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ADSORBENT DEACTIVATION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Water is often added to the eluent in high-performance adsorption chromatography to improve isotherm linearity and therefore increase the amount of sample which can be separated without affecting retention or plate height. Water is especially inconvenient as a deactivating agent in saturated hydrocarbon solvents because of its low solubility. First, it is difficult to prepare eluents with reproducible water content. Secondly, humidified solvents must be freshly prepared because the water content changes rapidly due to equilibration with the walls of the container. Thirdly, large volumes of eluent must be passed through the column to establish equilibrium. In order to circumvent these problems, the use of acetonitrile and methylene chloride as substitutes for water has been studied. The maximum sample capacity, $\theta_{.1}$, defined as the largest sample size (g sample/g adsorbent) which can be separated while maintaining k' to within 90% of that for a very small sample, is compared at several concentrations of each deactivating agent. Similarly, the maximum sample capacity is compared in terms of plate height. It is shown that acetonitrile is equal to or better than water with respect to sample capacity, plate height, convenience, and equilibration time. Sample selectivity with acetonitrile is not identical to sample selectivity with water as deactivating agent. Methylene chloride is significantly less effective with respect to sample capacity.

INTRODUCTION

For many years non-linear isotherms were believed to be an unavoidable aspect of adsorption chromatography. Although water had been used since the 1940s as a deactivating agent to control retention[1], it was not until the 1960s that Snyder[2,3] demonstrated that adsorption isotherms, at low sample concentrations, are linear, and that water deactivation greatly extends the range of linearity. In classical (low-pressure) chromatography the use of water as a deactivating agent added to the adsorbent is convenient since the column is used only once. In high-performance liquid chromatography (HPLC), however, the column is re-used many times, and the solvent must contain an equilibrium concentration of deactivating agent (modifier) in order to maintain a constant level of adsorbent deactivation[4].

A number of problems become immediately apparent when water-deactivated solvents are used, particularly when the solvent is of low polarity. First, water is immiscible with non-polar solvents and the rate at which non-polar solvents, especially saturated hydrocarbons, reach water saturation is extremely slow[5]. Normally, water-saturated eluent is prepared by passing it through a column of silica gel which is saturated with water[6]. This procedure is time consuming, especially if large volumes of solvents are needed. Even reagent quality solvents contain significant but variable quantities of water, so it is necessary to dry quantities of eluent for blending with water-saturated eluent in order to achieve a reproducible water content. Dry solvent can be prepared by passing the solvent through a column of dry silica or molecular sieves, but again the procedure is time consuming. Because it is difficult to measure low levels of water in saturated hydrocarbons, it is often not clear that the drying process has been successful. Wet solvents must be stored in containers which have no affinity for water or the water content will change significantly in a matter of hours. Glass containers are particularly unsuitable since dry glass can adsorb significant quantities of water, decreasing the final degree of water saturation by 50% or more[7]. Similarly wet glass containers can significantly increase the solvent water content. Because the concentration of water in water-modified saturated hydrocarbons is so low (usually <100 ppm[8]), great quantities of solvent must be passed through the column before equilibrium (constant retention time) is achieved. This conditioning process can take several hours[5,9].

The use of organic modifiers has been advocated by many chromatographers as a means of circumventing these problems[5,9–11]. However, when alcohols are used, selectivity changes[10], efficiencies decrease[5], and some rather unusual peak shapes occur at certain modifier concentrations[9]. In addition, column equilibration is not fast[5].

No systematic evaluation of the use of organic modifiers in HPLC has yet appeared, and little information is available on the use of organic modifiers with saturated hydrocarbons. For this reason we investigated the use of organic modifiers on silica with hexane as the solvent.

THEORY

The adsorption process has been described by the Langmuir equation[2]:

$$\theta_x = \frac{N_x K_x}{1 + N_x K_x} \tag{1}$$

where θ_x is the fraction of the adsorption sites covered by adsorbed sample molecules, x; N_x is the mole fraction of x in solution; and K_x is the thermodynamic equilibrium constant for the adsorption–desorption process. At low N_x, $\theta_x \approx N_x K_x$ and therefore the fraction of sites covered is proportional to the concentration in solution. This is the linear isotherm region of chromatographic interest. The chromatographic distribution coefficient K_x' is given by

$$K_x' = \frac{\theta_x}{N_x} = \frac{K_x}{1 + N_x K_x} \tag{2}$$

In the linear isotherm region $K_x' = K_x$, but at higher concentrations, K_x' becomes smaller than K_x, corresponding to a shift to lower retention volume upon sample overloading.

This simple approach does not take into account adsorbent deactivation. The primary difference between solvent and modifier is that sample molecules can compete with solvent molecules for adsorption sites, but cannot compete with modifier. What distinguishes a modifier from a solvent is its much greater attraction to the surface. If we define θ_y as the fraction of sites covered by modifier at equilibrium with modifier in solution, remembering that θ_y will be unaffected by N_x, then

$$\theta_y = \frac{N_y K_y}{1 + N_y K_y} \tag{3}$$

where N_y is the mole fraction of modifier in the eluent and K_y is its thermodynamic equilibrium constant.

