SOME ASPECTS OF ION-EXCHANGE CHROMATOGRAPHY EMPLOYING ADSORBED ION EXCHANGERS ON REVERSED-PHASE COLUMNS

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SUMMARY

The nature of the retention of ionic solutes in the presence of alkyl sulphonates and quaternary alkylammonium halides is examined, and it is shown that the ion-exchange reagents are adsorbed on the hydrocarbon stationary phase and act as adsorbed ion-exchange agents. Thus, in the system examined the major mechanism of retention is normal ion-exchange chromatography. This paper demonstrates that a layer of solvent exists over the surface of the bonded phase which can be shown by the nature of the adsorption isotherm of methanol on the bonded phase; this is also confirmed chromatographically. In a similar manner, the adsorption isotherm of sodium octane sulphonate is also determined, and the presence of the adsorbed layer of the octane sulphonate confirmed by chromatographic measurements. By the use of conductivity and nuclear magnetic resonance chemical shift measurements, it is shown that in the system examined and under the conditions where solutes are retained by an ion-exchange mechanism on the surface of the stationary phase, both solute and ion-exchange reagent are ionized.

INTRODUCTION

There appear to be some differences of opinion concerning the mechanism of the so called "reversed-phase ion-pair chromatography" carried out with chemically bonded stationary phases. Some publications on this subject follow the concept of "ion-pair" distribution where a charged solute is associated with a lipophilic detergent in the mobile phase and the neutral ion pair interacts with the stationary phase[1,2]. It has also been suggested that the conversion of the stationary phase into an ion exchanger by adsorption of the lipophilic detergent may explain the phenomenon[3,5]. This paper presents some evidence indicating that ion exchange can be the major retention mechanism in "reversed-phase ion-pair chromatography" where the ion exchanger is adsorbed on the stationary phase.

EXPERIMENTAL

The nature of the surface of a reversed bonded phase in contact with methanol–water mixtures

Before dealing with the interaction of either a lipophilic ion-exchange reagent or a solute with a bonded-phase surface, it is necessary to determine what part the solvent plays in modifying the reversed-phase surface.

Previously, it was shown[6] that at high concentrations of organic solvent in the water–solvent mixture, the reversed phase was covered with a layer of organic solvent: this was true for all solvent concentrations that wetted the bonded phase. Wetting occurred at approximately 60% (w/v) of organic solvent in the mixture, but in most instances, ion-exchange chromatography with the reversed bonded phases is carried out at significantly lower organic solvent concentrations.

To determine the nature of the surface at lower organic solvent concentrations, the adsorption isotherm was determined in a manner similar to that previously described[7]. To determine the adsorption isotherm of methanol in water in contact with the reversed phase, it is necessary to use an octadecyl-bonded phase that would wet with pure water. An appropriate bonded phase was ODS, manufactured by Whatman (Clifton, N.J., U.S.A.), which had a carbon content of approximately 5%. As this is an octadecyl-bonded phase, it is clear that there are a significant number of free silanol groups present, otherwise the material would not wet with pure water, but these silanol groups could also influence the distribution of methanol between the water mixture and the bonded phase. Adsorption isotherms were, therefore, determined for bonded ODS and Partisil 10 to ensure that the adsorption isotherm reflected the interactions of methanol with the bonded phase and not with the silica.

About 10 g of the ODS were placed in a 70-ml flask and 50 ml water added. Methyl alcohol in 0.5-ml quantities was added to the mixture and allowed to come into equilibrium at 25°C for 30 min with stirring. After each equilibrium, a sample was taken and analyzed by gas chromatography (GC) using the technique previously described[6]. This experiment was repeated for Partisil 10 silica gel, and the results from both adsorption isotherm experiments curve-fitted to the function $y = x/(A + Bx)$. The results are shown in Fig. 1. The correlation coefficients for the data fitting the two curves for ODS and silica gel were 0.983 and 0.979, respectively, and the curves in Fig. 1 were obtained from the theoretical values from the curve fit and the points are experimental.

