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

II. INFLUENCE OF ORGANIC CO-SOLVENTS ON RETENTION AT 25°C

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SUMMARY

The retention of *n*-alcohols and *n*-carboxylic acids on octyl-agarose in partly aqueous eluents, with *n*-alcohols, urea, ethylene glycol and poly(ethylene glycol), sucrose, acetone and acetonitrile as co-solvents, was measured. The activity coefficient of *n*-octanol in the stationary layer of octyl-agarose is calculated from experimental data on the solubility of *n*-octanol in the eluent and the composition of the co-existing liquid phases of the ternary system octanol–water–additive. On the basis of the magnitude of the activity coefficient of *n*-octanol, the retention mechanism is discussed and compared with that on octyl-silica.

INTRODUCTION

This paper deals with the influence of some organic additives to the eluent on the chromatographic retention of homologous *n*-alcohols and neutral *n*-carboxylic acids on octyl-agarose. In a previous paper[1], this adsorbent was found to sorb the co-solvent in a mixture of water and an organic additive. As a consequence, the properties of the adsorbent probably depend on the composition of the eluent. Our aim is to unravel the contributions of the eluent and the adsorbent to the retention in various media, *i.e.* to determine the activity coefficient of the chromatographed compound in the eluent (γ_{aq}) and in the layer of octylglycidyl groups and sorbed co-solvent (γ_{og}).

In the first part of this paper, the influence of additives on the specific retention volumes at 25°C is discussed. The chosen additives (methanol, ethanol, propanol, butanol, pentanol, ethylene glycol, poly(ethylene glycol), sucrose, acetone, acetonitrile and urea) differ widely with respect to molecular size and polarity. Taking into account the sorbed amounts of additive, we tested the partition model, which already has proved successful before[1,2].

The second part deals with the relation between the mole-fraction solubility in the eluent (X_{aq}^{sat}), γ_{aq} and γ_{og}, of one of the test solutes, *i.e.* octanol. We measured the

solubility of octanol in the eluent, and the composition of co-existing phases of the ternary systems octanol–water–additive for four additives (methanol, ethylene glycol, urea and propanol) at different concentrations. It will be shown that γ_{aq} can be calculated from these experimental data. This permitted us to evaluate γ_{og} and to discuss its magnitude.

THEORETICAL

Chromatographic retention and the partition model
    The specific retention volume V_g of a test substance B reflects the influence of additives to the eluent on the interaction of B with the adsorbent. It is given by

$$V_g = w_0^{-1}(V_e - V^0) \tag{1}$$

where V_e and V^0 are the elution volumes of B and a supposedly unretarded compound, respectively, on a column containing w_0 g of octylglycidyl (OG) groups.
    The partition model is formulated in terms of the partition constant of B, $K_{X,B}$, based on the mole fraction X of B in both the organic phase (og) and the aqueous phase (aq). The former phase is composed of OG groups (treated as free molecules) and sorbed additive, A, and the latter consists of water and A.
    $K_{X,B}$ can be derived from chromatographic data as follows. For a very dilute solution of B, $X_{B,og}$ is given by

$$X_{B,og} = \frac{n_{B,og}}{w_0(M_0^{-1} + W_{s,A}^0 M_A^{-1})} \tag{2}$$

where $n_{B,og}$ denotes the number of moles of B in the organic phase in the column, M_0 and M_A are the molecular weights of OG and A, respectively, $W_{s,A}^0$ is the sorbed amount of A on 1 g of OG, and w_0 is the mass of OG in the column. In a similar way,

$$X_{B,aq} = \frac{n_{B,aq}}{n_{H_2O} + n_{A,aq}} \tag{3}$$

where $n_{B,aq}$, n_{H_2O} and $n_{A,aq}$ denote the numbers of moles of B, water and A, respectively, in the aqueous phase in the column. If the agarose and its sorbed water behave essentially like a dead volume in the column, thus not causing retention of the model substances (this was verified: *cf.* Results and Discussion), it holds that

$$\frac{n_{B,og}}{n_{B,aq}} = \frac{V_e - V^0}{V^0} \tag{4}$$

    From eqns. 1–4 it can be derived that

$$K_{X,B} = \frac{X_{B,og}}{X_{B,aq}} = V_g \frac{(n_{H_2O}/V^0) + (n_{A,aq}/V^0)}{(M_0^{-1} + W_{s,A}^0 M_A^{-1})} \tag{5}$$

Note that n_{H_2O}/V^0 and $n_{A,aq}/V^0$ are equal to the numbers of moles of water and A, respectively, in 1 ml of eluent. It is seen that $K_{X,B}$ can be evaluated from chromatographic data, provided the sorption isotherm of the additive is known.

Activity coefficients

Adopting the pure liquid at 25°C and 1 atm as a reference state, $\log K_{X,B}$ at infinite dilution can be expressed as[3]

$$\log K_{X,B} = \log\frac{X_{B,og}}{X_{B,aq}} = -\log\frac{\gamma^{\infty}_{B,og}}{\gamma^{\infty}_{B,aq}} \tag{6}$$

Here, γ^{∞} denotes the activity coefficient at infinite dilution. It is seen from this equation that the activity coefficient of B in the layer of OG, $\gamma^{\infty}_{B,og}$, can be evaluated if $K_{X,B}$ and $\gamma^{\infty}_{B,aq}$ are known. The latter can be determined as follows.