The adsorption of sample will be affected by N_y. The fraction of sites available for sample adsorption is now $1 - \theta_y - \theta_x$, and at equilibrium the rates of desorption and adsorption are equal so that

$$k_d \theta_x = N_x k_a (1 - \theta_y - \theta_x) \tag{4}$$

where k_d and k_a are the rate constants for desorption and adsorption, respectively.

Combining eqns. 3 and 4, and remembering that $K_x = k_a/k_d$ gives

$$K'_x = \frac{\theta_x}{N_x} = \frac{K_x}{(1 + N_x K_x)(1 + N_y K_y)} \tag{5}$$

It has been long recognized that the adsorbent surface is inhomogeneous[2]. There exists a distribution of adsorption sites each of which attracts molecules with different strengths. If we assume that all sites can be classified as either strong or weak, we can repeat the derivation to obtain an equation which more adequately describes the real case. Taking the approach of Snyder[12] and extending it (as above) to include deactivation, we define K_{1x} and K_{2x} as the thermodynamic equilibrium constants for sample on strong and weak sites, respectively. Similarly, K_{1y} and K_{2y} are defined for the modifier on the two sites. According to eqn. 5, the fraction of strong sites covered by sample, θ_{1x}, is given by

$$\theta_{1x} = \frac{N_x K_{1x}}{(1 + N_x K_{1x})(1 + N_y K_{1y})} \tag{6}$$

Similarly, the fraction of weak sites covered by sample, θ_{2x}, is given by

$$\theta_{2x} = \frac{N_x K_{2x}}{(1 + N_x K_{2x})(1 + N_y K_{2y})} \tag{7}$$

The total fraction of sites covered by sample is then

$$\theta_x = N_1 \theta_{1x} + N_2 \theta_{2x} \tag{8}$$

where N_1 and N_2 are the fractions of strong and weak sites, respectively. The distribution coefficient then becomes

$$K'_x = \frac{\theta_x}{N_x} = \frac{N_1 K_{1x}}{(1 + N_x K_{1x})(1 + N_y K_{1y})} + \frac{N_2 K_{2x}}{(1 + N_x K_{2x})(1 + N_y K_{2y})} \tag{9}$$

Eqn. 9 describes how retention varies with sample concentration and modifier concentration. At high sample concentration K_x' is a function of sample size, but at low N_x, eqn. 9 reduces to

$$K_x^\infty = \frac{N_1 K_{1x}}{1 + N_y K_{1y}} + \frac{N_2 K_{2x}}{1 + N_y K_{2y}} \tag{10}$$

where K_x^∞ is defined as the linear isotherm value of the distribution coefficient.

The assumption leading to eqns. 9 and 10 is that the sample does not compete with deactivating agent for adsorption sites. This requires $K_{1y} \gg K_{1x}$ and $K_{2y} \gg K_{2x}$.

Eqn. 10 is composed of two terms. The first term gives the contribution to retention resulting from strong sites and the second term relates to weak sites. Therefore, $K_{1x} > K_{2x}$ and $K_{1y} > K_{2y}$. As N_y increases, the magnitude of the first term diminishes more rapidly than that of the second. Thus, as modifier concentration is increased, the importance of strong sites diminishes more rapidly than that of weak sites.

Eqns. 9 and 10 describe the behavior of a hypothetical adsorbent with only two distinct adsorption site types. Actual adsorbents are believed to exhibit several adsorption site types and each of these may be energetically heterogeneous. While this model may be oversimplified, it should at least give some qualitative insight into the nature of inhomogeneous adsorbents.

Fig. 1 shows the variation of the linear isotherm distribution coefficient, K_x^∞, with N_y, calculated from eqn. 10. The fractional coverage of strong and weak sites (θ_{1y} and θ_{2y}) is also shown in Fig. 1. The parameters used to calculate the curves shown in Fig. 1 were not entirely arbitrary. First, it has been reported that the relative concentration of strong sites on wide-pore silica is generally $<5\%$[13]. For this reason $N_1 = 0.05$ and $N_2 = 0.95$ were used. The values of K_{1x} and K_{1y} were chosen to give K_x^∞ of the order of magnitude observed in Experimental. It is not yet possible to predict the values of K_{1y} and K_{2y}, but the shape of the curves shown in Fig. 1 would be identical for all values providing the ratio K_{1y}/K_{2y} is constant. The only difference would be a displacement along the N_y axis. In any case, Fig. 1 is representative of the type of curve shape expected in real systems.

In Fig. 1, at low N_y, no significant fraction of either strong or weak sites is covered by modifier, and K_x^∞ (and therefore retention volume) remains fairly constant. At higher N_y the strong sites become increasingly covered by modifier and K_x^∞ diminishes. At the highest levels of N_y, K_x^∞ drops off less rapidly as the influence of strong sites gives way to weak sites. In Fig. 1, at $N_y \approx 10^{-3}$, the strong sites are about 90% covered, while 90% of the weak sites remain uncovered. At this point the curve begins to level off and deactivation is essentially complete without significant loss of weak sites. The shape of Fig. 1 indicates that in order to obtain reproducible retention in deactivated systems, it is important to maintain a sufficient level of modifier to cover most of the strong sites. By working in the lower (highly deactivated) region of the curve, retention is not so strongly influenced by small changes in modifier concentration.

