column-switching arrangement shown in Fig. 1 proved to be capable of avoiding this drawback.
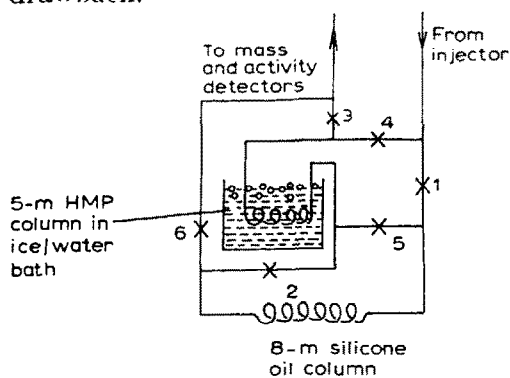

Fig. 1. Schematic diagram of the column-switching apparatus.

The on/off valves 1–6 were of the Hoke type (No. 465J) and the columns and interconnecting tubes made of ¼in. O. D. copper or stainless steel. Connections and T joints were made with "Swagelok" fittings.

This somewhat complex arrangement was necessitated by the fact that the sensitivity of the proportional flow counters[3] employed for the assay of ^{18}F is dependent upon the flow rate of the sample. In order that the flow rate should remain as nearly constant as possible whilst a sample was actually within the sensing chamber of the counter, the total length of column interposed between the injection port and the detectors was kept the same throughout the analysis.

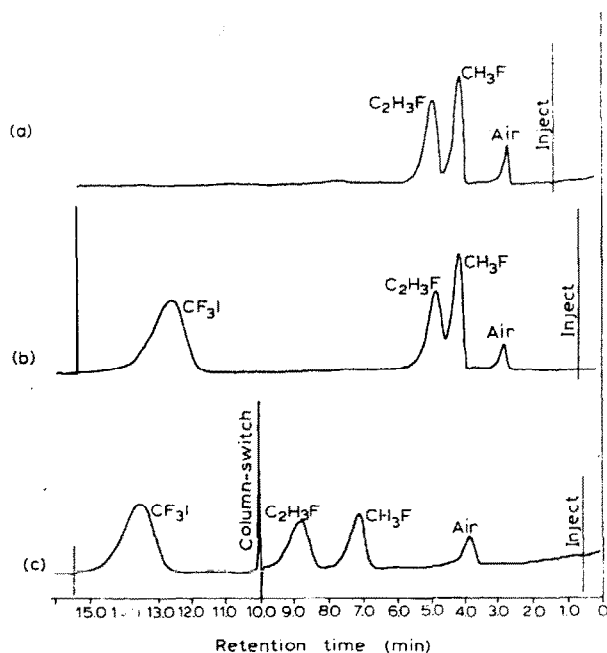Thus with valves 1, 2 and 3 open and 4, 5 and 6 closed the sample enters the

Fig. 2. Chromatograms of air–CH_3F–C_2H_3F–CF_3I mixtures on (a) the hexamethylphosphoramide (HMP) column (0°, flow rate 105.6 ml/min); (b) silicone oil column (25°, flow rate 97.4 ml/min); (c) HMP–silicone combination column in the switching apparatus (0° (HMP) and 25° (silicone), flow rate 104.2 ml/min).

silicone column and the more rapidly moving CF_4, CH_3F and C_2H_3F components pass on into the HMP column and thence to the detectors.

If now, following elution of C_2H_3F, valves 1, 2 and 3 are closed and 4, 5 and 6 opened the direction of gas flow within the HMP column is reversed whilst the flow within the silicone column continues in the same direction. Thus, apart from a short period of flow instability following the column-switch the flow rate remains constant within 1–2 % and the CF_3I is eluted with only slightly greater retention volume compared with an analysis using the silicone column alone.

Fig. 2 shows a comparison between chromatograms of mixtures of air, CH_3F, C_2H_3F and CF_3I obtained using the HMP (a) and silicone columns (b) separately and in the column-switching apparatus (c). These analyses were performed using macro-quantities of material and a Gow-Mac thermistor detector.

The degree of separation of CH_3F and C_2H_3F is approximately the same on the separate HMP and silicone columns, and in each case is considerably worse than the separation observed with the series arrangement of these columns.

The CF_3I peak has substantially the same shape on both the silicone and the complex column arrangement whilst the retention volume for this compound appears to have been increased from 1,150 ml to 1,350 ml due to the switching process. This presumably arises because of the time taken to stabilise the flow as the direction of the gas stream in the HMP column is reversed.

In applying this technique generally there appear to be only a few limitations. For example the retention volume of species X ($e.g.CF_3I$) on the first column A should be great enough to allow the other components of the mixture to elute from column B before X actually enters B. On the other hand if a component is weakly retained on A and strongly (but not irreversibly) retained on B then switching the columns before Y is eluted from B simply causes Y to re-pass through B in the reverse direction and thence through A once more. Provided the correct columns are chosen Y can be made to elute after X.

The author wishes to thank Professor R. WOLFGANG of Yale University for helpful discussions during the course of this work.

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A rapid and simplified method for quantitative analysis by thin-layer chromatography

Cylindrical thin-layer chromatography

In quantitative evaluations by the usual thin-layer chromatographic techniques, the sample solution has to be applied with a capillary pipette in a streak in order to expect good separation. The capillary pipette and ordinary thin-layer plate usually used, however, are not suitable for quantitative assay since the capillary pipette disturbs the surface of the thin layer, resulting in uneven solvent flow and unsatisfactory separation, thus causing errors. Furthermore, the use of the pipette often causes the removal of a portion of the sample together with the adsorbent from the layer so that a large amount (ml grade) of sample solution cannot be applied to the thin layer on the plate.

Several devices have been described for overcoming these difficulties[1–6]. This paper deals with a new simple apparatus for use in quantitative assays by which the application of a large amount of sample solution (ml grade) is possible without disturbing the surface of the thin layer.

Apparatus

The apparatus, depicted in Fig. 1, consists of: (1) an inner cylinder which is like a test tube, the bottom of which has a small hole (about 1 mm in diameter) that is used for exuding the sample solution and developing solvent from the inside to the outside; and (2) an outer cylindrical cover which is used as the developing chamber and has an inlet and outlet that are used for introducing gas while applying the sample solution.

Operation

Preparation of thin-layer cylinder

45–50 g of adsorbent is suspended in 100 ml of chloroform–methanol (2:1, v/v) as in PEIFER's method[7]. A homogeneous slurry is immediately prepared by stirring the suspension with a glass rod or by shaking the suspension in a glass-stoppered flask.

The inner cylinder described above is dipped into the homogeneous slurry, the hole in the cylinder being stoppered with a glass rod the end of which is covered with a rubber tube (as depicted in Fig. 2). The cylinder is slowly withdrawn from the slurry and a cylinder the outside of which is freshly coated with a thin layer of adsorbent is obtained. The wet cylinder is left standing in the atmosphere to dry. The solvent quickly evaporates. The thin-layer cylinder thus prepared is sufficiently stable and is ready for use. The dried thin layer on the cylinder may be exposed to steam for a short time and dried at 105° for about an hour for activation.