Consider a ternary system, comprising B, water and A, with two co-existing liquid phases. This is illustrated in Fig. 1. At equilibrium, the mole fractions of B in the co-existing phases Q and Q' are related by

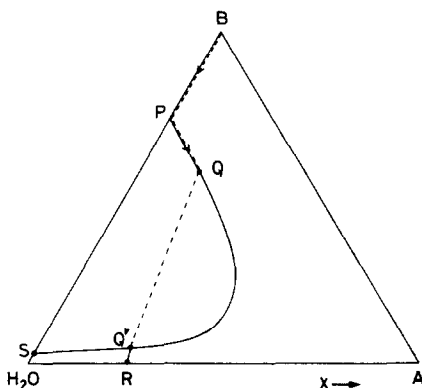$$\gamma_{B,or}X_{B,or} = \gamma^{sat}_{B,aq}X^{sat}_{B,aq} \tag{7}$$

Fig. 1. Phase diagram of a liquid ternary system, comprising additive (A), water (H_2O) and test solute (B). With B = octanol, the distance S–H_2O, where S represents a saturated aqueous solution of octanol is highly exaggerated for clarity. P is octanol, saturated with water. Q and Q' represent two co-existing liquid phases. The point R represents a binary phase system with the same mole fraction ratio of A and H_2O as in Q'.

Here, $X^{sat}_{B,aq}$ denotes the solubility of B in the aqueous phase and $\gamma^{sat}_{B,aq}$ the related activity coefficient. If $X^{sat}_{B,aq}$ is small, say less than 10^{-3}, the solute molecules (if not too large) are completely surrounded by a multilayer of A and water, and $\gamma^{sat}_{B,aq}$ is not expected to differ much from $\gamma^{\infty}_{B,aq}$ at the same ratio of the amounts of water and A. In this case, $\gamma^{\infty}_{B,aq}$ is given by

$$\gamma^{\infty}_{B,aq} = \frac{\gamma_{B,or}X_{B,or}}{X^{sat}_{B,aq}} \tag{8}$$

If the organic phase consists of pure B, then $\gamma_{B,or}X_{B,or} = 1$, and eqn. 8 reduces to the well-known relationship $\gamma_{B,aq}^{\infty} = (X_{B,aq}^{sat})^{-1}$. In this case, log $\gamma_{B,og}^{\infty}$ can be obtained from the solubility of B and $K_{X,B}$ only (eqn. 6).

In the general case, which must be considered here (see Fig. 1), substitution of eqn. 8 into eqn. 6 leads to the equation

$$\log \gamma_{B,og}^{\infty} = \log \frac{\gamma_{B,or}X_{B,or}}{K_{X,B}X_{B,aq}^{sat}} \tag{9}$$

Thus, in the general case, additional knowledge about the composition of the organic phase of the ternary system B–water–A is required and $\gamma_{B,or}$ has to be evaluated.

Calculation of $\gamma_{B,or}$

The low vapour pressure of a solute such as octanol prohibits the determination of log $\gamma_{B,or}$ via vapour pressure data (the same statement holds for the determination of log $\gamma_{B,aq}$). Unfortunately, solution theories fail to describe the organic phases of the ternary system B–water–A quantitatively if constituents with very different polarities are present. However, the Gibbs–Duhem relation provides an indirect way to evaluate activity coefficients. This method, suitable for all chromatographic systems with binary eluents and slightly soluble solutes, is outlined below.

The basic requirement is that the introduction of B into the aqueous eluent does not influence the activity coefficients of the other constituents ($\gamma_{H_2O,aq}$ and $\gamma_{A,aq}$) in an appreciable way. If X^{sat} is small, say less than 10^{-3}, and if the size of a molecule of B is not too large, then the fraction of the molecules of water and A in contact with B is small too. The influence of B on $\gamma_{H_2O,aq}$ and $\gamma_{A,aq}$ must then be small also. This condition is likely to be met for solutes such as octanol*. In this case, $\gamma_{H_2O,aq}$ and $\gamma_{A,aq}$ in the ternary system at the point Q´ in Fig. 1 can be taken to be equal to the values of γ in the corresponding binary system at R (*i.e.* before the introduction of B). (The determination of the latter data is described later in the notes accompanying Table IV.)

From the values of the activity coefficients in the aqueous phase at Q´ and the (known) mole fractions in both phases (see later: Table III), values of the activity coefficients of H_2O and A in the organic layer ($\gamma_{H_2O,or}$ and $\gamma_{A,or}$ at Q) can be calculated:

$$\gamma_{H_2O,or}X_{H_2O,or} = \gamma_{H_2O,aq}X_{H_2O,aq}$$

$$\gamma_{A,or}X_{A,or} = \gamma_{A,aq}X_{A,aq} \tag{10}$$

If Q and Q′ are moved along the phase boundary, the value of the activity coefficients of two of the three constituents of the organic phase of the ternary system can be

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* We estimated the change of log $\gamma_{A,aq}$ due to the introduction of octanol qualitatively, with the three-suffix Scatchard–Hamer equation for ternary systems[3,4]. In 3 *M* propanol, where the volume fraction of octanol at saturation is 0.01, log $\gamma_{A,aq}$ was found to decrease by only 0.03. In other systems, mentioned later in this paper, the decrease was even smaller.

calculated as a function of its composition. Then the change in the third one, *i.e.* B, can be calculated from the Gibbs–Duhem relation. From

$$X_{B,or}\left(\frac{\partial \log \gamma_{B,or}}{\partial X_{B,or}}\right)_{p,T} + X_{H_2O,or}\left(\frac{\partial \log \gamma_{H_2O,or}}{\partial X_{B,or}}\right)_{p,T} + X_{A,or}\left(\frac{\partial \log \gamma_{A,H_2O}}{\partial X_{B,or}}\right)_{p,T} = 0 \quad (11)$$

it can be derived that, for an isobaric, isothermal process:

$$\log \gamma_{B,or} = -\int_0^{X_{H_2O}} \frac{X_{H_2O,or}}{X_{B,or}} \, d \log \gamma_{H_2O,or} - \int_0^{X_A} \frac{X_{A,or}}{X_{B,or}} \, d \log \gamma_{A,or} \quad (12)$$

where the mole fractions, given as integration limits, refer to the corresponding values of log γ_{or}.