The sample linear capacity for an adsorbent in a chromatographic system, $\theta_{.1}$, is defined as the maximum weight of sample per gram of adsorbent which can be separated without reducing retention (k' or K_x°) by more than 10%[2,12]. If we set eqn.

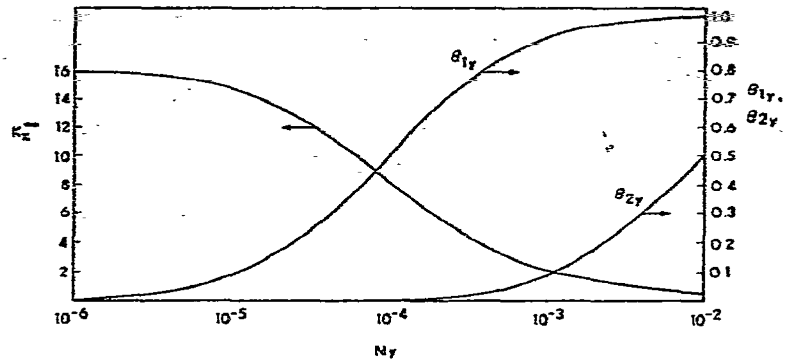

Fig. 1. Variation of the linear isotherm equilibrium constant, K_x^∞, and the fractional surface coverage of strong sites, θ_{1y}, and weak sites, θ_{2y}, with modifier concentration, N_y, according to eqns. 6, 7, and 10. $K_{1x} = 300$; $K_{2x} = 1$; $K_{1y} = 10^4$; $K_{2y} = 10^2$; $N_1 = 0.05$; $N_2 = 0.95$.

9 equal to 0.9 K_x^∞, combine with eqn. 10, and solve for N_x, we get the following quadratic expression for N'_x, the concentration of sample which results in $K'_x = 0.9\ K_x^\infty$

$$(N'_x)^2 (-0.9\ K_x^\infty\ K_{1x}\ K_{2x}\ C) + N'_x\ [K_{2x}\ A + K_{1x}\ B - 0.9\ K_x^\infty\ C\ (K_{1x} + K_{2x})] + \\ + A + B - 0.9\ K_x^\infty\ C = 0 \qquad (11)$$

where

$$A = N_1\ K_{1x}\ (1 + K_{2y}\ N_y)$$

$$B = N_2\ K_{2x}\ (1 + K_{1y}\ N_y)$$

$$C = (1 + K_{1y})\ (1 + K_{2y})$$

The solution of this equation gives a single positive value of N'_x for a given value of N_y. N'_x defines the beginning of the non-linear portion of the isotherm and therefore this value should be proportional to $\theta_{.1}$ on a given column. Table I shows the variation

TABLE I

EFFECT OF DEACTIVATING AGENT ON N_x

$K_{1x} = 300$; $K_{2x} = 1$; $K_{1y} = 10^4$; $K_{2y} = 10^2$; $N_1 = 0.05$; $N_2 = 0.95$.

N_y	K_x^∞	N'_x ($\times 10^3$)
0	16.0	0.40
0.00003	12.5	0.40
0.0001	8.44	0.42
0.0003	4.67	0.47
0.001	2.23	0.65
0.003	1.22	1.1
0.01	0.62	2.3
0.03	0.29	4.2
0.1	0.10	5.9
0.3	0.04	6.8

of N_x' with N_y calculated from eqn. 11 using the same parameters K_{1x}, K_{2x}, etc., used to calculate Fig. 1. As shown, the value of N_x', and thus sample capacity, increases with N_y. This increase is a consequence of the distribution of adsorption site strengths. At low concentration the strong sites (first term of eqn. 11) dominate the retention but because they are present at low concentration, relatively little sample is required to overload them. As the strong sites become covered with modifier, the weaker sites become more important and because there are more weak sites, N_x' (linear capacity) increases. Thus, highly deactivated systems allow larger amounts of sample to be separated as well as constant retention time.

The requirements of a desirable deactivating agent are: (1) $K_{1y} \gg K_{1x}$ and $K_{2y} \gg K_{2x}$; (2) useful concentration range, $\geqslant$0.01 vol.%; (3) common solvent properties.

Requirement 1 is a re-iteration of the definition of a deactivating agent. Since the modifier is much more strongly adsorbed, only small concentrations will be required to achieve adequate deactivation. If modifier concentrations >1% are required to cover the strong adsorption sites, then requirement 1 is probably not fulfilled.

Requirement 2 recognizes the relationship between modifier concentration and the volume of eluent which must be passed through the column to achieve equilibrium. As K_y becomes larger, the concentration of modifier, N_y, required to achieve a given degree of deactivation decreases proportionally. While requirement 1 indicates K_y values should be large, requirement 2 indicates they should not be too large. Experience has shown that modifier concentrations below 0.01 vol.% are slow to reach equilibrium[5,9]. Requirements 1 and 2 indicate that K_y values should be of such a magnitude as to allow adequate deactivation in the range 0.01–1 vol.%.

Requirement 3 concerns some practical limitations of the modifier. First, the modifier should be low boiling in order to simplify the recovery of collected fractions. Secondly, it should not be UV absorbing or otherwise interfere with sample detection. Finally, the modifier should be chemically stable. These are all common solvent properties and therefore it is likely that successful modifiers will be found among the list of the more polar LC solvents.