Application of sample solution

The thin-layer cylinder is put into the outer cover-cylinder. A sample solution can be applied to the thin-layer cylinder (see Fig. 3) by two different methods. One of them is the "total volume method" and the other the "partial volume method".

Total volume method. The sample solution (V_p ml) is pipetted into the thin-layer cylinder with a pipette of ml grade such as is used in ordinary volumetric analysis.
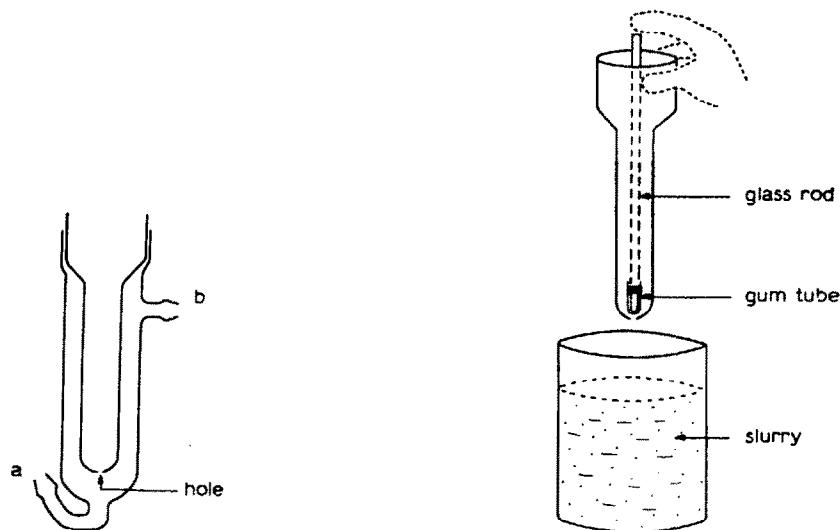
Fig. 1. Apparatus for cylindrical thin-layer chromatography. a = Inlet; b = outlet.

Fig. 2. Preparation of thin-layer cylinder.

During the course of this procedure, a flow of gas (e.g., N_2, H_2, CO_2) is introduced through the inlet a or the air is sucked through from the outlet b by a water aspirator. After almost all the sample solution in the thin-layer cylinder has exuded from inside to the outer thin layer through the small hole, a little solvent is poured into the thin-layer cylinder and allowed to stand for a few minutes to wash out the rest of the sample solution in the cylinder to the outer thin layer. This washing procedure (pour-
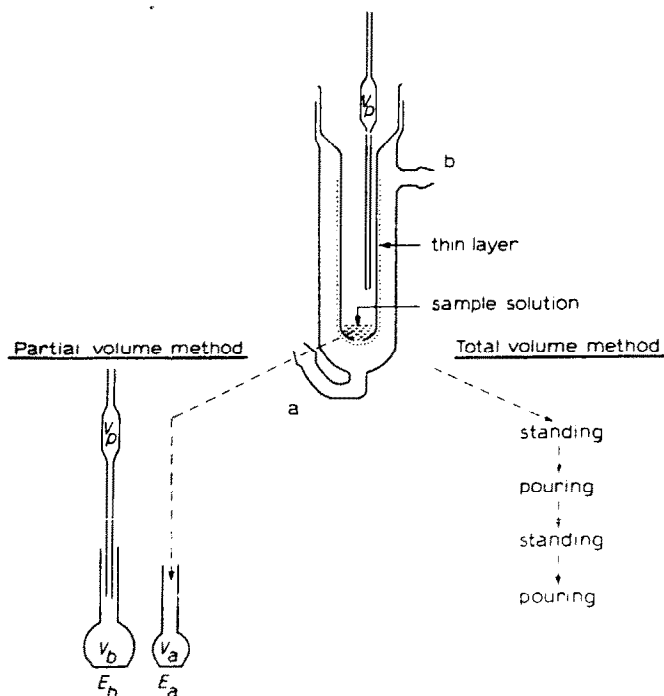

Fig. 3. Application of sample solution. a = Inlet; b = outlet.

ing and standing) is repeated until the whole sample solution in the cylinder has completely exuded to the outer thin layer.

Partial volume method. The sample solution (V_p ml) is pipetted into the thin-layer cylinder as described above and left standing for a suitable time. After an optional amount of the sample solution has exuded to the outer thin layer, the rest* of the sample solution in the cylinder is removed into a volumetric flask (V_a ml) and the cylinder is washed with solvent. The removed sample solution and washings are combined and the whole made up to V_a ml with the solvent (V_a solution). V_p ml of the original sample solution is pipetted into a volumetric flask (V_b ml) and the whole made up to V_b ml with the solvent (V_b solution). The optical densities of the V_a and V_b solutions are determined at a suitable** wave length giving optical densities E_a and E_b, respectively. In this case it is advisable to adjust the volumes V_a and V_b so that E_a and E_b are nearly equal***.

The volume (V) of the sample solution exuded to the outer thin layer is calculated from the following formula:

$$V \text{ (ml)} = \frac{(1 - E_a \times V_a)}{E_b \times V_b} \times V_p$$

Development

After the application of the sample solution to the thin layer as described above, the gas flow is stopped, and a developing solvent is poured into the bottom of the outer cover-cylinder through the inlet a and the outlet b is stoppered. The developing solvent is then poured into the thin-layer cylinder (Fig. 4). The time of development
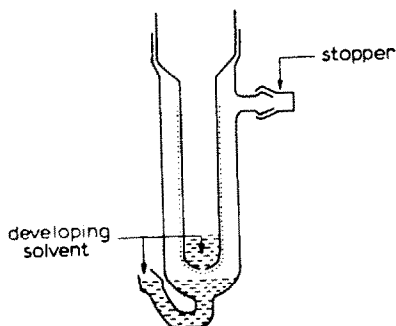

Fig. 4. Development.

depends on the size of the hole in the cylinder. After development, the thin-layer cylinder is dried in the air or in an inert gas and examined under ultraviolet light or visualised by other detection methods (*e.g.*, iodine vapor).

The band in the form of a ring, detected on the cylindrical thin layer, is scraped off with a flat spatula while rotating the cylinder and the sample is extracted with a suitable solvent. The substance in the extract is then measured quantitatively.

* If the sample solution contains an internal standard as in the case of a technique similar to quantitative gas chromatography, it is not necessary to measure the volume of the remaining sample solution.

