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DISTRIBUTION EQUILIBRIA OF SOLVENT COMPONENTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHIC COLUMNS AND RELATIONSHIP WITH THE MOBILE PHASE VOLUME

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SUMMARY

Various experimental methods for measuring the adsorption of solvent components from a mixed mobile phase in a liquid–solid chromatographic column are discussed in terms of the surface excess description.

The adsorption from aqueous mixtures of acetonitrile and methanol on to reversed-phase packings was measured. As the derivation of individual isotherms for both solvent components from these data is impossible, two models for the adsorption process, *viz.*, exclusive adsorption of organic modifier and adsorption of a mixture of constant composition, are discussed. The former model is discarded, partly because it cannot explain the occurrence of an azeotropic point. The second model can explain the data over a limited range of mobile phase compositions.

The discussion shows that simple experimental estimates of the mobile phase (zero) volume, such as the retention volumes of a mobile phase disturbance or of isotopically substituted solvent components, are in general unreliable. Their validity under certain conditions can be argued only from the full adsorption isotherm, accepting specific assumptions about the adsorption process.

INTRODUCTION

Several workers have studied the dependence of the capacity factor, k', on the percentage of carbon in an alkyl-modified silica. The general trend is that the retention increases with increasing chain length of the bonded alkyl group. Hemetsberger *et al.*[1] showed that the increase in retention depends on the type of anchoring group in the applied modifying reagent. However, even with homologous modifying reagents of the same type, various workers found different shapes for the plots of k' *versus* the carbon number, n, of the alkyl-modified silica. For example, Roumeliotus and Unger[2] found a linear relationship between k' normalized on the surface area of the adsorbent and

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the total hydrocarbonaceous surface area (HSA) of the alkyl chain bonded to the silica. On the other hand, Hennion *et al.*[3,4] reported a linear relationship for polyhydroxybenzenes as solutes, but for alkylbenzenes the relationship became linear only for a plot of log k' *versus* chain length. Berendsen[5] found a saturation effect when k' was plotted against chain length. The chain length corresponding to the point beyond which the increase in k' is small, zero or even negative increases when larger solute molecules are used. Berendsen explained this in terms of the possibility of penetrating between the alkyl chains of the adsorbent. When a solute molecule can penetrate completely between the alkyl chains of the modified silica, a further elongation of the alkyl chain is not effective. His results fit well with those obtained by Lochmüller and Wilder[6].

In order to obtain more insight into the influence of the chain length and the nature and concentration of the organic modifier, it is interesting to study the adsorption isotherms for some modifiers on reversed-phase (RP) materials. The problem of the proper definition and experimental determination of the mobile phase volume arises in the determination of adsorption isotherms. Only a few workers[5,7-9] have dealt with this problem. This is not surprising, as for practical applications the "true" mobile phase volume is not of great importance. The influence of the mobile phase volume on the calculated capacity factor is illustrated in Table I. The capacity factor is calculated using the equation

$$k'_i = \frac{t_{Ri} - t_{R0}}{t_{R0}} = \frac{V_{Ri} - V_{R0}}{V_{R0}} \tag{1}$$

where t_R is the retention time, V_R is the retention volume, the subscript i refers to the solute and the subscript 0 to the unretained component. When $k' \geqslant 5$ the error is proportional to the capacity factor, which in most instances is not of much importance.

TABLE I

INFLUENCE OF THE RETENTION TIME OF THE "UNRETAINED" COMPONENT ON THE CALCULATION OF THE CAPACITY FACTOR, k', WITH EQN. 1

t_{Ri}	t_{R0} *(sec)*		
	55	*60*	*65*
65	0.18	0.08	0
90	0.64	0.50	0.38
120	1.18	1.00	0.85
360	5.54	5.00	4.54
660	11.00	10.00	9.15
1260	21.90	20.00	18.4

However, for the study of retention mechanisms it is generally advantageous to vary the conditions over wide ranges such that a significant part of the data will correspond to low capacity factors. The values then can only be compared with the higher values and interpreted physically in a correct way if a clear definition of the mobile phase volume is given and if a corresponding and accurate experimental determination is used.

A rather extreme example of the importance of the determination of mobile phase volume is provided by ref. 10. The retention data reported there can be given an entirely different physical interpretation (solvent interaction and competition) on changing the mobile phase volume by about 10%.

THEORETICAL

The methods for the determination of mobile phase volumes are directly related to the distribution equilibria of the solvents used in liquid chromatography (LC). When the term mobile phase volume, V^m, is used, the assumption is made that a sharp boundary exists between the two phases (Fig. 1a). It is more likely, however, that there is a gradual change in properties on going from the mobile to the stationary phase (Fig. 1b). The position of the boundary (dividing plane) is not a major problem for a liquid–liquid system, because the thickness of the stationary liquid phase is usually much larger than the thickness of the interface. However, for an adsorption system, the situation generally is different. As the surface-to-volume ratio is extremely high, the choice of the location of the boundary is very important. A change in the thickness of the adsorption layer by 0.1 nm corresponds, for example for an adsorbent with a specific surface area of 400 m^2/g, to 40 μl. This is already 2–3% of the mobile phase volume (Table II).

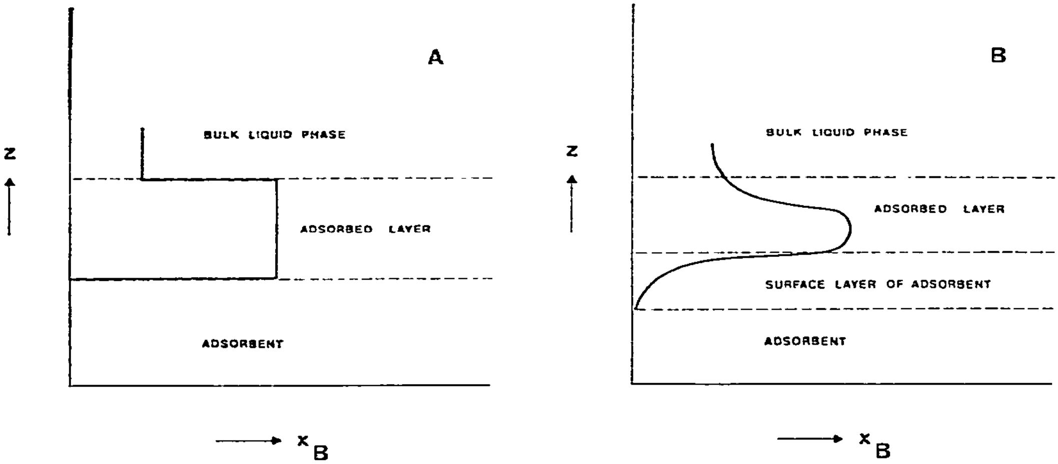

Fig. 1. Schematic representation of the molar fraction of B as a function of the distance from the adsorbent surface (z) for (a) a stepwise change and (b) a gradual change in x_B.

To avoid in the first instance the problem of defining the mobile phase volume and the dimensions of the adsorbed layer, we can treat the adsorption in terms of the excess compared with the mobile phase. The advantage of the use of surface excess concentrations is the possibility of defining the excess in terms of experimental quantities without the introduction of any assumptions of a model[11]; this approach is common practice in physico-chemical discussions of adsorption from the liquid phase. Starting with this treatment of adsorption data we compare various methods of estimating the mobile phase volume.

TABLE II

ILLUSTRATION OF THE INFLUENCE OF CHANGES IN THE POSITION OF THE BOUNDARY BETWEEN THE MOBILE PHASE AND THE ADSORBED LAYER ON THE CAPACITY FACTOR, k'

Specific surface area of adsorbent, 400 m^2/g; 0.1 nm ≈ 40 μl.

k'		
$V^m = 1460$ μl	$V^m = 1500$ μl	$V^m = 1540$ μl
0.54	0.5	0.46
1.05	1.0	0.95
5.16	5.0	4.84
10.3	10.0	9.7
20.6	20.0	19.5

In the thermodynamics of adsorption[12], the surface excess concentration of component B, $\Gamma_B^{(n)}$, is commonly used; $\Gamma_B^{(n)}$ is the difference, per unit area of solid–liquid interface, between number of moles of component B in the system and the amount of B in a reference system, containing the same total number of moles n^1 and in which the mole fraction of B, x_B^1, equal to that in the bulk liquid in the real system, is maintained throughout:

$$\Gamma_B^{(n)} = \frac{n_B - n^1 \cdot x_B^1}{A_s} \tag{2}$$

where x_B^0 is the molar fraction before the adsorbent is added, A_s is the surface area of the adsorbent and x_B is the molar fraction of B in the system, before the adsorbent is added. In experimental work the equation

$$\Gamma_B^{(n)} = \frac{n^1(x_B^0 - x_B^1)}{A_s} = \frac{n^1 \Delta x_B^1}{A_s} \tag{3}$$

is often used, where x_B^0 is the molar fraction before the adsorbent is added.