The integration must be performed from B along the axis representing the B–water binary system to the point P representing B, saturated with water and from there along the phase boundary to Q (see Fig. 1). Note that no data are available along the line BP, *i.e.* for $X_{H_2O} = 0$ to $X_{H_2O} = X_{H_2O}^0$, the mole fraction of water in B, saturated with water. However, the value of log $\gamma_{B,or}$, obtained by integrating *only* from $X_{H_2O} = 0$ to $X_{H_2O} = X_{H_2O}^0$ is equal to log $\gamma_{B,or}^0$, the activity coefficient of B in B, saturated with water. Therefore, it is convenient to split the first integral of eqn. 12 into two parts, one of which equals log $\gamma_{B,or}^0$. (For octanol, the calculation of log $\gamma_{B,or}^0$ is described in the notes accompanying Table IV.) The other part (an integration from $X_{H_2O} = X_{H_2O}^0$ to X_{H_2O}) plus the integral concerning A, reflect the change of log $\gamma_{B,or}$ with respect to log $\gamma_{B,or}^0$. These integrals can be evaluated graphically from plots of $X_{H_2O,or}/X_{B,or}$ *versus* log $\gamma_{H_2O,or}$ and $X_{A,or}/X_{B,or}$ *versus* log $\gamma_{A,or}$ as the area under the curves.

Once $\gamma_{B,or}$ has been obtained, $\gamma_{B,aq}$ can be found from eqn. 8, and $\gamma_{B,og}$ from eqn. 9.

EXPERIMENTAL

Chemicals and column packing material

All solutes had the highest available purity. Twice-distilled water was used. The *n*-alcohols were from Baker (Deventer, The Netherlands), the other chemicals were from Merck (Darmstadt, F.R.G.). Octyl-Sepharose Cl-4B was obtained from Pharmacia (Uppsala, Sweden).

Chromatographic experiments

The experiments were performed at 25°C, using binary solvents as the eluent. No buffer was added: with the carboxylic acids the pH of the eluent and sample solutions was adjusted to 2.5 with hydrochloric acid. The procedure has been described before[2], but the following changes were made.

(1) As the differential refractive index detector showed baseline instability at an elution volume of about V^0 (owing to sample introduction) we focused our attention to the higher homologues and hence reduced the bed volume to *ca.* 30 ml.

(2) V^0 was determined experimentally using various substances such as water, potassium bromide and acetone. The values obtained generally agreed within experimental error. (Calculation of V^0 from the column content is difficult is case of appreciable sorption of A.)

(3) w_0 was evaluated from the total column contents after each series of measurements with the same additive. As before, the degree of substitution was assumed to be 0.40 mol OG per mol disaccharide[5].

(4) The sample dose was kept as low as possible, *i.e. ca.* 100 μg.

(5) Baseline stability of the detector was improved and bubble formation in the detector avoided by storing the eluent at a higher temperature (30°C).

Determination of solubilities

A sensitive and accurate procedure for the measurement of small concentration differences has been described before[1]. It was also applied for the determination of the solubility of octanol in binary aqueous mixtures. For that purpose, the refractive index signal $S-S^0$ of an eluent, saturated with solute, was compared with the signal of an undersaturated one of known concentration. The baseline was obtained with the eluent. The temperature was 25°C. Only repeated distilled octanol was used immediately after distillation*. Saturated samples were obtained by shaking a very small excess of solute in the eluent for 10 min (a longer equilibration time did not result in a different signal), followed by centrifugation. The refractive index n was also determined. In this way, graphs were obtained of n as a function of the composition of the eluent.

Determination of the composition of co-existing phases

Some 25-ml flasks, containing known amounts of water, additive and octanol, were shaken for 1 h and centrifuged. The weight and the refractive index of the aqueous phase were determined. From the graph of n *versus* the aqueous additive concentration (obtained previously) the composition of the aqueous phase was determined. From the total amounts present, the composition of the organic phase was calculated.

RESULTS AND DISCUSSION

Chromatographic retention and the partition model

In Table I, the specific retention volumes V_g (ml/g) of some n-alcohols and n-carboxylic acids, calculated from eqn. 1, are listed for various additives and concentrations C_A (g of A per g of mixture).

Control experiments with unsubstituted Sepharose were performed using water and aqueous mixtures of methanol or butanol as the eluents. The elution volumes of

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* An unknown impurity seems to form spontaneously[6], capable of inducing a Tyndall effect far below the solubility limit of octanol. Freshly distilled octanol gives clear solutions. An abrupt change of the turbidity, accompanied with a phase separation, occurs at the solubility limit. This is in accordance with the fact that alcohols do not form micelles in aqueous solutions[7].

TABLE I

SPECIFIC RETENTION VOLUMES V_g (ml/g) OF n-ALCOHOLS AND n-CARBOXYLIC ACIDS, AND SORBED AMOUNTS $W_{s,A}$ OF CO-SOLVENT (g/g) FOR VARIOUS ADDITIVES AND MASS FRACTIONS $C_{A,aq}$ (g/g)