It is seen that the methyl alcohol exhibits a Langmuir-type isotherm when distributed both between the water and a reversed phase, and water and silica gel. This would appear to be in conflict with previous adsorption isotherms for polar solvents on silica gel. However, this adsorption isotherm is for methanol adsorbed on silica gel fully deactivated with water and thus represents the formation of a layer of methanol on the first layer of water. In fact, it is probably the adsorption isotherm for the second layer of a bilayer adsorption system. Taken separately, the adsorption isotherm of the second layer is Langmuir in type. It is also seen that relatively small quantities of methanol associate with the silanol groups on the silica gel, compared with the octadecyl groups on the ODS. It follows that a layer of solvent molecules form on the bonded-phase surface, and the extent of the layer increases with the concentration of methanol in the solvent mixture.
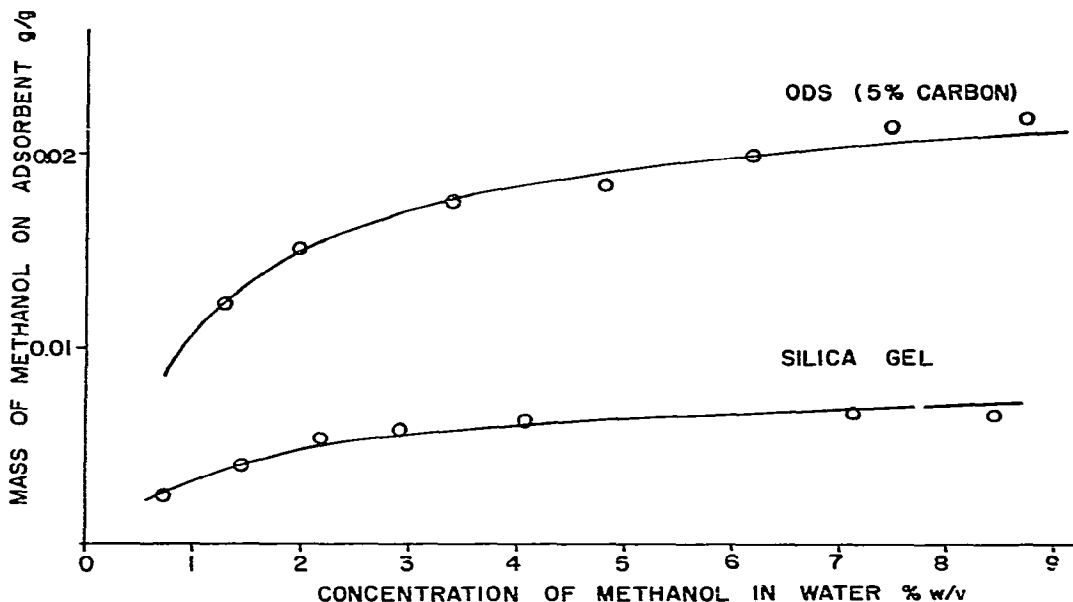
Fig. 1. Adsorption isotherms for methanol on ODS and silica gel.

From the Langmuir function, the relationship between solvent concentrations can be expressed by:

$$X_s = \frac{X_m}{A + BX_m} \tag{1}$$

Where X_s is the concentration of solvent on the surface in g/g, X_m is the concentration of solvent in the mobile phase in g/ml and A and B are constants; when $X_m \rightarrow \infty$, $X_s = 1/B$.

Now X_s will be proportional to the surface area covered by the solvent (A'_s) i.e., $A'_s = \alpha X_s$ and the total surface area will be A_s, where $A'_s = \alpha/B = A_s$ and α is a constant.

Thus the surface area exposed =

$$A_s - A'_s = \alpha \left(\frac{1}{B} - X_s\right) = \alpha \left[\frac{1}{B} - \frac{X_m}{A + BX_m}\right]$$

$$= \alpha \left[\frac{A + BX_m - BX_m}{B(A + BX_m)}\right] = \alpha \left[\frac{A}{B(A + BX_m)}\right] \tag{2}$$

Now $V' = K(A_s - A'_s)$, where $V' =$ the corrected retention volume of the solvent when chromatographed on the bonded phase with a solvent mixture containing X_m g/ml methanol in water and $K =$ distribution coefficient.

Thus

$$V' = K\alpha \frac{A}{B(A + BX_m)} \tag{3}$$

and

$$\frac{1}{V'} = A' - B'X_m \tag{4}$$

where $A' = B/K\alpha$ and $B' = B^2/\alpha KA$.

From eqn. 3 it can be seen that the corrected retention volume is proportional to the area of bonded phase available for interaction which, as shown by eqn. 4 will be inversely proportional to the concentration of the organic solvent. However, from an experimental point of view, the organic solvent when used as a solute will be retained to different extents, depending on the solvent composition; thus, the measurement of V' will require the accurate measurement of the dead volume of the column.