As discussed in various texts (*e.g.*, ref. 13), it is impossible to derive individual isotherms for the solvent components from measurements of the extent of adsorption, Γ, *i.e.*, it is possible to explain the experimental results with differing models, *e.g.*, monolayer of pure B or multiple layers of a mixture.

As a model for the real system one can consider a layer containing n^s moles, with molar fraction x_B^s of component B, in equilibrium with the mobile phase (Fig. 1a). The experimental quantity $\Gamma_B^{(n)}$ is related to the parameters of this model by

$$\Gamma_B^{(n)} = \frac{n^s(x_B^s - x_B^1)}{A_s} \tag{4}$$

When only molecules of component B are adsorbed ($x_B^s = 1$)

$$n^s = n_B^s = \frac{\Gamma_B^{(n)} A_s}{1 - x_B^1} \tag{5}$$

However, it should be noted that in many instances, the assumption $x_B^s = 1$ is unrealistic.

In chromatographic practice expressions based on concentrations are more usual and more convenient. The surface excess concentration, $\Gamma_B^{(v)}$, of B, based upon concentrations, is defined as the difference, per unit area of solid–liquid interface, of the number of moles n_B in the system and the amount of B in a reference system containing the same volume, V^1, of liquid and in which a constant concentration, equal to that in the bulk liquid in the real system, is maintained throughout:

$$\Gamma_B^{(v)} = \frac{(n_B - V^1 \cdot c_B^1)}{A_s} \tag{6}$$

V^1 is defined as the total volume of the system, but excluding the volume of the adsorbent. In chromatography, therefore, V^1 should be taken as the difference between the total volume of the column and that of the packing, where it is assumed that the latter is not changed by the adsorbtion process. The surface excess based upon concentration introduces an uncertainty[13], because volume changes may occur on adsorption and the partial molar volumes may also vary with composition.

Insufficient wetting leads to incomplete pore filling, and V^1 is not constant. In other words, the additivity of volumes is no longer valid and the concentration-based excess is not suitable. In that event the mass- or molar-based excess description still furnishes an unambiguous description of the system. However, from the experimental point of view, this is an awkward expedient, as it would require the determination of input and output mass to the column.

There are various ways of estimating V^1 values: helium pycnometry, calculation of the volume of the packing and subtraction from the total volume of the column tube[5]; or pycnometry with a liquid[10]. For convenience, the last method has been applied using pure acetonitrile and methanol.

Assuming (see remarks below) that n_B and V^1 can be determined with sufficient accuracy and precision, the surface excess can again be related to the parameters of a model in which the adsorbed phase has a homogeneous composition. With the added assumption that the molar volumes in the adsorbed state are the same as those in the liquid state, from eqn. 6 it can be derived that

$$n_B^s = \frac{\Gamma_B^{(v)} A_s c_B^s}{c_B^s - c_B^m} \tag{7}$$

with $x_B^s = 1$:

$$n^s = n_B^s = \frac{\Gamma_B^{(v)} A_s \varrho_B/M_B}{\varrho_B/M_B - c_B^m} \tag{8}$$

where c_B^s and c_B^m are the concentrations in the stationary phase and mobile phase, respectively, and ϱ_B and M_B are the specific density and the molecular weight of B, respectively.

For the volume of the adsorbed layer it follows that

$$V^s = V_B^s = n_B^s \cdot \frac{M_B}{\varrho_B} \tag{9a}$$

and for the volume of the mobile phase (see Fig. 2)

$$V^m = V^l - V^s \tag{9b}$$

The model (Fig. 1a) in which a sharp boundary between two phases exists is of only limited physical reality, which is obvious if one considers that the dimensions of the solute and solvent molecules are of the same order of magnitude as the thickness of the interfacial layer. It is therefore not surprising that different sample molecules, all of which can be expected to be unretained from their polarity, can penetrate to different extents into the transition region and yield V^m estimations that differ significantly.

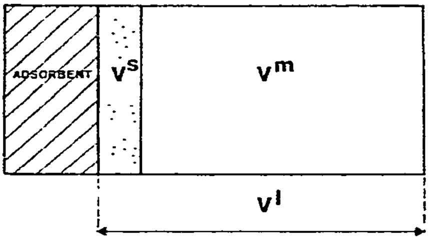

Fig. 2. Schematic representation of the adsorption model, assuming a stepwise change in the properties of the liquid on going from the mobile to the adsorbed phase.

Further complications arise, of course, as a result of size or electrostatic exclusion of molecules from the pores. However, in this discussion we shall neglect these effects.

The question of the penetration of the solvent molecules into the alkyl layer can be disregarded in the measurement of the excess (provided that the assumption of the additivity of volumes and constant partial molar volumes for all compounds involved remains valid). In the interpretation of the data this phenomenon may, of course, play an important role. However, the interpretation is speculative anyhow, as indicated above.

DISCUSSION OF EXPERIMENTAL METHODS

The difficulty of defining and interpreting of the mobile phase volume, V^m, is unavoidable, as it is intrinsic to the special conditions in high-performance liquid chromatography (HPLC).

However, apart from this aspect, it is useful to consider the various experimental methods[5,7–10] for determining V^s and V^m in relation to the surface excess description (which is freer from assumptions) and the model described in eqns. 8 and 9. The following methods are considered: (i) batch method; (ii) breakthrough method; (iii) minor disturbance method; (iv) method with the use of isotopically labelled components; and (v) use of other "unretained" compounds.

Batch method

With this static method, the change in the concentration of the solvent when the adsorbent is added to the solution is measured. The excess amount can be calculated with eqns. 2 and 6. For RP-HPLC this method[9] is not very attractive because the high concentrations lead to a very small difference in the concentration before and after adsorption. These are difficult to measure with sufficient precision, as the phase ratio in a batch experiment is always smaller than that in a column.

Breakthrough method

In this method[10,14] the composition of the solvent, with respect to B, is changed stepwise. From the solvent passed through the column between the solvent change and the step on the recorder, the amount adsorbed can be calculated. With the retention volume for the breakthrough from zero to concentration $c_{B,1}$, V^{R1}_{bt}, the number of moles B in the column, n_B, can be calculated:

$$n_{B,1} = V^{R1}_{bt}\, c_{B,1} \tag{10}$$

The excess amount based on concentrations is

$$n^{\sigma,v}_{B,1} = A_s \Gamma^{(v)}_{B,1} = (V^{R1}_{bt} - V^{l})c_{B,1} \tag{11}$$

The surface excess concentration is:

$$\Gamma^{(v)}_{B,1} = \frac{n^{\sigma,v}_{B,1}}{A_s} \tag{12}$$

More generally, for a step from concentration $c_{B,n}$ to concentration $c_{B,n+1}$ for component B, the number of moles of B in the column is

$$n_{B,n+1} = V^{R(n+1)}_{bt} \Delta c_B + n_{B,n} \tag{13}$$

where $\Delta c_B = c_{B,n+1} - c_{B,n}$, $n_{B,n}$ and $n_{B,n+1}$ are the total number of moles of B in the column before and after the step and $V^{R(n+1)}_{bt}$ is the retention volume of the step. Hence

$$n^{\sigma,v}_{B,n+1} = [V^{R(n+1)}_{bt} - V^{l}]\Delta c_B + n^{\sigma,v}_{B,n} \tag{14}$$

With the assumption, that in the adsorbed layer $x^s_B = 1$, the volume of the adsorbed layer can be calculated to be

$$V^s = n^{\sigma,v}_{B,n+1} \cdot \frac{1}{\varrho_B/M_B - c^m_{B,n+1}} \tag{15}$$

where ϱ is the density and M is the molecular weight, and the volume of the mobile phase as:

$$V^m = V^l - V^s \tag{16}$$

A special problem occurs with alkyl-modified column materials and aqueous mobile phases: here the wetting characteristics may invalidate the assumption that V^l is a constant. The same holds for the following method.

