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# Variations in the Chromatographic Properties of Different Silica Gel Samples

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# Variations in the Chromatographic Properties of Different Silica Gel Samples

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## Summary

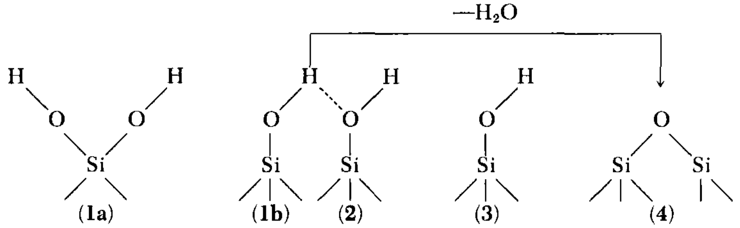
Silica gels for adsorption chromatography differ widely in their performance, depending upon the nature of the starting silica and its subsequent treatment. A general theory of the origin of these differences (and their control) is proposed in terms of the structure of the silica surface. Variations in the activity, selectivity, and linear capacity of different silica samples are readily understandable in terms of this theory. Details are provided for the initial evaluation, optimum treatment (activation and deactivation), and final standardization of silica adsorbents.

Adsorbents with the general formula $SiO_2 \cdot xH_2O$, described variously as silica, silica gel, or silicic acid (and to a lesser extent, porous glass or quartz), have found quite widespread usage in chromatography. The chromatographic properties of each of these substances (which we shall refer to collectively as "silica") can vary greatly with the method of preparation and with differences in subsequent thermal processing or "activation." The resulting adsorbent is (intentionally or otherwise) commonly used in a partially hydrated or water-deactivated form, and the water content of the final adsorbent has a further profound effect upon its behavior as an adsorbent. The relationship between chromatographic behavior and the history and/or physical properties of the final adsorbent is obviously quite complex. Several workers have contributed to our understanding of this area through the correlation of various chromatographic phenomena with fundamental studies of the silica surface. Wren (*1*) has reviewed early attempts of this sort. More recently Kiselev et al. (2) have discussed the gas-chromatographic properties of silicas of varying surface area and pore size. Several workers [for example, (*3,4*)] have examined the importance of adsorbent pore size in chromatographic separation on silica. Pitra et al. (*5*) and Klein (*6*) have discussed the optimum hydration of silica adsorbents in terms of the structure of the silica surface.

The fundamental study of the silica surface, and of the nature of interactions between surface groups and adsorbing molecules, has been in progress for several years. However, only recently has a fairly complete picture begun to emerge (*7–12*) [see particularly the discussion in (*12*)]. It is proposed in the present paper: (1) to review the recent work insofar as it appears related to the chromatographic properties of silica (2) to provide some new data on how the chromatographic properties of silica vary between different adsorbent samples, and (3) to offer a comprehensive theory of the chromatographic properties of silica as a function of the composition of the silica surface and the prior treatment of the adsorbent.

## SIGNIFICANT ADSORPTION SITES AND THE STRUCTURE OF THE SILICA SURFACE

All silica samples are characterized by a similar surface structure, one where the surface groups (**1**) to (**4**) occur in varying proportions (*9,12*):



*Geminal* silanol groups (**1a**), where a single surface silicon atom bears two hydroxyl groups, can occur either at edge positions in crystalline silica (*13*) or as defect structures in semicrystalline or amorphous silica (*9*). *Bound* silanol groups (**1b**) are so positioned on the silica surface as to permit hydrogen bonding with adjacent silanols (or other oxygen atoms). The terms "geminal" and "bound" have been used interchangeably to describe silanols of similar structure (*9*), and we shall not distinguish here between these two possibilities. *Reactive* silanol groups (**2**) possess more reactive, acidic protons than other silanol types by virtue of hydrogen bonding by another silanol to the oxygen of a reactive silanol (*12*). ***Free*** silanol groups (**3**) are isolated from other surface groups, and are believed characteristic of a relatively crystalline or ordered surface. ***Siloxane*** bridges (**4**) or surface oxide atoms result from the dehydration of adjacent surface silanols as indicated above.

The relative proportions of these groups on the silica surface are determined by the method of initially preparing the adsorbent, and by its subsequent treatment. The surfaces of hydrogels prepared by precipitation from aqueous solution, without subsequent heating above 120°C, are covered with varying proportions of the various silanol groups [(**1**) to (**3**)]. Fine-pore silicas (average pore diameters ~20 A) show a preponderance of bound (**1**) and reactive (**2**) silanols, whereas larger-pore silicas show increasing amounts of free (**3**) silanols (*12*). Varying amounts of physically adsorbed water may be present on the silica surface, and can be removed without affecting the underlying surface groups by heating the adsorbent at 120° in air, or by drying at lower temperatures in vacuum or over a desiccant (*14*). Heating silicas at temperatures up to 400° results in progressive loss of bound silanols (and probably reactive silanols) with formation of siloxane groups, but without removing free silanols (*2*). Drying temperatures below 400° cause little reduction in surface area, but heating above 400° leads to significant loss in surface area (*15*).

Unsaturated and polar molecules are selectively adsorbed on a normal, hydrated silica surface, by virtue of hydrogen bonding and other interactions between these adsorbates and surface silanols (*16*). On a severely dehydrated silica, where the surface has been converted predominantly to siloxane groups, the preferential adsorption of polar and unsaturated molecules disappears (*7*). Surface siloxane groups can therefore be regarded as inactive in the *selective* adsorption of various components from solution onto silica. Previous studies have shown that unsaturated molecules are adsorbed most strongly on reactive silanol sites (*12*), with free silanols the next strongest adsorption sites for these substances (*10,11*).

The relative proportion of reactive to total silanol groups $S_r/S_t$, and the silica surface area, can be determined readily by silanization techniques (*12*). These procedures will be described in detail later. Alternatively, the average pore diameter of a silica defines $S_r/S_t$ fairly closely (*12*). Silicas with low $S_r/S_t$ values are believed to possess surfaces that are relatively crystalline or ordered, whereas silicas with high $S_r/S_t$ values are believed to possess more amorphous surfaces.

## ADSORBENT ACTIVITY AS A FUNCTION OF SURFACE COMPOSITION, ACTIVATION TEMPERATURE, AND EXTENT OF WATER DEACTIVATION

A previous treatment (*17*) has related over-all adsorbent activity or adsorption affinity to two primary parameters: adsorbent surface area and average surface energy. For elution of a particular solute by a given eluent from an adsorbent sample of interest, the equivalent retention volume of the solute $R°$ (ml/g) is given by

$$\log R° = \log V_a + \alpha f(S, E) \tag{1}$$

Here $V_a$ is the adsorbent surface volume (ml/g) or volume of an adsorbed solvent monolayer (proportional to adsorbent surface area, corrected for partial coverage by added water). $\alpha$ is the adsorbent activity function (proportional to average surface energy), and $f(S, E)$[*] is a function of the solute and eluent used. The value of $V_a$ for a particular adsorbent sample can be readily calculated from its starting surface area and the amount of water added for partial deactivation: $\alpha$ can be measured chromatographically using a standard solute/eluent combination and Eq. (1). It is of interest to examine how the adsorbent surface energy function $\alpha$ varies with differences in the surface composition of the starting adsorbent, its subsequent thermal activation, and final water deactivation.

### The Importance of Adsorbent Structure and Relative Water Deactivation

Table 1 summarizes experimental data for the elution of naphthalene by pentane from three silicas of varying type and water content. These samples, Davison silicas of varying pore diameter, bracket the extremes of silica surface type found in various chromatographic adsorbents. $S_r/S_t$ varies from 3% for the wide-pore Code 62 silica to 71% for the narrow-pore Code 12 silica. The retention volume data of Table 1 permit the derivation of $\alpha$ values for each of these adsorbents. These values of $\alpha$ are plotted in Fig. 1 versus the per cent coverage of the adsorbent surface by added water. The 0% $H_2O$-$SiO_2$ samples of Table 1 and Fig. 1 show $\alpha$

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[*] Also referred to previously as $(S° - \epsilon° A_s)$.

