a standard solution of a concentration about equal to the expected value of C'_A. This inlet was provided with a small filter to prevent ingress of gel particles. The detector signal, S, was recorded.

Calibration plots of $S - S^0$ *versus* ΔC were linear for ΔC up to $6 \cdot 10^{-3}$ g g^{-1}. The linearity was tested for values of $C_{A,ref.}$ ranging from 0 to 0.350 g g^{-1}.

Thus, for each sample, two standard solutions had to be prepared. C'_A was calculated as

$$C'_A = C'_{A,ref.} + \frac{S^s - S^0}{S^{cal} - S^0} \cdot \Delta C \tag{7}$$

where S^s denotes the signal of a sample solution and S^{cal} the signal of a standard solution with a concentration difference ΔC from $C_{A,ref.}$. If $S^{cal} - S^0$ covers the full scale of a recorder and if ΔC is about $4 \cdot 10^{-3}$ g g^{-1}, we estimate the uncertainty in C'_A to be smaller than $4 \cdot 10^{-5}$ g g^{-1}.

It should be noted that every care should be taken to avoid evaporation. Adsorption on glass, measured by transferring some reference channel standard into a glass flask, followed by measuring the concentration with respect to the same standard as described above, was negligibly small.

Chemicals

Methanol, propanol, butanol and pentanol (analyzed grade chemicals) were obtained from Baker (Deventer, The Netherlands). Sepharose and octyl-Sepharose were obtained from Pharmacia (Uppsala, Sweden). Twice distilled water was used.

Preparation of standard and sample solutions

Standard solutions were prepared by weight. Methanol and propanol were added as such, whereas butanol and pentanol were added as their nearly saturated stock solutions. These stock solutions were prepared by pre-extracting butanol and pentanol with water, dissolving an excess of each in 2 l of water, discarding the remaining organic phase and slightly diluting the saturated solution. The alcohol content was determined from measurements on a dilute solution according to the procedure described above. A diluted solution of the non-purified alcohol was employed as a reference. Uncertainties in the concentrations (with respect to the stock solutions) were smaller than 4×10^{-5}; this uncertainty determined the precision of $W^0_{s,A}$.

For the batch experiments, three quantities should be known with sufficiently high accuracy, *i.e.*, the amounts of alcohol, W_A, water, W_{H_2O}, and gel matrix, W_{GEL}. From the latter, W_0 can be calculated[1].

As dry octyl-Sepharose does not swell in water, the gel must be added in wet form. Addition as a slurry introduces an intolerable uncertainty in W_{H_2O}. We found a modification of the gel that is much easier to handle than a slurry. To prepare this hypo-humid gel ("hypo-gel"), octyl-Sepharose CL-4B was washed with water until the supernatant showed no RI signal with respect to water. It was then sucked dry on a glass funnel. A whitish solid was obtained, containing about fifteen times its dry weight of water. It was transferred to a wide bottle, crumbled and stirred until a white, homogeneous, snow-like powder was obtained. This hypo-gel is easily transferred and swells reversibly (showing no loss of retention when used in chromatography). As it slowly loses water, it must be stored in an air-tight vessel in a cool place and be well stirred before use. The weight of the hypo-gel was found to be proportional to the dry weight, W_{GEL}, within 0.1%.

Constituents were weighed into a 25-ml flask. About 7 g of hypo-gel were used

(less in nearly saturated solutions). The flasks were sealed and their contents equilibrated by occasional shaking during 2 days. Corrections for air buoyancy were not necessary.

Chromatographic technique

Chromatographic experiments, using pure water as an eluent, were performed as described before[1]. Void volumes, V^0, were determined with KBr and acetone.

RESULTS AND DISCUSSION

Control experiments using unsubstituted Sepharose CL-4B and methanol or propanol as additives showed C'_A to be increased somewhat, independent of the nature of the alcohol and approximately proportional to C'_A. We also noted an increase in weight of well dried Sepharose and octyl-Sepharose on standing in air, approximately proportional to their Sepharose content. This strongly points to the adsorption of water by the gels, a property also displayed by Sephadex gels[5]. In addition, sugars in solution also are hydrated [6]. As a consequence, the bulk amount of water in the flasks containing octyl-Sepharose had to be corrected for the amount of water adsorbed by the Sepharose. We assumed the Sepharose to adsorb as much water as unsubstituted Sepharose. This is reasonable as the degree of substitution is relatively low. With butanol or pentanol as additives, no change in C'_A was observed, perhaps because C'_A is small for these alcohols.