EXPERIMENTAL

Solvents were ACS reagent grade or better. Dry hexane and methylene chloride were prepared by passing them through a 120 × 2.6 cm I.D. glass column filled with Davidson Code 62 silica which had been heated to 190° for 4 h. With this procedure up to four gallons of solvent could be dried. Water-saturated hexane was prepared by passing the solvent through a similar column filled with Code 62 silica containing 30 wt.% water. Water-modified hexane was prepared by blending wet and dry hexane immediately before use. The chromatograph consisted of a Waters Assoc. M-6000 pump, a Varian 5000 p.s.i. stopped flow injector, and an Isco UA-5 UV-visible absorbance detector equipped with 10-mm path cells, and operated at 254, 280, or 310 nm. Columns were 50 cm × 2.1 mm I.D. packed with LiChrosorb Si-60 (10 μm), and were purchased from Varian (Palo Alto, Calif., U.S.A.). Samples were dissolved in dry hexane except when methylene chloride was used as a modifier. In the latter case, samples were dissolved in the eluent in order to eliminate a bandspreading effect in the injector caused by a difference in density between the eluent and the sample solvent. The sample injection volume varied from 1–25 µl. It was determined that the volume of injection, up to 25 µl, had no measurable effect on efficiency for a given sample weight. A flow-rate of 3.0 ml/min was used throughout this work. In order to ensure that equilibrium of the eluent and adsorbent had been attained, the retention time of *m*-diphenoxybenzene was measured at 20–50 column volume (V^0) intervals until successive k' differed by no more than 1%.

RESULTS AND DISCUSSION

In order to compare the predicted influence of modifier to a real system, the retention of *m*-diphenoxybenzene was determined at several modifier concentrations. Fig. 2 is a plot of the capacity factor, k', *versus* modifier concentration. The eluents were dry hexane modified with acetonitrile and dry hexane modified with dry methylene chloride. The capacity factor is directly proportional to the distribution coefficient

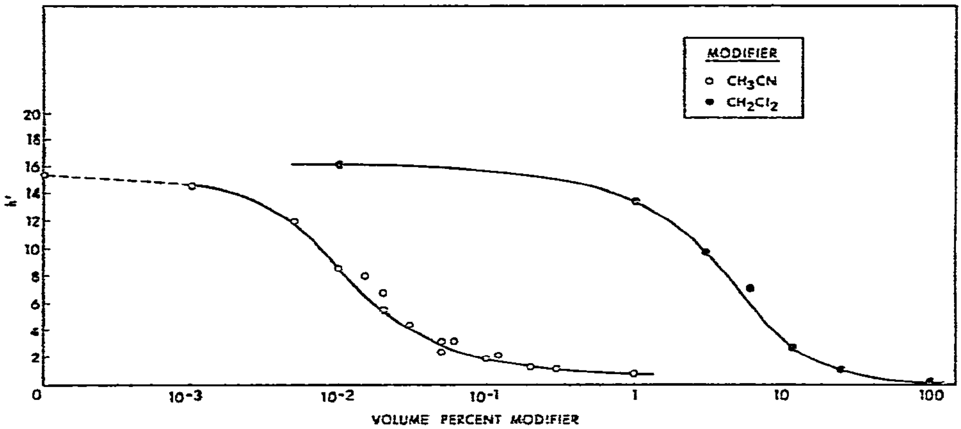

Fig. 2. Variation of capacity factor k' with modifier concentration. Sample, *m*-diphenoxybenzene; column, 50 cm × 2.1 mm I.D., LiChrosorb Si-60 (10 µm); solvent, dry hexane; flow-rate, 3.0 ml/min. Modifiers, acetonitrile and methylene chloride, as indicated. Column temperature, 24°; pressure, 200 atm.

$$k' = \frac{W}{V^0} K_x^\infty \tag{12}$$

where W is the weight of adsorbent in the column and V^0 is the retention volume of a non-retained component. Thus, the curve shape of Fig. 2 should be comparable to that of Fig. 1. Indeed the curves of Fig. 2 do show the anticipated shape. Comparing the curves of Figs. 1 and 2 suggests that an acetonitrile concentration of about 0.05 vol.% corresponds to nearly complete removal of strong sites. However, nearly 10 vol.% methylene chloride is required for the same degree of deactivation. This high concentration suggests that K_{1y} for methylene chloride is not sufficiently large to warrant its classification as a deactivating agent in this system.

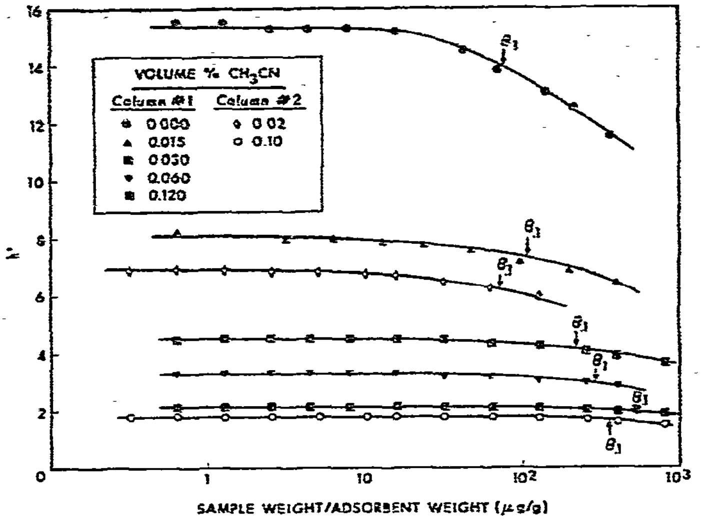

Fig. 3. Variation of k' with sample size for acetonitrile-modified dry hexane. Conditions, same as Fig. 2.