**TABLE 1**

Retention Volume and Linear-Capacity Data for Elution of Naphthalene by Pentane from Various Silica Samples (Initial Activation at 195°C)

| | | Linear capacity[a] | |
|---|---|---|---|
| Silica | $R°$, ml/g | g/g | g/m$^2$ |
| Davison Code 12[b] | | | |
| 0% $H_2O$-$SiO_2$ | 51.9 | $0.9 \times 10^{-4}$ | $1.1 \times 10^{-7}$ |
| 2.0% $H_2O$-$SiO_2$ | 25.1 | 7.5 | |
| 7.5% $H_2O$-$SiO_2$ | 6.3 | 29 | |
| 16.0% $H_2O$-$SiO_2$ | 1.84 | 26 | |
| Davison MS[c] | | | |
| 0% $H_2O$-$SiO_2$ | 46.4 | $1.0 \times 10^{-4}$ | $1.2 \times 10^{-7}$ |
| 2.1% $H_2O$-$SiO_2$ | 18.0 | 2.5 | |
| 7.9% $H_2O$-$SiO_2$ | 5.0 | 15 | |
| 16.8% $H_2O$-$SiO_2$ | 2.16 | 25 | |
| Davison Code 62[d] | | | |
| 0% $H_2O$-$SiO_2$ | 6.5 | $1.9 \times 10^{-4}$ | $6.0 \times 10^{-7}$ |
| 0.8% $H_2O$-$SiO_2$ | 4.42 | 2.9 | |
| 2.9% $H_2O$-$SiO_2$ | 2.74 | 11 | |
| 6.2% $H_2O$-$SiO_2$ | 1.77 | 4.2 | |

[a] Corrected for varying $R°$ and band width as in (*20*).

[b] Starting surface area by silanization 801 m$^2$/g; $S_r/S_t = 0.71$.

[c] Starting surface area by silanization 866 m$^2$/g; $S_r/S_t = 0.25$.

[d] Starting surface area by silanization 313 m$^2$/g; $S_r/S_t = 0.03$.

decreasing with decreasing concentration of reactive silanols (i.e., $S_r/S_t$). This same trend in adsorbent activity has been demonstrated for a much wider range of silica samples (*12*), and simply reflects the fact that reactive silanols are the strongest adsorption sites on the silica surface (at least for the adsorption of unsaturated molecules).

Upon addition of water to the various silicas of Table 1, there is a general lowering of the average surface energy $\alpha$. This presumably reflects preferential adsorption of water molecules on the strongest surface sites (i.e., reactive silanols). In the case of the Code 62 silica (see Fig. 1), water addition results in an initial slight decrease in $\alpha$, after which $\alpha$ remains essentially constant with further addition of water. This can be correlated with the composition of the surface of this silica: 3% reactive silanols and the balance primarily free silanols. Because of their small concentration on the Code 62 surface, reactive silanols make a relatively small contribution to $\alpha$, and their selective removal by initially added water (up

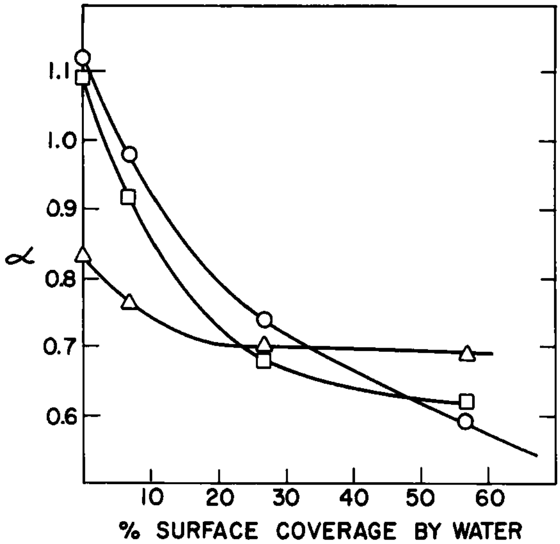

**FIG. 1.** Variation of adsorbent activity function $\alpha$ with relative surface coverage by added water for different silicas; initial activation in air at 195° for 16 hours: Davison Code 12, Davison MS, and Davison Code 62.

to 15% surface coverage by water) has a correspondingly small effect on average surface energy and $\alpha$. Beyond about 15% surface coverage by water only free silanols of equal energy remain, and further addition of water simply results in the nonselective coverage of these groups, without further decrease in average surface energy. Consequently, further addition of water has no effect on $\alpha$. The Code 12 silica of Fig. 1 shows a quite different response to water deactivation: $\alpha$ decreases strongly with water addition without leveling out as in the case of the Code 62 sample, and beyond 35% surface coverage by water the Code 12 sample shows lower values of $\alpha$ than for the Code 62 silica at comparable surface coverages. Again this can be correlated with the composition of the silica surface, which in the case of Code 12 silica consists almost exclusively of reactive and bound silanols. These groups are not energetically uniform, because they are associated with an amorphous surface. Consequently, after each incremental addition of water, which selectively adsorbs on the strongest surface sites, there always remains a range of site energies in the uncovered surface. Further addition of water again selectively covers the strongest sites, so that average surface energy and $\alpha$ continue to decline with increased water addition, and never reach a plateau as in the case of Code 62 silica. Presumably after 35% addition of water the remaining uncovered surface is composed primarily of bound silanols, which are weaker adsorption sites than free silanols.* The MS silica of Fig. 1 is intermediate in composition ($S_r/S_t$ equal 25%) and exhibits an intermediate dependence of $\alpha$ on surface coverage by water. This is better seen by comparing the ratio of $\alpha$ for 0% $H_2O$-$SiO_2$ to the value for 57% surface coverage by water for the three silicas: 1.90 (Code 12), 1.76 (MS), and 1.20 (Code 62).

The preceding data suggest that the experimentally determined value of $S_r/S_t$ is the key to the variation of $\alpha$ for different silicas as a function of added water. The data of Fig. 1 were cross-plotted to provide values of $\alpha$ for adsorbents of varying $S_r/S_t$ as a function of water addition, and these smoothed data are presented in Table 2. These latter values permit a more rational estimate of the gross chromatographic properties (i.e., "activity") of different silica samples in advance of their actual use.

**TABLE 2**

Adsorbent-Activity-Function ($\alpha$) Values as a Function of Silica Type and Water Content

| $\left[\frac{\%\ \text{Added } H_2O}{V_a}\right]^b$ | $\alpha^a$ | | | | | | |
|---|---|---|---|---|---|---|---|
| $S_r/S_t =$ | 0.00 | 0.03 | 0.05 | 0.10 | 0.20 | 0.40 | 0.70 |
| 0 | 0.69 | 0.83 | 0.88 | 0.97 | 1.07 | 1.12 | 1.12 |
| 3 | 0.69 | 0.80 | 0.84 | 0.91 | 0.98 | 1.05 | 1.06 |
| 6 | 0.69 | 0.77 | 0.80 | 0.85 | 0.92 | 0.98 | 1.00 |
| 10 | 0.69 | 0.74 | 0.76 | 0.80 | 0.84 | 0.89 | 0.93 |
| 15 | 0.69 | 0.72 | 0.73 | 0.76 | 0.78 | 0.81 | 0.85 |
| 20 | 0.69 | 0.70 | 0.70 | 0.71 | 0.72 | 0.75 | 0.80 |
| 30 | 0.69 | 0.70 | 0.69 | 0.68 | 0.67 | 0.68 | 0.72 |
| 40 | 0.69 | 0.70 | 0.68 | 0.66 | 0.65 | 0.65 | 0.67 |
| 50 | 0.69 | 0.69 | 0.67 | 0.65 | 0.63 | 0.63 | 0.62 |
| 60 | 0.69 | 0.69 | 0.67 | 0.65 | 0.62 | 0.61 | 0.58 |
| 70 | 0.69 | 0.69 | 0.67 | 0.65 | 0.62 | 0.59 | 0.54 |

[a] Silica activated at 200°C before addition of $H_2O$.