Sorption on Sepharose CL-4B

If we suppose exclusive adsorption of water, the amount of water adsorbed on 1 g of Sepharose, W^0_{s,H_2O}, may be calculated. At equilibrium, C'_A is given by:

$$C'_A = \frac{w_A}{w_A + (w_{H_2O} - w_{s,H_2O})} \tag{8}$$

Rearranging and dividing by the amount of adsorbent, $w_{SEP.}$, leads to:

$$W^0_{s,H_2O} = \frac{w_{H_2O} + w_A}{w_{SEP.}} - \frac{w_A}{C'_A w_{SEP.}} \tag{9}$$

A special case occurs if a constant amount of water is adsorbed. In this case, a plot of $y = w_{SEP.}^{-1}[C'_A(w_{H_2O} + w_A) - w_A]$ *versus* C'_A should be linear passing through the origin with the adsorbed amount equal to the slope of the line. Moreover, methanol and propanol must give the same result. Such a plot is presented in Fig. 1. It is seen that the results are well described by a straight line (calculated with the least-squares method), corresponding to the adsorption of 0.234 g water per g Sepharose. On a molar basis, 1 mol of disaccharide adsorbs 4.0 ± 0.3 mol of water, perhaps on both sides of the ring planes.

Sorption on octyl-Sepharose CL-4B

The $W^0_{s,A}$ values of methanol, propanol, butanol and pentanol on octyl-Sepharose CL-4B were calculated from eqn. 2. The amount of the octylglycidyl groups,

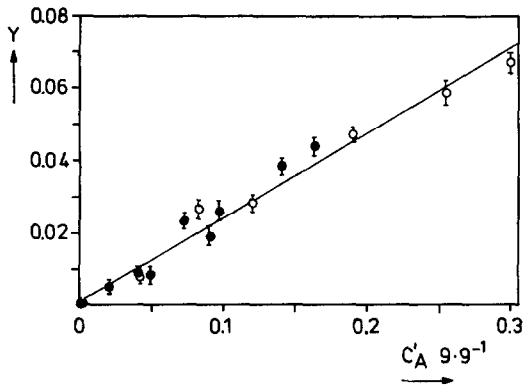

Fig. 1. Plot of $y = w_{SEP}^{-1}[C'_A(w_{H_2O} + w_A) - w_A]$ *versus* C'_A for control experiments with unsubstituted Sepharose. Slope: 0.234 ± 0.012. Additives: ○, methanol; ●, propanol.

w_0, was calculated from the known dry weight of the hypo-gel and its known degree of octyl substitution. w_{H_2O} was calculated from the amount of water added and hypo-gel and corrected for adsorption of water, assumed to be 4.0 mol per mol of disaccharide. This correction turned out to be insignificant for butanol and pentanol, due to their low C'_A and large $W^0_{s,A}$.

In Fig. 2 $W^0_{s,A}$ is plotted *versus* C'_A. The errors indicated were estimated from the experimental accuracy and include the uncertainty introduced by the adsorption of water on the Sepharose.

For comparison, Fig. 3 presents our data for methanol and pentanol sorption and data of Slaats *et al.*[4] and of Wahlund and Beijersten[7] for methanol and pentanol adsorption on octyl-silica RP-8, respectively. The latter were determined graphically and recalculated to a common basis of 1 g of octyl groups. It was assumed that 1 g of octyl-silica contains 0.14 g of octyl groups[8]. Note that Wahlund used a phosphate