Fig. 3 shows the variation of k' with sample size for dry hexane modified with various concentrations of acetonitrile. As shown, the sample capacity, $\theta_{.1}$, for column 1 increases with deactivating agent concentration from approx. 78 μg/g to approx. 530 μg/g. A similar behavior is exhibited with water-deactivated hexane, shown in Fig. 4, as expected. Within experimental error, methylene chloride seems to have little effect on $\theta_{.1}$, as shown in Fig. 5.

In order to compare the values of $\theta_{.1}$ derived from Figs. 3–5, it is important to realize that $\theta_{.1}$ is a function of k'. $\theta_{.1}$ would be expected to increase as k' decreases

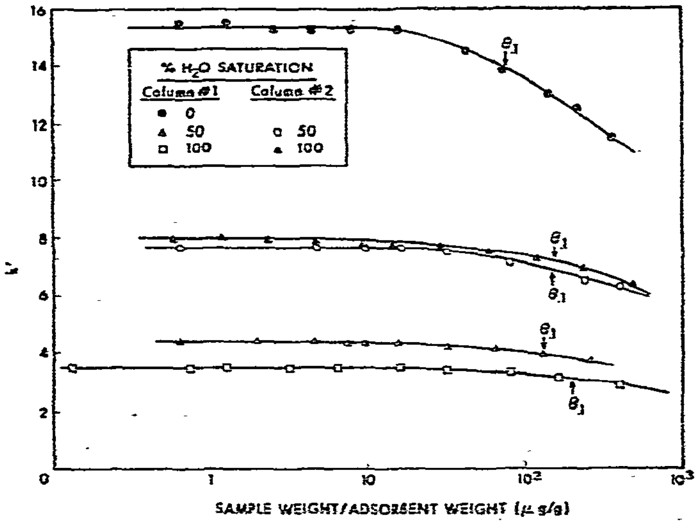

Fig. 4. Variation of k' with sample size for water-modified hexane. Conditions, same as Fig. 2.

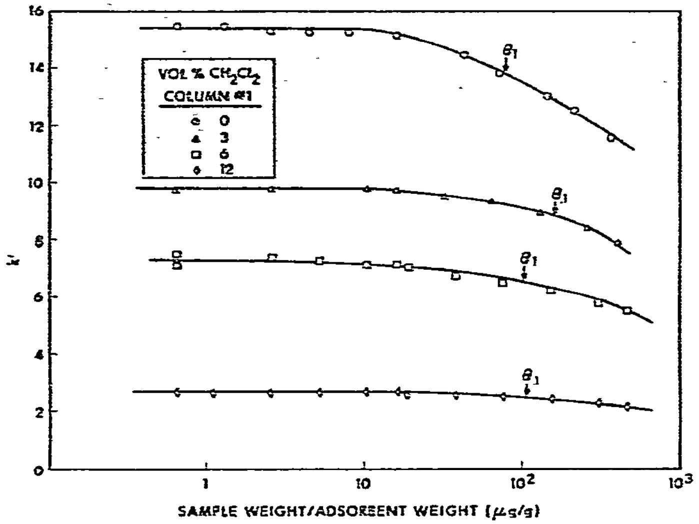

Fig. 5. Variation of k' with sample size for methylene chloride-modified hexane. Conditions, same a Fig. 2.

(even if all adsorption sites were homogeneous) because at lower k' the equilibrium is shifted so that a smaller fraction of sample is in the stationary phase. At $k' = 0$, $\theta_{.1}$ would be large, but no separation would occur. The fraction of solute in the stationary phase, p, is given by $k'/(1 + k')$, thus the product $p\theta_{.1}$ is a better measure of relative sample capacity.

Fig. 6 is a plot of the corrected sample capacity *versus* the reciprocal of k' for wo columns. When plotted in this way, the data for acetonitrile are linear. With the

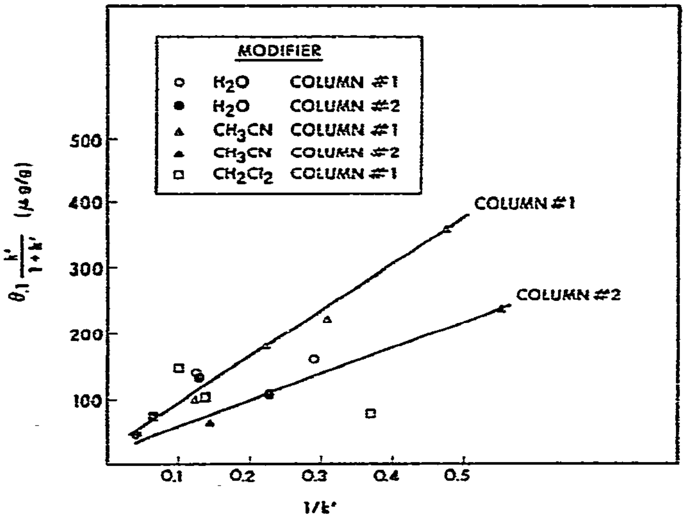

Fig. 6. Comparison of sample capacity for different modifiers.