** It is advisable that the absorption curve of the sample solution should be nearly flat at the wave length chosen.

*** Because it is possible that the substance may have a different molecular absorption at different dilutions.

$\Delta\alpha$ values are plotted *versus* Q°_k for elution by pentane (closed circles), and *versus* an equivalent quantity, the *net* adsorption energy of k, for elution by stronger eluents. The net adsorption energy is simply Q°_k minus the adsorption energy of the eluent molecules displaced upon adsorption of k (equal the effective area of the group k, a_k, times the eluent strength ε°). The dashed line of Fig. 2 is the function $1.00 - f(Q^\circ_k)/0.45$ (the "fractional delocalization"). The experimental data of Fig. 2 correlate closely with the relative delocalization of k, confirming the importance of solute localization in the failure of eqn. (1) for these solutes. Thus eqn. (1) applies for solutes which are not localized (net adsorption energy of k less than 1.00), but becomes a progressively poorer approximation as the localization of k increases (net adsorption energies of k increase beyond 1.0). The only significant exception in the correlation of the 17 solutes of Fig. 2 is the one aliphatic solute, propyl sulfide. This can be explained in terms of the known difference in localization functions for aromatic *versus* aliphatic solutes on alumina[13] (and presumably on silica as well).

The reason for preferential adsorption of water and of nonlocalized solutes on "reactive" silanol sites (but not of localized solutes) can be rationalized in terms of the proposed structure[12] (I) of these strong adsorption sites:

(I)
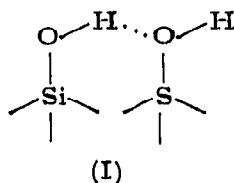
Because of the possibility of strong interactions between adjacent adsorbed water molecules (by hydrogen bonding), water will tend to be adsorbed as polymeric aggregates. Both protons of the site (I) can then be utilized, following rupture of the hydrogen bond of the site:
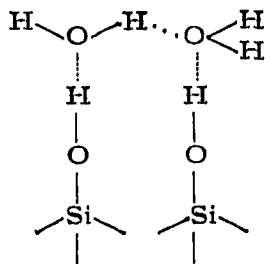
Similar rupture of the "reactive" silanol hydrogen bond can reasonably be postulated in the adsorption of aromatic hydrocarbons, leaving both silanols free to adsorb with the polyatomic hydrocarbon ring:
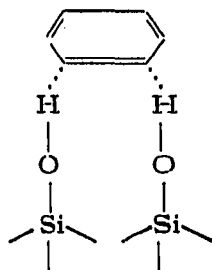
Localized adsorption suggests partial formation of a chemical *bond* between the localized group *k* and some adsorbent site or surface group; for example with the silanol group *per se*:
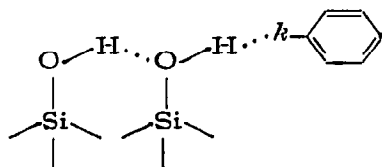
Other types of strong sites for the preferential adsorption of such groups *k* can be postulated (*e.g.*, free hydroxyls), but in any case (with the assumption of a single bond between k and a single adsorption site) the special advantage of "reactive" silanols (I) in the adsorption of water or of aromatic hydrocarbons is lost. That is, we are postulating that "reactive" silanols are strong adsorbent sites (for water and aromatic hydrocarbons) because of their polyfunctional character, but localized groups *k* can utilize only a single site.

Fig. 2 permits us to estimate the applicability of eqn. (1) for a particular chromatographic system as adsorbent activity is varied. This in turn is useful in a practical way, since whenever eqn. (1) is not obeyed, separation reversals are possible when adsorbent activity is changed. The ability to predict changes in separation order in such cases is valuable whenever two compounds of interest are not well separated by a silica of given activity. Since the extent of separation reversal in such cases is proportional to the difference in adsorbent α values, and since the potential range of α values is much greater for narrow than for wide pore silicas[7], narrow pore silicas offer greater possibility of changing sample separation order by means of varying adsorbent activity. On the other hand eqn. (1) will be generally more accurate in predicting separation order on wide pore silicas. It should be pointed out that the failure of eqn. (1) for the adsorption of polar solutes on silica is of relatively limited practical importance, since this failure is usually observed only at fairly large $\underline{R}^{\circ}$ values (compare Table II). For the majority of column separations, and for almost all thin layer separations, the present phenomenon may therefore be ignored.

Secondary adsorbent activity effects

On alumina there is a tendency for preferential adsorption of the longer (or more linear) of two or more isomers in the case of certain solute types (notably the aromatic hydrocarbons)[2]. This effect (so-called "weak localization") is quite pronounced for active alumina, but barely noticeable for heavily deactivated alumina. A previous paper[5] has shown the absence of such effects for adsorption on silica, both linear and nonlinear hydrocarbon isomers being adsorbed to the same extent. Since most of these data were obtained for heavily deactivated silica, however, it could be argued that "weak localization" on silica has not been conclusively refuted. In the present study the two isomeric hydrocarbons picene (linear) and 1,2,3,4-dibenzanthracene (nonlinear) were eluted from both 0.8 and 18 % H_2O–SiO_2 by ether–pentane solutions as eluent (2 % v ether in the latter case, and 5 % v ether in the former). For elution of these same two solutes from alumina by similar eluents, the log $\underline{R}^{\circ}$ values differ by 0.23 (low activity) to 1.10 (high activity). On silica the corresponding

differences were only 0.02 (0.8 % H_2O–SiO_2) and —0.01 (18 % H_2O–SiO_2). Clearly the marked tendency toward preferential adsorption of linear hydrocarbon isomers on alumina is *not* duplicated in adsorption on silica. That is, weak localization does not occur on silica.

ROLE OF THE ELUENT

The effect of different eluents on solute retention volume is also described by eqn. (1). The primary eluent effect is given by the term $—A_s\varepsilon°$. A_s is the effective size of the solute, or the relative area it appears to cover upon adsorption. $\varepsilon°$ is the eluent strength or adsorption energy per unit area of adsorbent surface. The derivation[6] of the term $—A_s\varepsilon°$ ignores the solution energies of solute and eluent, and assumes that the *net* adsorption energy of the solute is equal to the adsorption energy of solute minus that of the eluent molecule(s) displaced by the solute upon adsorption. Some less important contributions of eluent to retention volume are included in the term $\Sigma \varDelta_{eas}$, which is generally a function of both solute and eluent type. The following discussion of eluent effects in adsorption on silica is broken down into a separate examination of each of these three eluent related parameters.

Solute size A_s

For adsorption on alumina, solute A_s values are normally observed to be proportional to the calculated areas of the adsorbed solute[6,14,15]. This is also true for the adsorption of nonlocalized solutes on silica[4] and Florisil[11]. With increasing localization of a solute group k on these latter two adsorbents, however, the apparent A_s value of the solute (inferred from the effect of eluent on retention volume—equation 1) begins to exceed the value calculated from the physical dimensions of the solute. That is, localized solute groups appear to require a larger area for adsorption than indicated by their actual size. We fill first consider why this is so, and then present some additional experimental data which are of interest in this connection.