Minor disturbance method

In the minor disturbance method[15,16], a small disturbance to the solvent composition is made at the top of the column. The eluted volume between applying the disturbance and the occurrence of the corresponding signal on the detector is a measure of the change in the amount adsorbed resulting from the concentration change in the solvent. When there is a proportional relationship between the excess surface concentration $\Gamma_B^{(v)}$ and the concentration of B in the mobile phase, the retention volume of a small disturbance of B in this region is

$$V_{R_{\mathrm{md}}} = \frac{n_B}{c_B^m} \tag{17}$$

where n_B is the total amount of component B in the system and c_B^m is the concentration of B in the bulk liquid or mobile phase. In terms of surface excess the retention volume in that instance is

$$V_{R_{\mathrm{md}}} = V^1 + \frac{(n_B - V^1 \cdot c_B^m)}{c_B^m} = V^1 + \frac{n_B^{\sigma,v}}{c_B^m} \tag{18}$$

However, for the case of a non-linear relationship between the surface excess and the concentration of B in the bulk phase, eqns. 17 and 18 are no longer valid. In that event we have to use the derivative of the isotherm as described, *e.g.*, by Huber and Gerritse[15]. A sufficiently small change in the concentration of B causes a proportional change in the surface excess, which results in

$$V_{R_{\mathrm{md}}} = \frac{\mathrm{d}n_B^{\sigma,v}}{\mathrm{d}c_B^m} = V^1 + \frac{\mathrm{d}\Gamma_B^{(v)} A_s}{\mathrm{d}c_B^m} \tag{19}$$

With the assumption of an adsorbed layer with $x_B^s = 1$ and a corresponding mobile phase volume V^m, this becomes

$$V_{R_{\mathrm{md}}} = V^m + \frac{\mathrm{d}a_B}{\mathrm{d}c_B^m} \cdot A_s \tag{20}$$

where a_B is the surface concentration of B in the stationary phase. It should be noted that a disturbance of component B in a binary solvent system is the same as a disturbance of the other component A in the solvent system with the opposite sign. Thus, injection of a small amount of B or A gives the same retention volume. Eqns. 19 and 20 demonstrate that the observed retention volume on injection of B or A results in a bad estimate of the mobile phase volume[2,7,9], as it depends strongly on the slope of the adsorption isotherm.

When the retention volumes of the eluent disturbance, $V_{R_{\mathrm{md}}}$, are measured for the whole concentration range of component B, it is possible to calculate the adsorption isotherm by numerical integration of eqn. 19. This equation can be rearranged into

$$\frac{\mathrm{d}\Gamma_B^{(v)} A_s}{\mathrm{d}x_B^m} = (V_{R_{\mathrm{md}}} - V^1) \cdot \frac{\mathrm{d}c_B^m}{\mathrm{d}x_B^m} \tag{21}$$

which is convenient when molar fractions are used. $V_{R_{md}}$ and V^1 are measured, and dc_B^m/dx_B^m values for the binary solvent can be calculated with assumption of the additivity of the volumes (no contraction) or can be taken from the literature for most solvent combinations (*e.g.*, refs. 17 and 18).

Method with the use of isotopically labelled components

The use of ^2H_2O has become popular for the determination of the mobile phase volume $(V^m)^{7,9,19}$. However, as will be discussed in this section, the method has several disadvantages.

When a labelled form of an eluent component is injected, a different value for the retention volume is found than the value found in the case of the eluent disturbance in the previous section. Of course, when the labelled compound is injected in such a way that the total concentration of B is also disturbed, a disturbance will migrate through the column in the way described above. However, the elution profile of the isotope does not coincide with this peak, and in principle we can see two peaks (this phenomenon has led chromatographers to exaggerated claims about isotopic fractionation by LC). We can remove the disturbance by adjusting the injected concentration in such a way that the total concentration of B is not disturbed. In this instance, or when detection is selective to the isotope, we observe only one peak. The retention of this isotope is a measure of the total amount of compounds present in the column into which the isotope distributes in a time that is short compared with the retention time and provided that the isotope effect in the distribution constants can be neglected. This amount generally includes all (ad)sorbed amounts of B and compounds with which rapid isotopic exchange occurs. The latter effect is especially important with deuterated or tritiated hydroxyl groups, which give rise to exchange with water and other molecules containing hydroxyl groups.

The retention volume V_{RB*} corresponds to the species velocity[20] and is given by*

$$V_{RB*} = \frac{n_{exch}}{c_B} \tag{22}$$

where n_{exch} is the total amount in moles of exchanging compounds in the column and c_B is the total concentration of B.

If labelled B is injected and no exchange with other compounds occurs, the measurement of V_{R_B*} is a convenient way of finding the isotherm. Fig. 3 illustrates this and also the difference from the minor disturbance method described above.

For C^2H_3CN in acetonitrile–water and C^2H_3OH in methanol–water systems, the situation is relatively simple as no isotopic exchange occurs with other compounds and the total amount of B can be found without complications with eqn. 22. However, for ^2H_2O, H^2HO or CH_3O^2H an exchange with all protons bound to oxygen atoms occurs. A further complication arises from the exchange with accessible silanol groups, which in our experience is also "rapid". As a consequence the retention volume of H^2HO, being the same as for CH_3O^2H, is determined by the total effect of water, methanol and accessible silanol groups.

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* In the special case where isotopic exchange occurs, "species" should be understood as "atoms".

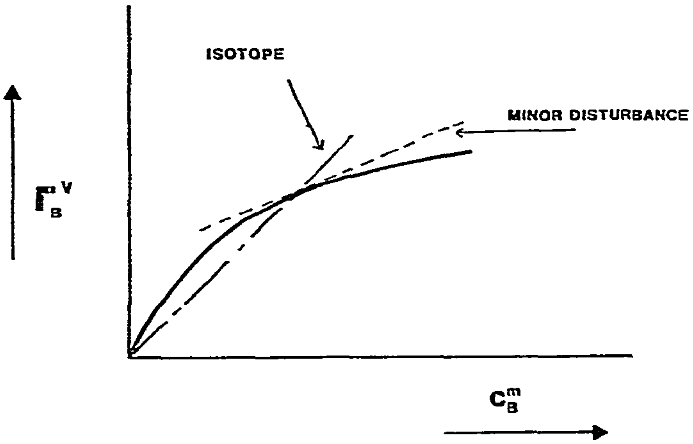

Fig. 3. Illustration of the difference in the measured slope of the distribution isotherm between the minor disturbance and the method where an isotopically labelled solvent component is injected.

The equilibrium reaction for H^2HO in methanol–water systems has, on statistical grounds, an equilibrium constant $K = 0.5$. This value has been experimentally verified by Halford and Pecherer[21]. From the equilibrium reaction, it therefore follows that

$$\frac{c_{CH_3O^2H}}{c_{CH_3OH}} = 0.5 \cdot \frac{c_{H^2HO}}{c_{H_2O}} \tag{23}$$

Little is known about the equilibrium constant for the exchange with the residual silanol groups. However, comparison of numerous comparable systems[22] suggests that a significant deviation from the statistical prediction occurs only if one of the phases is a gas. Assuming the equilibrium constant to be 0.5, eqn. 17 becomes

$$V_{R_{H^2HO}} = V_{R_{CH_3O^2H}} = \frac{n_{H_2O} + 0.5 \cdot n_{CH_3OH} + 0.5 \cdot n_{SiOH}}{c_{H_2O}} \tag{24}$$

When the retention volume of H^2HO or CH_3O^2H and the weight of the liquid in the column, W^1, are known, the amount of H_2O and methanol (MeOH) can be calculated:

$$n_{H_2O} = \frac{V_{R_{H^2HO}}[c_{H_2O} + 0.5 \cdot c_{MeOH}] - 0.5 \cdot \frac{W^1}{M_{MeOH}}}{\left[1 - 0.5 \cdot \frac{M_{H_2O}}{M_{MeOH}}\right]} - 0.5 n_{SiOH} \tag{25}$$

$$n_{MeOH} = W^1 - n_{H_2O} \tag{25a}$$

where M is molecular weight. With eqn. 6 the surface excess can be calculated.

Use of other "unretained" compounds

Other compounds have also been used for the determination of the mobile phase volume. The possibilities are discussed extensively by Berendsen[5]. The drawback of the methods presented by Berendsen is that their choice cannot be justified in a physical picture.

EXPERIMENTAL

Apparatus

The liquid chromatograph was constructed from custom-made and commercially available components and consisted of a reciprocating membrane pump (DMP 515; Orlita, Giessen, G.F.R.), a flow-through Bourdon-type manometer as damping device, a high-pressure sampling valve (Model 7125; Rheodyne, Berkeley, CA, U.S.A.) and a refractometer detector (Model R-401; Waters Assoc., Milford, MA, U.S.A.). In all experiments stainless-steel 316 columns (length 25 cm, I.D. 4.6 mm and O.D. $\frac{1}{4}$ in.) were used. The chromatograms were recorded on a linear potentiometric recorder (Kompensograph III; Siemens, Karlsruhe, G.F.R.).

Chemicals and materials

In all experiments doubly distilled water and organic solvents of analytical-reagent grade were used. ^2H_2O, C^2H_3CN, CH_3O^2H (Aldrich, Beerse, Belgium) and C^2H_3OH (MSD, Quebec, Canada) were used as solutes. LiChrosorb RP-2, RP-8 and RP-18 (10 μm) (Merck, Darmstadt, G.F.R.) were used as column materials.