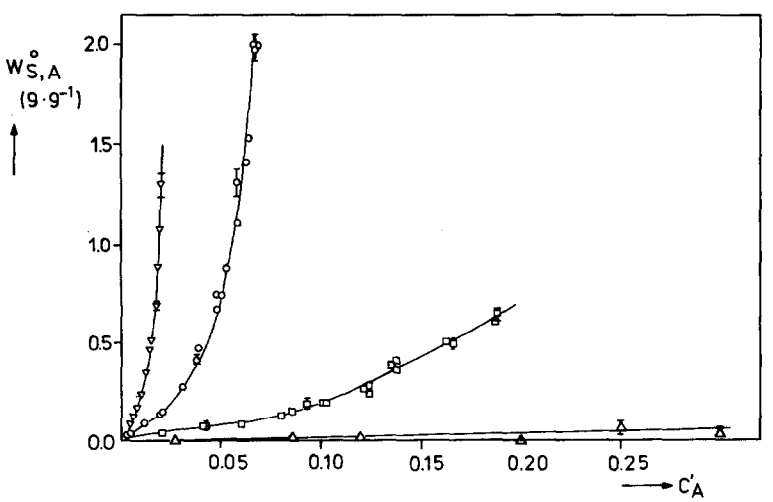

Fig. 2. Sorbed amounts per gram octylglycidyl groups at 20°C *versus* aqueous concentration of the alcohols at equilibrium. Additives: △, methanol; □, propanol; ○, butanol; ▽, pentanol.

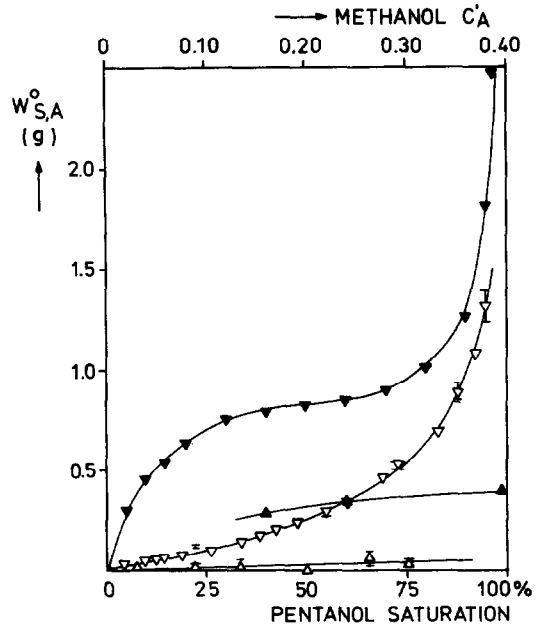

Fig. 3. Sorption isotherms of pentanol (▽, ▼) and methanol (△, ▲) on octyl-agarose (open symbols) and octyl-silica (closed symbols) *versus* their aqueous concentrations at equilibrium.

buffer, which decreased the solubility of pentanol somewhat compared to that in water. Therefore, in Fig. 3 the concentration of pentanol relative to that at saturation in water or buffer is used.

It is seen from Fig. 2 that sorption on octyl-agarose strongly depends on the nature of the additive. Near saturation, butanol and pentanol are very strongly absorbed. The more polar species show much less sorption and the sorption of methanol is barely detectable. In fact, the latter sorption is an upper limit. Should octylglycidyl groups hinder water adsorption by the Sepharose matrix, then w_{H_2O} is too small and $W^0_{s,A}$ becomes even smaller.

A comparison of Figs. 2 and 3 reveals a large difference between the properties of octyl-agarose and octyl-silica. No Langmuir-type isotherm is observed on octyl-agarose. In adsorption terms, a low energy surface seems to exist and especially at low concentrations sorption is very much lower. Note that, in the horizontal parts of the isotherms on octyl-silica, nearly identical numbers of moles of methanol and pentanol are adsorbed. This is consistent with the explanation of Wahlund *et al.* and Slaats *et al.*, *viz.*, the formation of a monolayer, with the long axes of the alcohols pointing perpendicularly to the adsorbent surface. This is not observed on octyl-agarose.

Wahlund *et al.* explain the steep increase in $W^0_{s,A}$ at higher concentrations as the formation of a multilayer of pentanol, perhaps due to capillary condensation. It is interesting to note the nearly identical shapes of the isotherms at $C'_A \geqslant 75\%$, despite the absence of pores on octyl-agarose. (It presents rather a convex surface!) So, either capillary condensation does not occur on octyl-silica or a different mechanism operates on octyl-agarose. Such a mechanism may be partition. As it is possible

to calculate partition equilibria from data at infinite dilution, this hypothesis can be tested by comparing experimental $\log K_{X,A}^{exptl.}$ values and calculated ones, $\log K_{X,A}^{calc.}$.

Calculations on partition equilibria

In the convention where the activity coefficient $\gamma \to 1$ when $X \to 1$, the partition constant at equilibrium is given by:

$$\log K_{X,A} = \log \frac{X_{A,or}}{X_{A,aq}} = -\log \frac{\gamma_{A,or}}{\gamma_{A,aq}} \tag{10}$$

Eqns. 3 and 10 relate $W_{s,A}^0$ to the activity coefficients in both phases, $\gamma_{A,or}$ and $\gamma_{A,aq}$. The prediction of sorption is possible by calculation of the activity coefficients[9,10].