exception of one point for water-modified hexane on column 2, Fig. 6 indicates that the substitution of acetonitrile for water as modifier results in no loss in sample capacity. In addition, k' can be varied over a wider range and higher values of $\theta_{.1}$ can be attained with acetonitrile. Methylene chloride appears to give equivalent sample capacity at low concentration (low $1/k'$) but falls short of the values obtained with acetonitrile at high concentration. As indicated above, this may be due to a failure to meet requirement 1. Fig. 6 also serves to point out that fairly large differences in overall sample capacity can occur between different columns even though the dimensions and column packing are nominally the same. Differences of this sort may be a result of variations in adsorbent surface heterogeneity from batch to batch.

A useful deactivating agent should not degrade performance with respect to plate height. Figs. 7–9 show the variation of H with sample size for two columns and several modifier concentrations. All curves show a constant low level of H at low sample loads, but rise rapidly as sample capacity is exceeded. All modifier concentrations resulted in nearly identical values of H for a given column at low sample load. This result is in contrast to an earlier study, where it was found that the lowest value of H for dry diethyl ether was substantially higher than that for water-modified ether[9].

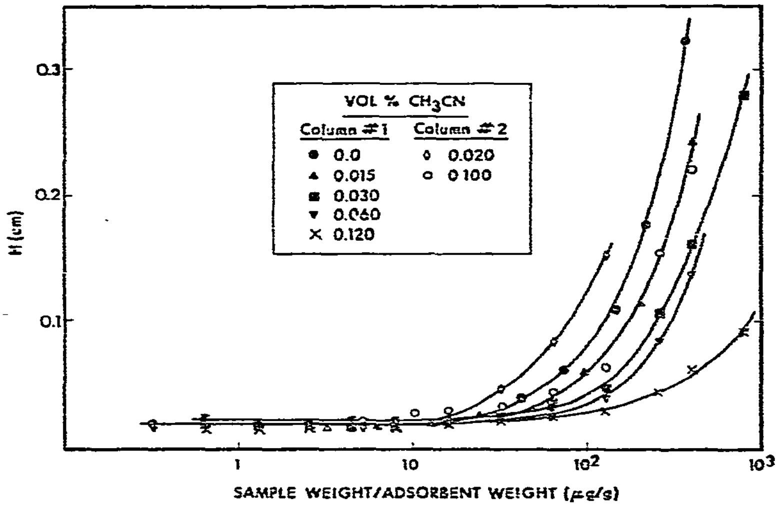

Fig. 7. Variation of H with sample size for acetonitrile-modified dry hexane. Conditions, same as Fig. 2.

In order to compare sample capacity with respect to plate height, we define θ_{2H} as the sample weight per gram of adsorbent which causes H to double. This definition was chosen rather than the conventional definition (10% increase in H) because a minor amount of scatter in the data makes it difficult to accurately determine the sample size corresponding to a 10% increase in H. Values of θ_{2H} taken from Figs. 7–9 are compared in Fig. 10. The correction for small k' is applied in Fig. 10 as in Fig. 6. Again, we find acetonitrile gives sample capacities equivalent to water over the full range of k', but methylene chloride fails at higher concentration.

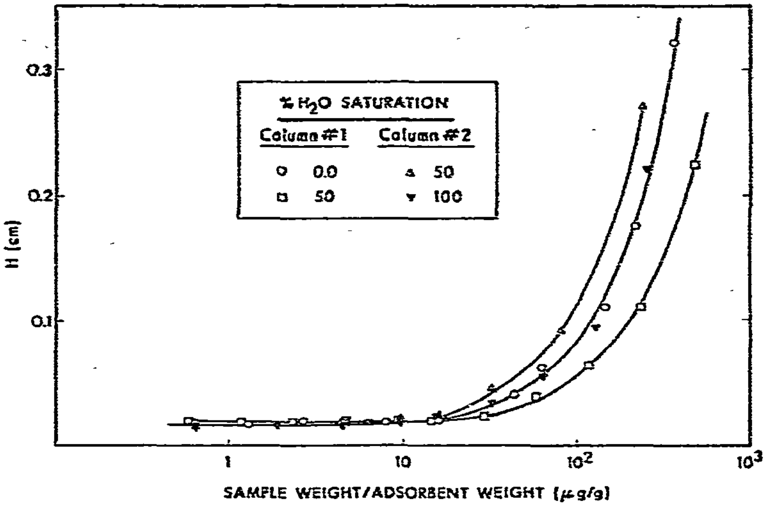

Fig. 8. Variation of *H* with sample size for water-modified hexane. Conditions, same as Fig. 2.

