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INFLUENCE OF WATER CONTENT OF THE MOBILE PHASE ON CHROMATOGRAPHIC PERFORMANCE IN ADSORPTION CHROMATOGRAPHY*

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SUMMARY

Silicas (LiChrosorb Si 60 and Si 100) and aluminas (Alox T, Woelm, A5Y Spherisorb) with different specific surface areas were investigated with chloroform and dichloroethane as mobile phases containing various amounts of water in the range 0–600 ppm. The water content of the eluent was adjusted by adding a measured amount of water and was determined by the Karl Fisher method. Measurements were carried out with different mobile phase velocities and reduced plate heights were determined. From the measured data, the capacity ratio (k'), selectivity (α), number of theoretical plates (N_{max}) and resolution (R_S) were determined and plotted as a function of the water content of the mobile phase.

It was established that in order to evaluate the influence of the water content of the mobile phase on the chromatographic performance of an adsorbent packing, not only changes in the capacity ratios but also the changes in the selectivities and efficiencies and from these data the changes in resolution should be determined.

INTRODUCTION

It has been long recognized that retention on active stationary phases such as silica and alumina with non-polar and moderately polar eluents is strongly influenced by the water content of the eluent[1,2]. Water is often added to the eluent in adsorption chromatography to improve isotherm linearity and column efficiency[3–10]. These investigations have shown that the different adsorbents can be adjusted to defined activity levels by changing the water content of the eluent.

The above studies, however, were directed mainly to investigations of the change in capacity ratio (k') as a function of the water content of the mobile phase. Some indications were given of the variation of relative retention, *i.e.*, selectivity, by

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changing the water content[4–6,8–10], but no definite conclusions were drawn. It was generally stated that on increasing water content of the mobile phase the column efficiency increases[1,2,4,6]; even an optimum range was indicated[8], but the values given in the literature are contradictory and not supported by experimental evidence. No systematic evaluation of the influence of water content on the chromatographic performance, *i.e.*, the resolving power of the different adsorbent packings, has yet appeared. For this reason we investigated the influence of the water content of the mobile phase on the resolution of columns packed with silicas and aluminas with different characteristics.

EXPERIMENTAL

Equipment

A Varian Aerograph Model 5000 liquid chromatograph equipped with a Valco injector valve (10-μl loop) and an LDC SpectroMonitor III UV detector were used.

The water content of the eluent was determined by means of a coulometric Karl Fischer titrator (Automate Bizot et Constant, Prolabo, Paris).

Columns

The column tubes used were 15 cm × 4.6 mm I.D. for each packing. Table I summarizes the main physical characteristics of the packings used. The silicas were activated at 180°C for 6 h and the aluminas at 400°C for 12 h. The columns were filled by the slurry technique. For silica packings the suspending solvent was a mixture of carbon tetrachloride (90%) and methanol (10%), forming a dilute suspension (2 g of silica per 20 ml of solvent).

For alumina packings, the slurry for the balanced-density technique was prepared by using a mixture of 3 g of alumina and 20 ml of solvent. Different solvents were tried (acetone, methanol, ethylene glycol–acetone, ethylene glycol–dioxane, triethylene glycol–acetone, glycerol–methanol, tetrabromoethane–dioxane). The best results were obtained by using 80% tetrabromoethane–20% dioxane, giving a mixture of density equal to that of the alumina (balanced-density slurry). With the other

TABLE I

CHARACTERISTICS OF THE INVESTIGATED PACKINGS

Packings	*Particle size (μm)*	*Mean particle size (μm)*	*Particle shape*	*Surface area (m^2/g)*	*Producer**
LiChrosorb Si 60	5–7	6	Irregular	482	Merck
LiChrosorb Si 100	4–7	5	Irregular	309	Merck
Alox T	5–7	6	Irregular	68	Merck
Alumina Woelm N 3-6	3–6	5	Irregular	145	Woelm
Spherisorb A5Y	3–7	5	Spherical	110	Phase Separations

* Merck, Darmstadt, G.F.R.; Woelm, Eschwege, G.F.R.; Phase Separations, Queensferry, Great Britain.

solvents indicated no stable suspensions were obtained; on the other hand, by using high-viscosity solvents (glycols, glycerol) the packing procedure became much slower, resulting in a poorer column efficiency.

Ethanol was used as the supporting liquid for both silicas and aluminas. After packing, the columns were flushed with 300–400 ml of ethanol to eliminate the slurry liquid.

Reagents

Chloroform and 1,2-dichloroethane of LiChrosolv quality (Merck, Darmstadt, G.F.R.) were used as eluents.

The solutes used were of analytical-reagent grade and had different polarities in order to cover a broad enough range of capacity factors for each adsorbent.

Procedures

The filled columns were conditioned by eluting them with 300–400 ml of chloroform.

The measurements were started at a low water content with solvents dried over molecular sieve 5A. The water content of the solvent was then adjusted by adding a measured amount of water and equilibrated overnight by recycling the eluent. The water content of the eluent was subsequently determined by the Karl Fischer method at different intervals during the measurements. In addition, the capacity ratios were measured and checked for constancy.

The solutes were dissolved in the mobile phase and 10-μl samples were injected with the sampling valve. In all experiments toluene was used as the unretained component.

Measurements with a given water content of the eluent were carried out on the same day; then the water content was adjusted for the next measurements and equilibrated as described above.