Eluent composition ($C_{A,aq}$)	*Alcohols,* $C_nH_{2n+1}OH$					$W_{s,A}$ (g/g)
	$n = 5$	$n = 6$	$n = 7$	$n = 8$	$n = 9$	
Methanol						
0.000	17	61	220	816		0.000
0.098	13	46	159	545	1839	0.015
0.199	11	37	111	340	1000	0.035
0.303	8	22	60	153	390	0.055
0.413		12	27	58	124	0.085
Ethanol						
0.032	14	57	201	731		0.010
0.094	15	51	174	607		0.040
0.190	13	43	125	367		0.080
0.240	13	37	89	241		0.095
0.290		19	50	113	243	0.110
Propanol						
0.061	17	61	218	765		0.095
0.123	22	66	206	646		0.290
0.138	23	62	180	496		0.380
0.154	25	65	158	392		0.440
0.186		42	95	179	316	0.600
0.218		31	51	82	125	0.720
Butanol						
0.015	20	68	244	879		0.100
0.037	25	87	313	1080		0.400
0.060	48	163	549	1730		1.250
Pentanol						
0.004			67	251		0.070
0.009			83	298		0.180
0.013			104	378		0.360
0.019			173	630		0.850
Ethylene glycol						
0.092	17	56	202			
0.152	17	54	190	680		
0.299	17	47	150	499		
0.440	15	37	105	297		
Poly(ethylene glycol)$M = 400$						
0.147	13	47	163	568		
0.380		40	110	282		
Sucrose						
0.162	20	68	249	914		
Acetone						
0.118	12	37	119	374		
0.241		19	50	129		
Acetonitrile						
0.084	16	49	158	503		
0.170		30	80	211		
0.259		15	33	71		
Urea						
0.059	16	59	212	788		
0.172	15	51	182	634		
0.279	14	47	157	526		
0.380	13	42	130	386		

TABLE I (*continued*)

Eluent composition ($C_{A,aq}$)		Carboxylic acids, $C_nH_{2n-1}O_2H$					$W^{\circ}_{s,A}$ (g/g)
		n = 5	n = 6	n = 7	n = 8	n = 9	
Methanol	0.000	19	70	257	959	3450	0.000
	0.148	17	48	153	491	1600	0.028
	0.303		24	61	163	437	0.055
Ethanol	0.142		56	176	569		0.060
	0.215		43	117	317	833	0.090
	0.264		30	76	179	436	0.100
	0.315		19	37	77	150	0.120
Propanol	0.061	20	73	252	821	2820	0.095
	0.123	22	67	214	630	2038	0.290
	0.154	21	63	147	428	1019	0.440
	0.186	20	45	94	188	342	0.600
Butanol	0.015	20	77	278	1014		0.100
	0.030	24	85	307	1106		0.260
	0.044	30	104	374	1333		0.590
	0.057	38	129	459	1451		0.960
	0.064	53	186	615	1825		1.600

potassium bromide, acetone, *n*-pentanol and *n*-heptanol are identical and are very close to the calculated volume of the eluent in the column. This indicates that Sepharose behaves essentially as a dead volume for these solutes.

In Fig. 2, log V_g of a representative solute, 1-heptanol, is plotted *versus* φ_A, the volume fraction of the additive before mixing. For the solid additives, the volume fraction after mixing was taken, the partial molar volume of water was supposed to be 18.0 ml.

As can be seen from eqn. 5, the methylene increments of log V_g are identical with those of log $K_{X,B}$. In Fig. 3, these increments are presented as a function of φ_A. In most cases, the difference between the values of the methylene increment in the two homologous series was not larger than the experimental error (estimated from duplicate experiments to be 0.7 ml for low values of V_g or 0.9% for high values). For most of the eluent compositions, the methylene increment is independent of the chain length of the solute up to $n = 9$, but at high concentrations of some of the additives it seems to decrease with increasing chain length. This is especially clear with butanol.

Fig. 4 shows log $K_{X,B}$ of heptanol, calculated according to eqn. 5. Values of $W^{\circ}_{s,A}$ of methanol, propanol, butanol and pentanol were obtained from experimentally determined sorption isotherms[1]. Values at the highest concentration of methanol and propanol were found by extrapolation. For ethanol, experimental values of $W^{\circ}_{s,A}$ are not available. We estimated $W^{\circ}_{s,A}$ for this co-solvent with limiting activity coefficients, obtained from P–x data[8,9] according to the procedure described before[1]. The uncertainties in the calculated values are probably not large, as this method led to good results with methanol and propanol.
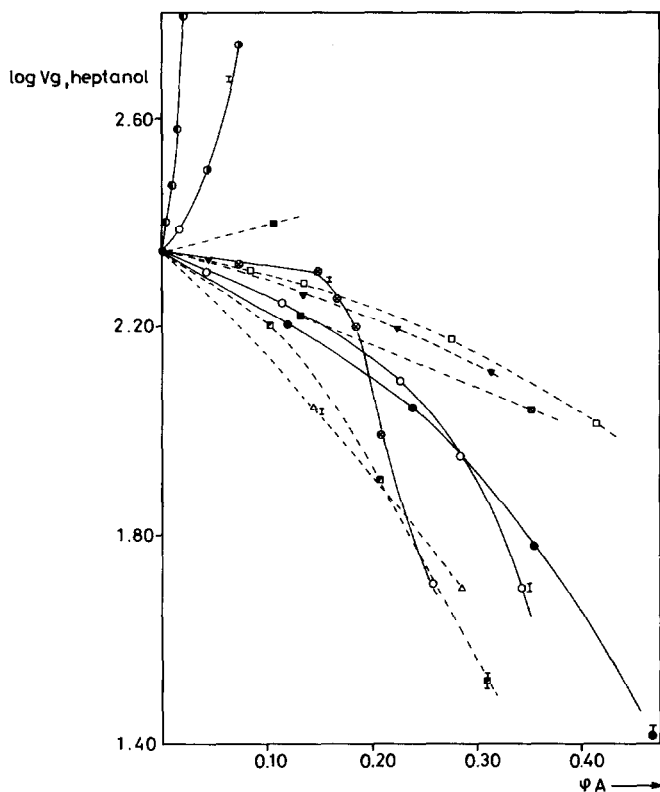
Fig. 2. Log V_g of n-heptanol as a function of the volume fraction, φ_A, of the additive. Data points: ● = methanol; ○ = ethanol; ⊗ = n-propanol; ◑ = n-butanol; ◐ = n-pentanol; △ = acetone; ■ = sucrose; □ = ethylene glycol; ⊠ = poly(ethylene glycol) M = 400; ▼ = urea; ▨ = acetonitrile.