The original derivation of the term $—A_s\varepsilon°$ of eqn. (1) assumes a uniform surface; no notice is taken of the possibility that certain portions of the surface may function as stronger sites than other parts of the surface. The fact that certain solutes localize on strong adsorbent sites complicates this otherwise simple picture of adsorption. It is clear that the adsorption of a solute group k on a strong site (locali-

(a) NONLOCALIZED ELUENT ADSORPTION
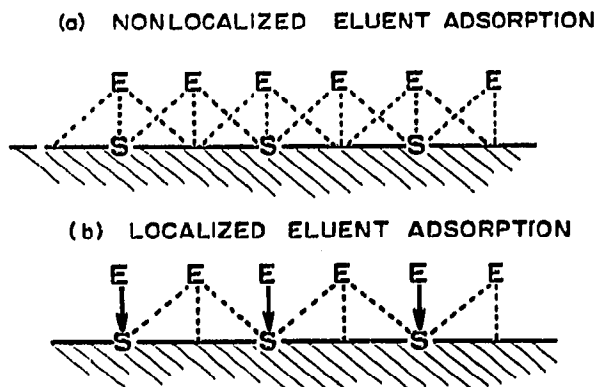

(b) LOCALIZED ELUENT ADSORPTION

Fig. 3. Hypothetical adsorption of eluent molecules (E) on an adsorbent surface covered by strong sites (S).

zation) results in a greater adsorption energy than does its adsorption elsewhere on the adsorbent surface. The same may or may not be true of the adsorption of eluent molecules. That is, eluent molecules may or may not adsorb with some localization on strong adsorbent sites. These two possibilities are illustrated in Fig. 3 for the adsorption of eluent molecules E on a surface covered with strong sites S. Where the eluent shows no tendency toward localization, its adsorption energy need not be widely different at different points on the surface; both strong and weak adsorbent sites can simultaneously contribute to its adsorption energy, and one strong site may interact with several adjacent eluent molecules. When the eluent can localize on particular strong sites, however, the adsorption energy of localized eluent molecules will generally be much larger than of nonlocalized eluent molecules.

Consider first the case of eluent adsorption without localization (Fig. 3a). An adsorbing solute group k may displace an eluent molecule(s) from the immediate vicinity of a strong site S (if k is localized on S), or from some other position on the adsorbent surface (if k is not localized). In either case, due to the lack of *specific* interaction between eluent molecules and S, the energy required to displace an eluent molecule (equal to the adsorption energy of E) is roughly the same. Thus the net energy of adsorption of k is reduced by the average adsorption energy of the eluent per unit area of surface, times the area required by k on adsorption. That is, eqn. (1) holds with A_s proportional to the size of the solute. Considerable evidence has been amassed[9] which suggests that the eluent is generally delocalized for adsorption on alumina, so that the proportionality of A_s and solute size for all solutes adsorbed on alumina is reasonable.

Consider next the case of eluent adsorption with localization (Fig. 3b). Adsorption of a solute group k which localizes on S requires the desorption of a localized eluent molecule. The similar adsorption of a nonlocalizing solute, or of the remainder of the solute molecule attached to a localized group k, does not. Even if the areas of localized and nonlocalized solute groups are identical, therefore, the eluent has a greater effect on the adsorption of the localized group k. That is, the eluent desorption energy (which affects the net adsorption energy of a solute group k) will be greater for the adsorption of a localized group k, relative to a nonlocalized solute group of similar size (which displaces the same number of eluent molecules as k). This is mathematically equivalent to the localized solute possessing a greater molecular area or value of A_s. From this we conclude that in adsorption on silica some localization of the eluent generally occurs.

The apparent localization of eluent molecules on silica but not alumina seems related to the importance of surface hydroxyls as adsorption sites on silica, and of aluminum atoms or other less exposed sites on alumina[1,6]. Thus localization should be easier to effect when the adsorbent site (*i.e.*, hydroxyl) is more accessible. A number of other localization phenomena have similarly been correlated with these differing views of the silica and alumina surfaces[6].

We have seen in the preceding section that as the activity of narrow pore silica is increased, the average surface energy increases, but the energy of sites for localized adsorption remains relatively constant. This suggests that the difference in adsorption energies of localized and nonlocalized eluent molecules should become less marked as adsorbent activity increases, and that the apparent solute A_s values for localized solutes should approach the "normal" values (those predicted from so-

TABLE III

PRIMARY ELUENT EFFECTS: THE APPARENT SIZE OF THE SOLUTE (A_s)

Solute	$Log\ \underline{R}°$ 1.6% H_2O-SiO_2					$Log\ \underline{R}°$ 6.2% H_2O-SiO_2					
	P[a]	10% M-P[b]	25% M-P[c]	50% M-P[d]	A_s	P[a]	10% M-P[b]	25% M-P[c]	50% M-P[d]	M°[e]	A_s
1,2-Benzanthracene						1.60	0.69	—0.15			
3,4-Benztetraphene	2.58	1.21	0.38			1.95	0.85	0.06			
1-Methoxynaphthalene						1.50	0.69	0.10			10.5
Nitrobenzene	2.43	1.59	0.99	0.45	8.2	2.04	1.29	0.70	0.13		10.8
1-Nitronaphthalene						2.32	1.41	0.77	0.04		12.6
Acetophenone						2.26	1.66	1.10	0.53		11.8
Phenol						2.33	1.77	1.28	0.70		10.8
$\alpha\varepsilon$° (calc.)[f]	0.000	0.116	0.185	0.237		0.000	0.078	0.134	0.180	0.224	
(expt.)[g]	0.000	0.098	0.157			0.000	0.077	0.140			

[a] Pentane.
[b] 10% v CH_2Cl_2-pentane.
[c] 25% v CH_2Cl_2-pentane.
[d] 50% v CH_2Cl_2-pentane.
[e] CH_2Cl_2.
[f] Eqn. (2), ref. 14.
[g] Eqn. (1) using hydrocarbon solutes.

lute area). This is in fact observed, as seen from the data of Table III. For adsorption on 1.6 and 6.2 % H_2O-SiO_2 the eluent strengths $\alpha\varepsilon$° of a series of eluents were evaluated from elution of nonlocalized solutes (aromatic hydrocarbons). Comparison of these values with calculated values (eqn. 2, ref. 14) shows reasonable agreement. Using these eluent strength values the A_s values of the various (localized) nonhydrocarbons of Table III could then be evaluated from their $\underline{R}$° values (by eqn. 1). Comparison of these A_s values with previously measured values for 16% H_2O-SiO_2 shows the expected decrease in A_s for localized solutes with increasing silica activity. For example, nitrobenzene has A_s values of 13.4, 10.8, and 8.2 for adsorption, respectively, on 16.0, 6.2, and 1.6 % H_2O-SiO_2. The latter value (8.2) is close to the "normal" values observed on alumina (8.1) or calculated from the molecular dimensions of the molecule (7.3). The apparent excess area of groups localized on silica (equal to apparent group area or contribution to A_s, minus calculated group area) has been correlated with the localization function $f(Q°_k)$ for the group [4]. Fig. 4 shows the
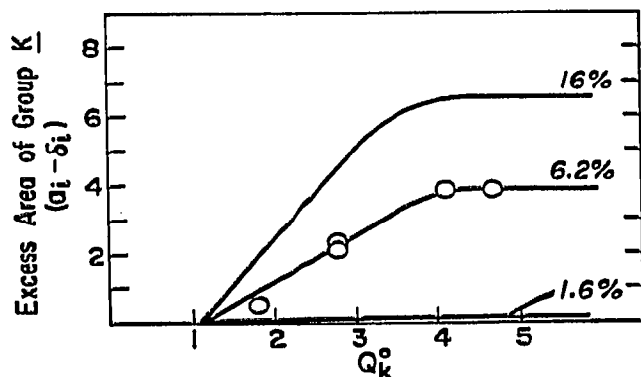

Fig. 4. Variation of solute group excess area with solute group adsorption energy for silicas of differing activity. O = experimental data for 6.2 % H_2O-SiO_2.

similar correlation of the data of Table III, along with the average curve for 16.0 % H_2O–SiO_2[4]. Group excess areas appear to decline smoothly to zero as adsorbent activity increases to 0 % H_2O–SiO_2.