RESULTS AND DISCUSSION

Water adsorption isotherms

For the silica gels and aluminas used water adsorption isotherms were available, having been determined in our laboratory. The mobile phase (solvent + water) is percolated in a closed circuit into a chromatographic column connected to two small columns containing a weighed adsorbent mass. When equilibrium is attained (solute retention times are constant) the mobile phase is removed with a syringe through a septum and then the two small columns are removed from the circuit. The water contents of the mobile and stationary phases are then determinated by Karl Fischer titration. This dynamic method gives a rapid equilibrium owing to good contact between the two phases[11,12]. In this work we tried to evaluate and interpret the influence of the water content of the mobile phase on the chromatographic characteristics of the different adsorbents on the basis of the shapes and characteristics of the water adsorption isotherms.

The water adsorption isotherms are shown in Fig. 1. The amount of water adsorbed is given by the number of moles of water adsorbed per unit weight of the sorbent (nH_2O) and the weight percentage of water in the sorbent; the ab-

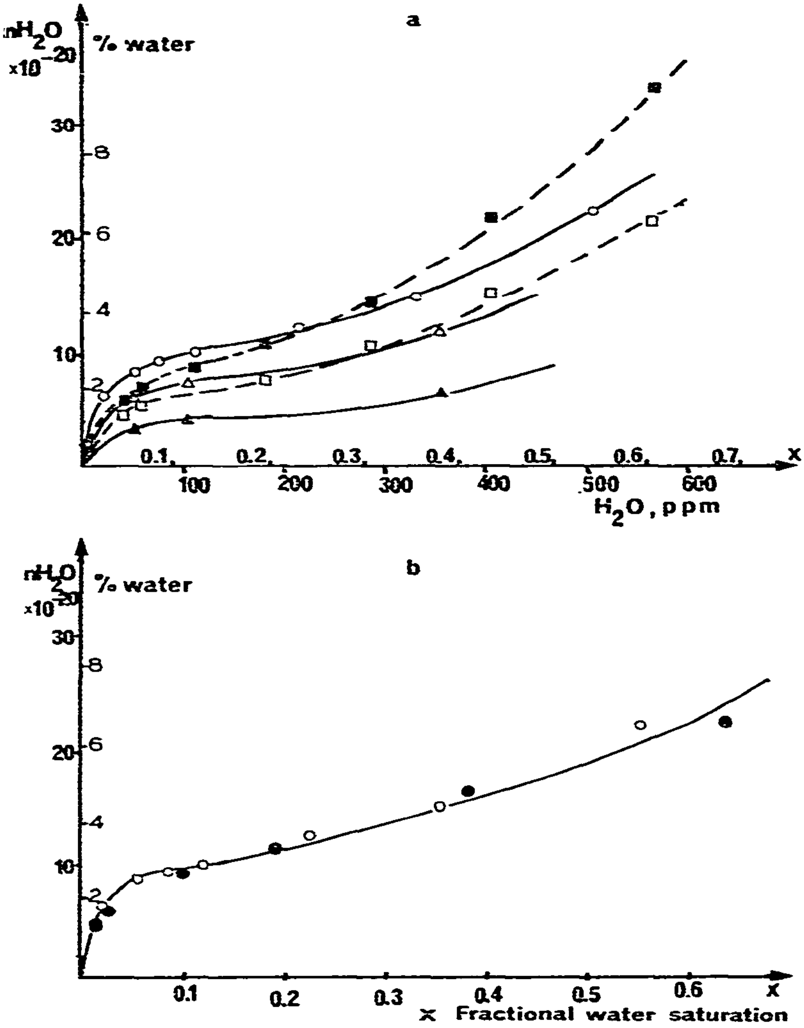

Fig. 1. Water adsorption isotherms. (a) On different adsorbents in chloroform as solvent: □, Si 100; ■, Si 60; ○, Woelm alumina; ▲, Alox T; △, A5Y. (b) On Woelm alumina: ○, in chloroform; ●, in dichloroethane.

scissas indicate the water content of the mobile phase (Fig. 1a) and the fractional water saturation (Fig. 1b). Fig. 1a shows that each isotherm consists essentially of two distinct ranges. At low water contents, a concave part can be observed, which can be linearized with the Langmuir equation[11]; at higher water contents a convex part characteristic of multilayer adsorption can be distinguished. The transition from the concave to the convex curve occurs at about 120 ppm of water in chloroform and 220 ppm of water in dichloroethane, corresponding to monomolecular coverage. Corresponding to the difference between the surface areas, the amount of water adsorbed on Si 60 is much higher than that adsorbed on Si 100. For the alumina packings the water adsorption isotherms also reflect the differences in the surface areas as given in Table I.

By comparing the water adsorption isotherms in Fig. 1a it can be seen that at low water contents the amounts of water adsorbed on the aluminas are much higher than those obtained on the silicas in spite of the lower surface areas, indicating a more pronounced activity of the alumina surface. At higher water contents the amount of water adsorbed increases more rapidly for the silicas than for the aluminas.

In Fig. 1b the water adsorption isotherms measured for Woelm alumina in chloroform and in dichloroethane are represented as a function of the fractional water saturation (X) of the solvents:

$$X = \frac{\text{ppm } H_2O \text{ (acutal)}}{\text{ppm } H_2O \text{ (saturation)}}$$

The saturation values were found to be 930 ppm for chloroform and 1860 ppm for dichloroethane at 20°C. In Fig. 1b it can be seen that the points of the isotherms measured in the two solvents are practically on the same curve, indicating that the quality of these two similar solvents has no influence on the amount of water adsorbed at the same fractional water saturation. The limit of the validity of the Langmuir equation was determined to be about $X = 0.12$ in both solvents, corresponding to monomolecular coverage of the adsorbent by water.