The measurement of the column dead volume was achieved by measuring the retention volume of methanol using a refractive index detector over a range of solvent compositions; the results are shown in Fig. 2. It is seen that as the surface of the bonded phase is progressively covered by the organic solvent, the retention volume of the organic solvent decreases and attains a constant minimum value at high concentrations of organic solvent in the mobile phase. This constant value was taken as the dead volume of the column, and thus V' was taken as the difference between the retention volume and the dead volume of the column. It is interesting to note that the extrapolated values for the dead volume of the column shown in Fig. 2 were the same as the dead volume values obtained by injecting samples of potassium nitrate onto the columns.
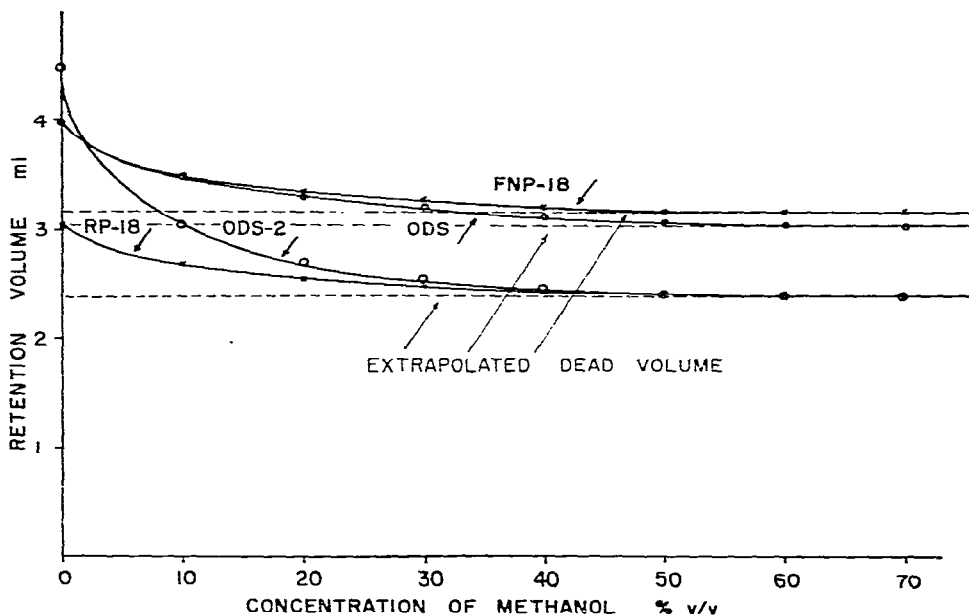

Fig. 2. Graphs of retention volume of methanol against solvent composition for different reversed phases.

The curves in Fig. 2 show the dead volume of different columns (each 25 cm × 4.6 mm I.D.) packed with four different bonded phases. It is seen that the Silarex FNP-18, supplied by Lion Technology (Dover, N.J., U.S.A.) and ODS, supplied by

Whatman, provided a high column dead volume as the particles are fully permeable to the mobile phase. However, for the RP-18 octadecyl packing provided by Merck (Darmstadt, G.F.R.) and the ODS-2, another octadecyl packing provided by Whatman, the dead volume is significantly smaller and, in fact, is close to the interparticle volume for a column packed with plain silica gel of the same particle size. It would appear that the pores of the parent silica gel particles in the ODS-2 and RP-18 bonded phases are completely blocked and, in fact, these bonded phases act as a type of micropellicular packing.

Using the data obtained in Fig. 2, the corrected retention volumes of methanol and acetonitrile were measured at different solvent concentrations on reversed-phase packings ODS, ODS-2, and FNP-18. The results obtained are shown as curves relating $1/V'$ against solvent composition in Fig. 3. It is seen, as predicted by eqn. 4, that a linear relationship exists between $1/V'$ and solvent composition and that this is true for methanol on different bonded phases and for the alternative organic solvent acetonitrile on ODS-2.
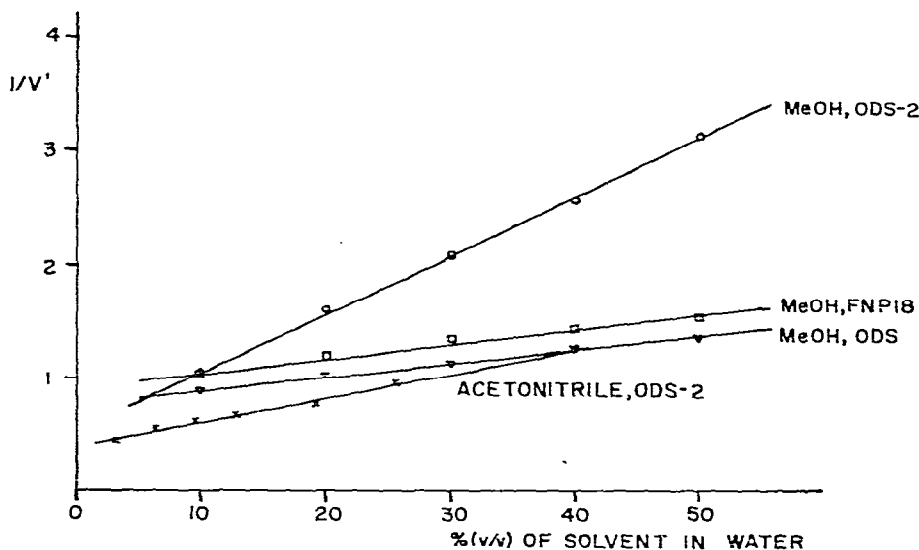

Fig. 3. Graphs relating the reciprocal of the net retention volume of different solvents to the composition of the mobile phase. MeOH = Methanol.

The combined results shown in Figs. 1 and 3, involving both gravimetric and chromatographic experiments, confirm that when a reversed bonded phase is used in conjunction with an organic solvent water mixture, the organic solvent is adsorbed on the surface of the bonded phase in a manner that can be described by a Langmuir-type isotherm and that at high concentrations [excess of 50 or 60% (w/v) organic solvent in water] the layer of solvent on the surface of the bonded phase is complete.