Eluent strength $\varepsilon°$

Eluent strength values for adsorption on alumina are available for over 50 pure solvents[9]. For adsorption on silica, $\varepsilon°$ values have been reported for only four pure solvents[4]. In this connection it should be noted that such values are measured by comparing $\underline{R}°$ values for the same solute eluted by two eluents (in terms of eqn. (1)), which permits the difference in $\varepsilon°$ values for the two eluents to be obtained. This procedure is much more difficult to apply in the case of silica, relative to alumina.

TABLE IV

EXPERIMENTAL ELUENT STRENGTH VALUES FOR VARIOUS SOLVENT MIXTURES; 18 % H_2O–SiO_2

Eluent (B) Solute	Measured $\alpha\varepsilon°$ values for pentane solutions (% v) of eluent at left						log R_p[a]	A_s[a]
% v B	2	5	15	30	60	100		
Ethyl ether								
Picene	0.081						1.24[b]	14.0
3,4-Benztetraphene	0.087						1.25[b]	14.0
Acetophenone	0.105	0.128	0.165				2.74	15.2
m-Nitroacetophenone			0.140	0.170	0.195		4.10	22.4
m-Hydroxyacetophenone				(0.170)	0.210		4.94	23.4
Average	0.091	0.128	0.152	0.170	0.207			
Calc.[c] ($\varepsilon° = 0.38$, $n_b = 10$)	0.070	0.106	0.149	0.177	0.206			
Ethyl acetate								
Acetophenone		0.146					2.74	15.2
m-Nitroacetophenone			0.162	0.187			4.10	22.4
Calc.[c] ($\varepsilon° = 0.38$, $n_b = 10$)		0.116	0.160	0.187				
Chloroform								
Acetophenone						0.139	2.74	15.2
m-Nitroacetophenone						0.170	4.10	22.7
Calc.[c] ($\varepsilon° = 0.26$)						0.156		
Dioxane								
m-Nitroacetophenone		0.175					4.10	22.4
Calc.[c] ($\varepsilon° = 0.49$, $n_b = 10$)		0.177						
Acetone								
m-Nitroacetophenone		0.173					4.10	22.4
Calc.[c] ($\varepsilon° = 0.47$, $n_b = 10$)		0.171						
Acetonitrile								
m-Nitroacetophenone		0.193[d]					4.10	22.4
Calc.[c] ($\varepsilon° = 0.50$, $n_b = 10$)		0.192						

[a] Ref. 4.
[b] Experimental values for this system.
[c] Eqn. (2), ref. 4.
[d] 5 % v acetonitrile–benzene.

Because the A_s values of strongly adsorbed solutes (on water deactivated adsorbent) are generally large, $\underline{R}^{\circ}$ decreases quite rapidly with increases in ε°. This makes it difficult to compare different eluents with respect to the elution of the same solute. There are also few suitable solutes for the study of strong eluent systems on silica. Most strongly adsorbing solutes are hydroxyl or carboxyl substituted, which introduces serious complications (see following section).

With the foregoing difficulties in mind, the data of Table IV were obtained for the purpose of measuring or estimating ε° values for several additional pure solvents on silica. Where it was possible to elute one of the solutes of Table IV by the pure eluent, the calculation of ε° was straightforward (e.g., chloroform). For the remaining strong eluents of Table IV, it was necessary to estimate ε° for the pure solvent from data on a binary solution with a weaker eluent (e.g., pentane). This was possible by means of a previous relationship (eqn. (2), ref. 14) which relates the eluent strength of the binary to the strengths of the two components, the mole fraction of each component, and the size of the strong eluent component n_b (equal to A_s for the eluent as solute). For the strong eluents ethyl ether and ethyl acetate of Table IV it was found that the best fit to this previous relationship was obtained not with the "normal" or calculated n_b values, but with a much larger value (equal about 10). This has also been observed for the adsorption of very strong eluents on alumina[9], and appears to result from "extreme" localization of the eluent. Since the strong eluent component of the remaining binaries of Table IV are each quite strong, n_b was assumed equal 10 for these solvents as well. The experimental binary solvent $\alpha\varepsilon^{\circ}$ values of Table IV agree well with calculated values, particularly for solvents containing more than 5 % of the strong eluent component*. Table V summarizes the derived ε° values for the

TABLE V

EXPERIMENTAL ELUENT STRENGTH VALUES ON SILICA

Eluent	ε° (SiO_2)	ε° (Al_2O_3)	$0.77\ \varepsilon^{\circ}$ (Al_2O_3)
Pentane	0.00	0.00	0.00
Carbon tetrachloride	0.11	0.18	0.14
Benzene	0.25	0.32	0.25
Chloroform	0.26	0.40	0.31
Methylene chloride	0.32	0.42	0.32
Ethyl ether	0.38	0.46[a]	0.35
Ethyl acetate	0.38	0.58	0.45
Acetone	0.47	0.56	0.43
Dioxane	0.49	0.56	0.43
Acetonitrile	0.50	0.64	0.49

[a] Apparent value for nonhydrocarbons[9].

pure strong eluent components of Table IV, along with previously measured ε° values for some other solvents. These ε° values for silica are plotted *versus* the corresponding values for alumina in Fig. 5, and a reasonable correlation is noted: the

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* This is related to the relative coverage of adsorbent surface by the strong eluent component[9]; the theoretical relationship is most applicable when more than 50% of the adsorbent surface is covered by strong eluent. For the stronger eluent components dioxane, acetone, and acetonitrile, more than 50% of the surface should be covered even with 5 % v solutions.

silica $\varepsilon°$ values appear equal to 0.77 times the alumina $\varepsilon°$ values, Table V also compares the silica $\varepsilon°$ values with values calculated in this fashion from the alumina $\varepsilon°$ values. A standard deviation of only ± 0.04 units between experimental and calculated $\varepsilon°$ values for silica is found. This suggests that $\varepsilon°$ values for other eluents on silica can be calculated from data for alumina with similar precision. As a check on this postulate,
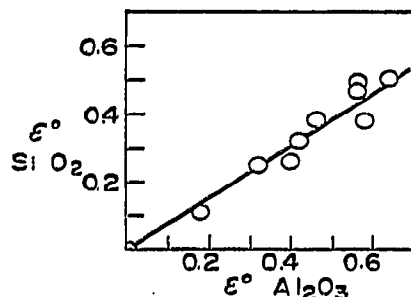