[b] Value of $V_a$ for starting (i.e., undeactivated) adsorbent; $V_a = 0.00035$ surface area ($m^2/g$).

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[*] The fact that $S_r/S_t$ is much higher than 35% (i.e., 71%) does not actually mean that 71% of the surface must be covered by water before all reactive silanols are covered, because the method of measuring $S_r/S_t$ (*12*) may include two other silanol groups for every reactive silanol actually determined.

In a related study (*18*) it has been shown that polar sample groups (e.g., the nitro group of nitrobenzene) apparently show less tendency to preferentially adsorb on reactive silanols (particularly from weak eluents). As a practical result the relative separation of polar samples on different silicas (or on silicas of varying water content) is occasionally less sensitive to adsorbent $\alpha$ values than is the separation of nonpolar compounds.

### Variation of Adsorbent Activity with Activation Temperature

Silica samples for chromatography are frequently activated at 110 to 125° before use, and occasionally at temperatures as high as 400°. It is of interest to examine the effect of activation temperature over this range. Figure 2 shows the variation of adsorbent activity (as measured by $R^\circ$ for naphthalene eluted by pentane) for Code 12 silica versus initial activation temperature. Adsorbent activity is seen to rise with higher activation temperatures below 150°, remains constant (±2% in $R^\circ$) between 150 and 250°, and then gradually declines between 250 and 400°. Past 400° the decline in adsorbent activity becomes more rapid [see, for example (*10*)].

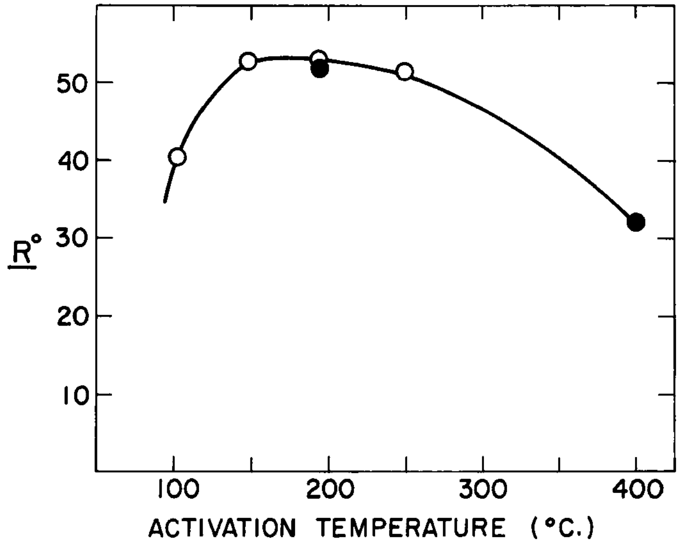

**FIG. 2.** Variation in silica activity as a function of activation temperature; elution of naphthalene by pentane; ○, activation in stream of nitrogen for 15 minutes; ●, activation in air for 16 hours.

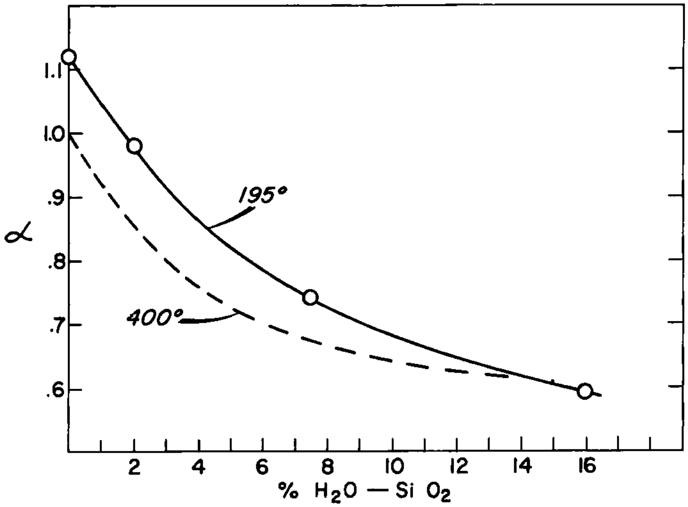

**FIG. 3.** Effect of initial activation temperature on adsorbent activity versus per cent water deactivation; Code 12 silica.

Presumably the initial rise in adsorbent activity with temperature reflects the removal of physically adsorbed water (the reverse of water deactivation as in Fig. 1). Between 250 and 400° bound and reactive silanols are lost from the surface, leading to a gradual decline in adsorbent activity. With major loss of all silanol groups from the silica surface at temperatures above 400°, decrease in adsorbent activity is expected to become quite marked. Because silica surface area is little affected by thermal activation up to 400° (*15*), and $V_a$ is proportional to surface area, the changes in silica activity between 250 and 400° can be attributed solely to changes in surface energy or $\alpha$. For wider-pore silicas than the Code 12 sample of Fig. 2, or silicas with smaller $S_r/S_t$ values (i.e., most other silicas), the loss in bound and reactive silanols from the surface should be less marked (because their relative concentration is less). Consequently, relative adsorbent activity (and chromatographic behavior in general) should change less with changes in activation temperature (up to 400°) for wide-pore silicas. This is, in fact, observed in Figs. 3 and 4, where the silica $\alpha$ values versus per cent added water are plotted for Code 12 and Code 62 silicas at 195° (data of Fig. 1) and 400° [data of (*19*)]. For the Code 12 silica (Fig. 3), $\alpha$ is less for the 400° sample at all water coverages up to 57% surface coverage by water. For the Code 62 silica, $\alpha$ is virtually the

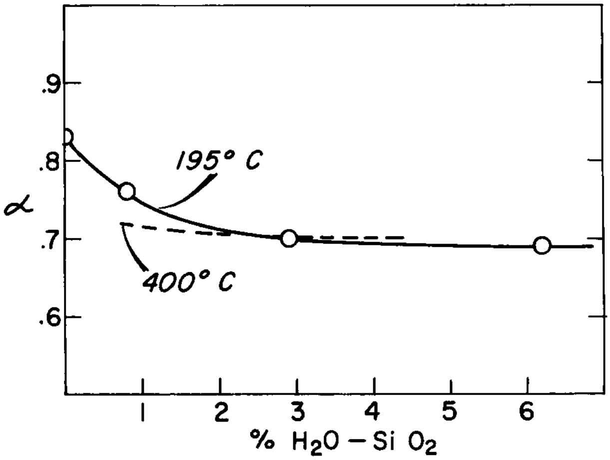

FIG. 4. Effect of initial activation temperature on adsorbent activity versus per cent water deactivation; Code 62 silica.

same for the 195 and 400° samples at all water coverages past about 10% surface coverage by water.

## SILICA LINEAR CAPACITY AS A FUNCTION OF SURFACE COMPOSITION AND WATER CONTENT

The linear capacity of an adsorbent, defined as the maximum amount of sample that can be applied to a chromatographic column (or plate) before nonlinear isotherm separation occurs (i.e., before $R^\circ$ for a given compound decreases by 10% from the linear isotherm value) is an important chromatographic property. A previous treatment (*20*) has shown that adsorbent linear capacity per unit of surface area is greater the more homogeneous the surface (i.e., the smaller the energy differences between various sites). We have seen that the Code 62 silica is relatively homogeneous (i.e., most surface sites approximately the same), and the Code 12 silica is quite heterogeneous (i.e., surface sites show a wide range in energies). Consequently, the linear capacities per unit of surface area for the 0% $H_2O$-$SiO_2$ samples are expected to be much higher for the Code 62 silica than for the Code 12 silica, with the MS silica intermediate. As seen in Table 1, this is in fact observed.