Calculation of $\gamma_{A,or}$ and $\gamma_{A,aq}$. In our case, among the relatively simple approaches, only the three-suffix Scatchard-Hamer (SH)[11] or Wilson[12] equations are suitable. The latter is thought to be better[13], but its use is hampered by the lack of Wilson constants at 20°C. Therefore, we tested the applicability of the SH equation to data of Hirata *et al.*[13] for related systems and as the result was good, we used this equation for all our systems except the one mentioned later.

According to Scatchard and Hamer, the activity coefficient of A in the non-diluted range in a binary mixture of A and B is related to the activity coefficients at infinite dilution as follows:

$$\log \gamma_A = (1 - \varphi_A)^2 \left[\log \gamma_{A,B}^{\infty} + 2\varphi_A \left(\log \gamma_{B,A}^{\infty} \cdot \frac{\bar{V}_A}{\bar{V}_B} - \log \gamma_{A,B}^{\infty} \right) \right] \tag{11}$$

Here, $\bar{V}$ is the molar volume of a pure constituent, φ_A the volume fraction of A before mixing, $\gamma_{A,B}^{\infty}$ the activity coefficient of A at infinite dilution in pure B and $\gamma_{B,A}^{\infty}$ the same for B in pure A. In the organic phase, B represents the octylglycidyl groups and in the aqueous phase, B denotes water. Provided the limiting γ^{∞} values are known, $\gamma_{A,aq}$ and $\gamma_{A,or}$ can be calculated as follows.

For a given value of $X_{A,aq}$ and the corresponding value of $\varphi_{A,aq}$, $\gamma_{A,aq}$ is calculated with eqn. 11. Then, as the activities of A in both phases are equal, we know the value of $X_{A,or}\gamma_{A,or}$ in the organic phase. Using eqn. 11 now for the organic phase, we have to find the particular values of $\varphi_{A,or}$ and $X_{A,or}$ which yield $\gamma_{A,or}$ so that $X_{A,or}\gamma_{A,or}$ equals the already found value of $X_{A,aq}\gamma_{A,aq}$. This is easily performed by iteration. Thus, the activity coefficients of A in both phases at equilibrium are known and, hence, $\log K_{X,A}^{calc.}$.

Limiting activity coefficients. For the aqueous phase, γ_{A,H_2O}^{∞} was calculated from free energies of transfer at infinite dilution from a data compilation by Abraham[14]. No correction for the small temperature difference from 20°C was made. $\gamma_{H_2O,A}^{\infty}$ was evaluated from P–X (pressure–mole fraction) diagrams[13,15] and extrapolated to 20°C. Only a slight dependence on temperature exists. The activity of water in butanol was obtained from Hála *et al.*[9] and that in pentanol from mutual solubility data. γ_{A,H_2O}^{∞} and $\gamma_{H_2O,A}^{\infty}$ are listed in Table I.

Unfortunately, the liquid analogue of octylglycidyl groups, *i.e.*, 2-hydroxy-*n*-propoxyoctane, is rather exotic. This limits the availability of physical data. There

TABLE I

LIMITING ACTIVITY COEFFICIENTS IN THE AQUEOUS PHASE

Additive	*log* γ^{∞}_{A,H_2O}[14]	*log* $\gamma^{\infty}_{H_2O,A}$[9,13,15]
Methanol	0.17	0.25
Propanol	1.15	0.55
Butanol	1.72	0.60
Pentanol	2.32	0.60

are various ways round this: (1) $K^{0}_{X,A}$ and γ^{∞}_{A,H_2O} can be used with eqn. 1 to evaluate $\gamma^{\infty}_{A,O}$; (2) the Flory–Huggins–Hildebrand–Scott (FHHS) equation[16] can be used for *a-priori* calculation of $\gamma^{\infty}_{A,O}$ and $\gamma^{\infty}_{O,A}$. This method has already been successfully adopted by Tewari *et al.*[17].