Fig. 5. Correlation of experimental eluent strength values for silica *versus* alumina.

relative eluent strength series ("eluotropic series") from four previous studies on silica are compared in Table VI with $\varepsilon°$ values from Table V or values estimated from corresponding alumina values[9] (as in Table V). Recalling the uncertainty of ± 0.04 units in estimated $\varepsilon°$ values, only one of the 23 solvents compared in Table VI is significantly out of line with the $\varepsilon°$ values shown. On the basis of calculated $\varepsilon°$ values it appears that acetone in the series of reference 16 should precede both ethanol and *sec.*-butanol. Possibly this reflects an erroneous assignment of the acetone position in the series of reference 16. Alternatively alcohols may actually be weaker eluents on silica than on alumina, relative to other solvents. Another possibility is variability of the apparent eluent strengths of alcohols due to secondary eluent effects (see ref. 9 and following discussion).

In conclusion the data of Tables V and VI suggest that $\varepsilon°$ values for a variety of solvents as eluents for silica can now be reliably estimated.

Secondary eluent effects $\Sigma \Delta_{eas}$

A variety of secondary eluent effects not covered by the term $-A_s\varepsilon°$ of eqn. (1) have been observed on alumina[9]. For ethers as eluents, localized solutes are eluted somewhat faster than predicted from $\varepsilon°$ values measured by nonlocalized solutes. This has been attributed to localization of the eluent. A similar effect is suggested in Table IV, where the $\alpha\varepsilon°$ value for 2 %v ether–pentane is significantly lower when measured for the aromatic hydrocarbon solutes, compared to the localized aceto-phenone. Since almost all nonlocalized solutes are eluted quite rapidly by even dilute ether solutions, however, the ether anomaly is not of practical significance in adsorption on silica. On alumina basic eluents (*e.g.*, diethyl amine, pyridine) preferentially retard the relative migration of acidic or potentially acidic solutes, relative to other solute types. This effect was not studied for elution from silica in the present investigation. On the basis of the mechanism proposed for the basic eluent anomaly[9], and the preferential adsorption of acidic solutes on alumina[3], it seems likely that a basic eluent anomaly may not be observed for elution from silica.

In adsorption on alumina HEŘMÁNEK *et al.*[20] have observed that eluents capable of hydrogen bonding with certain solutes selectively increase the migration

TABLE VI

COMPARISON OF ELUOTROPIC SERIES FOR SILICA WITH CALCULATED ε° VALUES

Series of ref. 16		Series of ref. 17	
Eluent	ε°	Eluent	ε°
n-Heptane	0.01	Cyclohexane	0.03
Diisobutylene	0.05	Carbon tetrachloride	0.11
Benzene	0.25	Carbon disulfide	0.20
Isopropyl chloride	0.22	Chlorobenzene	0.23
Isopropyl ether	0.21	Ethyl benzene	0.20
Ethyl ether	0.38	Chloroform	0.26
Ethyl acetate	0.38	Nitrobenzene	0.40
sec.-Butanol	0.56	Ethyl ether	0.38
Ethanol	0.68	Ethyl acetate	0.38
Water	?	Acetone	0.47
Acetone	0.47	Acetic acid	?
Methanol	0.73	Methanol	0.73
Pyruvic acid	?		

Series of ref. 18		Series of ref. 19[a]	
Eluent	ε°	Eluent	ε°
Carbon tetrachloride	0.11	Cyclohexane	0.03
Toluene	0.23	Benzene	0.25
Benzene	0.25	Chloroform	0.26
Tetralin	?	Butyl acetate	0.33
Chloroform	0.26	Ethyl ether	0.38
		Ethyl acetate	0.38
Nitrobenzene	0.40	Acetone	0.47
Dioxane	0.49	Methanol	0.73
Cyclohexanone	?		
Aniline	0.48⁻		

[a] The adsorbent was not explicitly stated, but appears to have been silica.

rates of those solutes. This has been attributed to simple interaction of solute and eluent in solution, an effect which is normally of minor importance in determining solute retention volumes. Since this phenomenon is not related to the adsorbent surface, similar effects would be expected for other adsorbents. In the case of silica, WAKSMUNDSKI et al.[18] have demonstrated that this is indeed the case.

Activation of adsorbent by solvent

Previous papers[9,21] have noted that dry eluents can remove water from water deactivated alumina during separation. This can result in certain undesirable effects, (band splitting, sample reaction, increased separation time, variations in R° values etc.), particularly where the same column is used for more than one separation. The activation of water deactivated silicas by solvent washing has also been observed[22]. Fig. 6 illustrates the magnitude of this effect in the elution of a typical solute from 6.2 % H_2O–SiO_2 by methylene chloride. Separation was carried out (first run), and repeated (second run) under the same conditions on the same column. The difference

in $\underline{R}°$ values for the two runs permits an evaluation of the relative column drying or wetting by the eluent between the two runs (see Fig. 2 of ref. 9 and related discussion). In the example of Fig. 6, methylene chloride which is 34 % saturated with water* appears to be in equilibrium with the column. None of the data reported here or previously[4] used water equilibrated eluents, but this should have little effect on reported $\underline{R}°$ values since only one run per column was ever made, and most of the eluents used would be less likely than methylene chloride to give adsorbent drying.
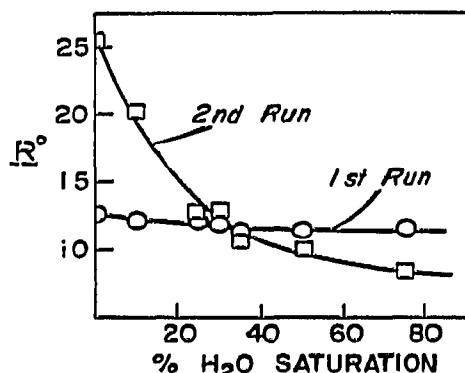

Fig. 6. Drying of the adsorbent by the eluent during separation; elution of dimethyl phthalate from 6.2 % H_2O–SiO_2 by CH_2Cl_2 of varying water content. ◯ = first run; ☐ = second run.

In Fig. 6 it is seen that the $\underline{R}°$ values for the first run (our normal procedure for measuring $\underline{R}°$ values) are very little dependent on eluent water content. The problem of adsorbent drying by the eluent appears much more serious with silica than in the case of alumina. Eluents such as methylene chloride are incapable of drying aluminas with less than a monolayer of added water.