Addition of water to an adsorbent should markedly increase its linear capacity when the surface is initially heterogeneous, up to the point where the strongest sites are covered by water and the

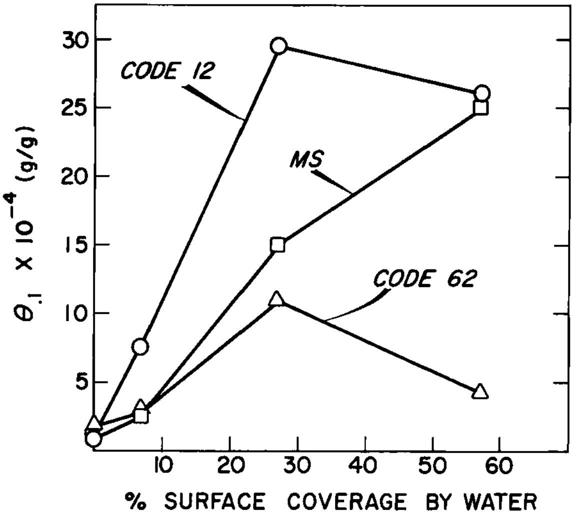

**FIG. 5.** Adsorbent linear capacity versus added water for different silicas.

remaining surface is homogeneous. Further addition of water should then decrease adsorbent linear capacity (*20*). These effects for the three adsorbents of Table 1 are examined in Fig. 5. As expected, addition of small amounts of water to all three adsorbents increases their linear capacity, and the effect is greatest for Code 12 and least for Code 62 silica. After coverage of the small number of reactive silanols on the Code 62 surface, however, further addition of water to the homogeneous free silanol surface should markedly reduce linear capacity, and this is in fact observed. In the case of the MS and Code 12 silicas, linear capacity does not increase as rapidly after initial water addition, but no marked decrease in linear capacity results below 57% coverage of the surface by water.

Higher activation temperatures (e.g., 400 versus 195°) would be expected to increase linear capacity for nondeactivated Code 12 silica (because of loss of reactive silanols). This effect should be less noticeable in wide-pore silicas.

## VARIATIONS IN SAMPLE SELECTIVITY WITH CHANGING SURFACE STRUCTURE

Numerous factors can affect adsorbent selectivity or relative sample separation on silica and other adsorbents. Changes in the eluent used are perhaps the commonest cause of reversals in separation order [see, for example, (*18,19*)]. In one of the few studies on the effect of silica type on separation selectivity, Klein (*3*) and Klein and Szczepanik (*21*) have demonstrated some interesting changes in the relative separation of various sterol acetates on silicas of varying pore size. These effects were originally interpreted as arising from restricted adsorption of the large sterol molecules in small adsorbent pores. An alternative explanation in terms of silica surface composition seems possible, because we have seen that average pore diameter and surface composition are interrelated.

The concept that the adsorption of large organic molecules is sensitive to adsorbent pore diameter is fairly common [see the review in (*4*)] and superficially attractive. Thus with decreasing pore diameter and average radius of curvature it would seem that large molecules should find increasing difficulty in adsorption: "It is only when the pore is large with respect to the major axis of the adsorbed molecule that the molecule can, in effect, be considered to be attracted by a flat surface" (*3*). A critical test of this hypothesis is afforded by the adsorption of large, planar molecules such as the condensed aromatic hydrocarbons on silicas of differing pore diameter. The rigidity of these molecules would presumably require a quite planar surface for optimum interaction upon adsorption [see the discussion in (*22*)]. As already shown (*4,19*), however, the experimental evidence is quite substantial that the condensed aromatics are *not* hindered in their adsorption on fine-pored silicas. Thus the relative adsorption of the larger fused aromatics is relatively *less* on wide-pore than on narrow-pore silicas (compared to smaller aromatics), and there is no preferential adsorption of narrow versus wide isomers on silica (e.g., chrysene versus triphenylene). From this we can conclude that only in extreme cases (i.e., pore diameters less than 20 A, or very large molecules) will pore diameter, per se, affect the relative adsorption of different compounds on silica.

The major effect of reduced pore diameter on the separation of the sterol acetates studied by Klein et al. appears to be an expansion of their range of retention volume values. Thus relative to cholesterol acetate, sterol retention volumes range from 0.89 to 1.69 on Code 62 silica (wide pore), and (for the same sterols) from 0.74 to 2.42 on Code 12 silica (narrow pore). This effect is examined quantitatively in Fig. 6, where the relative sterol retention volumes (cholesterol equal 1.00 throughout) on the various silicas studied

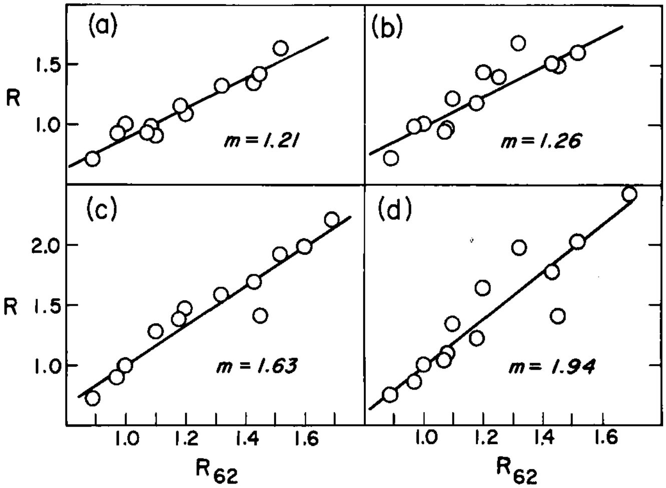

**FIG. 6.** Relative retention volumes of sterols studied by Klein and Szczepanik (*21*) on different silicas versus values on Code 62 silica. (a) Davison MS, (b) Davison Code 950, (c) Mallinckrodt, (d) Davison Code 12.

by Klein et al. are plotted against the relative retention volumes for Code 62 silica. The least-squares slopes **m** of these plots increase regularly with decreasing pore size. The various plots of Fig. 6 suggest a simple increase in adsorbent activity $\alpha$ in going from large- to small-pore silicas, as described by Eq. (1). This is not the case, however, as can be seen from the absolute retention volume data for cholesterol acetate itself (Table 3). The data of Table

**TABLE 3**

Failure of **m** to Correlate with Silica Activity[a]

| Silica | Av. pore diameter, A | $R^{ob}$, ml/g | $\alpha/\alpha_{62}{}^{c}$ | **m** expt. | **m** pred.[d] |
|---|---|---|---|---|---|
| Code 62 | 170 | 93 | (1.00) | 1.00 | (1.00) |
| MS | 67 | 180 | 0.94 | 1.21 | 0.98 |
| 950 | 27 | 142 | 0.90 | 1.26 | 0.96 |
| Code 12 | 20 | 102 | 0.85 | 1.94 | 0.94 |

[a] Data of Klein and Szczepanik (*21*).

[b] Calculated as retention volume per ml column volume divided by bulk density of adsorbent.

[c] Assumes $V_a$ values equal those of 0% $H_2O$-$SiO_2$.

[d] From ($\alpha/\alpha_{62}$) values.

3 permit an approximate estimate of the relative $\alpha$ values for these various silica samples, which in turn permits an estimate of **m** for each silica (assuming adsorbent activity is responsible for the variations of **m** with pore size). As seen in Table 3, the silica $\alpha$ values predict a slight decrease in **m** with decreasing pore size, whereas a large *increase* is actually observed. Additional data which we shall refer to shortly [see also (*18*)] corroborate the general unimportance of adsorbent activity in affecting selectivity on silica.