Thomas *et al.*[9] studied these conditions and defined isohydric solvents for improving reproducibility in liquid–solid chromatography.

Influence of water content on retention

In order to investigate the influence of the water content on retention the capacity ratios (k') of different solutes were determined. The results are shown in Figs. 2 and 3.

In Fig. 2a the capacity ratios determined on the silica packings are shown as a function of the water content in chloroform. At low water contents there is a steep decrease in the k' values corresponding to a small increase in the water content. This effect has also been observed by other workers[4,8,10]. By increasing the water content a quasi-linear slower decrease can be observed. The two parts of the curves can be approximated by straight lines, indicating a transition at about 120 ppm of water corresponding to the validity of the Langmuir equation, *i.e.*, to the formation of a monolayer of water on the silica surface.

In Fig. 2b the k' values determined on aluminas A5Y and Alox T are shown. Corresponding to the higher activity of the alumina surface and the shape of the water isotherm at low water contents, the decrease in k' values is much steeper than on silica. There are considerable differences among the capacities of the aluminas investigated in accordance with the differences in the specific surface areas. It is interesting that at higher water contents (above 300–400 ppm) these differences become very small, indicating a more uniform surface activity due to the larger amount of water adsorbed.

In Fig. 3 the effect of the solvent on the capacity ratios can also be observed. As the amount of water adsorbed is the same at a given fractional water saturation for both solvents, the influence of the solvent strength can be studied under these "isohydric" conditions. It can be seen that the k' values obtained in the "weaker" chloroform are much higher than those obtained in dichloroethane. The curves in Figs. 2

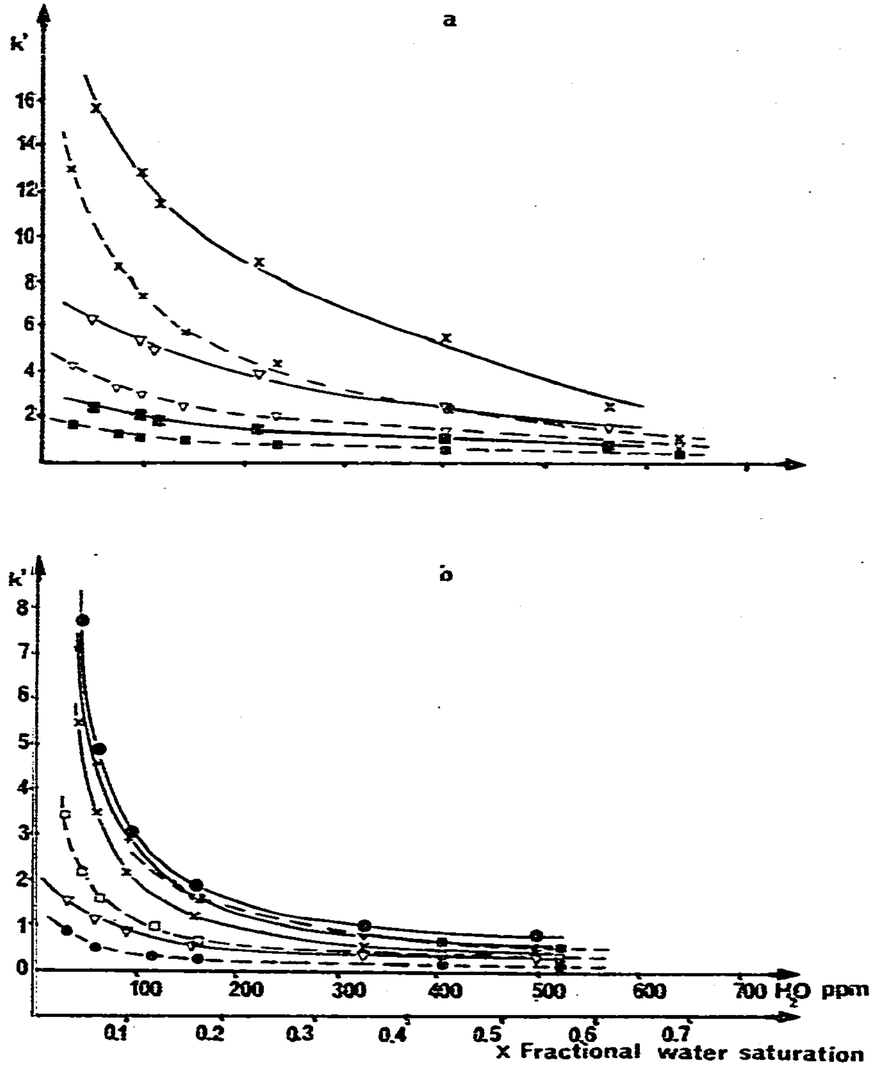

Fig. 2. Changes in capacity ratios as a function of the water content of chloroform. (a) On silica packings: ———, Si 60; — — —, Si 100; ■, ethyl benzoate; ▽, acetophenone; ×, dibutyl phthalate. (b) On alumina packings: ———, A5Y; — — —, Alox T; ▽, acetophenone; ×, dibutyl phthalate; +, diethyl phthalate; ●, dimethyl phthalate; □, *o*-nitroaniline; ■, *p*-nitroaniline.

and 3 demonstrate the importance of controlling the water content at low water contents in order to obtain the reproducibility required for the determination of *k'* values. The results obtained at higher water contents indicate, however, that in spite of the steep increase in the water adsorption isotherms, *i.e.*, the amount of water adsorbed in this range, the activity of the surface of all of the adsorbents investigated hardly decreases for the different solutes investigated, resulting in a very small decrease in the *k'* values with increasing water content of the mobile phase.