Procedures

The columns were packed by means of a balanced slurry technique, using 5% methanol in tetrachloromethane[23] as slurry solvent. For the determination of the geometrical zero volume of the column, V_0, the column was dried with dry nitrogen at 140°C overnight. The column volume was determined by pycnometry using methanol, acetonitrile and *n*-heptane as solvents.

The retention volumes of the solutes were calculated from the first moments and corrected for the dead volume between the injection valve and the detector.

The sample concentrations were chosen as low as possible while maintaining a suitable signal-to-noise ratio.

The solvent mixture compositions were adjusted by weighing the constituents.

For the numerical integration required in the minor disturbance method, a procedure described by Smit *et al.*[24] was found to be convenient. An improved trapezoidal rule was used and the derivatives required in the integration procedure were determined from a quadratic fit through three points.

RESULTS AND DISCUSSION

General

For the experimental determination of adsorption isotherms we relied on two methods, *viz.*, the minor disturbance method and the isotope method. The breakthrough method was discarded because it is expected to give the same results as the minor disturbance yields after integration and it was less practical and less precise. Fig. 4 shows the correlation between the results obtained with the minor disturbance method and numerical integration, and the isotope method, with C^2H_3CN and H^2HO as solutes, using RP-2, RP-8 and RP-18 as column materials and acetonitrile–water as the mobile phase. It can be seen that there is a very good correlation between the two methods. Results for methanol–water, using C^2H_3OH in the isotope method, are shown in Fig. 5. For RP-2 sufficient precision could not be obtained; for RP-18 the

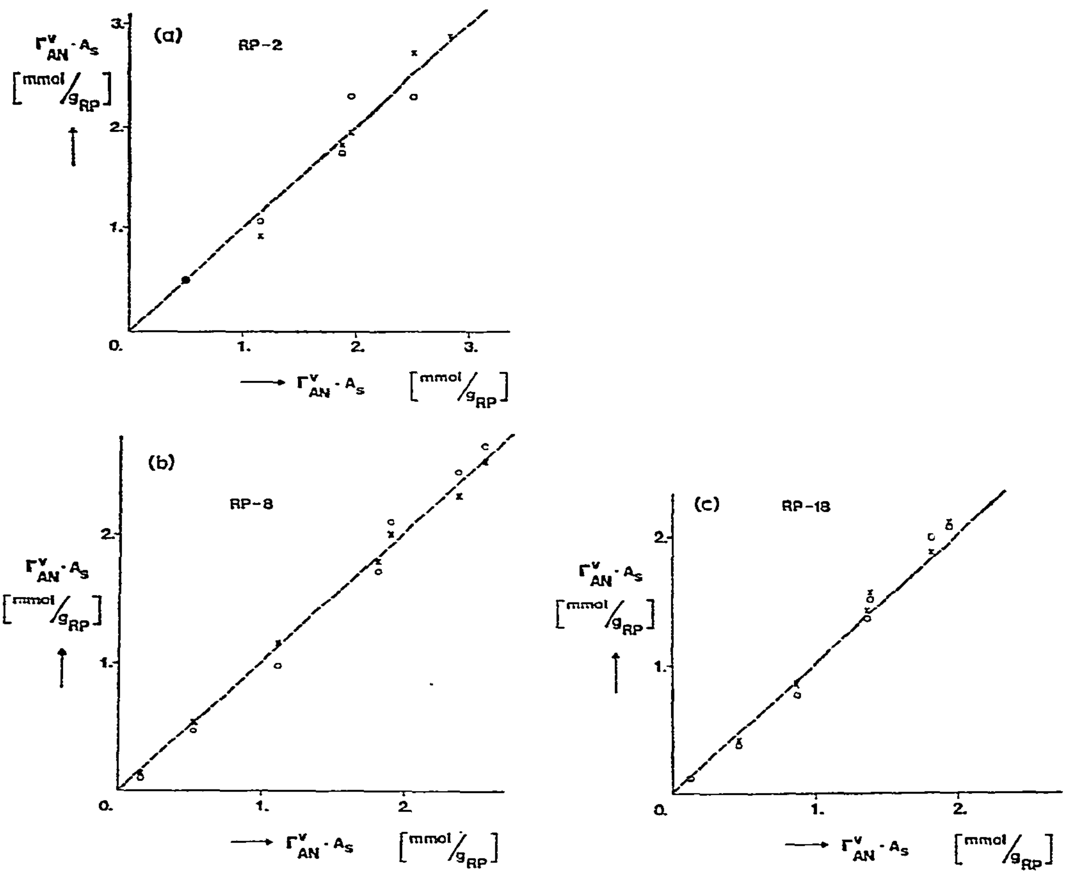

Fig. 4. Correlation of the experimental results for the surface excess amount of acetonitrile in aqueous mixtures with the minor disturbance method (abscissa) and the method with C^2H_3CN (×) and ^2H_2O (○) (ordinate) on (a) RP-2, (b) RP-8 and (c) RP-18 at 22°C.

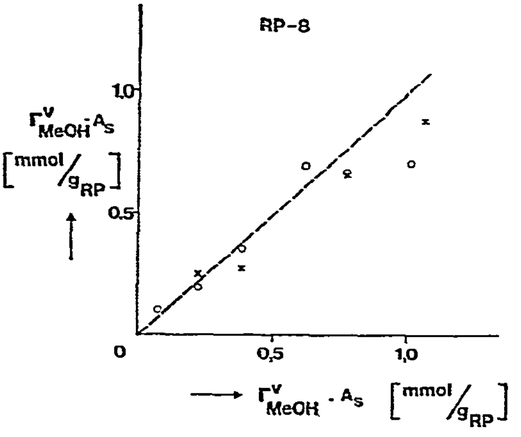

Fig. 5. Correlation of the experimental results for the surface excess amount of methanol in aqueous mixtures with the minor disturbance method (abscissa) and the method with C^2H_3OH (×) and ^2H_2O (○) (ordinate) on RP-8 at 22°C.

number of points was too small. In both instances the elimination of the eluent peak was hardly possible. Also for the RP-8 material this problem leads to a decrease in precision, and consequently the agreement between the isotope method and the minor disturbance method is worse in Fig. 5 than in Fig. 4b. When H^2HO or CH_3O^2H are used as tracer, residual silanol groups present on the adsorbent influence the retention volume. In those instances we used a correction term as described in eqn. 24. The surface concentrations of residual silanol groups, α_{OH}, were determined from a ^2H_2O experiment with low H_2O concentrations ($\leqslant 1\%$) and appeared to be 1.8–2.0 μmol/m^2. The possibility of the precise and accurate determination of α_{OH} with this method is under study.

With the minor disturbance method, the term $V_{R_{md}} - V^1$ is sensitive to changes in V^1 when the difference is smaller than about 50 μl. If the amount adsorbed is small, as with methanol–water systems, this problem is more serious. The geometrical volume of the column, V^1, is fairly constant when solvents such as methanol, acetonitrile and *n*-heptane are used. However, for mixtures that contain a high proportion of water ($>80\%$, v/v) the geometrical volume changes. The high surface tension of the solvent prevents its penetration into the very small pores. For this reason the integration procedure was started from pure organic solvent.

Adsorption in acetonitrile–water systems

The adsorption isotherms were measured for acetonitrile–water systems on RP-2, RP-8 and RP-18 and the results are given in Tables III–V. In Fig. 6a–c the surface excess amounts are plotted against the molar fraction. Also plotted are the amounts of acetonitrile adsorbed, assuming exclusive adsorption of acetonitrile (AN) ($x^s_{AN} = 1$). The plots show the great importance of the adsorption effect. The large changes in the excesses as a function of the mobile phase composition are also remarkable. However, the interpretation of the results by assuming an adsorbed layer consisting of acetonitrile exclusively (7th, 8th and 9th columns in Tables III–V; dashed lines in Fig. 6) are not tenable. First, the decrease in the amount adsorbed at the higher percentages of acetonitrile is very unlikely. Secondly, this interpretation leads to a negative amount of acetonitrile adsorbed for mobile phase compositions near 100%. Indeed, it can be seen in Fig. 6 that the three curves for $\Gamma^{(v)}$ show azeotropic points, which indicate concentrations where the preferential adsorption of one compound changes to that for the other compound. This indicates that water is also adsorbed. It follows that the exclusive adsorption of acetonitrile is not likely.