If adsorbent activity is not responsible for the apparent effect of pore size on the relative adsorption of the sterol acetates, the relative proportions of different silanol types cannot be responsible per se, because these proportions determine adsorbent activity. An alternative aspect of the silica surface which varies continuously with pore diameter is its relative regularity or order, which arises from the relative crystallinity of the silica surface. We have noted that free silanols are associated with a near-crystalline surface, the free silanols occupying definite positions in the crystal lattice [see, for example, (9)]. Thus Code 62 silica is largely "regular" in this sense, and Code 12 silica is quite "irregular." The ratio $S_r/S_t$ furnishes a quantitative index of surface regularity. To see how surface regularity can affect the relative adsorption of the sterol derivatives, it is easier first to examine the relative adsorption of benzene, dibenzyl, and the fused aromatic hydrocarbons on silica. A previous paper (*4*) has shown that the adsorption energy of dibenzyl is about twice that of benzene on different silicas (i.e., adsorption energy per aromatic carbon is constant), whereas fused aromatic hydrocarbons have a lower adsorption energy per aromatic carbon atom (relative to benzene or dibenzyl). These facts have been explained in terms of the localization of the benzene ring (or of each phenyl group in dibenzyl) on surface silanol sites. Similar localization of all aromatic carbons for compounds such as naphthalene is not generally possible, because with one ring localized (as in benzene) the additional carbon atoms cannot generally adopt a similar configuration (i.e., localized) on adjacent silanol groups [see Fig. 4 of (*4*)]. The relative adsorption energy of the additional four carbons of naphthalene will depend upon the proximity of neighboring silanol groups. On a regular surface such as Code 62 silica, each silanol will be situated about 5.0 A from neighboring silanols (*23*), which is too distant for optimum interaction of two silanols with *each* ring of naphthalene. On an irregular surface (e.g., Code 12) the distance between adjacent silanols will be variable, and some silanols may be sufficiently close for optimum interaction with each ring of naphthalene. Consequently, we expect the adsorption energy per carbon atom of the "extra" carbon atoms (i.e., past the first six of each aromatic nucleus) in the fused aromatic hydrocarbons to be greater on the irregular Code 12 surface than on the regular Code 62 surface. Some data relative to this point are summarized in Table 4, where the experimentally determined adsorption energies of the extra carbons in fused polyaromatic hydrocarbons are reported for various cases (as a percentage of the adsorption per carbon atom in benzene or dibenzyl). As predicted, the adsorption energy per extra carbon atom in the polyaromatic hydrocarbons is substantially higher for adsorption on the irregular Code 12 silica relative to the regular Code 62 silica. Decreasing adsorbent activity (see Table 4), as by adding water or by selective reaction of reactive silanols by trimethylchlorosilane (*12*), does not decrease the relative adsorption energy of the extra carbon atoms (compared to those in benzene). As in the case of the sterol derivatives, the preferential adsorption of these extra carbon atoms on small pore silicas is in the opposite direction predicted by the over-all activity of the silica

**TABLE 4**

Adsorption Energy of "Extra" Carbon Atoms in Fused Polyaromatic Hydrocarbons for Different Silica Samples; Relative Separation Order of Dibenzyl

| | $R^\circ$, ml/g; pentane eluent | | | | |
|---|---|---|---|---|---|
| Adsorbent[a] | Naphthalene | Fluoranthene | Dibenzyl | Adsorption energy of "extra" carbons, %[b] | $n_a$ of dibenzyl |
| Code 62 | | | | | |
| 195° | 6.5 | 16.9 | 45.0 | 37 | 22.2 |
| 195°—TMCS | 3.5 | 7.9 | 16.2 | 39 | 21.4 |
| 400°—4%[c] | | | | 39 | 21.3 |
| Code 12[c] | | | | | |
| 400°—1.6% | | | | 48 | 18.6 |
| 400°—8.0% | | | | 53 | 17.3 |
| 400°—16.0% | | | | 56 | 16.8 |

[a] Temperature of initial activation given; % figures refer to added water; TMCS refers to treatment with trimethylchlorosilane to remove reactive silanols.

[b] Adsorption energy of extra carbons in polycyclic hydrocarbons, as a percentage of adsorption energy per carbon in benzene.

[c] Data of (*19*).

surface. The effect of this change in the relative adsorption of the extra carbons of the fused polyaromatic hydrocarbons with silica pore size is further illustrated by the $n_a$ values of dibenzyl in each case (Table 4). $n_a$ refers to the number of carbon atoms in the hypothetical fused hydrocarbon, which has the same $R^\circ$ value as dibenzyl. Thus on Code 62 silica, dibenzyl separates with fused hydrocarbons having about 22 carbon atoms (e.g., picene and dibenzanthracene), and on Code 12 silica with fused hydrocarbons having about 18 carbon atoms (e.g., triphenylene and chrysene).

Returning to the case of the sterol derivatives of Klein et al., these may be likened to the above hydrocarbons as follows. The principal difference in the structure of these sterols, as far as $R^\circ$ values are concerned, is the presence of varying numbers of strongly adsorbing double bonds.* The sterols with no double bonds are analogous to benzene in having only one adsorbing group (the acetate group), whereas sterols with one double bond are analogous to dibenzyl in having two functional groups separated by an aliphatic chain. Thus all strongly adsorbing groups (acetate, double bond) in the latter two sterol types are capable of simultaneous localization on silanol groups upon adsorption, and we would expect to see little difference in the relative $R^\circ$ values for these compounds on small- versus large-pore silicas [just as in the case of benzene versus dibenzyl on various silicas (*19*)]. The observed average ratios of retention volume values (relative to cholesterol) of the sterols with no and one double bond on the Code 12 relative to Code 62 silica are 0.89 and 0.98 ± 0.07, respectively (i.e., close to 1.00 in each case, as predicted). For sterols containing more than one double bond, however, the additional double bonds are much less able to localize on separate silanol groups, because two groups (or points) of the molecule are already fixed on the silica surface (i.e., localized on two silanol groups). Additional double bonds thus bear a resemblance to the extra carbons of the fused hydrocarbons. Accordingly, we expect to see stronger *relative* adsorption of the second and third sterol double bonds on fine-pore silicas, and correspondingly greater adsorption of the sterols themselves. This is, in fact, the case; the ratios of retention volume values (relative to cholesterol) of sterols with two and three double bonds on the Code 12 versus Code 62 silica are 1.34 ± 0.11 and 1.37, respectively (i.e., signifi-

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* As noted by Klein and Szczepanik (*21*), sterically hindered double bonds in the 22,23 position contribute little to adsorption and may be ignored (i.e., not counted) as far as their contribution to adsorption.

cantly larger than 1.00, as predicted). The relatively greater adsorption of the stronger adsorbing sterols on fine-pore adsorbents is simply a result of the higher adsorption energies of the second and third double bonds on an irregular surface, just as in the case of the preferential adsorption of fused hydrocarbons on fine-pore silica (relative to benzene and dibenzyl). The correlations of Fig. 6 are simply an alternative expression of this argument. The parameter **m** should correlate smoothly with surface regularity and with $S_r/S_t$. As seen in Fig. 7 this is indeed the case.

Other sample types differing in the number and position of double bonds or aromatic rings should exhibit separation effects on silica (of varying pore size), similar to those for the sterols and aromatic hydrocarbons. In the case of polyfunctional molecules with adsorbing groups other than double bonds, it is difficult to estimate the extent to which similar generalizations hold. Localization of polar groups on silica seems less severe than in the case of hydrocarbon double bonds (*19*), and there is evidence that different adsorption sites are involved (*18*).