ROLE OF SOLUTE STRUCTURE

The general role of solute structure in determining sample separation ($\underline{R}°$ values) on silica *versus* alumina has been reviewed recently[1], and discussed in detail elsewhere[3–5,7]. A few major points remain uncertain, and these will be re-examined in the present section in the light of some recent experimental data (Table VII). The major contribution of solute structure to $\underline{R}°$ is given by the parameter $S°$ of eqn. (1): the dimensionless adsorption energy of the solute in a standard chromatographic system (where α equals 1.00 and $\varepsilon°$ and $\Sigma \Delta_{eas}$ equal 0.00). Solute structure partially determines the term $\Sigma \Delta_{eas}$, but the role of solute structure in this connection has already been discussed. For adsorption on silica $S°$ is related to solute structure[4] by:

$$S° = \overset{i}{\Sigma} Q°_i - 0.4 \, f \, (Q°_k) \overset{i \neq k}{\Sigma} Q°_i - c \, (n - 6 \, r) + \overset{j}{\Sigma} q°_j \tag{2}$$

The first term of eqn. (2) is the sum of adsorption energies $Q°_i$ of the various solute groups i. The second and third terms represent solute adsorption energy losses which arise from the delocalization of certain solute groups (which in turn result

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* *i.e.*, made up from 34 parts methylene chloride (water saturated) plus 66 parts dry methylene chloride.

TABLE VII

ADSORPTION ENERGIES OF VARIOUS SOLUTES ON 18% H_2O–SiO_2

Solute	$Log\ \underline{R}^{\circ}$ (CH_2Cl_2–pentane eluent[a])						A_s	(S°)[b]
	$0\%\ v$ CH_2Cl_2	$2.0\%\ v$ CH_2Cl_2	$5.5\%\ v$ CH_2Cl_2	$11.0\%\ v$ CH_2Cl_2	$18.5\%\ v$ CH_2Cl_2	$100\%\ v$ CH_2Cl_2		
1,2,3,4-Dibenzanthracene	1.25	0.99	0.68	0.39	—0.08		(14.0)	
1-Phenyl-3-thiaoctane			0.79	0.36	—0.12		15.7	4.02
1-Phenyl-2-chloroethane	0.50	0.43	0.25	—0.04			8.2	2.52
1-Phenyl-2-bromoethane	0.55							2.52
1-Phenyl-2-iodoethane	0.48							2.40
1-Phenyl naphthalene	0.56							2.53
2-Phenyl naphthalene	0.76							2.87
m-Terphenyl	0.92							3.13
1,2-Naphthoquinone					0.48			
1,4-Naphthoquinone					—0.10			
Nitrobenzene				0.49				4.70
p-Nitrotoluene				0.54				4.95
Eluent strength $\alpha\varepsilon^{\circ}$								
(exptl.)	0.000	0.020	0.044	0.066	0.102			
(calc.)[c]	0.000	0.017	0.043	0.070	0.097	0.192		

[a] ε° values for eluent of indicated composition.
[b] Eqn. (1); $\alpha = 0.60$, $\log V_a = -0.96$.
[c] Eqn. (2), ref. 14.

from the localization of other solute groups). The last term represents the sum of adsorption energy contributions q°_j caused by the intramolecular interaction of two or more solute groups, and is hence a function of solute geometry. The following discussion will examine each of these terms further.

Solute group adsorption energies and localization terms

A fairly complete treatment of solute group adsorption energies on alumina and silica has been given recently[3]. Values of Q°_i for the adsorption of numerous groups i on silica were tabulated. The localization term $-0.4\ f(Q^{\circ}_k)\ \overset{i \neq k}{\Sigma}\ Q^{\circ}_i$ of eqn. (3) reflects the delocalization of all solute groups i other than k, as the result of localization of a polar, strongly adsorbing group k. Values of the localization function $f(Q^{\circ}_k)$ *versus* Q°_k have been tabulated for the adsorption of both aliphatic and aromatic solutes on alumina (Table II of ref. 13). It has been shown[4] that these values for alumina may be used in eqn. (3) for the adsorption of *aromatic* groups k on silica. It has not previously been verified that this is true in the case of aliphatic groups adsorbing on silica. This point can be checked by means of S° for the solute 1-phenyl-3-thiaoctane in Table VII. In this solute the strongly adsorbing aliphatic sulfide group should localize with corresponding delocalization of the phenyl ring, which is attached to the sulfide group through a —$(CH_2)_2$— link. From the latter S° value we calculate that $-0.4\ f(Q^{\circ}_k)\ \overset{i \neq k}{\Sigma}\ Q^{\circ}_i$ equals —0.30 (calculated S° for this solute with no localization equals 4.30). $\overset{i \neq k}{\Sigma}\ Q^{\circ}_i$ equals 1.48 (the value of S° for the delocalized benzene ring), and $f(Q^{\circ}_k)$ is then calculated equal to 0.50, for Q°_k equal 3.0. The corresponding value of $f(Q^{\circ}_k)$ for adsorption on alumina[13] is 0.40, which is reasonably close to the

latter value (0.50) for silica. We conclude that $f(Q°_k)$ values previously tabulated for adsorption on alumina can be used in eqn. (3) for adsorption on silica, in the case of both aliphatic and aromatic solutes.

With the latter premise assumed, the values of $S°$ for the various 1-phenyl-2-halo ethanes of Table VII can be used to calculate values of $Q°_i$ for the corresponding aliphatic halo groups: (R)-Cl, 1.25; (R)-Br, 1.25, (R)-I, 1.13. These values are significantly lower than $Q°_i$ values for the same groups on alumina, despite a general similarity of $Q°_i$ values for most groups on both alumina and silica[3]. Similarly $Q°_i$ values for aromatic halo groups adsorbed on silica are also lower than corresponding values on alumina[2]. There is thus a definite tendency for the stronger adsorption of halogen derivatives on alumina versus silica, relative to other compound types.

The localization term $c(n - 6\,r)$ represents a delocalization of aromatic carbon atoms beyond the first six in solutes such as naphthalene or phenanthrene. This has been treated in detail elsewhere[4,6,7] and does not require further comment.

Solute geometry terms

Solute geometry effects can be broken down into (i) solute planarity, (ii) steric hindrance to adsorption, (iii) chemical interaction between adjacent solute groups, (iv) concerted adsorption of adjacent groups, (v) electronic effects, and (vi) miscellaneous other effects of lesser importance.