The distribution of ion-exchange reagents between aqueous methanol and a reversed bonded phase

As the retention of ionic solutes may result from interaction with adsorbed ion exchangers in reversed-phase systems, then the ion-exchange reagents must form a

layer of adsorbed material on the surface of the stationary phase. Such a phenom-
enon could be measured experimentally by determining the adsorption isotherm of
the ion-exchange reagent on the surface of the bonded phase. The adsorption isotherm
for sodium octane sulphonate on a C_{18} reversed-phase was determined in the
following way. The solvent system used was 20% (v/v) methanol in water, and the
stationary phase was Partisil ODS. A 10-g amount of the bonded phase was in-
troduced into a 100-ml container and 70 ml of the solvent mixture added. Known
masses of sodium octane sulphonate were added to the mixture, which was stirred at
25°C, and the quantity of octane sulphonate present in the solvent mixture measured
gravimatically by weighing the residue from a known volume of sample, after eva-
porating down to dryness.

The mass adsorbed on the bonded phase was calculated as the difference
between the mass of octane sulphonate added and that remaining in the solvent
mixture. The results obtained are shown in Fig. 4 as a curve relating mass of octane
sulphonate on the reversed phase against the concentration of octane sulphonate in
the mobile phase. The points were curve-fitted to the Langmuir adsorption isotherm
function which gave a correlation coefficient of 0.997. In Fig. 4 the curve is con-
structed from the theoretical values obtained from the curve fit procedure, and the
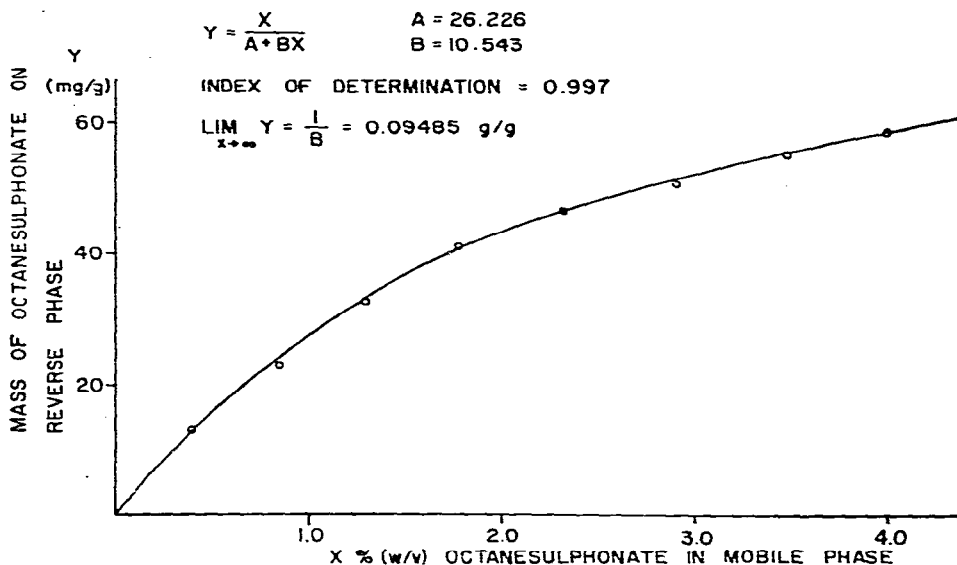points are experimental.

Fig. 4. Adsorption isotherm for sodium octane sulphonate between C_{18} reversed phase and 20%
(v/v) methanol in water at 25 C.

The results in Fig. 4 clearly demonstrate that a layer of the ion-exchange
reagent, octane sulphonate, forms on the surface of the bonded phase, and the greater
the concentration of octane sulphonate in the mobile phase, the more octane sul-
phonate resides on the stationary phase.

This layer of ion-exchange reagent on the surface of the bonded phase can be
confirmed chromatographically by similar experiments to that used to confirm solvent
layer formation. Eqns. 3 and 4 apply equally to the adsorption of ion-exchange

reagents, and therefore, if the corrected retention volume of the reagent is measured at different sulphonate concentrations, a graph relating $1/V'$ against ion-exchange reagent concentration would give a straight line.