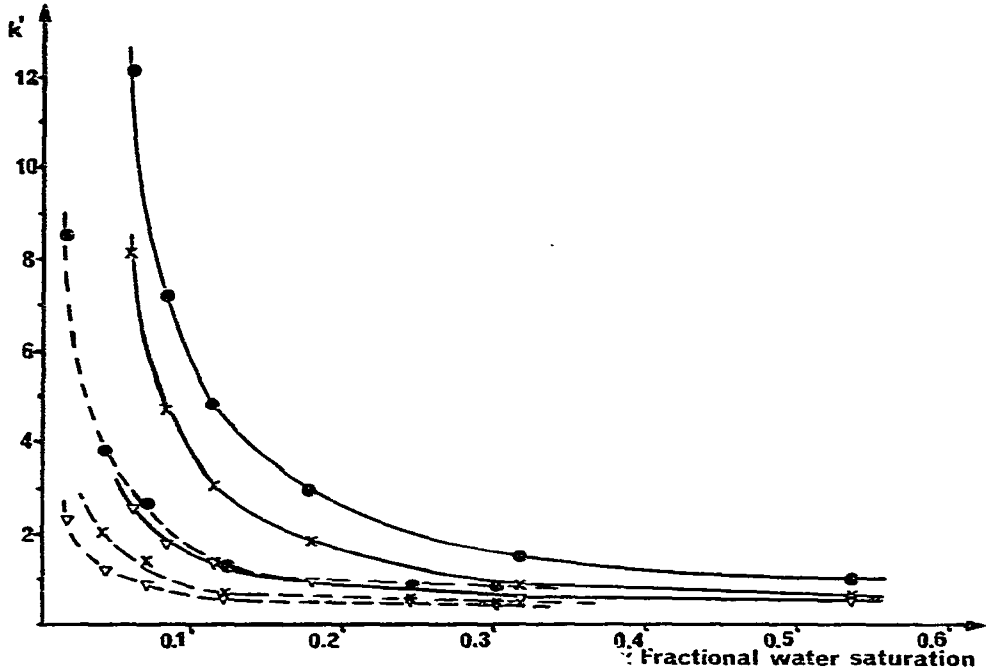

Fig. 3. Changes in capacity ratios as a function of the water content of the mobile phase on Woelm alumina. ———, Chloroform; — — —, dichloroethane; ▽, acetophenone; ×, dibutyl phthalate; ●, dimethyl phthalate.

Influence of water content on selectivity

In the literature, some general comments can be found on the influence of the water content of the mobile phase on the relative retention, *i.e.*, selectivities of different solutes[1–4,7–10]. It was generally observed that the retentions of different solutes vary differently on increasing the water content, sometimes resulting in a considerable change in selectivity (α). For a given pair of solutes even variations in the order of elution are possible[1,10]. However, no systematic investigation of the variation in selectivity has been published. As the selectivity has a considerable effect on the resolution, *i.e.*, the chromatographic performance of the column, the influence of the water content on the selectivity is of paramount importance for selecting the optimum conditions of the separation.

In Figs. 4 and 5 relative retentions, *i.e.*, selectivity values are shown for different pairs of solutes. In Fig. 4a the α values determined on silica packings are shown. As with the curves obtained for the capacity ratios, two parts of the curves can be distinguished. At low water contents there is a steeper decrease in the α values corresponding to a small increase in the water content of the chloroform. Again, the two parts of the curves can be approximated by straight lines, indicating a transition at about 120 ppm of water, corresponding to the formation of a monolayer of water on the silica surface. For the solutes investigated the α values always decrease with increasing water content of the chloroform.

In Fig. 4b the selectivities determined on A5Y and Alox T alumina packings are shown. In accordance with the shapes of the curves obtained for the k' values, the