For the other co-solvents, we also lack experimental data about sorption. From octanol–water partition data on urea[10], ethylene glycol[11], sucrose and poly(ethylene glycol) M_w = 400 (the last two being estimated from Rekkers hydrophobic fragmental constants[6]) it was found that these additives show much less affinity for octanol (and thus, probably, for OG groups) than methanol. As a consequence, sorbed amounts are probably much lower than those of methanol and can be neglected. For acetone and acetonitrile, no reliable estimates about $W_{s,A}^*$ can be made and therefore, no log $K_{X,B}$ values can be calculated.

Influence of additives on log V_g

Fig 2 and Table I show that the change of V_g with the additive concentration strongly depends on the nature of the additive. The decrease of V_g observed with most co-solvents is in accordance with the experience that methanol, ethylene glycol, propanol and urea bring about desorption of adsorbed proteins on octyl-agarose[12–14], and that methanol and acetonitrile weaken hydrophobic interaction in reversed-phase high-performance liquid chromatography (HPLC)[15,16]. It might be of practical interest to note that, at constant φ_A, acetone is as effective as acetonitrile in decreasing
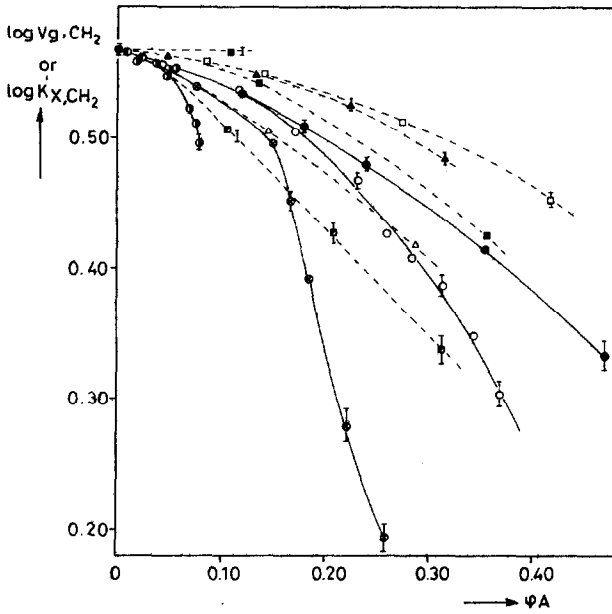
Fig. 3. The methylene increments of log V_s or log $K_{X,B}$ (all data except for $n = 5$) *versus* φ_A. Symbols as in Fig. 2. Errors are indicated with bars.
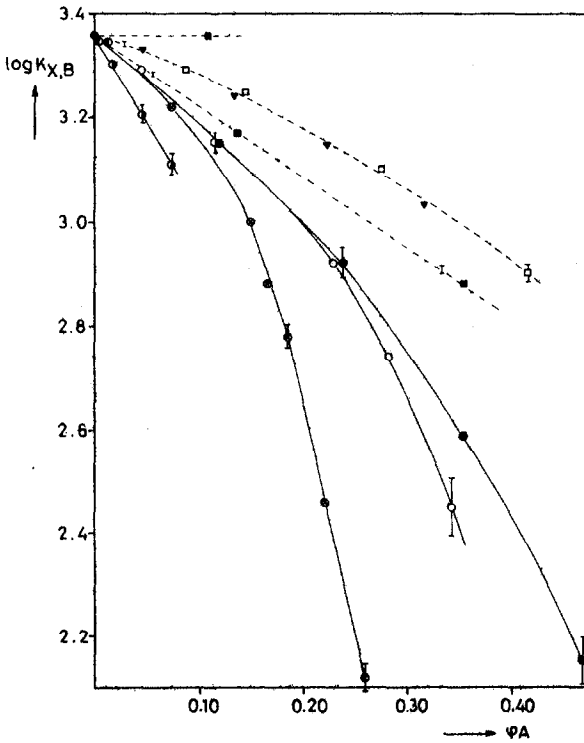

Fig. 4. Log $K_{X,B}$ of *n*-heptanol as a function of φ_A. Symbols as in Fig. 2.

the retention. The larger the alkyl group of B, the larger is the influence of the additive (Table I).

On the other hand, butanol, pentanol, sucrose and (at low C_A and for low-molecular-weight alcohols as test compounds) propanol seem to strengthen the interaction with OG. With pentanol, this is contrary to the results of Wahlund and Beijersten[17,18] on octyl-silica, as is shown in Fig. 5 for some organic compounds. This discrepancy probably stems from a different retention mechanism. In our system, retention is probably governed by a kind of partition mechanism. The mass increase of the stationary phase, caused by the uptake of pentanol, contributes to the volume of the stationary phase. If the additive has about the same properties as the OG groups, the sorbed amounts will effectively contribute to the retention of solutes. With octyl-silica, the retention is probably governed by an adsorption mechanism, at low concentrations of pentanol. With sucrose, results seem consistent with the observed depression of the solubility of octanol in aqueous mixtures of other sugars at low concentration[19].

The influence of the amount of sorbed eluent on the retention of test compounds can be eliminated in two ways, as can be seen from eqn. 5. First, the ratio of the specific retention volumes of two test compounds can be taken. This ratio is equal to the corresponding ratio of partition constants, $K_{X,B}$, and is thus independent of the amount of stationary phase. If the two test compounds are successive members of a homologous series, this ratio yields the methylene increment of log V_g or log $K_{X,B}$, denoted as log V_{g,CH_2}. Second, from eqn. 5, the specific retention volume of a solute and the sorbed amount of the additive, the partition constant $K_{X,B}$ can be calculated, which does not depend on the amount of stationary phase. The influence of additives on log V_{g,CH_2} and log $K_{X,B}$ is discussed in the following paragraphs.

