Chapter 3

Adsorption Chromatography: Mechanism and Materials

3.1. INTRODUCTION

Adsorption may be defined as the concentration of solute molecules at the interface of two immiscible phases. In liquid–solid adsorption chromatography (LSAC) the mobile phase is a liquid while the stationary phase is a finely divided, usually porous solid. The atoms in the bulk of the solid are subjected to equal forces in all directions, whereas the surface atoms experience unbalanced forces which can attract molecules from the surrounding solution to restore the balance.

In a multicomponent system selective adsorption occurs due to competition between the solutes and the mobile phase for the surface. It is governed by the differences in the strengths of the adsorption forces between the adsorbent and the adsorbates. In general, polar compounds are more strongly adsorbed by polar solids than are nonpolar compounds. Adsorption of a polar compound is enhanced in a nonpolar medium, but reduced in a polar medium, due to increased competition of the mobile phase for the surface.

A convenient method of studying adsorption is by measurement of adsorption isotherms.

3.2. ADSORPTION ISOTHERMS

An adsorption isotherm describes the equilibrium concentration relationship between the adsorbed and unadsorbed solute at a given temperature. It is a plot of the concentration of solute in the adsorbed phase versus

S. G. Perry et al., *Practical Liquid Chromatography*

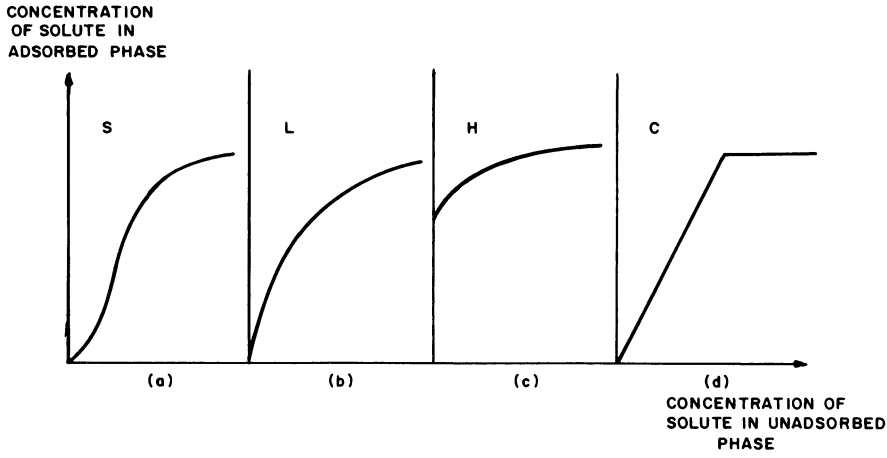


Fig. 3.1. Basic types of adsorption isotherms between a liquid and a solid surface.

its concentration in the unadsorbed phase. Giles *et al.*(1) classified the adsorption of solute from a solvent onto a solid surface according to the following scheme (see Fig. 3.1).

1. The *S*-shape isotherm represents the situation in which, as adsorption proceeds, it becomes easier for the solute molecules to be adsorbed; those already adsorbed on the surface at the most active sites assist further adsorption by intermolecular bonding. It is found that such isotherms are generally, but not always, given by flat molecules standing edge-on to the adsorbent surface, e.g., phenol adsorbed on alumina, where the hydroxyl group probably forms a hydrogen bond to surface oxygen atoms on the alumina, and the aromatic nucleus associates with other solute molecules.

2. The *L* (or normal Langmuir) isotherm is the most common one met with in LSAC. As adsorption proceeds, the most active sites are first covered by adsorbate and the ease with which adsorption takes place decreases until finally the monolayer is complete, and all the adsorption sites are occupied. This type of isotherm is usually obtained when molecules are adsorbed flat and when there is no intermolecular bonding.

3. The *H* (high-affinity) isotherm starts at a positive value on the ordinate axis, showing that all the solute has been removed from dilute solution. This isotherm is typical of chemisorption.

4. The *C* (constant-partition) isotherm is linear. This indicates that, as adsorption proceeds, the ease with which it takes place remains constant. This type of isotherm, though common in partition chromatography, is rarely observed in LSAC.