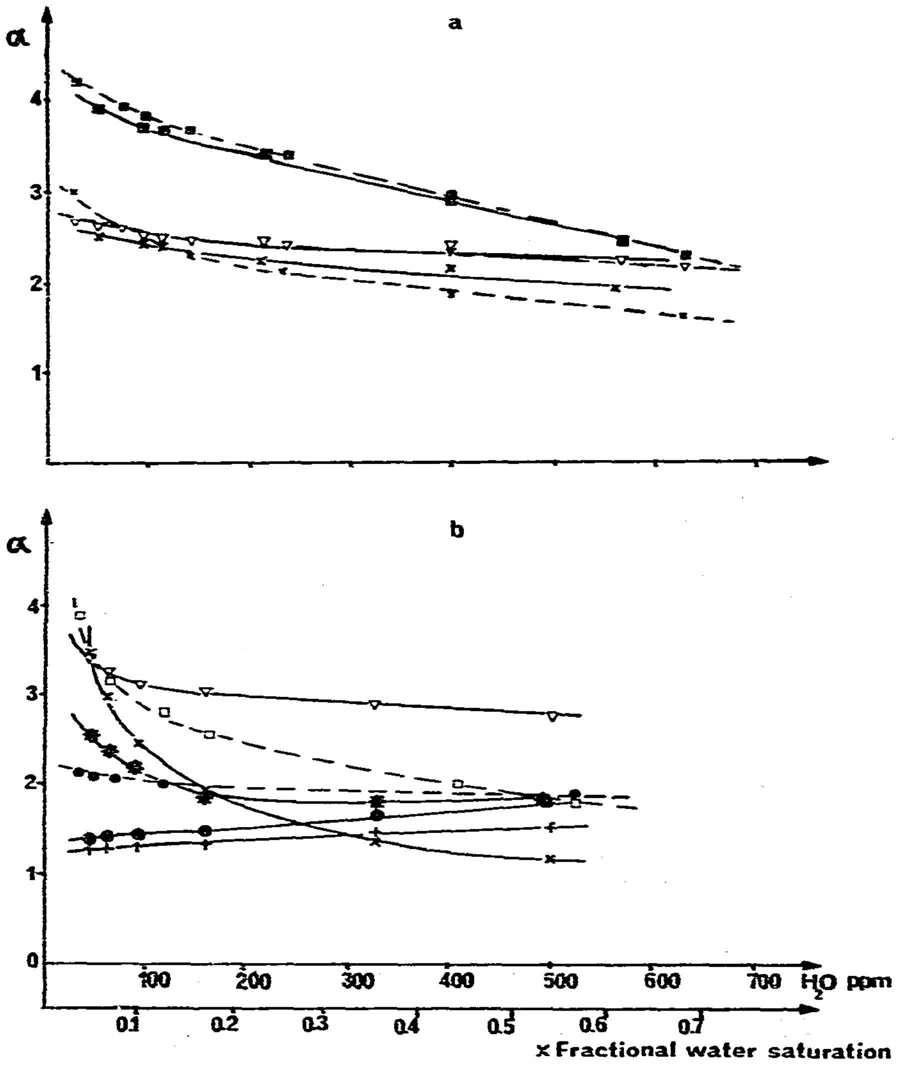

Fig. 4. Changes in selectivities as a function of the water content of chloroform. (a) On silica packings: ———, Si 60; – – –, Si 100; ■, ethyl benzoate–nitrobenzene; ▽, acetophenone–ethyl benzoate; ×, dibutyl phthalate–acetophenone. (b) On alumina packings: ———, A5Y; – – –, Alox T; ▽, acetophenone–nitrobenzene; ×, dibutyl phthalate–acetophenone; +, diethyl phthalate–dibutyl phthalate; ●, dimethyl phthalate–dibutyl phthalate; ★, acetophenone–benzonitrile.

decrease in the α values is much more significant at low water contents than that on the silicas. On the aluminas, however, the change in selectivity with increasing water content is ambiguous. For some pair of solutes (phthalic acid esters) a small but continuous increase in the selectivity can be observed with increasing water content. This indicates a special mechanism of phase exchange for these compounds on the alumina surface covered partly or completely with adsorbed water. Again, at higher water contents the decrease in selectivity becomes negligibly small, indicating a nearly

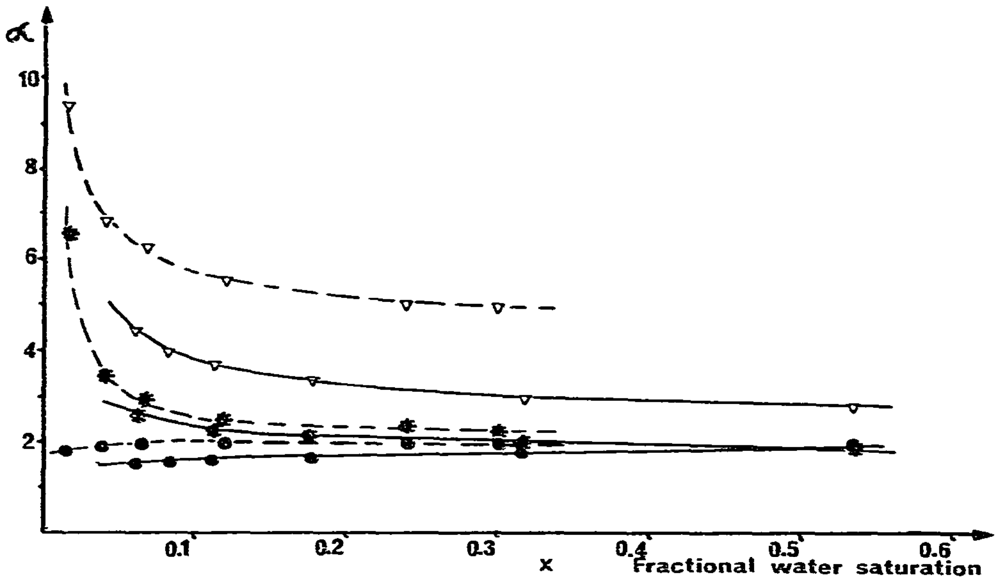

Fig. 5. Changes in selectivities as a function of the water content of the mobile phase on Woelm alumina. ——, Chloroform; – – –, dichloroethane; ▽, acetophenone–nitrobenzene; ●, dimethyl phthalate–dibutyl phthalate; ★, acetophenone–benzonitrile.

constant activity of the surface covered with adsorbed water.