As pointed out in the Introduction, it is not possible to derive individual isotherms for the two components from the excess isotherm with certainty. However, for the special case, where the adsorption isotherm displays a linear section, Schay[13] pointed out that the linear section is probably due to the constant composition of the adsorbed phase in that part of the curve. As Fig. 7 shows, such a linear section between 30 and 60 mol-% (50–80%, v/v) of acetonitrile, it is useful to calculate the corresponding size and composition of the adsorbed phase. With some substitutions for Δx, eqn. 3 can be written[12] as

$$A_s\Gamma^{(n)}_{AN} = \frac{n_0 \Delta x_{AN}}{A_s} = n^s_{AN}(1 - x^1_{AN}) - n^s_{H_2O} \cdot x_{AN} \tag{26}$$

When the composition of the adsorbed phase is constant, this results in a straight line.

TABLE III

EXPERIMENTAL DATA OBTAINED FOR AN ACETONITRILE–WATER SYSTEM ON RP-2 AT 22°C

Mobile phase composition		*Surface excesses (mmol/g)*				*With assumption $x^s_{AN} = 1$*			*Retention volumes normalized on empty column volume*		
% (v/v)	*Mol-%*	$\Gamma^{(v)}_{AN} A_S$	$\Gamma^{(n)}_{AN} A_S$	$\Gamma^{(v)}_{AN} A_S$		*Amount adsorbed*		*Mobile phase porosity, ε_m*	$V_{R_{C^2H_3CN}}$	$V_{R_{^2H_2O}}$	$V_{R_{md}}$
		Minor disturbance		*Isotope, C^2H_3CN*	*Isotope, ^2H_2O*	n^s_{AN} *(mmol/g)*	V^s_{AN} *(μl/g)*				
						Minor disturbance					
20	7.75	1.95	2.24	1.96	2.31	2.24	127	0.671	1.000	0.670	0.948
35	15.7	2.82	3.70	2.88	–	3.70	230	0.618	0.950	–	0.759
50	26.4	2.51	3.83	2.70	2.31	3.83	272	0.596	0.875	0.626	0.582
60	34.8	1.87	3.18	1.83	1.76	3.18	255	0.605	0.814	0.636	0.542
70	45.4	1.13	2.17	0.93	1.05	2.17	208	0.629	0.770	0.669	0.550
80	58.4	0.50	1.08	0.50	0.49	1.08	136	0.666	0.752	0.716	0.590
90	75.8	0.04	0.09	−0.15	0.05	0.09	20	0.725	0.731	0.818	0.652
99	97.1	−0.03	−0.06	–	$\equiv -0.03$	–	–	–	0.730	1.69	0.836
100	100	0	0	0	0	0	–	–	0.733	–	0.870

TABLE IV

EXPERIMENTAL DATA OBTAINED FOR AN ACETONITRILE–WATER SYSTEM ON RP-8 AT 22°C

Mobile phase composition		*Surface excesses (mmol/g)*				*With assumption $x^s_{AN} = 1$*			*Retention volumes normalized on empty column volume*		
		$\Gamma^{(v)}_{AN} A_S$	$\Gamma^{(n)}_{AN} A_S$	$\Gamma^{(v)}_{AN} A_S$		*Amount adsorbed*		*Mobile phase porosity, ε_m*			
% (v/v)	*Mol-%*	*Minor disturbance*		*Isotope, C^2H_3CN*	*Isotope, ^2H_2O*	*n^s_{AN} (mmol/g)*	*V^s_{AN} (μl/g)*		$V_{R_{C^2H_3CN}}$	$V_{R_{^2H_2O}}$	$V_{R_{md}}$
						Minor disturbance					
20	7.75	1.89	2.17	2.01	2.08	2.35	123	0.669	0.961	0.670	0.865
35	15.14	2.56	3.33	2.56	2.70	3.92	205	0.632	0.897	0.635	0.761
50	26.45	2.38	3.64	2.29	2.48	4.95	259	0.608	0.828	0.613	0.607
60	34.8	1.81	3.08	1.78	1.70	4.72	247	0.613	0.792	0.632	0.563
70	45.4	1.11	2.12	1.15	0.98	3.89	203	0.633	0.762	0.660	0.570
80	58.4	0.52	1.11	0.53	0.47	2.67	140	0.661	0.740	0.693	0.608
90	75.8	0.15	0.27	0.13	0.10	0.94	49	0.702	0.728	0.754	0.658
99	97.1	−0.02	−0.02	0	0	–	–	–	0.724	1.25	0.777
100	100	0	0	0	0	0	0	0.724	0.724	–	0.7

TABLE V

EXPERIMENTAL DATA OBTAINED FOR AN ACETONITRILE–WATER SYSTEM ON RP-18 AT 22°C

Mobile phase composition		*Surface excesses (mmol/g)*				*With assumption $x^s_{AN} = 1$*		*Mobile phase porosity, ε_m*	*Retention volumes normalized on empty column volume*		
		$\Gamma^{(v)}_{AN} A_S$	$\Gamma^{(n)}_{AN} A_S$	$\Gamma^{(v)}_{AN} A_S$		*Amount adsorbed*					
% (v/v)	*Mol-%*	*Minor disturbance*		*Isotope, C^2H_3CN*	*Isotope, ^2H_2O*	n^s_{AN} *(mmol/g)*	V^s_{AN} *(µl/g)*		$V_{R_{C^2H_3CN}}$	$V_{R_{^2H_2O}}$	$V_{R_{md}}$
						Minor disturbance					
20	7.75	1.38	1.59	1.56	1.50	1.72	90	0.623	0.879	0.626	0.793
35	15.7	1.94	2.54	2.10	2.07	3.01	157	0.589	0.826	0.592	0.700
50	26.5	1.81	2.76	1.88	1.99	3.75	196	0.568	0.766	0.571	0.568
60	34.8	1.37	2.34	1.42	1.35	3.59	187	0.574	0.730	0.591	0.535
70	45.4	0.87	1.67	0.85	0.77	3.06	160	0.588	0.701	0.617	0.550
80	58.4	0.46	0.98	0.40	0.36	2.36	123	0.606	0.682	0.649	0.565
90	75.8	0.12	0.29	0.11	0.10	1.19	62	0.638	0.684	0.706	0.606
99	97.1	0.00	0.00	0.00	0	–	–	0.669	0.669	1.27	0.678
100	100	0	0	0	0	0	0	0.669	0.669	–	0.71

From extrapolation in Fig. 6, according to eqn. 26, to $x^1_{AN} = 0$ and $x^1_{AN} = 1$, values for n^s_{AN} and $n^s_{H_2O}$ can be found for this model. The results are presented in Table VI.

It is useful to consider the consequences that such a (hypothetical) composition would have for the chromatographic distribution of solutes. The polarity of the adsorbed phase probably is not only a function of the nature of the adsorbent, determined in the present instance by the surface concentration of the alkyl groups, but also a function of the composition of the adsorbed liquid. To illustrate this, the composition of the stationary phase was calculated, considering the alkyl chains as a part of the stationary phase having liquid properties. The surface area of the silica before