The deviation of the experimental data from the curves of Fig. 6 (average deviation ±9%, maximum 25%) reflects additional con-

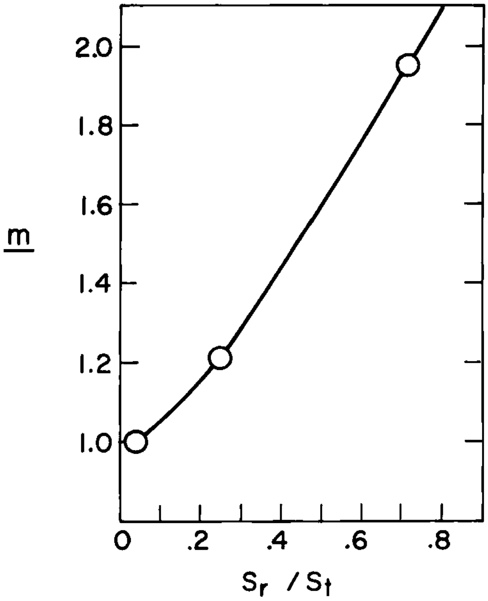

**FIG. 7.** Silica selectivity factor **m** versus $S_r/S_t$.

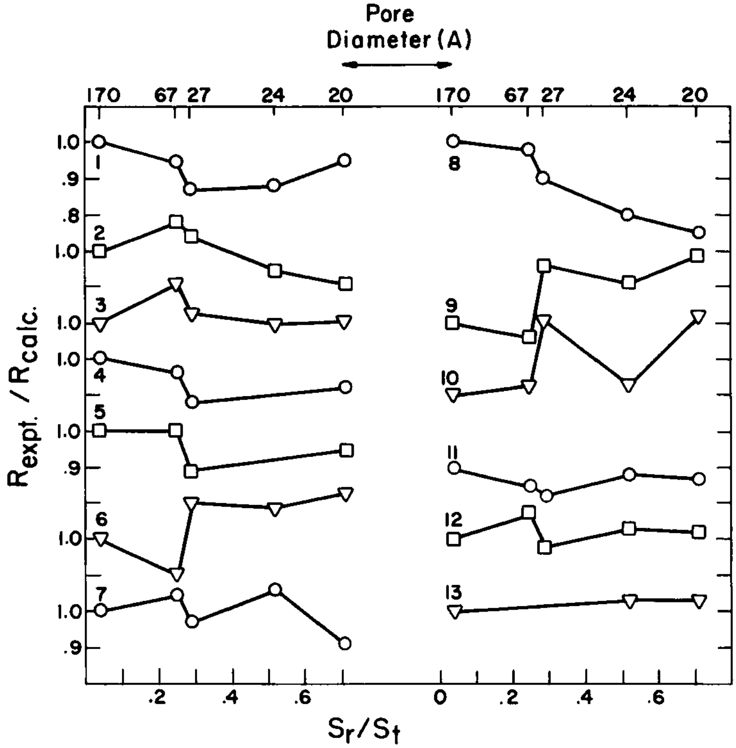

FIG. 8. Variation of relative sterol retention volumes [data of Klein and Szczepanik (*21*)] versus silica type, after correction for variations in silica surface regularity.

tributions of silica type to sample selectivity, beyond those covered by surface regularity. This residual selectivity or ratio of experimental to calculated retention volume values is plotted versus $S_r/S_t$ (or pore diameter) in Fig. 8, for each of the sterols studied by Klein et al.[*] The resulting variations appear to be completely random, and have so far defied all attempts to classify them according to sterol structure. Thus it seems probable that this residual contribution of silica type to sample selectivity represents a random matching of each sterol and surface, which is a specific function of

[*] The numbers correspond to the order of sterols in Table 2 of (*21*): 1, dihydrolanosterol; 2, dihydrocholesterol, etc.; $S_r/S_t$ values for the Code 950 and Mallinckrodt silicas studied by Klein et al. were estimated from their m values (Fig. 6) and Fig. 7.

each surface and each sterol. That is, the specific features of each silica (e.g., concentrations of various silanols, their relative arrangement on the surface, etc.) are more or less well tailored to the adsorption of specific molecules in a random (i.e., unpredictable) fashion.

## DETERMINATION OF $S_r/S_t$ AND SURFACE-AREA VALUES FOR SILICA BY SILANIZATION

A previous communication (*12*) has indicated in a general way how the concentrations of reactive ($S_r$) and total ($S_t$) surface silanols may be determined by selective silanization. The quantity $S_t$ may in turn be related to silica surface area with a precision of about ±10%. Because of the fundamental importance of the surface area and value of $S_r/S_t$ of a silica in understanding its chromatographic properties, a detailed description of these silanization methods will be given.

Figure 9 is a schematic diagram of the all-glass silanization unit. A wrap-around design permits the unit to be housed in a beaker or similar vessel with a diameter of 6 to 8 inches. The beaker is filled with a suitable heat-transfer oil and maintained at 195 ± 5° (easily accomplished using a simple, unthermostated heater). The

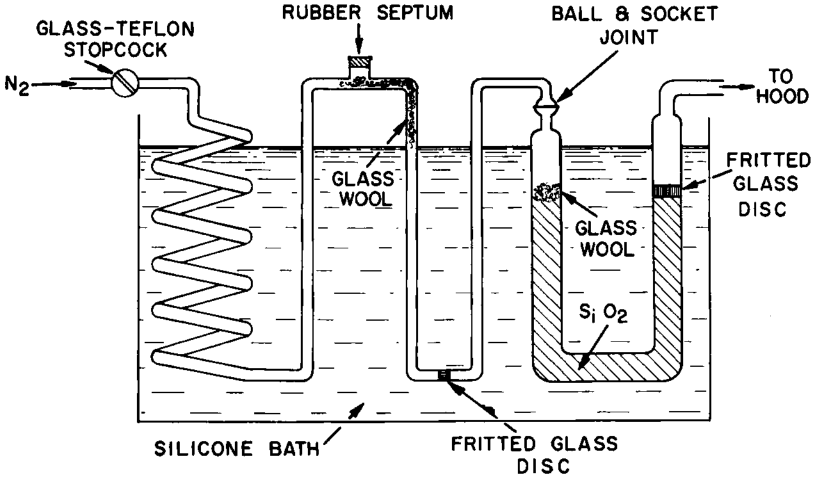

FIG. 9. Silica silanization apparatus.

tared silica tube is filled with sample and weighed, attached to the unit, and a nitrogen flow of about 2 ml/sec (determined by water displacement) is begun. After 15 minutes the silica tube is removed, cleaned (externally), and reweighed to obtain a dry adsorbent weight $W_d$. The procedures for determining $S_r$ or $S_t$ are quite similar, differing only in the use of trimethylchlorosilane* (TMCS) for the determination of $S_r$, and hexamethyldisilazane* (HMDS) for the determination of $S_t$. In either case, after the above predrying operation, the silica tube is reattached to the unit, a certain volume of silane $V_s$ is slowly injected through the rubber septum (as in gas chromatography), and nitrogen flow is continued through the unit for 15 minutes. The silica unit is then cleaned and reweighed. The process is repeated at least three times for the determination of $S_t$, using a value of $V_s$ equal to about 0.15 ml of HMDS per g of silica; only one silane injection is used in the determination of $S_r$ (see below). For the determination of $S_t$ the cumulative weight increase (relative to original dry adsorbent) after each silane addition is calculated. These values of the weight increase (in mg) are plotted versus the cumulative volume of added silane ($V_s$, $2V_s$, $3V_s$, etc.) in ml. A straight line through the origin with slope of 685 mg/ml is drawn. If the three data points fall on a reasonably straight line which intersects the *y* axis above the origin, no further data are needed. If the three data points fall on the line through the origin, or on a curve, additional data points are collected and plotted as previously. The last three points should define a reasonably straight line, which is then extrapolated back to the *y* axis. A point *P* is marked on this line halfway between the *y* axis and the original line through the origin. The weight increase (value of *y*) corresponding to this point is noted: $W_t$. Values of $S_t$ (meq/g) are then given as $W_t/72W_d$. This over-all procedure is illustrated in Fig. 10.

$S_r$ may be obtained by a similar procedure [see (*12*)], but a quicker, more reliable method is as follows. A single silane addition ($V_s$ equal to 0.125 times $W_dS_t$) is made, and the weight increase $\Delta W$ is determined as before. The ratio $S_r/S_t$ is then determined from Fig. 11. $S_t$ may also be calculated from the silica surface area and vice versa: surface area ($m^2/g$) $= 358S_t$. Determination of $S_t$ plus $S_r/S_t$ by silanization requires about 4 hours per sample.