Influence of additives on log V_{g,CH_2}

Fig. 3 shows that all the additives except sucrose weaken the interaction of a methylene group with the stationary phase. The sequence of the additives with respect to their effect on log V_{g,CH_2} is: sucrose, urea and glycol, poly(ethylene glycol), methanol, ethanol, acetone, acetonitrile, propanol and butanol. This sequence shows a steady decrease of polarity and reflects the increasing affinity of the eluent for the apolar solute. Values for acetonitrile agree well with literature data on reversed-phase HPLC[16].

Influence of additives on log $K_{X,B}$

This influence is shown in Fig. 4. For sucrose, the most polar additive, it is seen that log $K_{X,B}$, just like log V_{g,CH_2}, is constant. Because the sorbed amount of sucrose is extremely small, it can be anticipated that $\gamma_{B,og}^{\infty}$ in sucrose–water eluents is constant. It follows from eqn. 6 that $\gamma_{B,aq}^{\infty}$ is then also constant. It follows from eqn. 5 that the observed increase of log V_g is due to the decrease of $(n_{H_2O}/V^0 + n_{A,aq}/V^0)$, *i.e.* the number density of the eluent molecules, with the sucrose of the eluent. The increase in log V_g can thus be explained as merely arising from the large size of sucrose molecules with respect to water. It follows that the partition model (on the mole fraction scale) holds good in sucrose–water eluents.

With pentanol, the least polar additive, log $K_{X,B}$ and log V_{g,CH_2} are also approximately constant. A change of $\gamma_{B,aq}^{\infty}$ is not likely in the dilute pentanol solutions.
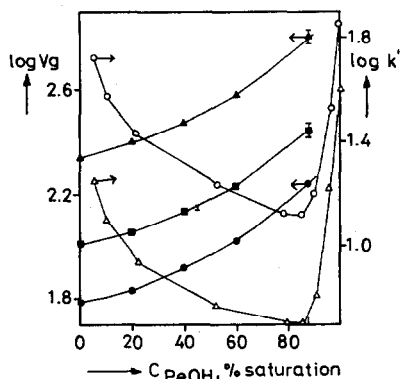
Fig. 5. A comparison of the behaviour of some test solutes with octyl-agarose or octyl-silica as an adsorbent and aqueous pentanol–water mixtures as eluents. Data points: ● = n-hexanol; ▲ = n-heptanol; ○ = benzoic acid; △ = 3-hydroxy benzoic acid; ■ = n-pentyl acetate, a non-alcohol as an illustration that the mechanism is not solute-dependent. Full symbols are values of log V_g on octyl-agarose (our data); open symbols are values of log k on octyl-silica (Wahlund and Beijersten)[18]. Both are presented as a function of the pentanol concentration relative to saturation.

It follows that $\gamma_{B,og}^{\infty}$ does not change much, in spite of the very large amounts of pentanol that are sorbed. The sorbed pentanol has apparently the same solvent properties as the OG groups. This is not unexpected, as the polarity of pentanol and OG groups must be about equal. We interpret this as evidence in favour of a partition mechanism. It explains the difference with octyl-silica as an adsorbent.

For the other additives of intermediate polarity, the situation is more complicated. Here, the possibility must be envisaged that $\gamma_{B,og}^{\infty}$ and $\gamma_{B,aq}^{\infty}$ change with additive content of the eluent. Therefore, $\gamma_{B,aq}^{\infty}$ and $\gamma_{B,og}^{\infty}$ must be evaluated as described in the theoretical section of this paper.

Activity coefficients

The solubilities of octanol in some binary eluents are listed in Table II. Methanol was chosen because of its frequent use in HPLC as a co-solvent, urea for its interesting properties as a co-solvent[20,21], ethylene glycol as a co-solvent that is often

TABLE II

SOLUBILITY, S, OF OCTANOL IN AQUEOUS ELUENTS WITH VARIOUS ADDITIVE (A) CONCENTRATIONS $C_{A,aq}$

A	$C_{A,aq}$ (g/g)	S (g/l)	A	$C_{A,aq}$	S
Methanol	0.000	0.572	Propanol	0.061	0.486
	0.098	0.730		0.123	0.492
	0.199	1.16		0.154	0.890
	0.303	2.57		0.186	2.70
	0.413	6.52		0.218	8.07
Ethylene glycol	0.152	0.680	Urea	0.059	0.601
	0.299	0.902		0.172	0.671
	0.440	1.48		0.279	0.845
				0.380	1.003

TABLE III

EQUILIBRIUM COMPOSITION (MOLE FRACTIONS) OF TERNARY SYSTEMS OCTANOL–WATER–A

System number	A	$X_{A,aq}$	$X_{A,or}$	$X_{H_2O,or}$
1	Methanol	0.000	0.000	0.249
2		0.062	0.085	0.250
3		0.124	0.163	0.256
4		0.172	0.229	0.258
5		0.213	0.273	0.265
6		0.261	0.329	0.275
7	Ethylene	0.051	0.008	0.244
8	glycol	0.090	0.026	0.237
9		0.173	0.054	0.229
10		0.288	0.097	0.206
11	Propanol	0.007	0.076	0.260
12		0.012	0.139	0.274
13		0.025	0.254	0.310
14		0.041	0.341	0.387
15		0.050	0.372	0.451
16		0.062	0.365	0.524
17	Urea	0–0.16	*ca.* 0	0.249

used to bring about desorption of proteins, and propanol because of its large influence on log V_g at higher concentrations and for being strongly sorbed by the OG groups. The experimental error, as estimated from duplicate measurements, is 1–2%. Our experimental value in pure water compares very well with the literature value[22].

The compositions of co-existing phases of some ternary systems octanol–water–additive are listed in Table III. The general shape of the phase diagram of the systems, consisting of n-octanol and water with methanol, propanol or ethylene glycol is shown in Fig. 1. The system with urea is in a class apart. In this case, the solubility of solid urea in n-octanol is so small that we could take the amount of urea in the organic layer equal to zero. From these experimental data, $\gamma_{B,or}$ (B = octanol) can be obtained from eqn. 12.