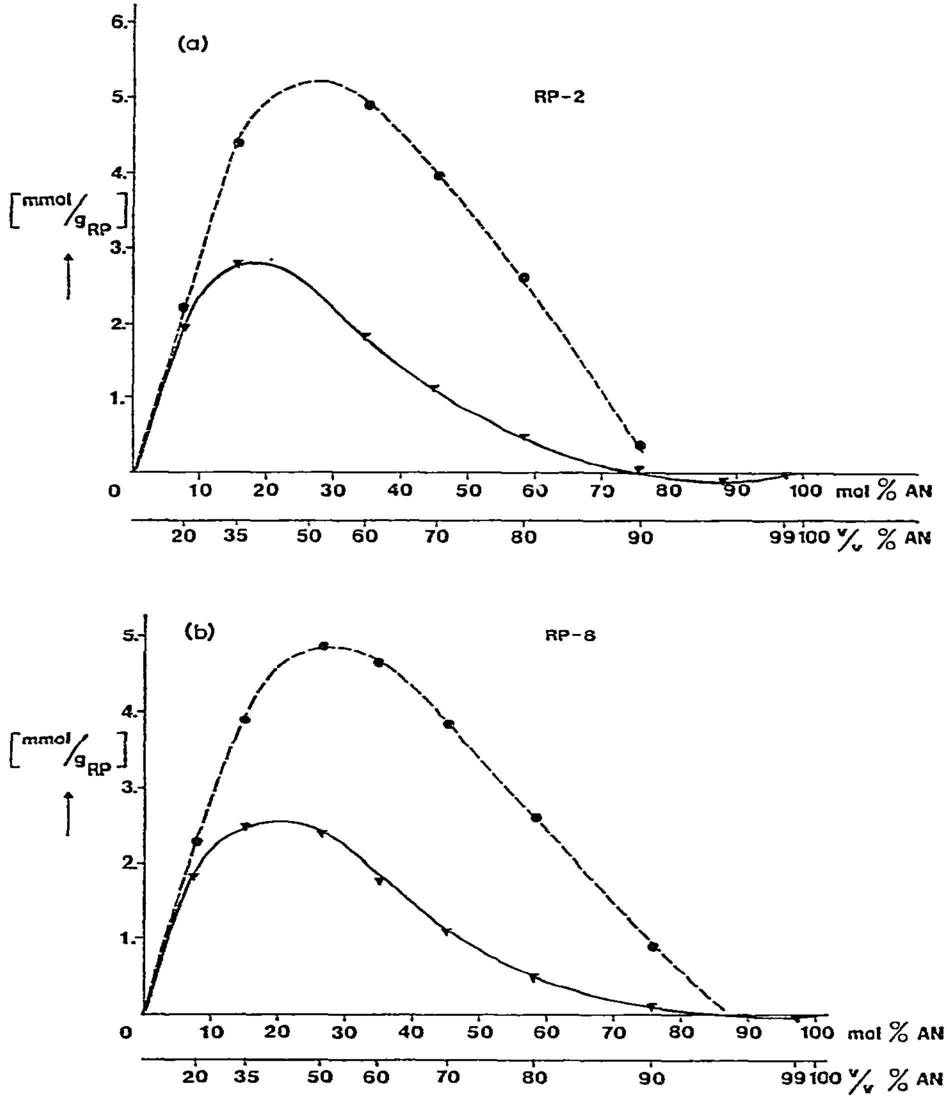

Fig. 6.

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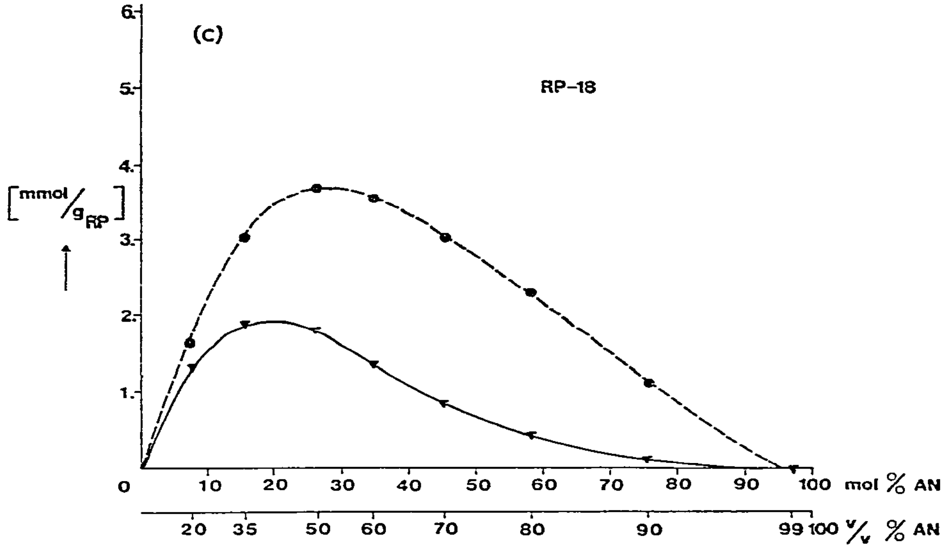

Fig. 6. The surface excess amount (▲) and the adsorbed amount assuming $x^s_{AN} = 1$ (●) for acetonitrile adsorbed at 22°C from an aqueous mixture on to (a) RP-2, (b) RP-8 and (c) RP-18 plotted against the molar fraction of acetonitrile in the liquid.

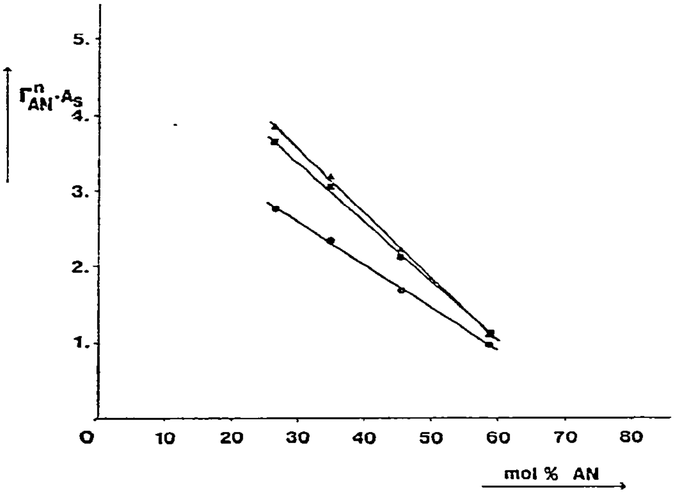

Fig. 7. Plot of straight section of the surface excess amount of acetonitrile on a molar base *versus* the molar fraction in an acetonitrile–water system on (▲) RP-2, (■) RP-8 and (●) RP-18 at 22°C.

modification is *ca.* 500 m^2/g for RP-2 and 400 m^2/g for RP-8 and RP-18. A surface coverage of 3.5 μmol/m^2 was assumed for the bonded alkyl groups and the composition of the adsorbed phase can be calculated. For the region of 50–80% (v/v) acetonitrile this results in the following compositions: RP-2, C_2:AN:H_2O = 29:62:9; RP-8, C_8:AN:H_2O = 40:53:7; RP-18, C_{18}:AN:H_2O = 65:32:3 (%, v/v).

TABLE VI

COMPOSITION OF THE ADSORBED LAYER FOR AN ACETONITRILE–WATER SYSTEM BY USING THE SCHAY MODEL

Alkyl chain length	n^s_{AN} *(mmol/g RP)*	n_{H_2O} *(mmol/g RP)*	x^s_{AN}	$V^a_{H_2O,AN}$ *(µl/g)*
C_{18}	4.27	1.37	0.76	250
C_8	5.80	2.24	0.72	345
C_2	6.16	2.54	0.71	370

These results show that there are large differences in the compositions of the adsorbed phase. Such differences have a strong influence on the solute activity in a phase, as is discussed in other papers[10,25]. Thus the change in composition in the adsorbed phase may be partly responsible for the increase in retention on going from RP-2 to RP-18, when using the same mobile phase composition. Comparison of the adsorption data for RP-2, RP-8 and RP-18 in Tables III, IV and V reveals that the adsorbed phase volume is not proportional to the chain length. The amount adsorbed seems rather to be correlated with the surface area of the modified silica, at least for RP-8 and RP-18 (surface areas are *ca.* 350, 250 and 150 m^2/g for Merck RP-2, RP-8 and RP-18, respectively). The increase in the capacity factor for a solute when a higher alkyl silica is used cannot be explained by the ratio of the stationary phase volume and the mobile phase volume (Table VI), and consequently a liquid–liquid partition mechanism is not likely. In other words, an adsorption mechanism is more acceptable.

Adsorption isotherms for methanol–water systems

The adsorption isotherms were measured for methanol–water systems on RP-8 and RP-18. The results are given in Tables VII and VIII. The results were plotted in the same way as for acetonitrile–water systems and Fig. 8a and b show the adsorption isotherms measured by means of the minor disturbance method. With the assumption of exclusive adsorption of methanol, the adsorbed amount is 2.5–3 times lower than for an acetonitrile–water system on the same RP-8 and RP-18 silica. Again, azeotropic points were found in these adsorption isotherms and therefore preferential adsorption of water occurs. Also, the linear sections of the plot of $\Gamma^{(n)}_{MeOH}$ *versus* x_{MeOH} were extrapolated, resulting in the data given in Table IX. This gives a rough impression of the adsorbed phase volume, provided that the treatment of Schay is applicable.

The same conclusion can be drawn for methanol–water as for acetonitrile–water systems, *i.e.*, that adsorption gives a better description than liquid–liquid partition.