The latter procedure has so far been applied successfully (*12*)

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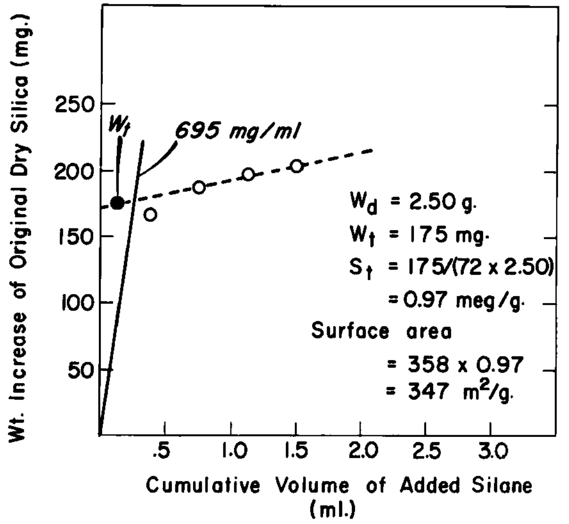

**FIG. 10.** Calculation of $S_t$ from HMDS silanization for hypothetical example.

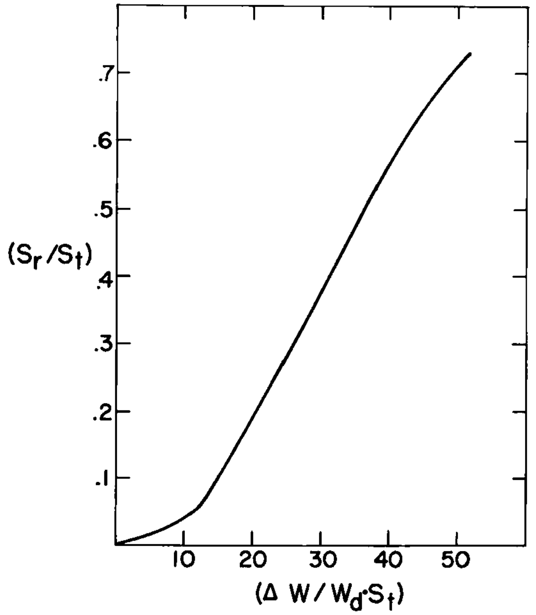

**FIG. 11.** Calculation of $S_r/S_t$ from TMCS silanization.

to a large number of silicas suitable for column chromatography (60–200 mesh). Attempts to extend these techniques to thin-layer silicas have not been successful. In the case of silicas with calcium sulfate binder, experimental values of $S_r$ and $S_t$ appear quite variable, and do not agree with values that can be inferred from surface area, pore diameter, and chromatographic-activity data. This suggests that calcium sulfate in some way interferes with the silica-silane reaction. For thin-layer silicas without binder, erroneously low values of $S_r/S_t$ were obtained, which is believed due to the low permeability of reaction tubes packed with these fine-particle silicas. As a result there is vapor bypass in the silanization unit, through small cracks in the packed silica, and uneven contacting of silica by silane. An alternative procedure for estimating values of $S_r/S_t$ for thin-layer silicas was devised, based on the correlation of $S_r/S_t$ with silica surface area and the $R^\circ$ value of a standard solute/eluent combination. This correlation is summarized in Table 5.

**TABLE 5**
Approximate Standardization of Thin-Layer Silicas

| 100 $R_s/SA$[a] | $S_r/S_t$ | Commercial silica | Surface area, m$^2$/g | $R_s$[a] | Estimated $S_r/S_t$ |
|---|---|---|---|---|---|
| 0.8 | 0.00 | | | | |
| 1.0 | 0.02 | | | | |
| 1.2 | 0.04 | | | | |
| 1.4 | 0.07 | | | | |
| 1.6 | 0.11 | Silica gel G[b] | 430 | 5.4 | 0.05 |
| 1.8 | 0.16 | Adsorbosil No. 2[c] | 343 | 3.2 | 0.01 |
| 2.0 | 0.24 | | | | |
| 2.2 | 0.32 | Woehlm TLC[d] | 545 | 8.9 | 0.12 |
| 2.4 | 0.39 | | | | |
| 2.6 | 0.47 | | | | |
| 2.8 | 0.55 | | | | |
| 3.0 | 0.62 | | | | |
| 3.2 | 0.70 | | | | |
| 3.4 | 0.77 | | | | |

[a] $R_s$, the $R^\circ$ value of fluoranthene eluted by 5%v benzene/pentane from the silica sample after initial activation at 195°; SA silica surface area (m$^2$/g).

[b] Research Specialties Co., Richmond, Calif.

[c] Applied Science Laboratories, Inc., State College, Pa.

[d] M. Woehlm, Eschwege, Germany.

Values of $S_r/S_t$ for several commercial thin-layer silicas were estimated from their surface areas (by BET) and chromatographic properties by means of the correlation of Table 5, and are shown there. All the commercial thin-layer silicas studied exhibited relatively low values of $S_r/S_t$ (0.01 to 0.12).

## PREPARATION AND STANDARDIZATION OF SILICA ADSORBENTS

The present discussion is intended primarily for liquid chromatography in columns, although some (less direct) applications to thin-layer chromatography will also be evident. By the preparation and standardization of the adsorbent we imply all the processes that lead to a final column packing: (1) selection of the initial adsorbent, and if necessary the measurement of its critical properties (i.e., surface area and $S_r/S_t$); (2) the manner and degree of initial activation of the adsorbent; (3) the manner and extent of subsequent water deactivation; and (4) the evaluation or standardization of the final product. Each of these various processes will be more or less important, according to the type of final separation anticipated: e.g., simple separation of an unknown mixture for the first time, attempted duplication of an earlier separation of the same type of sample, or precise quantitative analysis (where sample component $R^\circ$ values must be closely reproducible).

In the past most workers have paid little attention to the actual choice of one silica in preference to another. It is clear from the discussion of preceding sections that silicas vary widely in their potential chromatographic properties. For separations in general it appears that high-surface-area, high-$S_r/S_t$ silicas have a general advantage. Thus the linear capacities of moderately deactivated Code 12 or MS silica are generally $2\frac{1}{2}$ to 6 times greater than that of water-deactivated Code 62 silica (Table 1). This means that greater column loadings and/or separation efficiencies are possible with the former two silicas under normal separation conditions. Furthermore, for the separation of mixtures with varying numbers of double bonds (and possibly other functional groups), sample components are more spread out on high $S_r/S_t$ silicas and are hence better separated. In the preparative separation of easily resolved mixtures, other factors may be more important. Thus it is frequently convenient to determine the best separation conditions (i.e., best eluent, or series of eluents) by carrying out initial thin-layer separations under a variety of conditions. From these initial separations, an analogous large-scale separation by column chromatography is easily arrived at, providing that the adsorbents used in the thin-layer and column separations are sufficiently similar. At the present time it appears that the various commercial thin-layer silicas have $S_r/S_t$ values in the range 0.01 to 0.12 (Table 5). The general duplication of separation on thin-layer silicas by column procedures seems therefore to require a column silica with $S_r/S_t \approx 0.0$ to 0.2. In this connection we have carried out many preparative separations in our laboratory, of widely different sample types, on columns of Code 62 silica ($S_r/S_t$ equal 0.03), and as predicted have always found column separations identical (i.e., sample separation order unchanged) with those on plates of Silica Gel G (using the same eluents). An 8 foot × 1 inch column of Code 62 silica (direct from the shipping container) is generally equivalent to separation on an 8-inch plate, providing the sample/adsorbent ratio for the column separation is less than 1/50.