The retention volume of octane sulphonate was measured in a mobile phase when carrying 20% of methanol in water and different concentrations of octane sulphonate. The results obtained are shown as graphs relating $1/V'$ against concentrations of sodium octane sulphonate in Fig. 5. It is seen that a linear relationship is obtained confirming the Langmuir-type adsorption isotherm for the ion-exchange reagent on the reversed bonded phase.
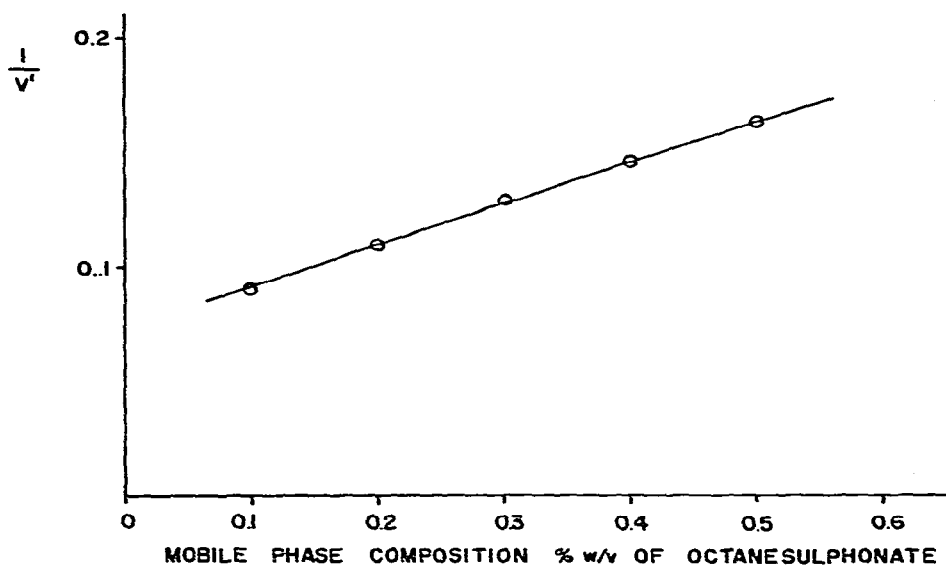

Fig. 5. Graph relating the reciprocal of the net retention volume of octane sulphonate to the percentage of octane sulphonate in the mobile phase.

The graphs relating the reciprocal of the corrected retention volume and the mobile phase composition at the lower concentrations of organic solvent in the mobile phase are useful for demonstrating the existence of adsorbed material on the surface of the bonded phase. To substantiate the results previously described, experiments were carried out to demonstrate how other ion-exchange reagents such as sodium hexane sulphonate and tetrabutylammonium iodide also form adsorbed layers on the bonded phase. It was also of interest to note that this phenomenon occurred with organic solvent water mixtures other than methanol. The corrected retention volumes of tetrabutylammonium iodide and sodium hexane sulphonate were determined for a column (25 cm × 4.6 mm I.D.) packed with ODS-2 over a range of mobile phase compositions consisting of mixtures of acetonitrile in water.

The results obtained were presented in the conventional manner as curves relating $1/V'$ against % (v/v) acetonitrile in water, and are shown in Fig. 6. It is seen that, again, linear plots are obtained indicating the formation of adsorbed layers of tetrabutylammonium iodide and sodium hexane sulphonate. The retention volume of sodium hexane sulphonate was also determined on similar columns packed with

ODS-2, ODS, and RP-18 using a mobile phase of methanol in water. In Fig. 6 an insert is shown demonstrating the reciprocal relationship between $1/V'$ and % (v/v) methanol in water on the three columns.
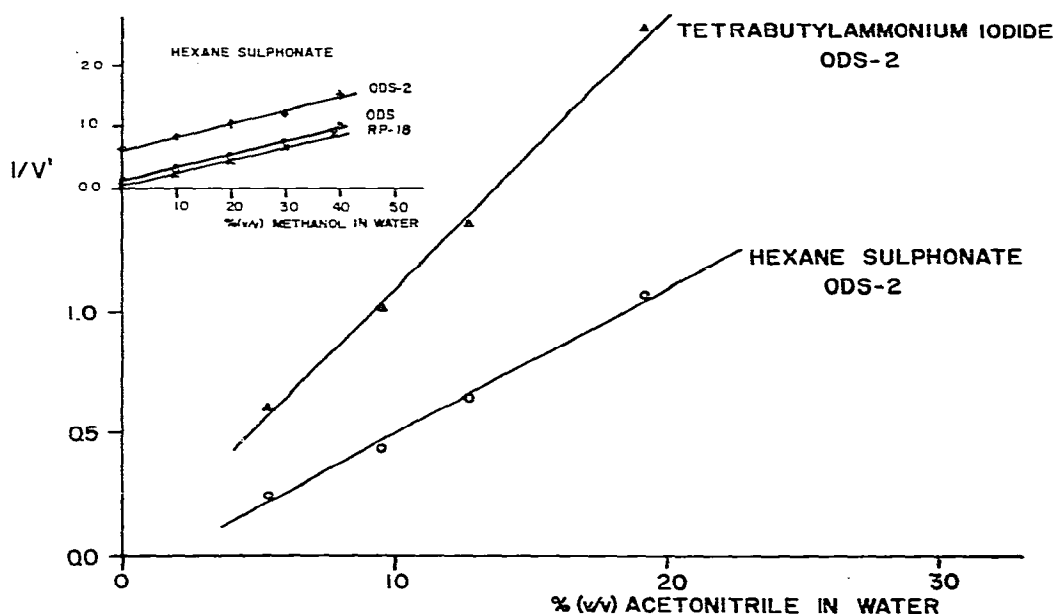

Fig. 6. Graphs relating the reciprocal of the net retention volume of different reagents to the composition of the mobile phase.