Adsorption isotherms can be further subdivided according to subsequent

inflections and plateaus. Usually, a further rise following an initial plateau, or merely an inflection, indicates the formation of a second layer on top of the first, or in some cases, a reorientation of the first layer.

Maxima have been observed in some isotherms, mainly in the *L* and *H* classes. The solutes involved associate in solution and it has been suggested that at high concentrations the association may draw some of the adsorbed solute back into solution.

Further discussion of nonlinear isotherms and their practical significance follows in the section concerned with sample size in adsorption chromatography.

3.3. NATURE OF ADSORPTION FORCES

The forces involved in adsorption chromatography can be classified as follows.

3.3.1. Van der Waals Forces (London Dispersion Forces)

These are intramolecular forces which hold neutral molecules together in the liquid or solid state. They are purely physical in character and do not involve the formation of any chemical bonds. Adsorption of this type is known as physical adsorption, and is characterized by low adsorption energies leading to the rapid establishment of equilibria and hence good chromatographic separation.

Dispersion forces account for all the adsorption energy in cases of adsorption of nonpolar solutes onto nonpolar adsorbents, e.g., hydrocarbons on graphite, while Snyder has shown[2] that the contribution of dispersion forces on alumina ranges from 100% for saturated hydrocarbons to less than 50% for polar molecules such as acetone or methanol.

3.3.2. Inductive Forces

These exist when a chemical bond has a permanent electrical field associated with it, e.g., a C–Cl or C–NO_2. Under the influence of this field, the electrons of an adjacent atom, group, or molecule are polarized so as to give an induced dipole moment. It seems[2] that induction forces make a major contribution to the total adsorption energy on alumina (but not on silica).

3.3.3. Hydrogen Bonding

These forces make an important contribution to the adsorption energy between solutes having a proton-donor group and a nucleophilic polar surface possessed by, for example, silica or alumina, which is normally

covered with hydroxyl groups. Similarly, the hydroxyl groups on the surface may react with other weakly electrophilic groups such as ethers, nitriles, or aromatic hydrocarbons.

3.3.4. Charge Transfer

This could occur, for example, when an electron is transferred from a solute S to a surface site A to form an adsorbed complex of the type S^+A^-. However, it has been shown[2] that forces due to charge transfer make an insignificant contribution to the adsorption energy of most compounds.

3.3.5. Covalent Bonding (Chemisorption)

This occurs when chemical bonds are formed between solute and adsorbent. These relatively strong chemical forces give rise to H-type isotherms and generally lead to poor separation in elution chromatography. Chemisorption is often exploited for the selective retention of certain compound types, e.g., the adsorption of amines by cation-exchange resins, the adsorption of olefins by silver nitrate-impregnated silica. On the other hand, H-type isotherms are not uncommon in high-efficiency elution chromatography. They can be attributed to the chemisorption of certain solutes onto those active sites on the surface of the adsorbent that have not been fully deactivated. For example, silica surfaces may contain some residual acidic sites which chemisorb bases. Similarly, alumina contains basic sites which strongly chemisorb acids. Florisil (magnesium silicate) also contains strong acidic sites and has been observed to chemisorb a wide variety of compounds, including aromatic hydrocarbons, basic nitrogen compounds, and esters, while magnesia chemisorbs polynuclear aromatic hydrocarbons.

As a consequence of chemisorption in columns, certain solutes give rise to strongly tailing elution bands, resulting in incomplete resolution and sample recovery, while in TLC part of the sample is seen to remain behind as a spot at the point of application.

For a further discussion of chemisorption see list of further reading at the end of this chapter.

3.4. CHOICE OF CHROMATOGRAPHIC SYSTEM IN LSAC

In designing a suitable chromatographic experiment in LSAC, the following factors have to be taken into consideration: (i) choice of adsorbent (ii) sample size and linear capacity, (iii) standardization of adsorbent, (iv) choice of mobile phase, (v) method of detection or quantitation.