Comparison of the estimates of the mobile phase volume

In the theoretical part of this paper the various methods for the determination of the mobile phase volume have been discussed. Fig. 9a and b shows a series of three peaks, obtained using RP-18 with 35:65 and 75:25 acetonitrile–water systems. Two of these peaks could at first sight be identified with the mobile phase volume. However:

TABLE VII

EXPERIMENTAL DATA OBTAINED FOR A METHANOL–WATER SYSTEM ON RP-8 AT 22°C

Mobile phase composition		*Surface excesses (mmol/g)*				*With assumption $x^s_{MeOH} = 1$*			*Retention volumes normalized on empty column volume*		
		$\Gamma^{(v)}_{MeOH} A_S$	$\Gamma^{(n)}_{MeOH} A_S$	$\Gamma^{(v)}_{MeOH} A_S$		*Amount adsorbed*		*Mobile phase porosity,* ε_m			
% (v/v)	*Mol-%*	*Minor disturbance*		*Isotope, C^2H_3OH*	*Isotope, ^2H_2O*	n^s_{MeOH} *(mmol/g)*	V^s_{MeOH} *(µl/g)*		$V_{R_{C^2H_3OH}}$	$V_{R_{^2H_2O}}$	$V_{R_{md}}$
						Minor disturbance					
20	9.8	1.01	1.14	–	0.71	1.26	51	0.705	–	0.721	0.743
30	15.6	1.06	1.27	0.89	–	1.50	61	0.701	0.782	–	0.723
50	32.2	0.78	1.15	0.66	0.67	1.69	69	0.698	0.763	0.716	0.696
60	40.0	0.62	0.94	–	0.70	1.57	64	0.700	–	0.725	0.692
70	52.1	0.39	0.71	0.28	0.36	1.49	60	0.701	0.735	0.728	0.694
80	64.8	0.22	0.45	0.25	0.20	1.28	52	0.706	0.734	0.738	0.698
90	80.3	0.08	0.19	–	0.11	0.97	39	0.712	–	0.738	0.703
95	95.7	–0.08	–	–	–	–	–	–	–	–	0.710
100	100	0	0	–	–	–	–	0.729	–	–	0.905

TABLE VIII

EXPERIMENTAL DATA OBTAINED FOR A METHANOL–WATER SYSTEM ON RP-18 AT 22°C

Mobile phase composition		*Surface excesses (mmol/g)*				*With assumption $x^s_{MeOH} = 1$*			*Retention volumes normalized on empty column volume*		
		$\Gamma^{(v)}_{MeOH} A_S$	$\Gamma^{(n)}_{MeOH} A_S$	$\Gamma^{(v)}_{MeOH} A_S$		*Amount adsorbed*		*Mobile phase porosity, ε_m*			
% (v/v)	*Mol-%*	*Minor disturbance*		*Isotope, C^2H_3OH*	*Isotope, ^2H_2O*	*(n^s_{MeOH} mmol/g)*	*V^s_{MeOH} (µl/g)*		$V_{R_{C^2H_3OH}}$	$V_{R_{^2H_2O}}$	$V_{R_{md}}$
						Minor disturbance					
10	4.9	0.73	0.78	–	–	0.82	33	0.652	–	–	0.711
30	15.6	0.87	1.04	0.71	–	1.24	50	0.644	0.718	–	0.661
50	32.2	0.66	0.95	0.39	–	1.41	57	0.640	0.696	–	0.638
60	41.5	0.46	0.75	–	–	1.27	51	0.643	–	–	0.636
70	52.1	0.32	0.63	0.34	–	1.20	50	0.644	0.742	–	0.639
80	64.8	0.19	0.37	0.20	–	1.10	45	0.647	0.741	–	0.640
90	80.3	0.06	0.14	–	–	0.72	29	0.654	–	–	0.657
95	95.7	−0.07	–	–	–	–	–	–	–	–	0.659
100	100	0	0	–	–	–	–	0.670	–	–	0.850

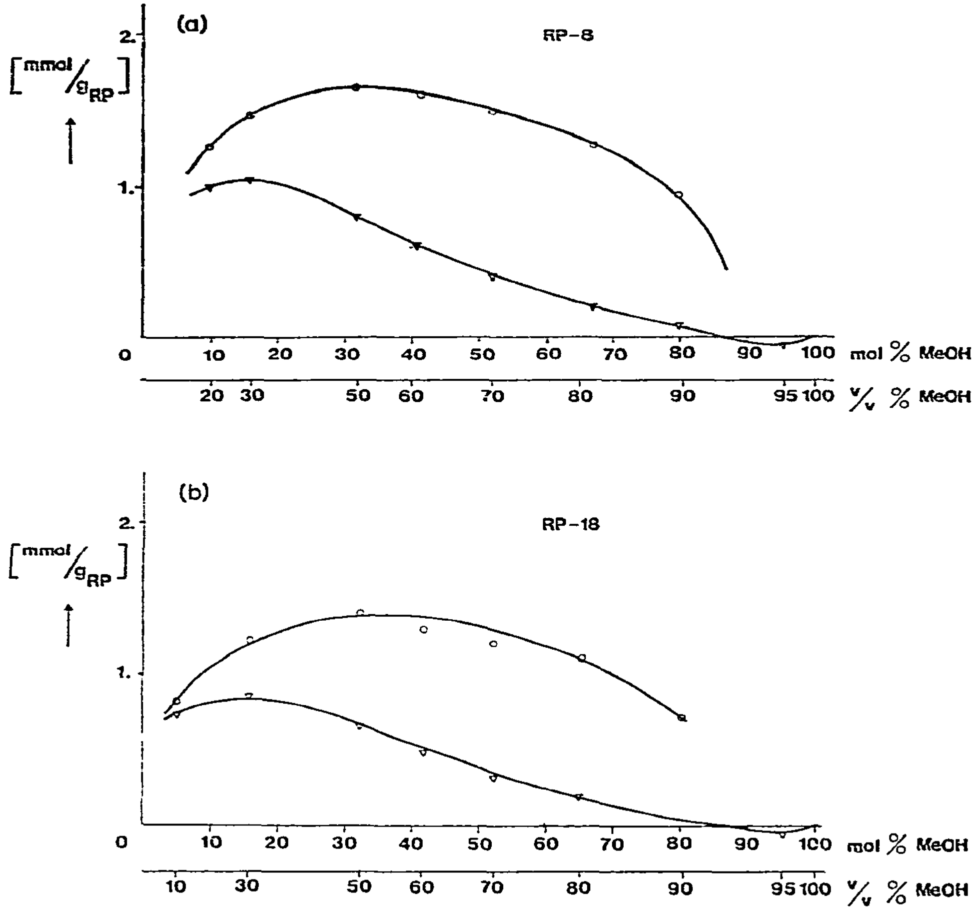

Fig. 8. The surface excess amount (▽) and the adsorbed amount of methanol (○) with the assumption $c^s_{MeOH} = 1$ plotted *versus* the molar fraction of methanol in a methanol–water system on (a) RP-8 and (b) RP-18 at 22°C.

TABLE IX

COMPOSITION OF THE ADSORBED LAYER FOR A METHANOL–WATER SYSTEM USING THE SCHAY MODEL

Packing	n^s_{MeOH} *(mmol/g)*	$n^s_{H_2O}$ *(mmol/g)*	x^s_{MeOH}	V^s_{MeOH,H_2O} *(µl/g)*
RP-18	1.49	0.22	0.87	63
RP-8	1.81	0.30	0.86	79

(a) The deuterated water peak (H^2HO, ^2H_2O) can be considered as "unretained" only for B solvents without OH groups and provided that one can assume that the organic modifier B is adsorbed exclusively and that the influence of the silanols can be neglected. Our results show that exclusive adsorption on the RP materials is highly improbable.

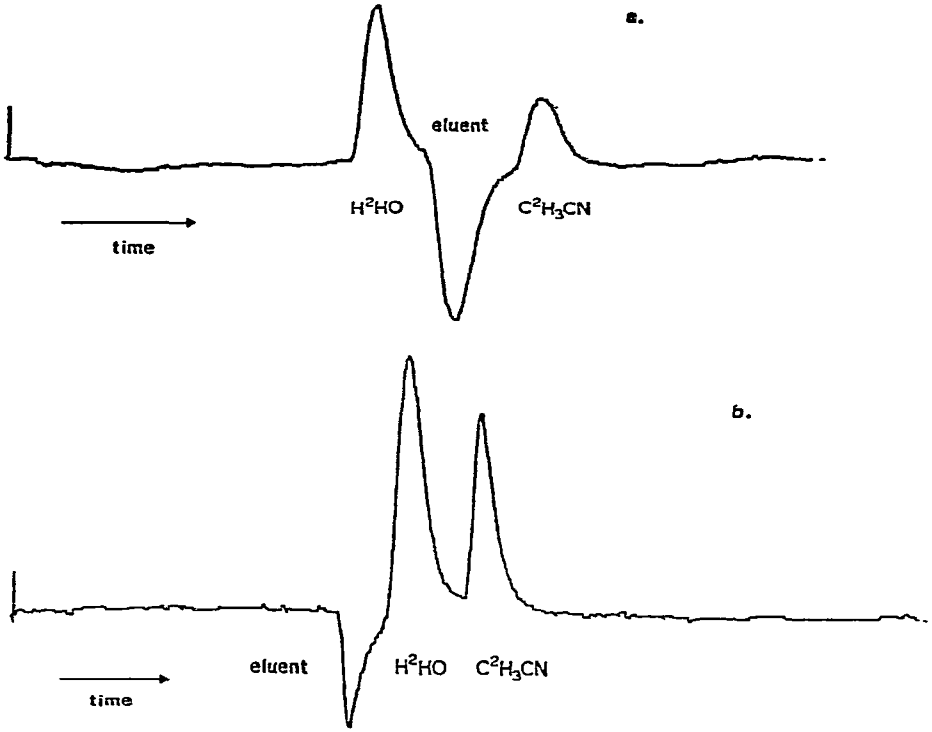

Fig. 9. Chromatogram of the eluent disturbance and ^2H_2O and C^2H_3CN as solutes in a chromatographic system consisting of 35:65 and 75:25 acetonitrile–water on RP-18.