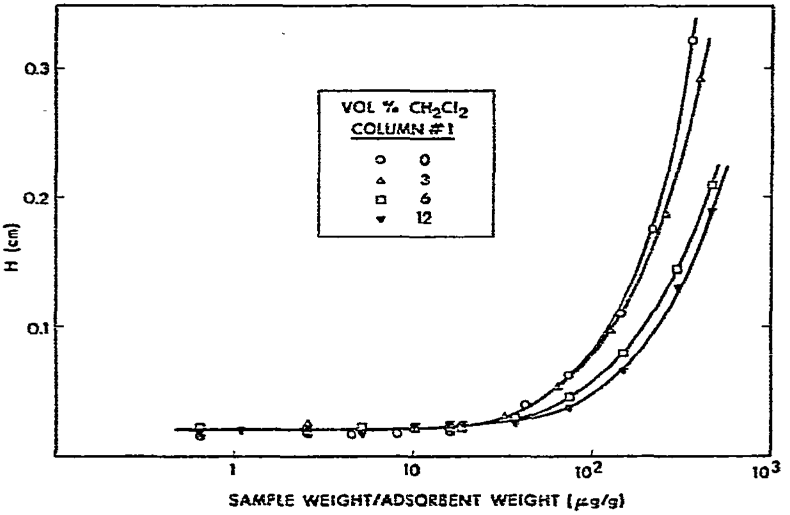

Fig. 9. Variation of *H* with sample size for methylene chloride-modified dry hexane. Conditions, same as Fig. 2.

Presumably, other modifiers, which are more strongly adsorbed than methylene chloride, would show a smooth transition between the roles of solvent and modifier. The large difference in the slopes of the lines for the two columns in Fig. 10 was unexpected. One possibility is that the adsorption sites are more homogeneous in column 2.

An important practical consideration in the choice of modifier is the volume of eluent which must be passed through the column to achieve equilibrium (constant retention). With water deactivation, 300 or more column volumes, $V°$, of hexane are

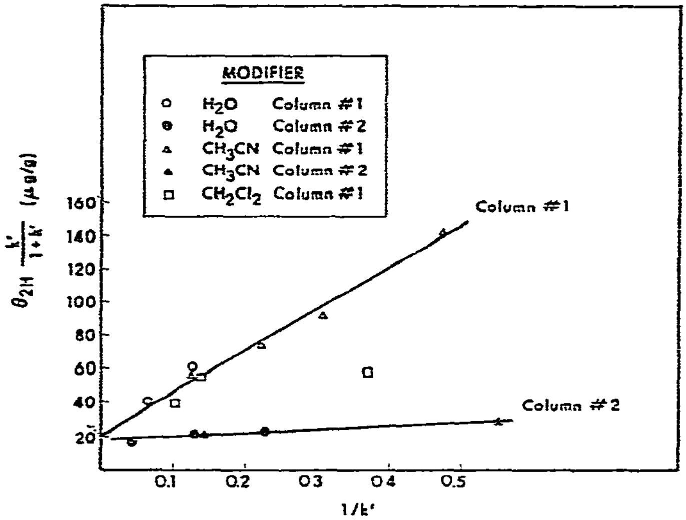

Fig. 10. Comparison of θ_{2H} for different modifiers.

required to reach equilibrium[5,9]. This is a consequence of the low concentration of water in hexane (<50 ppm) and the relatively high content of water which must be deposited on the silica before deactivation is achieved[12]. In typical LC systems it may take several liters of solvent and many hours to reach equilibrium. It was anticipated that equilibrium would be established more rapidly if the modifier is present at higher concentration. With acetonitrile we found that equilibrium was always achieved with 100 V°, and usually less than 50 V° was required. With methylene chloride, equilibrium was established even more rapidly. If the volume of solvent which must pass through the column in order to reach equilibrium is related to the amount of modifier which must be deposited on the adsorbent, then it should be possible to achieve equilibrium more rapidly by injecting an aliquot of pure modifier in slight excess of the amount required to coat the adsorbent. Once the excess is washed off with eluent, equilibrium should be quickly established. To test this possibility, a column was equilibrated with dry hexane and the retention of *m*-diphenoxybenzene was measured ($k' = 25.1$). Then the eluent was changed to 0.05 vol.% acetonitrile in hexane and 300 μl of pure acetonitrile were injected. The test compound was then repeatedly injected and k' was measured *versus* the volume of solvent passing through the column. Fig. 11 shows the results of this experiment. Initially k' dropped to 1.17 but quickly began to rise to its final equilibrium value of 2.65. Equilibrium was established after about 30 V° had passed through the column. At a flow-rate of 3.0 ml/min equilibrium was established in about 20 min.

Since water-deactivated solvents are inconvenient to prepare, and since larger volumes are needed to attain equilibrium, it might seem worthwhile to prepare and store large quantities. However, we have found that water-deactivated non-polar solvents (pentane, hexane, methylene chloride) are not stable when stored in glass bottles. It has been observed that the retention volume for a test compound varies with the storage time of the eluent. The retention time may drift in either direction. By analogy to a study of the changes in the water content of oil[7], we believe this