The last point, method of detection or quantitation, will be dealt with in the respective chapters on thin-layer and column chromatography,

although in column chromatography especially the choice of a particular mobile phase may be influenced by the detection system used. In addition, the method of detection determines the sample size required. Each of the other four factors will now be discussed in turn.

3.4.1. Choice of Adsorbent

(a) General Properties of Adsorbents

Adsorbents used for LSAC are finely divided, porous solids with a surface area usually greater than 50 $m^2\ g^{-1}$. Table 3.1 shows the more common materials used as adsorbents, placed approximately in order of increasing strength. Strong adsorbents, i.e., those adsorbents with a relatively high concentration of strongly active sites, are preferred for the separation of weakly adsorbed, chemically inert compounds such as hydrocarbons, while weak adsorbents are preferred for the separation of labile or strongly adsorbed compounds.

Although the nature of the adsorbent surfaces has been indicated, this is frequently modified by the presence of free acid or base left over from the manufacturing stage or by the deliberate addition of buffering agents.

Other materials that have been used as adsorbents include calcium sulfate, talc, polyamide, organo-clays, and molecular sieves. A number of group-selective adsorbents have been prepared by impregnating an adsorbent with a material that will form a complex with a specific organic functional group. For example, silver nitrate-impregnated silica gel has been used for the separation of unsaturates. Further examples will be discussed in the section on modified adsorbents.

Silica gel and alumina are by far the two most common adsorbents in use today. It is not now necessary for one to prepare one's own adsorbent since they are readily available commercially. In fact, one can go even further,

Table 3.1. Activated Adsorbents in Approximate Order of Increasing "Strength"

Adsorbent	Nature of active sites
Sucrose	Neutral
Starch	Neutral
Kieselguhr	Neutral
Silica	Acidic
Magnesium silicate	Acidic
Alumina	Acidic and basic
Fuller's earth	Acidic
Magnesia	Basic
Charcoal	Neutral and acidic
Ion-exchange resins	Acid or basic species

Table 3.2. Some Commercial Sources of Adsorbents [a]

Chemical name	Trade name	Supplier
Magnesium silicate	—	Hopkin & Williams, Ltd. Chadwell Heath Essex England
	—	M. Woelm, D-344 Eschwege, W. Germany
	Florisil	Floridin Co., Pittsburgh, Pa. 15235
Magnesia	—	Hopkin & Williams, Ltd. Chadwell Heath Essex England
Carbon	Graphon; Spheron	Cabot Corp. Cambridge, Mass.
Ion exchangers	Amberlyst	Rohm & Haas, Co., Independence Mall West Philadelphia, Pa. 19105
Surface-modified glass beads	Zipax; Permaphase	E.I. Du Pont de Nemours & Co. Wilmington, Del. 19898
	Corasil	Waters Associates, Inc. 61 Fountain Street Framingham, Mass. 01701.
Textured glass beads	Corning glass beads	Corning Glass Works, Corning, N. Y. 13840

[a] Excluding silica and alumina.

since precoated TLC plates are now widely used and prepacked, high-efficiency columns are gradually being introduced. For a list of suppliers of chromatographic silica and alumina, see Heftmann([35]). Table 3.2 lists some suppliers of other adsorbent materials referred to in this chapter.

In selecting a suitable adsorbent, one needs to consider "adsorbent type" (i.e., strength, polar or nonpolar, surface *p*H) and the surface area and pore diameter. These factors will now be considered in turn.

(b) Adsorbent Type

The various adsorbent types exhibit different selectivities toward different compound types. Polar adsorbents (metal oxides, magnesium silicate, etc.) selectively adsorb unsaturated, aromatic, and polar molecules such as alcohols, amines, and acids. Polar adsorbents may be further subclassified as acidic, basic, or neutral, according to the *p*H of the surface. Silica, magnesium silicate, and cation-exchange resins are acidic and thus chemisorb bases. While chemisorption is an effective concentration method, quantitative chromatographic separation may not be possible because of the difficulty of desorption. Bases are best separated on basic adsorbents such

as magnesia. Similarly, basic adsorbents chemisorb acids and these are best separated on acidic adsorbents. The alumina surface contains both acidic and basic sites, but this is an excellent adsorbent for unsaturated and aromatic compounds.