Calculation of $\gamma_{B,aq}^{\infty}$ and $\gamma_{B,og}^{\infty}$

Values of log $\gamma_{B,aq}^{\infty}$ can be calculated from eqn. 8. The required values of $\gamma_{B,or}X_{B,or}$ at the volume fractions used in the chromatographic experiments were obtained by interpolation of the values listed in Table IV. Also listed in that table and discussed are the values of the various auxiliary quantities, encountered in eqns. 8–12.

Values of log $\gamma_{B,og}^{\infty}$ were calculated from eqn. 9.

Fig. 6 shows a plot of log $\gamma_{B,aq}^{\infty}$ *versus* φ_A. Also presented are values of $-$log $X_{B,aq}^{sat}$ and of log $\gamma_{B,og}^{\infty}$. The need for the correction, applied by the calculation of $\gamma_{B,or}X_{B,or}$, is apparent from the differences between corresponding values of log $\gamma_{B,aq}^{\infty}$ and $-$log $X_{B,aq}^{sat}$.

Now, a remarkably simple picture emerges: the activity coefficient of octanol

TABLE IV

VALUES OF LOG $\gamma_{B,or}$ AND AUXILIARY QUANTITIES

System number	$\gamma_{A,aq}$[a]	$\gamma_{H_2O,aq}$[a]	Log $\gamma_{A,or}$[b]	Log $\gamma_{H_2O,or}$[b]	Log $\gamma_{B,or}$[c]	a_B
1	1.48	1.00	−0.09[e]	0.60[h]	0.02[j]	0.79
2	1.60	0.95	0.03	0.55	0.03	0.71
3	1.65	0.90	0.10	0.49	0.04	0.64
4	1.60	0.85	0.08	0.43	0.07	0.60
5	1.50	0.90	0.07	0.43	0.08	0.56
6	1.45	0.95	0.06	0.41	0.10	0.50
A[d]	0.45	1.00	0.74[f]	0.60	0.02	0.79
7	0.45	1.00	0.45	0.59	0.02	0.78
8	0.45	1.00	0.20	0.58	0.03	0.79
9	0.45	1.00	0.16	0.56	0.04	0.79
10	0.45	1.00	0.13	0.54	0.05	0.78
B[d]	14.1	1.00	−0.13[g]	0.60	0.02	0.79
11	13.8	1.00	0.10[i]	0.58	0.02	0.70
12	13.3	1.00	0.06	0.56	0.03	0.63
13	12.4	1.01	0.09	0.50	0.05	0.49
14	11.2	1.02	0.13	0.40	0.11	0.35
15	10.5	1.03	0.15	0.33	0.22	0.29
16	9.1	1.03	0.19	0.27	0.36	0.25
17					0.02[k]	0.79

[a] Values of $\gamma_{A,aq}$ and $\gamma_{H_2O,aq}$ at the point R in Fig. 1 for the systems mentioned in Table III. For A = methanol and propanol, they were obtained from $(p–X)$ diagrams at different temperatures[8,9], the lowest of which were near 25°C. For A = ethylene glycol, they were calculated from $(T–X)$ diagrams[9] at various values of p. We assumed the vapour phase to behave ideally.
[b] Calculated with eqn. 10 from γ_{aq} and mole fractions from Table III.
[c] Calculated according to eqn. 12 as the area under the straight line sections connecting experimental points.
[d] Auxiliary system with $X_A \approx 0$, in which log $\gamma^\infty_{A,or}$ for methanol, propanol (see ref. 1) and ethylene glycol[11] (e, g and f respectively) were calculated from distribution constants in the octanol–water system.
[e] log $\gamma^\infty_{A,or}$ for methanol, see also d.
[f] log $\gamma^\infty_{A,or}$ for ethylene glycol, see also d.
[g] log $\gamma^\infty_{A,or}$ for propanol, see also d.
[h] Calculated as the reciprocal mole fraction of water in octanol at saturation (Table III).
[i] Outlier, set equal to 0.06 in calculations (without consequences to the results) in order to obtain a smooth integration path.
[j] log $\gamma^i_{B,or}$, estimated from the Regular Solution Theory (although the mixture is not very regular) with $\delta_{octanol} = 20$ and $\delta_{water} = 48$ (J$^{\frac{1}{2}}$mol$^{-\frac{1}{2}}$cm$^{-3/2}$).
[k] In the systems with urea, the small amount of urea in the organic phase was neglected. The presented value was taken for all these systems.

in the stationary phase is nearly constant, and the whole of the variation of log $K_{X,B}$ with the composition of the eluents is caused by the variation of the activity coefficient of the solute in the eluent with its composition.