The experiments carried out clearly demonstrate that not only is there a layer of organic solvent on the surface of the bonded phase, but on the addition of ion-exchange reagents such as alkyl sulphonates and quaternary alkylammonium halides, these also adsorb on the surface of the bonded phase. Such materials would act as adsorbed ion-exchange reagents and permit separation by normal ion-exchange procedures.

The relationship between solute retention and the degree of ionization on reversed-phase systems

It has been demonstrated that ion-exchange reagents can form a layer on the bonded reversed phase and thus act as an adsorbed ion exchanger. However, the ion-exchange mechanism can only take place between ions and not between undissociated salts. Some workers[1,2] consider that ion pairs of undissociated salts are the entities that are exchanged between the two phases, but it has also been suggested that the mechanism of retention is solely by ion exchange[3–5]. It is, therefore, necessary to determine whether the solute and the ion-exchange reagent exist as ions or as associated salts under conditions where the solute is retained.

Ion–ion interactions are basically coulombic in nature, and any ion–ion association will be dependent on the charge, the distance which separates the two ions, temperature, and the dielectric constant of the solvent. Consequently, it is reasonable to assume that in a solvent of relatively low dielectric constant, the

attraction force between two adjacent ions of similar charge and size will be much greater than in a solvent with a relatively high dielectric constant; therefore, the ion pair formed under these conditions would have a good chance of existing for some time.

It is interesting to note that the extensive work of Kraus and Fuoss in 1933[8–11] where the dissociation constants and conductivity of various quaternary ammonium salts like tetraisoamylammonium nitrate and picrate were measured in different dioxane–water concentrations, showed no ion-pair formation of these salts between 0 and 80% dioxane in water. When the concentration of dioxane in water exceeded 80% (v/v) they demonstrated an anomalous conductivity drop and concluded that a neutral ion pair drastically decreased the conductivity of the mixture when the dielectric constant of the solvent was less than 12. Retention measurements, coupled with conductivity and chemical shift measurements, were therefore carried out to identify the ionization conditions under which retention does occur. Pyridoxine was employed as the solute for these experiments.

The column used was 25 cm × 4.6 mm I.D., packed with RP-18, 10 μm reversed phase and thermostated at 25°C. A range of mobile phases was employed from pure water to 90% methanol in water. The sample injector was a Valco high-pressure valve delivering 1-μl samples and the detector employed was the LDC UV monitor, operated at 254 nm. All solvents used contained 0.04 M NaH_2PO_4 as a buffer to control the pH at 5 and 0.005 M sodium octane sulphonate as the ion-exchange agent. The solute chosen was pyridoxine, a base that could associate with the sulphonate. The retention volume of this solute was measured for a series of different concentrations of methanol in water. The results are shown in the upper curve in Fig. 7.

Measurement of conductivity

The conductivity of pyridoxine, octane sulphonate, and mixtures of pyridoxine octane sulphonate at the same concentrations as those used in the retention volume measurements was determined using the Markson Science electromark analyzer equipped with an appropriately calibrated conductivity cell. All measurements were carried out at 25°C. The results obtained are shown as curves relating the conductivity in μS to the concentration of methanol in water, and are shown in the center diagram in Fig. 7.

Measurement of the chemical shift of the α-N-proton of pyridoxine

A series of mixtures of methanol in water were used, each mixture carrying 0.04 M NaH_2PO_4, 5 mM sodium octane sulphonate, and 5 mM pyridoxine. Each sample was placed in a nuclear magnetic resonance (NMR) tube employing an XL 100 Varian NMR spectrometer operating on protons at 100 MHz, and the chemical shift (δ ppm) was measured for the α-N-proton of pyridoxine. The results obtained are shown in the lower curve in Fig. 7, where the chemical shift is plotted against composition of the methanol–water mixture.