In Fig. 5 the α values determined in chloroform and dichloroethane are shown as a function of the fractional water saturation (X) of the mobile phase. The considerable differences in the α values measured in the two solvents are interesting. Surprisingly, for most of the pairs of solutes investigated, higher selectivities were obtained with the stronger dichloroethane eluent. This finding emphasizes the necessity for further investigations on the influence of the eluent on selectivities in liquid–solid chromatography.

Influence of water content on efficiency

Very few experimental data, and some contradictory observations could be found in the literature on the influence of the water content on column efficiency. It is generally accepted that with a "dry" mobile phase, *i.e.*, at low water contents, much lower efficiencies can be obtained because of the strong active sites of the adsorbent surface, leading to slow adsorption–desorption kinetics[1,4,8,13]. The variation in efficiency as a function of the water content of the mobile phase was studied by El Rassi *et al.*[8] on different silica packings using dichloromethane as eluent, but only for one solute, linuron. The HETP values given show a flat minimum in each instance but at widely different and fairly high water contents for the three silicas investigated (Si 60, Si 100, Partisil 5). In another study column efficiency was found to be virtually independent of the composition of the mobile phase[14].

In our study, column efficiency was investigated by determining the number of theoretical plates at different water contents. The measurements of efficiency were carried out at different mobile phase velocities and with high chart speeds in order to

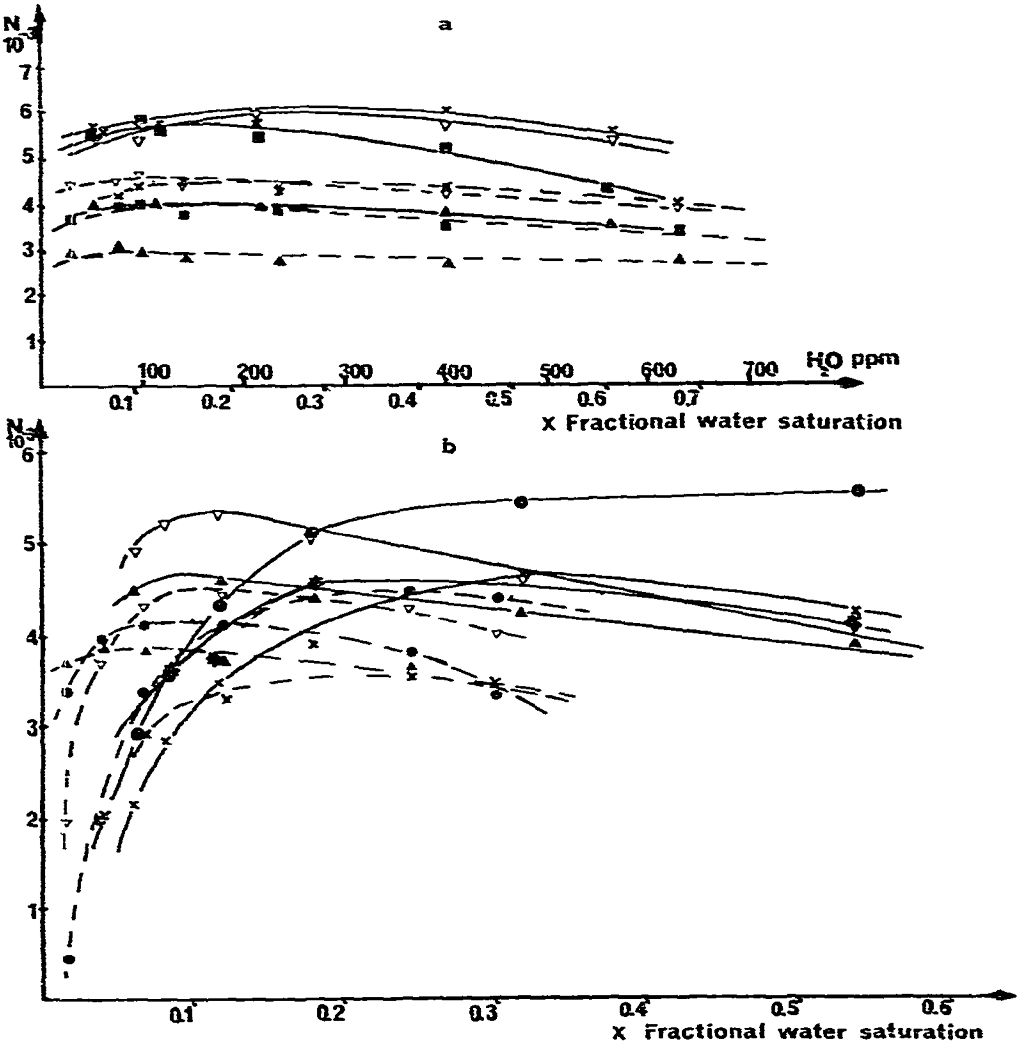

Fig. 6. Changes in number of theoretical plates as a function of the water content of the mobile phase. Flow-rate, 0.5 ml/min. (a) On silicas in chloroform: ———, Si 60; – – –, Si 100; ▲, nitrobenzene; ■, ethyl benzoate; ▽, acetophenone; ×, dibutyl phthalate. (b) On Woelm alumina: ———, chloroform; – – –, dichloroethane; ▲, nitrobenzene; ★, benzonitrile; ▽, acetophenone; ×, dibutyl phthalate; ●, dimethyl phthalate.

permit the reliable determination of peak characteristics. Peak widths measured at half-height were used for the calculation of the number of theoretical plates, *N*. It should be noted that *N* values were calculated by using the actual peak parameters and no correction was made for external effects. N_{max} values are shown in Fig. 6 as a function of the water content of the mobile phase. Depending on the success of the filling procedure, the columns investigated showed some differences in efficiency, but this had no influence on the trends observed.