For the co-solvents methanol, ethylene glycol and urea, this result is in agreement with a partition mechanism. These polar co-solvents are scarcely sorbed on octyl-agarose, and thus the solvent properties of the stationary phase are not expected to change when one of these co-solvents is added to the eluent. The small value of $\gamma^\infty_{B,og}$ indicates that the layer of OG closely resembles liquid n-octanol. This could not
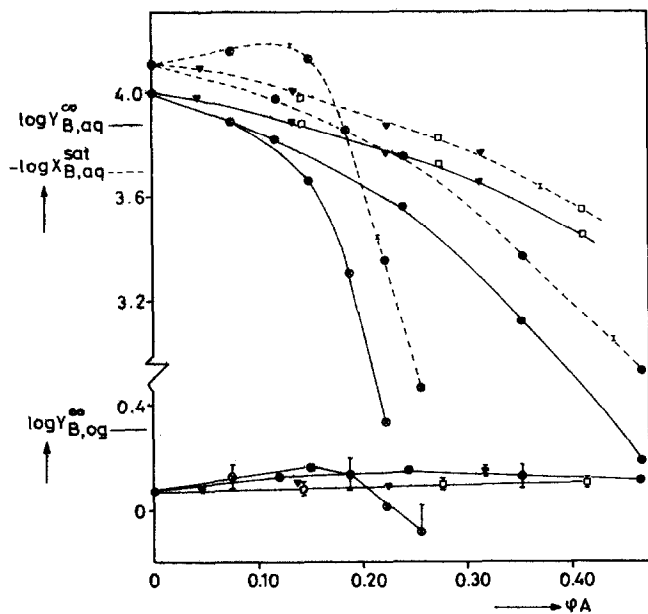
Fig. 6. Values of $\log \gamma_{B,aq}^{\infty} - \log X_{B,aq}^{sat}$ and $\log \gamma_{B,og}^{\infty}$ as a function of φ_A. Errors, estimated from the various contributions, are indicated with bars. Symbols as in Fig. 2.
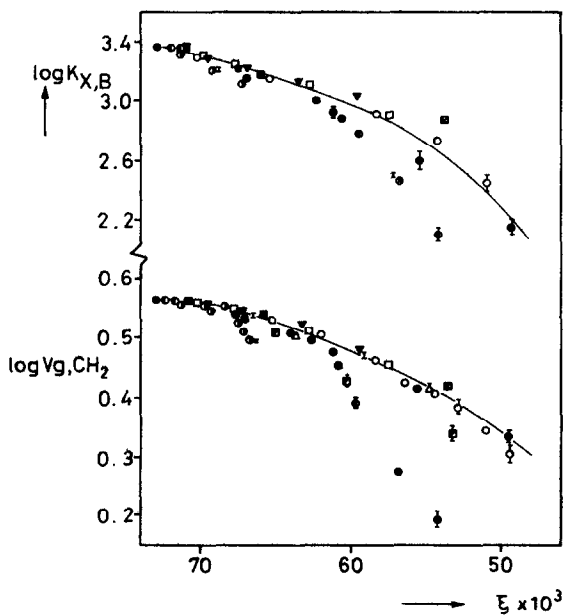

Fig. 7. Log $K_{X,B}$ of heptanol and log V_{g,CH_2} as a function of ξ. Symbols as in Fig. 2.

be expected from the relatively low degree of substitution. However, the compact secondary structure of agarose[23] may lead to high local densities of octyl groups.

It is striking, at first sight, that $\gamma^{\infty}_{B,og}$ is also approximately constant in mixtures of OG groups with propanol and pentanol. This can be explained as follows. The regular solution theory describes the organic layer surprisingly well. Its solubility parameter, δ_{og}, can be expressed as[24,25]:

$$\delta_{og} = (1 - \varphi_{A,og})\delta_{OG} + \varphi_{A,og}\delta_A$$

where δ_{og} denotes the value of δ of the OG groups, δ_A that of the additive and $\varphi_{A,og}$ the volume fraction of the sorbed additive in the stationary phase. Polar additives are scarcely sorbed, and $\varphi_{A,og}$ remains small. Strongly sorbed apolar additives have values of δ_A that are about equal to that of δ_{OG}. In both cases, δ_{og} nearly equals δ_{OG}.

The same results are expected for solutes with a value of δ_B close to that of $\delta_{octanol}$, such as heptanol or nonanol. As a consequence, the value of log V_{g,CH_2} is also approximately independent of the composition of the stationary phase.

For practical purposes, it would be convenient if solution theories could describe the eluent–solute system. However, to our knowledge, they can not. In addition, physical data about the mixtures we used are difficult to obtain. Therefore, we investigated the usefulness of empirical parameters. It resulted in the use of Rekkers f-values combined in a parameter ξ, which is given by $\xi = \sum_i n_i f_i$. Here, n_i denotes the number of moles of a molecular fragment i in 1 ml of eluent. The summation concerns all molecular fragments present in the eluent.

The parameter ξ strongly reduces the nature of the additives in their effect on the values of log $K_{X,B}$ and log V_{g,CH_2}, as can be seen in Fig. 7. High concentrations of propanol, butanol or acetonitrile (additives with large values of $\gamma_{A,aq}$) are outliners.

In contrast with this simple picture, reversed-phase chromatography with alkyl-silica is based on an adsorption mechanism. The influence of a co-solvent such as methanol is more complex, as it is strongly adsorbed itself[26]. As a consequence, the activity coefficient of the solute in the stationary phase is about equal to that in the bulk co-solvent. Thus, the properties of the adsorbent alkyl-silica change from one solvent to another.

CONCLUSIONS

(i) Retention in hydrophobic interaction chromatography of n-alcohols on octyl-Sepharose can be described by a partition model. The stationary phase has solvent properties very similar to those of liquid n-octanol.

(ii) The influence of the additives sucrose, ethylene glycol, urea, methanol, propanol and pentanol on the partition constant in hydrophobic interaction chromatography with octyl-agarose can be explained by a very simple mechanism: the change of the activity coefficient of the solute in the eluent. The activity coefficient in the stationary phase does not change.

(iii) Thus, the sequence of the additives, ordered with respect to their increasing effect on log V_{g,CH_2}, shows a steady decrease of polarity. It reflects the affinity of the eluent mixture for apolar solutes.

(iv) As a consequence, the retention of a solute can be influenced by organic additives in three distinct ways, that can be predicted at least qualitatively: (1) an increased amount of stationary phase, owing to sorption of the additive, leads to an increased retention (pentanol, butanol); (2) a larger size of polar additive molecules with respect to the size of water molecules leads to an increased retention (sucrose); (3) an increase of affinity of the eluent for the solute leads to a decrease of retention. The third way is always mixed with the second [glycol, poly(ethylene glycol), urea] and sometimes with the first (propanol).

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