Values of log $\gamma^{\infty}_{A,O}$ are listed in Table II; $K^{0}_{X,A}$ was evaluated from chromatographic data given in Table III. With a Hildebrand solubility parameter $\delta = 20.0$ (equal to that of octanol), the FHHS equation gives $\gamma^{\infty}_{A,O}$ in close agreement with values calculated with $K^{0}_{X,A}$. For comparison, values in octane[18,19] and octanol[20–22], obtained from recalculated partition data, γ^{∞}_{A,H_2O} and eqn. 10 are included in Table II. Values of log $\gamma^{\infty}_{O,A}$ were calculated with the FHHS equation ($\delta = 20$, and the estimated volume of the octylglycidyl groups, $V_0 = 206$ ml).

Comparison of experimental and theoretical values of log $K_{X,A}$. Values of log $K^{exptl.}_{X,A}$ on octyl-Sepharose were calculated according to eqn. 3 and plotted *versus* (relative) aqueous concentration in Fig. 4, with open symbols. Some negative values with methanol, arising from the low $W^{0}_{S,A}$, were omitted as their uncertainty on a mole fraction scale is very large. For comparison, log $K^{exptl.}_{X,A}$ for adsorption on octyl-silica is plotted with closed symbols. (Here, octyl groups also have been treated as free molecules.) At $C = 0$, log $K^{0}_{X,A}$ is plotted. These data points are in agreement with the limits of log $K^{exptl.}_{X,A}$, extrapolated to zero concentration. Full lines in Fig. 4 represent theoretical values, log $K^{calc.}_{X,A}$, calculated from Tables I and II. Here, log $\gamma^{\infty}_{A,O}$, evaluated from log K^{0}_{X}, was used (with the exception of that for methanol, which

TABLE II

LIMITING ACTIVITY COEFFICIENTS OF THE ORGANIC PHASE

OG denotes octylglycidyl groups. The value of log $\gamma^{\infty}_{O,A}$ pertaining to octane was estimated from closely related systems.

Additive	*log* $\gamma^{\infty}_{A,O}$				*log* $\gamma^{\infty}_{O,A}$	
	OG Chromatographic experiment	*OG FHHS*	*Octanol Partition*[20–22]	*Octane Partition*[18,19]	*OG FHHS*	*Octane P–X*[13]
Methanol	–	0.28	−0.09	1.53	2.2	
Propanol	−0.03 ± 0.09	0.11	−0.13	1.59	0.40	
Butanol	−0.05 ± 0.03	0.05	−0.06	1.61	0.18	
Pentanol	+0.08 ± 0.01	0.04	+0.04	1.59	0.10	0.4–0.5

TABLE III

NET RETENTION VOLUMES (ml) ON 100-ml BEDS OF OCTYL-SEPHAROSE AT 20°C

For comparison, data for a phosphate buffer are included.

Additive	*Pure water*	*Phosphate buffer I = 0.05, pH = 3.0*
Propanol	1.0 ± 0.2	1.1
Butanol	3.7	2.9
Pentanol	11.0	9.6
Hexanol	38.8	34.8
Gel content (g)	3.30	3.17