For the silica packings, the curves in Fig. 6a show flat maxima in the range

100–200 ppm of water, *i.e.*, at about the formation of a monolayer. It can also be seen that there is not much difference in efficiency in the range investigated.

The results obtained for the alumina packings were very different. Fig. 6b shows *N* values measured on Woelm alumina in the two solvents. At low water contents considerable peak broadening and consequently very low efficiency can be observed for nearly all of the solutes investigated. On increasing the water content the curves pass through maxima in the range 0.08–0.3 of fractional water saturation, except for dimethyl phthalate. At higher water contents the decrease in efficiency is generally slight and almost linear. The same types of *N* curves also were obtained on A5Y and Alox T aluminas.

Fig. 6b shows the effect of the viscosity of the eluent on column efficiency. For the less viscous chloroform (η = 0.57 cP) much higher efficiencies can be achieved than for dichloroethane (η = 0.79 cP) on the same column.

It can be concluded that peak broadening and consequently column efficiency may be different for the different solutes and different eluents and no *a priori* prediction of these values can be made. The very low and rapidly changing values at low

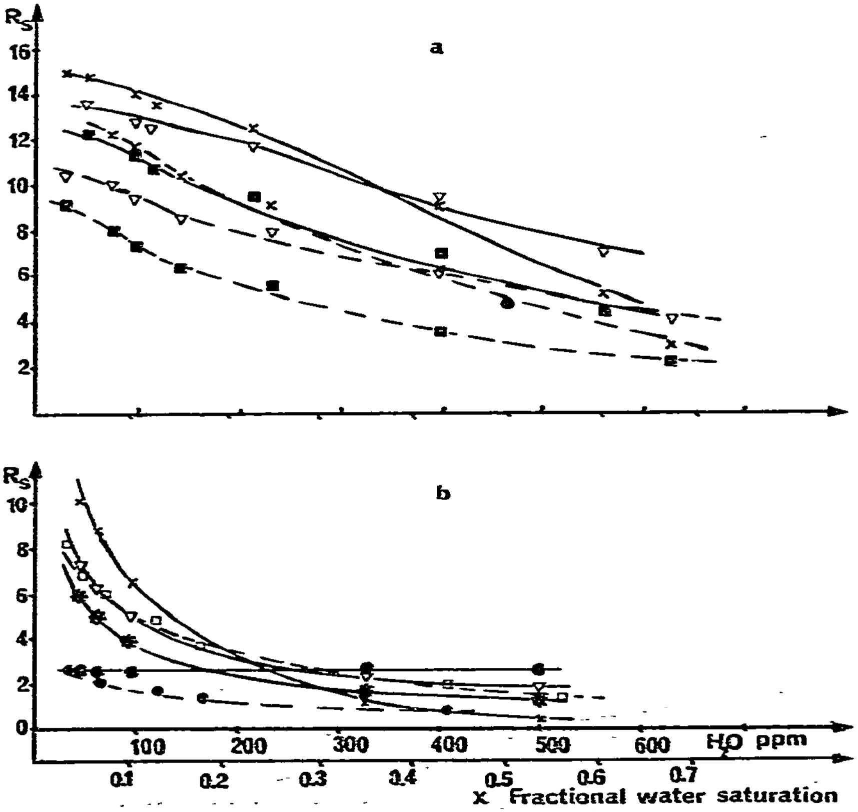

Fig. 7. Changes in R_s as a function of the water content of chloroform. (a) On silica packings: ———, Si 60; — — —, Si 100; ■, ethyl benzoate–nitrobenzene; ▽, acetophenone–ethyl benzoate; ×, dibutyl phthalate–acetophenone. (b) On alumina packings: ———, A5Y; — — —, Alox T; ▽, acetophenone–nitrobenzene; ×, dibutyl phthalate–acetophenone; ●, dimethyl phthalate–dibutyl phthalate; ★, acetophenone–benzonitrile; □, *o*-nitroaniline–dimethyl phthalate.

water contents again indicate the necessity for selection and control of the water content in the mobile phase.

Influence of water content on resolution

In every chromatographic separation the object is to separate the components of a mixture, and this separation is characterised by the resolution of two peaks (R_S). In the literature different equations are used for the determination of peak resolution. It was found that the peak resolutions determined from the chromatograms were best fitted by the values calculated by the following equation[15]:

$$R_S = \frac{\bar{N}^{1/2}}{2} \cdot \frac{\alpha - 1}{\alpha + 1} \cdot \frac{\bar{k}'}{1 + \bar{k}'}$$

where $\bar{N}$ and $\bar{k}'$ are the arithmetic mean values of the plate numbers and capacity ratios, respectively, measured for the two components being evaluated.

For this reason, eqn. 1 was used for the calculation of the R_S values shown in Figs. 7 and 8. No study has appeared in the literature on the influence of the water content of the mobile phase on peak resolution. As the terms in eqn. 1 change in different manners with changes in the basic characteristics (N,α,k'), obviously the superposition of these variations will give the variation of R_S with varying water content.