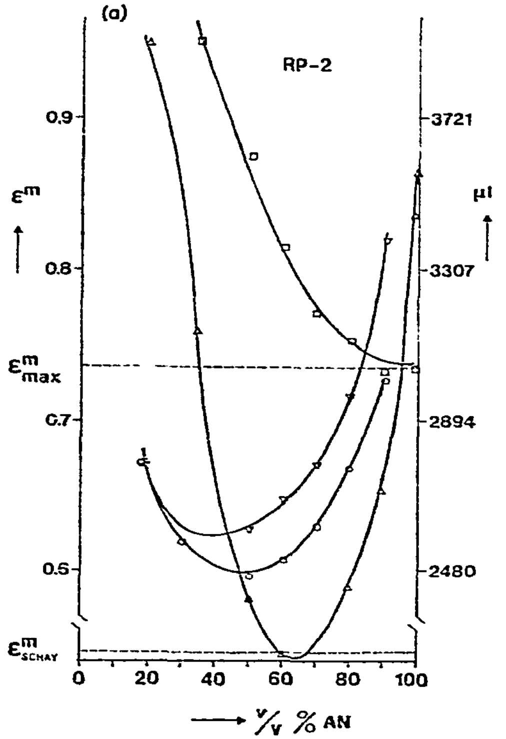

Fig. 10

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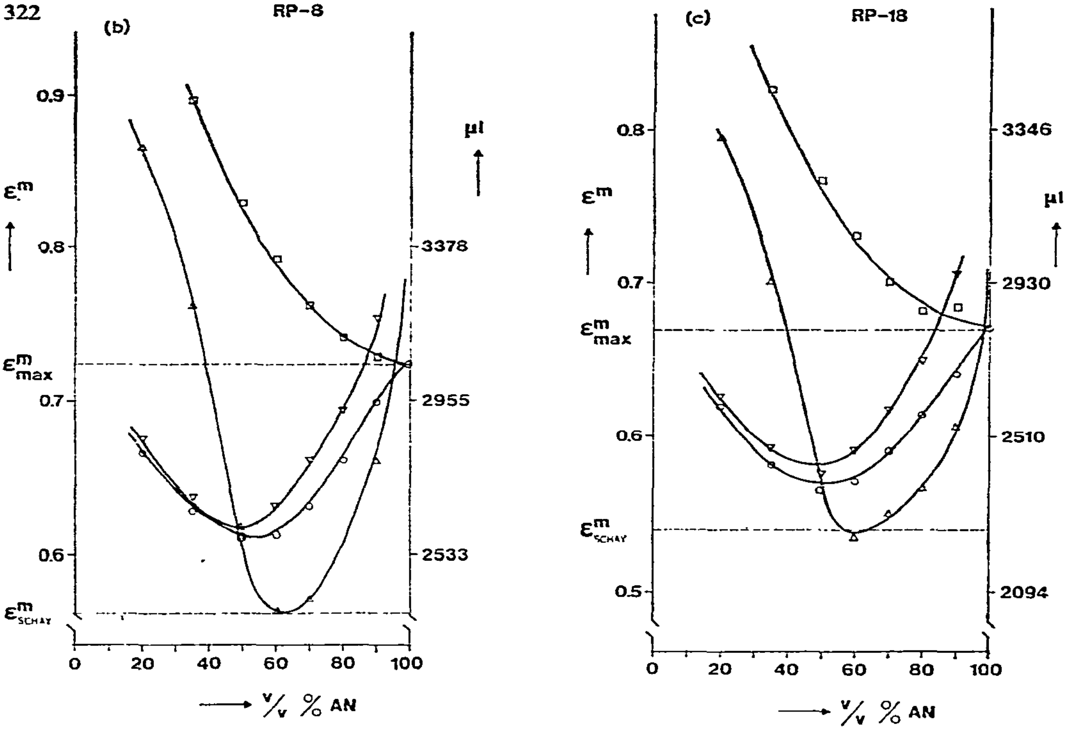

Fig. 10. Various estimates of the mobile phase volume, expressed as porosity factors, ε, as a function of the acetonitrile–water composition for (a) RP-2, (b) RP-8 and (c) RP-18. ○, $\varepsilon_m = V^m/V^{col}$ (with the assumption $x^s_{AN} = 1$, from data of the minor disturbance method); ▲, $V_{R_{md}}/V^{col}$; □, $V_{R_{C_2H_3OH}}/V^{col}$; △, $V_{R_{2H_2O}}/V^{col}$; $\varepsilon^m_{max} = V^1/V^{col}$; $\varepsilon^m_{Schay} = V^m/V^{col}$ (see eqn. 26); V^{col} = volume of empty column.

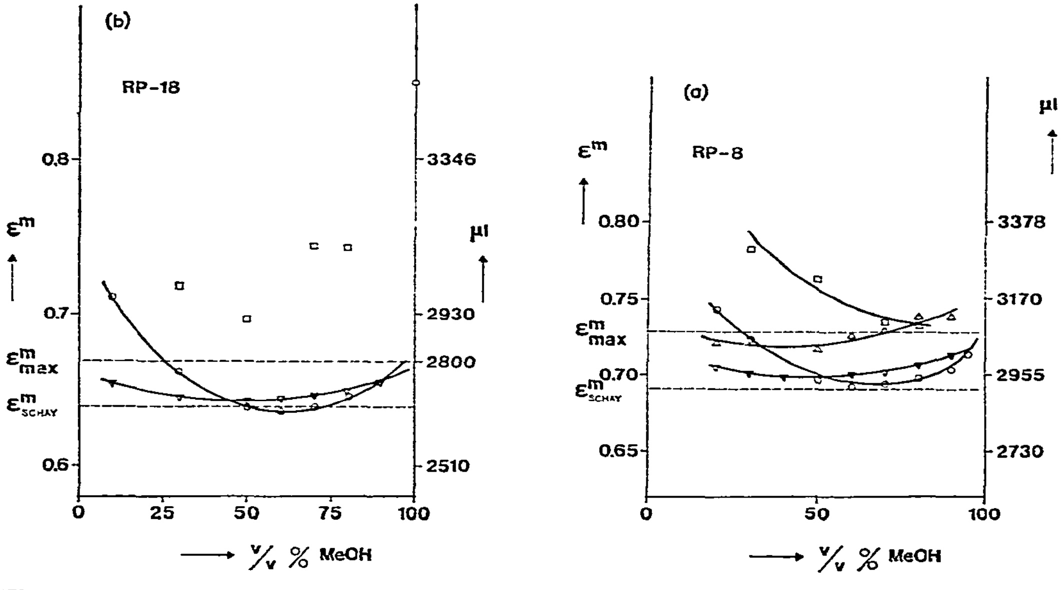

Fig. 11. Various estimates of the mobile phase volume, expressed as porosity factors, ε, as a function of the methanol–water composition for (a) RP-8 and (b) RP-18. △, $\varepsilon_m = V^m/V^{col}$ (with the assumption $x^s_{MeOH} = 1$, from data of the minor disturbance method); ○, $V_{R_{md}}/V^{col}$; □, $V_{R_{C_2H_3OH}}/V^{col}$; △, $V_{R_{2H_2O}}/V^{col}$; $\varepsilon^m_{max} = V^1/V^{col}$; $\varepsilon^m_{Schay} = V^m/V^{col}$ (see eqn. 26); V^{col} = volume of empty column.

(b) The eluent disturbance peak is in fact a measure for the derivative of the composite isotherm. Hence this method gives a realistic estimate of the mobile phase volume only if the amounts adsorbed can be considered to be constant, *i.e.*, if $\Gamma_{B}^{(v)}$ is a linear function of c_{B}^{m}. However, as has also been shown by Berendsen[5], for a number of instances the disturbance peak leads to excessively high estimates of the mobile phase volume. This occurs especially in the lower concentration range of B, where the isotherm is steep.

(c) A clearer way to arrive at an estimate of the mobile phase volume is to extrapolate the linear part of the excess isotherm and to calculate the corresponding adsorbed phase volume.

In Figs. 10 and 11 the results of the various methods are plotted. The volume was expressed as the fraction ε of the total column volume. It can be seen that there is only a small region [around 60% (v/v) of organic solvent] where the disturbance methods provide a useful value for the mobile phase volume, especially for acetonitrile–water.

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