A general treatment of solute planarity (i) as a factor in determining $\underline{R}°$ has been given[10], and shown capable of accurately correlating the $\underline{R}°$ values of nonplanar solutes adsorbed on alumina. In general, solutes which are nonplanar in solution will be adsorbed less strongly on alumina than are otherwise similar solutes (e.g., nonplanar 1-phenyl naphthalene is adsorbed less strongly on alumina than is planar pyrene, although each compound possesses 16 aromatic carbon atoms). Limited qualitative data for the adsorption of nonplanar solutes on silica[23] confirm the importance of solute planarity for adsorption on this adsorbent as well. Table VII provides $S°$ values for the nonplanar hydrocarbons 1- and 2-phenyl naphthalene (NP) and *m*-terphenyl (TP). For the moderately hindered solutes 2-NP and TP, the $S°$ values tend to be, if anything somewhat *larger* than $S°$ values for fused aromatic hydrocarbons[4] of equal carbon number; e.g., 16 carbon solutes, 2-NP (2.87), pyrene (2.57), fluoranthene (2.79); 18 carbon solutes, TP (3.13), chrysene (3.09), triphenylene (3.15). Apparently any adverse effect on the adsorption energies of 2-NP and TP as a result of their normal nonplanarity is more than offset by some other factor. This latter factor is undoubtedly the lesser delocalization of the various phenyl groups in 2-NP and TP, similar to the lesser (i.e., zero) delocalization of the phenyl groups in dibenzyl[4] (see also Fig. 10 of ref. 6). In the case of the more severely nonplanar 1-NP, its $S°$ value is significantly less than that of 2-NP and the various C_{16} fused aromatic hydrocarbons. It is interesting to compare the relative effects of solute nonplanarity in the adsorption of 1-NP on silica versus alumina. On alumina[10] the $S°$ values of naphthalene, 1-NP, and 2-NP are 3.10, 3.75 and 4.75, respectively. On silica the $S°$ values of these same solutes are 2.02, 2.53, and 2.87, respectively. Thus the phenyl ring in 2-NP, upon being subjected to the greater nonplanarity of the 1-phenyl isomer, suffers a reduction in adsorption energy of approximately 40 % on silica and 60 % on alumina. That is, solute planarity appears somewhat more important generally in adsorption on alumina than on silica, even disregarding the favored adsorp-

tion of phenyl groups on silica versus alumina. The relatively lesser importance of solute planarity in adsorption on silica is reasonable in terms of the more accessible nature of silica surface sites (relative to alumina)[1,6]. Thus the interaction of an exposed, flexible group with a phenyl group should be relatively insensitive to small rotations of the phenyl group in the plane of the adsorbent surface. On alumina the energy of interaction of a phenyl group with the surface is approximately proportional to the cosine of the angle between the phenyl group and the surface[10].

On alumina steric hindrance to adsorption (between groups on the same solute molecule) (ii) has been treated quantitatively for several solute types[2,24,25]. In general the adsorption energy of a solute group x adjacent to a blocking group y in the same molecule is reduced in proportion to the energy of x and the size or closeness of y. A similar situation is predicted for adsorption of hindered solutes on silica, and has been confirmed in the case of hindered phenols[26,27] and pyridine derivatives[28,29] adsorbed on silica. The complexity of these steric effects, and the present absence of detailed, reliable information concerning their importance in adsorption on silica, makes quantitative comparisons difficult at the present time.

Chemical interaction between neighboring solute groups (iii), most notably intramolecular hydrogen bonding, generally reduces the adsorption energies of isomers in which such interactions can occur (e.g., ortho disubstituted aromatics). Numerous examples of the preferential adsorption of nonhydrogen bonded isomers on silica have been cited (e.g., see discussion of ref. 1).

By concerted adsorption of adjacent solute groups (iv) we mean the simultaneous interaction of two solute groups (in the same molecule) with a single adsorbent site. On alumina, isomers which are capable of concerted adsorption are generally more strongly adsorbed[24]. On the basis of assorted studies from the literature, it has been tentatively concluded[1] that concerted adsorption of adjacent solute groups is less important on silica relative to alumina. Specifically, one study[31] indicates no preferential adsorption of ortho versus non-ortho-quinones, while ortho-quinones are more strongly retained on alumina[24]. Table VII gives the $S°$ values of 1,2- and 1,4-naphthoquinones (NQ). It is seen that (as in the case of alumina) the ortho-quinone is much more strongly adsorbed than the para-quinone. The difference in $S°$ values for adsorption on silica of 1,2- and 1,4-NQ is 0.97, while for adsorption on alumina[24] the difference in $S°$ values is 1.15. Thus these data suggest that concerted adsorption is about as important on silica as on alumina. Similarly other data[30] show the preferential adsorption of various cis-azobenzene derivatives on silica, relative to trans-isomers. This reflects concerted adsorption of the two nitrogen atoms in the cis-azobenzenes (see discussion of ref. 24).

Electronic effects (v) in adsorption refer here to the electronic activation of one solute group by another, with a resulting change in its adsorption energy. On alumina electron donating groups generally increase the adsorption energy of the strongest solute groups k, and hence increase the adsorption energy of the solute molecule (see discussion of ref. 1). In principle the same sort of effects, and of similar magnitude, would be expected in adsorption on silica. The $S°$ values of nitrobenzene and of p-nitrotoluene in Table VII permit a limited test of this assumption. In the absence of electronic activation of the nitro group in p-nitrotoluene by the methyl group, the $S°$ value of the latter compound would be increased by 0.13 units relative to nitrobenzene. The actual difference in $S°$ values is 0.25 units, from which the increase in

$S°$ of p-nitrotoluene due to electronic effects is 0.12 units. For adsorption on alumina[2], the contribution of electronic effects to the $S°$ value of p-nitrotoluene is 0.31 units. As predicted the methyl group does increase the adsorption energy of the nitro group on silica, but apparently to a lesser extent than on alumina (see also discussion of ref. 29).

Miscellaneous other contributions (vi) to adsorption energy on alumina and silica have already been reviewed in detail (see ref. 1).

CONCLUSION

With the completion of the present study we now possess a general theoretical model for the adsorption of complex organic molecules on both alumina and silica. In a great many cases it is possible to accurately predict $\underline{R}°$ for various sample components in a particular chromatographic system using either of these adsorbents. The factors which determine separation in such chromatographic systems are generally well understood, and can be deliberately varied to achieve desirable changes in sample separation order. The major deficiency in this theoretical treatment concerns the correlation of very strong eluent systems (e.g., the alcohols, their solutions, and other solvents of comparable eluent strength). Many of the relationships presented here (and previously) break down for such systems, and any calculations of $\underline{R}°$ in such systems must be regarded as qualitative at best. Whether this situation can be improved by further theoretical analysis of adsorption in strong eluent systems can only be answered by future work. In addition to this general limitation of our present theory, prediction of $\underline{R}°$ values for many solute types is hindered by their complexity (e.g., involving important steric interactions or solute geometry terms, particularly of type ii, iii, and iv). This can be remedied only by measuring $\underline{R}°$ or R_F values for each of these solutes, deriving their $S°$ values, and tabulating these $S°$ values for all solutes of interest.

With the completion of the present investigation it seems appropriate to terminate this series of papers, at least as regards the general title "Linear Elution Adsorption Chromatography". Any future work along these lines will therefore assume, as is the case in most other forms of chromatography, that linear isotherm separation is specifically understood unless otherwise stated.

SUMMARY

The various factors which determine sample separation order on silica have been further examined in the context of a previous theory for separation on alumina. Certain basic differences in separation on these two adsorbents can be rationalized in terms of differing adsorption mechanisms and the presence of two major site types on the silica surface. Eluent strength values for elution from silica and alumina appear related in a simple fashion, permitting the prediction of eluent strength for most solvents on silica.

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