In Fig. 7a the influence of the water content on the resolution determined for the silica packings is shown. In accordance with the shape of the curves given for the basic characteristics in Figs. 2, 4 and 6, roughly two parts can be distinguished on the curves in Fig. 7a. At low water contents the decrease in the resolution is more pronounced than at higher water contents. The change in the shape of the curves at very

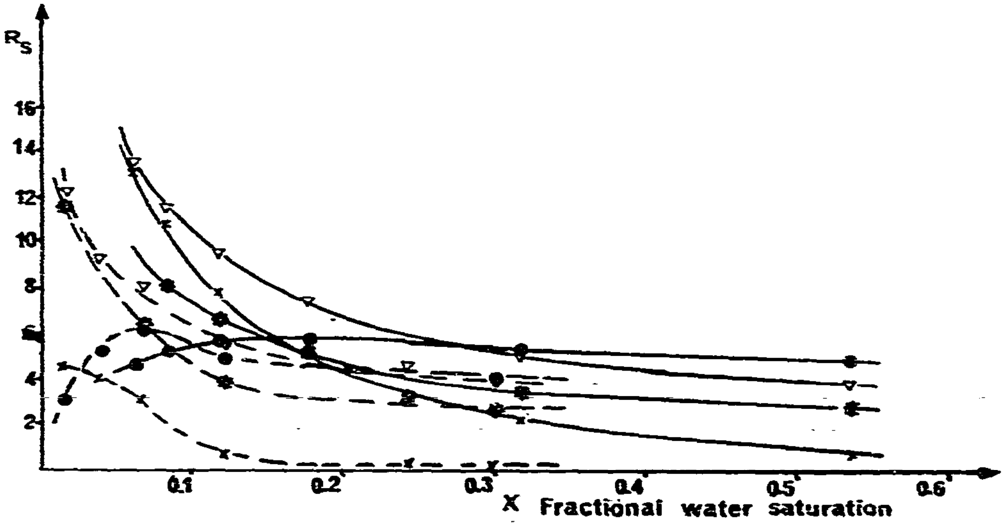

Fig. 8. Changes in R_S as a function of the water content of the mobile phase on Woelm alumina. ———, Chloroform; — — —, dichloroethane; ∇, acetophenone–nitrobenzene; ×, dibutyl phthalate–acetophenone; ●, dimethyl phthalate-dibutyl phthalate; ★, acetophenone–benzonitrile.

low water contents can be explained by the decreasing column efficiency in this region.

In Fig. 7b the resolutions obtained on A5Y and Alox T aluminas are compared. In accordance with the steep decrease in the k' and α values with increasing water content at low water contents, for most of the compounds investigated the decrease in R_S is more pronounced with increasing water content than with the silica packings. On the other hand, above a certain water content (about 200–300 ppm) the resolution decreases only very slightly.

Corresponding to the anomalous shape of the α curves obtained for some pairs of solutes (phthalic acid derivatives) and the low efficiencies obtained at low water contents, a very slight decrease, or even an increase, in resolution can also be observed with increasing water content.

The shape of the resolution curves shown in Fig. 8, obtained on Woelm alumina, reveal similar changes in resolution to those obtained on the other alumina packings. The different effects of the two eluents on the k', α and N values result in a higher resolution for the chloroform eluent.

CONCLUSION

In order to evaluate the influence of the water content of the mobile phase on the chromatographic performance of an adsorbent packing, not only changes in the capacity ratios but also changes in the selectivities and efficiencies and from these data the changes in resolution should be determined.

The changes in the chromatographic characteristics with increasing water content of the mobile phase can be explained qualitatively on the basis of the water adsorption isotherms measured on the different adsorbents.

For silica packings and the solutes investigated, an increase in the water content of the mobile phase results in a decrease in the k' and α values and flat maxima of the N values. In accordance with these variations, R_S decreases continuously with increasing water content but this decrease is more pronounced at low than at higher water contents. The transition occurs at about 120 ppm of water in chloroform, corresponding to the formation of a monolayer on the adsorbent surface. On the alumina packings investigated, in accordance with the higher activity of the alumina surface, k' and α values decrease more rapidly with increasing water content and for the α values reversed effects can also be observed. The plate numbers are very poor at low water contents and pass through flat maxima at higher water contents.

Corresponding to these effects, for most of the compounds investigated the decrease in R_S is more pronounced with increasing water content than that on silica packings, but beyond a certain limit R_S decreases only very slightly. For some pairs of solutes a very slight decrease, or even an increase, in resolution can also be observed with increasing water content.

The results obtained with two different solvents on Woelm alumina indicate the influence of the solvent on the chromatographic characteristics. The choice of the best solvent for a given separation is still a hardly exploited area in high-performance liquid chromatography. The effect of the water content on the chromatographic characteristics of different solutes may be quite different, indicating a possible diversity of mechanisms and the necessity for further investigations with a broad range of solutes.

The results of this study also reveal some possibilities of practical importance. Adsorbent packings with moderately polar solvents and a properly adjusted water content can be used advantageously for the separation of polar compounds. At higher water contents both the variation in the water content and the effect of this variation on the chromatographic performance are much less important, in many instances negligible, than at the low water contents generally applied in adsorption chromatography.

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