Nonpolar adsorbents such as graphitized carbon, which is a strong adsorbent, and kieselguhr, which is a weak adsorbent, show no selectivity for the adsorption of polar molecules. Kieselguhr is such a weak adsorbent that it has been used to provide an inactive solid support for the stationary phase in liquid partition chromatography (see Chapter 4). Further details on the nature of adsorbent surfaces are discussed in the section on individual adsorbents.

(c) Surface Area and Pore Diameter

The surface area and pore diameter of a given adsorbent vary widely with the method of manufacture. Probably no two commercial manufacturers produce silicas of the same surface area and pore diameter. The variation in properties of different batches of the same grade of adsorbent from one manufacturer is usually not great. Dramatic changes do occasionally occur, however, presumably due to alterations in process conditions. Attention must be drawn to those instances where the same manufacturer operates plants in more than one location. The properties of the adsorbents from the alternative locations are rarely identical. As a result, the reader is recommended to pay particular attention to the properties of his adsorbent and to standardize on one particular grade where possible.

Adsorbents for chromatography are porous solids with high specific surface areas usually in excess of 50 $m^2\ g^{-1}$ to provide high sample capacity. The surface area increases as the porosity increases and the average pore diameter decreases. The linear adsorption coefficient K° of a solute is independent of both these parameters provided the solute molecule is small enough to enter the pores unimpeded and provided the nature of the active surface sites is independent of the pore diameter. We shall see that in the case of silica gel, for example, large differences in pore diameter correspond to differences in surface structure. If the size of the solute molecule is comparable with or exceeds the pore diameter, then the phenomenon of exclusion rather than adsorption occurs. Exclusion is discussed fully in Chapter 5.

3.4.2. Sample Size and Linear Capacity

(a) Sample Size and Band Shape

In analytical LSAC relatively low sample concentrations are used, which means only the low-concentration regions of the isotherms need be

considered. In these regions the isotherms have three possible shapes: convex (*L*-type isotherm), concave (*S*-type isotherm), and linear (*C*-type isotherm). These three types give rise to elution bands or spot shapes as shown in Fig. 3.2.

Thus, in the case of convex isotherms, which are the normal ones encountered in LSAC, the adsorption coefficient, K decreases as the sample size increases. Only at very low sample loadings can the isotherm be considered to be approximately linear. The consequence of this is illustrated in Fig. 3.3, which shows the effects of increasing sample loadings upon the band shape, in column chromatography, and the retention value, in thin-layer chromatography, as is observed in practice.

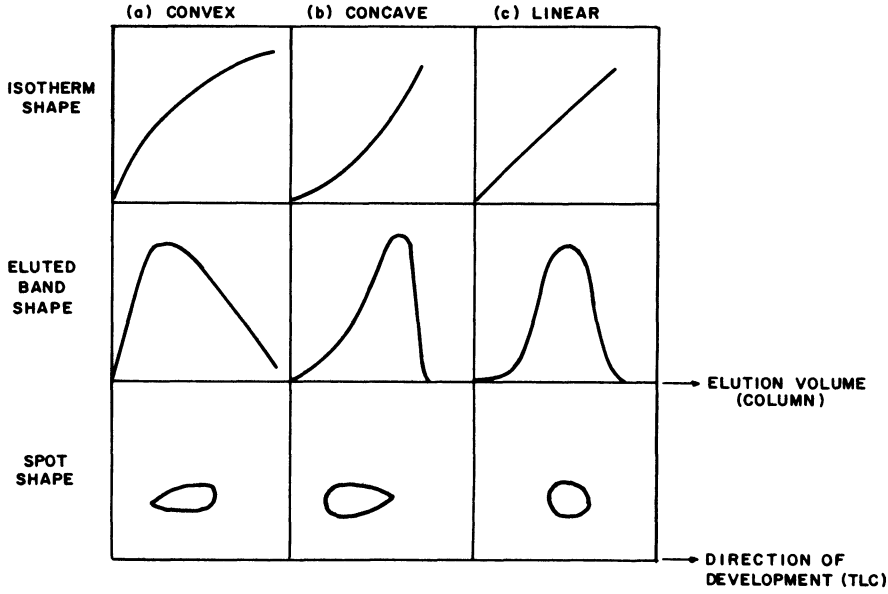


Fig. 3.2. Effect of isotherm shape on eluted band or spot shape.