DISCUSSION

The conductivity measurement of a 0.005 M mixture of pyridoxine and sodium octane sulphonate in various methanol–water mixtures, shown as the center curve in
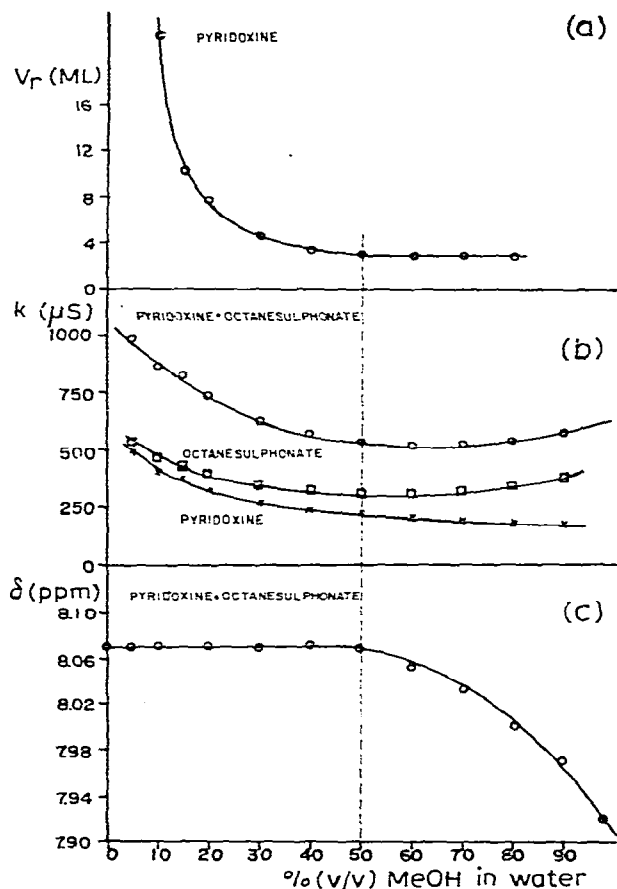
Fig. 7. Graphs relating solute retention, solute and reagent conductivity and NMR chemical shift of the α-N-proton of pyridoxine to the percentage of methanol in water. (a) Liquid chromatography retention volume (V_r). Conditions: column, 25 cm × 4.6 mm I.D., RP-18; 5 mM octane sulphonate, 0.04 M NaH$_2$PO$_4$, pH 5, 25 C; mobile phase, methanol–water. (b) Conductivity. Conditions: mixture of 5 mM pyridoxine – 5 mM octane sulphonate, pH 5, 25 C. (c) NMR, chemical shift. Conditions as in (b).

Fig. 7, indicates a conductivity increase at about 50% (v/v) methanol in water. The increase-in conductivity or the degree of the ionization of the salt with higher concentrations of water coincides with the concentrations of methanol where the pyridoxine begins to be significantly retained on the RP-18 reversed phase in methanol–water mobile phase with added octane sulphonate, shown in the upper curve in Fig. 7.

The proton NMR study of the same pyridoxine–octane sulphonate solutions carried out under the same conditions similarly demonstrates increasing protonation of the nitrogen atom with the increasing water content of the solution. It can be seen from the lower curve in Fig. 7 that the chemical shift of the α-N-proton of the pyridoxine nitrogen increases from 7.90 to 8.07 and levels off when the concentration of water in methanol exceeds 50% (v/v), which again indicates increasing ionization of the salt with increasing water concentrations. Above 50% water in the solvent mixture, it would appear from the NMR data that ionization was complete. It can be

seen that at relatively high concentrations of methanol in the mobile phase, and at lower dielectric constants, some ion pairing could be expected to occur. It should again be noted that the increase in retention shown in the upper curve corresponds to those methanol concentrations in the lower curve where ionization appears to be complete.

The relationship between the retention volume of an ionic solute and the retention of the ion-exchange reagent

Consider conditions where ionic interactions predominate in the retention mechanism and the concentrations of the ion exchange reagent in the mobile phase are relatively small so that ionic interactions in the mobile phase are minimal. Thus, the retention of a solute will depend on its interaction with the stationary phase and the following equation will apply:

$$V'_s = K_s A'_s = K_s A_s f \qquad (5)$$

Where V'_s is the corrected retention volume of the solute and A'_s is the fraction of the surface covered with ion-exchange reagent, where $A'_s = f A_s$. A_s is the total available surface area of the bonded phase and K_s is the distribution coefficient of the solute.

In a similar manner, the corrected retention volume of the ion-exchange reagent V'_d will be proportional to that area of bonded phase not already covered by the reagent.

$$V'_d = K_d A_s (1 - f) \qquad (6)$$

When $K_d =$ the distribution coefficient of the ion-exchange reagent. Substitution for f in eqn. 6 from eqn. 5:

$$V'_d = K_d A_s (1 - V'_s / K_s A_s)$$

$$= K_d A_s - \frac{K_d V'_s}{K_s} \qquad (7)$$

Thus, V'_d will be linearly related to V'_s over a range of concentrations of ionic reagent in the mobile phase, providing the concentration is not sufficiently high to render ionic interactions between the solute and the reagent in the mobile phase significant.

In the above argument only ionic interactions are being considered. To be precise, the corrected retention volume of the solute V'_s will be made up of two parts; one due to the dispersive and polar interactions with the stationary phase and the other due to ionic interactions with the stationary phase. It follows that eqn. 7 can be rewritten in the following form:

$$V'_d = K_d A_s - \frac{K_d}{K_s} (V'_s + V''_s)$$

or

$$V'_d = A - B V'_s \qquad (8)$$

where V_s' is the total retention volume of the solute and V_s'' is the retention volume of the solute in the absence of an ion exchanger and is due to polar and dispersive interactions of the solute with the stationary phase only. However, eqn. 7 is adequate to demonstrate the discussed relationships between V_d' and V_s'.