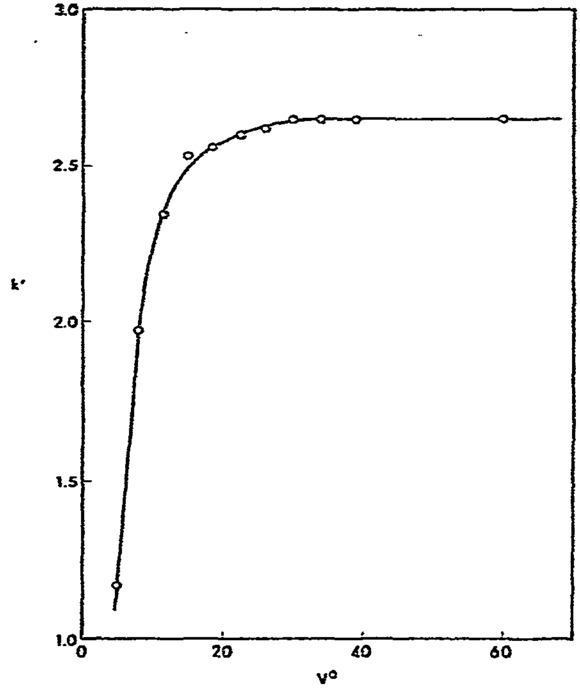

Fig. 11. Attainment of constant retention with 0.05 vol.% acetonitrile in hexane initially equilibrated with dry hexane, after injection of 300 μl of acetonitrile.

effect to be a consequence of the equilibration of water with the walls and head space of the container. It was found that the water content of a transformer oil could change by as much as 50% when stored in a glass bottle for one day[7]. Storage in humidified bottles lessened the problem but did not eliminate it. Presumably, polyolefin or Teflon® bottles would reduce the effect. However, storage is unnecessary with miscible deactivating agents because the eluent can easily be prepared immediately before use by simply shaking the appropriate volume of modifier with a measured volume of solvent.

We have been using acetonitrile-deactivated eluents for several months and have noted a marked improvement in the constancy of retention. Some variations still occur, but these appear to be due to changes in laboratory temperature. The effect of temperature on retention in adsorption systems with modified eluents is well known[11], but since equilibrium is more rapidly established it is believed that temperature will be less of a problem with organic modifiers such as acetonitrile.

The role of solvent composition in sample selectivity (relative retention) has been well established[12,14]. Eqn. 10 suggests selectivity will be independent of modifier composition providing K_{1y}/K_{2y} is constant. Earlier work indicates that this criterion is not met and that selectivity does change with modifier type[9,10]. The effect of acetonitrile on selectivity was tested for a few compounds and the results are shown in Table II. Compared to water, acetonitrile has a moderate but significant effect on selectivity. Although this effect will complicate the use of the wealth of data available

TABLE II

RETENTION OF VARIOUS COMPOUNDS ON SILICA EQUILIBRATED WITH DIFFERENT DEACTIVATING AGENTS

Compound	*Deactivating agent (in hexane)*			
	Water (saturated)		*Acetonitrile (0.05%)*	
	k'	*α*	*k'*	*α*
Thiophenol	0.74	0.28	0.82	0.32
Phenanthrene	0.72	0.27	1.05	0.42
1-Methoxynaphthalene	1.35	0.50	1.79	0.71
m-Diphenoxybenzene	2.68	1.00	2.53	1.00
2-Methoxynaphthalene	2.75	1.03	3.03	1.20
1,7-Dimethoxynaphthalene	7.58	2.83	7.38	2.92
Methyl benzoate	14.3	5.34	14.05	5.55
1-Cyanonaphthalene	15.6	5.82	16.61	6.57
2,6-Dimethylphenol	17.5	6.53	24.97	9.87

in the literature compiled with water-deactivated adsorbents, we believe the advantages of acetonitrile will far outweigh this disadvantage.

The use of alcohols as modifiers[5,9,10,15] has been suggested, however, alcohols have been shown to result in much lower efficiency[5], and unusual peak shapes[9]. Because alcohols are more strongly adsorbed than acetonitrile, their effective concentration range for non-polar solvents would probably be <0.01 vol.%. For example, 0.01% methanol was found to give equivalent deactivation to 60% water saturation on alumina, and equilibration time was long[5]. For these reasons, alcohols are not recommended as deactivating agents in saturated hydrocarbon solvents.

Adsorption sites on silica are believed to consist entirely of silanol groups, while differences in adsorption site strengths are believed to be a consequence of their spatial arrangement on the surface. The strongest adsorption sites "reactive silanol groups" are more reactive because of hydrogen bonding by another silanol group to the oxygen of the reactive silanol[16]. Different silicas possess different proportions of reactive and other types of silanol groups, small-pore silicas (high surface area) having the greatest proportion of strong sites[13]. If a silica could be prepared free of all reactive silanol groups, the resulting adsorbent might be sufficiently homogeneous to obviate the need for a deactivating agent. Heating silica above 400° may selectively remove reactive silanol groups[16,17], but no comparative HPLC studies relating sample capacity on heated silicas have appeared. Another approach might be the selective removal of reactive silanols by chemical reaction. The literature indicates that reactive silanols chemically react with trimethylchlorosilane more rapidly than other surface hydroxyls[16]. Thus, by controlling the mole ratio of silanizing reagent, it should be possible to produce a more homogeneous adsorbent. Until means for producing homogeneous adsorbents can be found, it appears that acetonitrile will serve as a suitable modifier with substantial advantages over water at least for the silica/hexane system. More work will be required to determine if acetonitrile has similar advantages in other adsorbent/solvent systems.

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