The initial activation of chromatographic silicas has been effected in a variety of ways: washing with polar solvents [see, for example, (*24*)], heating in air or vacuum at various temperatures, and storage over desiccants or solutions of constant water-vapor pressure [see, for example, (*6*)]. The most common and convenient procedure is simple heating in air. From Fig. 2 it appears that thermal activation at temperatures of 150 to 250° removes all physical water with little dehydration of the actual silica surface. There is at present no evidence that removal of surface silanol groups is beneficial, and Cahnman has in fact noted (*25*) that activation at 500° gives a quite undesirable silica. Consequently, it appears that thermal activation at 150 to 250° provides a reasonably reproducible activation procedure, with no deterioration in performance of the resulting adsorbent. Combined with the simplicity and ease of thermal activation, this surely is all that can be expected of any activation procedure.

Klein (*6*) and numerous other workers have devoted much attention to how a silica should be deactivated: i.e., addition of water as liquid versus vapor. It is widely assumed that vapor-phase deactivation is preferable, either because it gives more uniform coverage of active surface sites, or because liquid water can hydrolyze surface siloxane bonds with adverse effects on silica performance (*6*). Various experiments performed in our laboratory indicate an extremely rapid equilibration of added liquid water with the surface of most chromatographic adsorbents. Thus the chromatographic activity of such deactivated adsorbents changes hardly at all with time, after an hour's equilibration with added water. This implies no further redistribution of physically adsorbed water from strong to weak adsorption sites. In view of the obvious mobility of physically adsorbed water on the adsorbent surface (much of it can be removed by simple evacuation at room temperature), this is scarcely surprising. Thus water added either as liquid or vapor appears to distribute rapidly over the silica surface to give identical final product.

The second objection to addition of liquid water appears equally untenable. Hydrolysis of surface siloxane groups assumes first that they are present in significant quantities, and second that liquid water (in the short time it is in contact with the silica surface) is capable of reacting with these groups. Klein (*6*) observed approximately 20 to 25% siloxane-group concentrations on the various Davison silicas, using Iler's value (*26*) of 8 silanols per millimicron of fully hydrated silica surface. However, more recent work (*13*) indicates that a fully hydrated silica surface corresponds to 6 rather than 8 silanols per millimicron, from which Klein's data suggest very few (if any) siloxane groups on the various Davison silicas (as received). Other work (*10*) suggests that this is typical of silica hydrogels that have not been heated above 200°C. The data of Fig. 3 also belie any rapid reaction of liquid water with surface siloxane groups when these are present. Thus the decrease in activity of the 0% $H_2O$-$SiO_2$ (Code 12) activated at 400° suggests some conversion of surface silanols to siloxane groups. If the reverse reaction (hydrolysis of siloxanes) were significant in deactivation of this sample by liquid water, addition of the initial 1 to 2% of water should result in a competition between rehydration and simple physical adsorption, with a resulting discontinuity in the activity versus % $H_2O$ curve. Such a discontinuity is unobservable in the plot of Fig. 3. It thus appears that liquid or vapor deactivation by water gives identical silicas. The convenience and speed of direct liquid-water deactivation therefore make it the preferred procedure.

There is a further advantage of liquid-water deactivation, in the precise standardization of silica samples. After addition of a specified amount of water, the resulting adsorbent may be slightly under or over active (as discovered in a subsequent chromatographic standardization of the adsorbent). This may be corrected simply by comparing the actual adsorbent activity with a standard activity–

% $H_2O$-$SiO_2$ plot (see below), estimating the amount of additional water (or dry adsorbent) that must be added to the sample, and then adding it. Comparable vapor-deactivation procedures would be considerably less convenient.

The question of how much water should be added to a particular silica normally depends upon its surface area and surface type (value of $S_r/S_t$). Some workers [see, for example, (6)] have assumed that it is generally undesirable to add more than a monolayer of water to the adsorbent. Other workers (5) have claimed optimum separation (in terms of band width or separation efficiency) for addition of 1½ to 3 monolayers of adsorbed water. Three separate contributions to the optimization of silica performance by water deactivation should be distinguished: change in linear capacity, change in sample selectivity, and change in separation efficiency. From Fig. 5 it appears that maximum linear capacity is attained for the Code 12 and Code 62 silicas with addition of about 30% of a monolayer of added water. With addition of more than a monolayer of water, linear capacity would be expected to decrease markedly for every silica. Selectivity or the relative spacing of band centers on a column or plate is generally expected to decrease with increased water addition. This effect may be quite marked for alumina as adsorbent (27), but is less severe for silica (*19*). Increased addition of water, up to monolayer coverage, appears generally to increase separation efficiency (i.e., gives narrower bands) for all silicas (*6*). There is some indication (*5,6*) that this increase in separation efficiency with increased water deactivation continues past monolayer coverage for wide-pore, small-$S_r/S_t$ silicas. The over-all conclusion as to the optimum water deactivation of a silica therefore appears as follows: For relatively large-$S_r/S_t$, small-pore silicas (e.g., Code 12, MS), water deactivation should probably fall in the range 25 to 50% of monolayer coverage (7 to 14% $H_2O$-$SiO_2$). For small-$S_r/S_t$, large-pore silicas (e.g., Code 62) addition of 1 to 2 monolayers of water (10 to 20%) *may* give the best separation, but adsorbent linear capacity will probably be much reduced.

By the standardization of an adsorbent we mean to include both measurements that precisely define its chromatographic properties, and procedures that permit the exact duplication of an adsorbent used previously. Both of these processes have been discussed previously in connection with the use of alumina and silica for chromatography (*17,19,28*). From the present discussion it appears that a silica is adequately characterized when its initial value of

$S_r/S_t$ and its final values of $\alpha$ and $V_a$ are known. Where the starting silicas all have approximately the same values of $S_r/S_t$ and surface area, as is frequently the case for a particular silica from a given commercial source, the combined variability of $\alpha$ and $V_a$ can be lumped into an over-all "activity function," or nominal % $H_2O$-$SiO_2$ [i.e., single activity scale; see the discussion in (29)]. The latter can then be related to the $R°$ value of a standard eluent/solute combination. Table 6 summarizes values of these standard retention volume values $R_s$ for Code 12, MS, and Code 62 silicas of varying nominal % $H_2O$-$SiO_2$. The preparation of a silica of particular type and activity involves first the selection of the appropriate starting adsorbent, its activation at 150 to 200° for 8 to 16 hours in air, and the addition of the desired amount of liquid water. Measurement of $R_s$ for the resulting adsorbent may show a value of the activity (% $H_2O$-$SiO_2$ from Table 6) somewhat different than that desired, owing to minor variations in the starting adsorbent. This can be precisely corrected by adding enough water or activated adsorbent to give adsorbent of the desired (calculated from Table 6) activity. The resulting adsorbent can then be restandardized ($R_s$ remeasured), but this will seldom be necessary. Even the initial standardization of the adsorbent can be eliminated unless sample retention volumes are required to be precisely reproducible, as in so-called "linear elution adsorption chromatography" procedures [see, for example, (*30*)].

**TABLE 6**

Silica Standardization: $R_s$ Values versus Water Content

| | $R_s{}^b$, ml/g | | |
|---|---|---|---|
| % $H_2O$–$SiO_2{}^a$ | Code 12 | Code MS | Code 62 |
| 0 | 52 | 47 | 6.5 |
| 0.5 | 44 | 37 | 4.9 |
| 1.0 | 36 | 30 | 4.2 |
| 2.0 | 25 | 19 | 3.3 |
| 4.0 | 13.0 | 10.0 | 2.4 |
| 7.0 | 6.7 | 5.7 | 1.6 |
| 10.0 | 4.4 | 4.0 | 1.2 |
| 15.0 | 2.1 | 2.6 | |
| 20.0 | 1.1 | 1.7 | |

[a] Per cent water added to silica activated initially at 200°C.

[b] $R°$ value for elution of naphthalene by pentane.

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