Eqn. 7 can be confirmed experimentally providing the concentration of the ion-exchange reagent is kept sufficiently low so that the assumption that there are no interactions of the solute with the ion-exchange reagent in the mobile phase is valid. A column (25 cm × 4.6 mm I.D.) packed with ODS was operated employing a mobile phase consisting of 20% (v/v) methanol, 0.110 M sodium dihydrogen phosphate solution as a buffer, and varying concentrations of sodium octane sulphonate from 0.025 to 0.5% (w/v). Three substances were employed as solutes, methylnicotinamide, nicotinyl alcohol, and pyridoxine. At each concentration of ion-exchange reagent (sodium octane sulphonate) the retention volume of the three solutes was measured and also the retention volume of the octane sulphonate.

The results obtained are shown as curves relating the corrected retention volume of each solute against the retention volume of the octane sulphonate in Fig. 8. It is seen that up to concentrations of 0.3% (w/v) octane sulphonate in the mobile phase a linear relationship is obtained between the corrected retention volume of the solute and that of octane sulphonate, the curves having a negative slope as predicted by eqn. 7. However, at about 0.3% sodium octane sulphonate in the mobile phase the curves tend to flatten and above this begin to fall. At this point the surface of the reversed phase is almost completely covered by the ion-exchange reagent and, furthermore, the concentration of the octane sulphonate in the mobile phase has reached a level where interactions with the solute in the mobile phase would be significant.
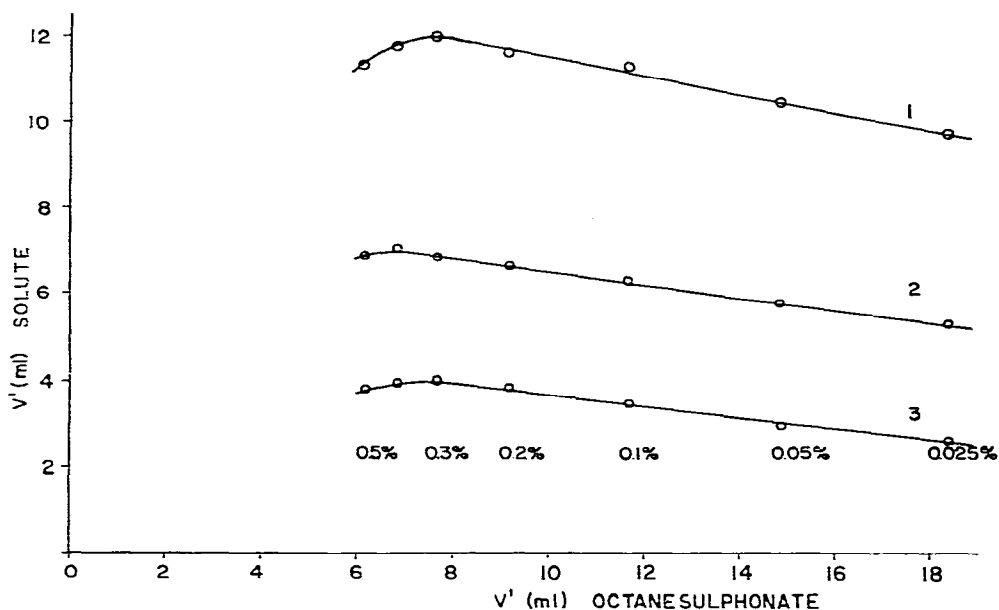

Fig. 8. Graphs relating the net retention volume of different solutes to the net retention volume of octane sulphonate measured at different octane sulphonate concentrations. 1 = N-Methylnicotinamide; 2 = nicotinyl alcohol; 3 = pyridoxine. Conditions: column, 25 cm × 4.6 mm I.D., ODS, 10 μm; 25°C; 20% (v/v) methanol, 0.11 M NaH$_2$PO$_4$, pH 5.

CONCLUSIONS

When employing ion-exchange chromatography to separate solutes in conjunction with reversed-phase systems, it would appear that the nature of the surface of the stationary phase is complex. In the first instance, at high concentrations of water, the surface is covered to varying extents with a layer of the organic solvent. On the addition of ion-exchange reagent such as alkyl sulphonate or quaternary alkyl-ammonium halide, some of the reagent is adsorbed on the surface of the bonded phase and can thus act as an adsorbed ion exchanger. Thus, the separation process can be predominantly ion-exchange chromatography, and the more ion exchanger that is adsorbed on the stationary phase, the greater the retention of the solute. Under such conditions, the retention volume of a given solute may, therefore, be largely controlled by the amount of ion-exchange reagent on the surface which will be, in turn, dependent on both the concentration of the ion-exchange reagent and the concentration of the organic solvent.

Experiments carried out in this paper support these conclusions in so much that they demonstrate that a layer of ion-exchange reagent is formed on the stationary phase and, furthermore, that the retention of an ionic solute increases as the quantity of ion exchanger adsorbed on the stationary phase increases. It is also shown that for the solutes examined and under conditions where the organic solvent content is less than about 50 % (w/v), most of the solute is ionized and there is minimal undissociated salt present: the exchange of ion pairs, as such, between the two phases under these conditions is therefore insignificant.

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