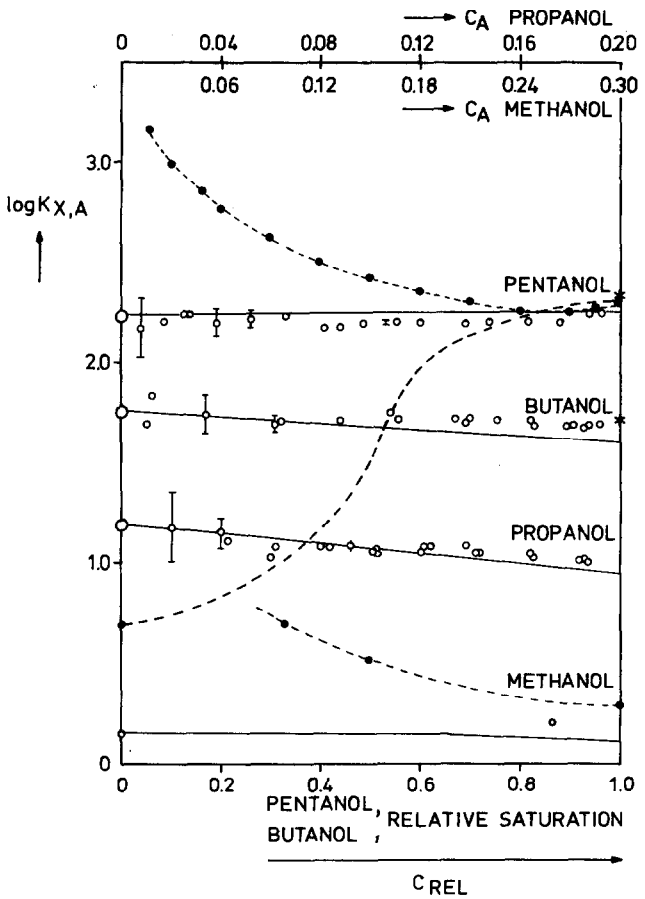

Fig. 4. Experimental log $K_{X,A}^{expt.}$ on octyl-agarose (○) and on octyl-silica (●), calculated log $K_{X,A}^{calc.}$ on octyl-agarose (full lines) and of a related system, octane–water (broken line). Reciprocal solubility is indicated with an asterisk. Values are plotted *versus* relative concentration units. At $C_{REL.} = 0$, chromatographically obtained log $K_{X,A}^{0}$ values are plotted.

we estimate to be zero from Table II). This implies that the lines are forced to pass through the data points representing log $K^0_{X,A}$. The broken line represents the distribution of pentanol between water and octane, calculated with the Wilson equation using log $\gamma^\infty_{O,A} = 0.5$ as estimated from closely related systems[13].

For sorption on octyl-Sepharose the agreement between log $K^{exptl.}_{X,A}$ and log $K^{calc.}_{X,A}$, is good. The maximum deviation is 0.07 for butanol at $C_{REL} > 0.8$. The agreement at low C_{REL} reflects the equality of the limit of log $K^{exptl.}_{X,A}$ extrapolated to zero concentration and log $K^0_{X,A}$. Alternatives such as octanol–water partition data or Rekker's hydrophobic fragmental constants[23] yield essentially the same results. For methanol, the predicted values of $W^0_{S,A}$ are in agreement, within experimental error, with experimental ones.

It is seen that the three curves for the systems pentanol–octane, pentanol–octyl-Sepharose and pentanol–octyl-silica merge into one, at saturation ending in a point nearly equal to the logarithm of reciprocal solubility of pentanol, denoted by an asterisk. This reflects the enrichment of pentanol in the organic systems, their character finally being dominated by pure pentanol. This also holds for butanol. It is, of course, no proof of a partition mechanism as the apparent $X_{A,O}$ in the case of multilayer formation also approaches unity. (Note that, in the systems pentanol–water and butanol–water, the organic phase has taken up a large amount of water. This reduces the corresponding values of log K_X to 1.43 for butanol and to 2.18 for pentanol.)

In RP-HPLC on alkyl-silica a mismatch between calculated partition constants and experimental capacity factors has been observed before[24,25]. Such a mismatch is also clearly seen in Fig. 4. For pentanol, the choice of octane as an analogue for octyl groups on octyl-silica leads to the dashed curve, which clearly is not in agreement with the experimental data at low concentrations. The choice of octanol instead of octane leads to $K^{calc.}_{X,A}$ values that are nearly indistinguishable from those calculated for octylglycidyl groups (solid line). This line also does not agree with the experimental data at low concentrations.

The origin of this difference in behaviour may lie in the properties of the surfaces of octyl-agarose and octyl-silica. The rigid surface of the latter is thought to be composed of methyl groups, and as such it closely resembles the octane–water interface. The activity coefficient of pentanol in this interface, calculated using the same convention from free energy of transfer data[18], leads to log $K_{X,A}$ (ads) = 3.7 at $C_{REL} = 0$. This compares favourably with log $K_{X,A}$ extrapolated to zero concentration in Fig. 4. Thus, on octyl-silica, as on the octane–water interface, pentanol manages to keep its polar head out of the octyl groups and the energy of dehydration of the polar head explains the difference between the partition data.

Octyl-agarose has a less rigid surface. Inspection of its synthesis reaction pathways[26] reveals the possibility of substitutions resulting in a chaotic layer with exposed hydroxyl groups. This might explain the low energy surface.

CONCLUSION

Adsorption of *n*-alcohols on octyl-agarose is a clearly different process from that on octyl-silica. A partition model satisfactorily explains the sorption isotherms on octyl-agarose but fails to do so on octyl-silica. The logarithm of the partition

constant for sorption of the alcohols on octyl-agarose can be predicted within 0.07. Chromatographic retention is highly indicative of the extent of sorption of the alcohols.

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