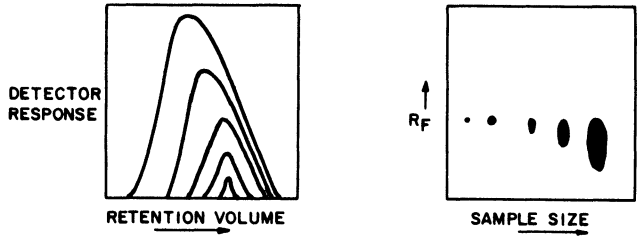


Fig. 3.3. Effect of sample size on band shape and retention values.

At very low sample loadings, V_r and R_F apparently remain constant. As the sample load is increased, however, a point is reached where V_r and R_F noticeably decrease. Increasing the sample size still further eventually leads to an increase in the amount of band tailing, loss of resolution, and even incomplete elution from the bed.

(b) Isotherm Linearity

On the theoretical side, assumption of isotherm linearity is essential to the development of any general theory of adsorption chromatography. From such a theory understanding of behavior in nonideal separations follows. On the practical side, a linear isotherm means that R_F, V_r, and K are constant for a given system at a given temperature, i.e., they are independent of concentration, and identification of separated zones by retention values is facilitated. Furthermore, linear isotherms give rise to Gaussian elution peaks or symmetric spots, enabling band resolution to be optimized.

Prior to the 1950's it was assumed that adsorption isotherms in LSAC were generally nonlinear. This was perfectly true with the large sample-to-adsorbent ratios which were and still often are being used. The development of TLC during the 1950's demonstrated that under the conditions in which this technique was used not only were faster and sharper separations obtained, but symmetric spots were common–suggesting that isotherm linearity was readily attainable using small sample loadings on deactivated adsorbents. Only during the past few years have the practical advantages of linear isotherm separations in columns been fully exploited.

(c) Linear Capacity

The linear capacity of an adsorbent has been defined[3] as the maximum weight of sample that can be applied to a gram of absorbent before the adsorption coefficient K falls more than 10% below its linear isotherm value $K°$. This means that as long as the sample size does not exceed this value, the V_r or R_F of the individual solutes will be within 10% of their linear isotherm values.

The linear capacity of most activated adsorbents is very low (less than 10^{-4} g g^{-1}). It is therefore desirable to find ways of increasing the linear capacity to aid in the detection and further handling of sample components. Before we do this, however, let us look at some of the reasons why isotherms are nonlinear in adsorption chromatography.

(d) Origins of Isotherm Nonlinearity

Isotherm nonlinearity arises from three main causes[2]:

Surface coverage. It can be shown from the theory of the Langmuir isotherm that this becomes nonlinear when 10% of the adsorbent surface is covered by sample. This fixes the maximum possible linear capacity of a given adsorbent for a given solute. It can be calculated that about 0.03 g of a typical organic compound would form a monolayer on 100 m^2 of surface. Thus the maximum linear capacity of an adsorbent with a surface area of 100 m^2 g^{-1} would be 0.003 g g^{-1}. In practice, however, much lower linear capacities are generally encountered.

Interaction between adjacent adsorbed sample molecules. Such interactions are insignificant at the low surface coverage imposed by the above restriction and therefore need not be considered further.

Adsorbent heterogeneity. This arises because not all adsorption sites on a surface are equivalent. Some sites are said to be more "active" than others. The greater the heterogeneity, the smaller the linear capacity becomes. A small number of very active sites greatly reduces the linear capacity because these sites are occupied by solute molecules first. Departure from linearity occurs when 10% of these active sites have been covered. Heterogeneity is therefore seen to be the major cause of low linear capacities and ways must be sought to overcome this.

(e) Maximization of Linear Capacity

The more active sites of a heterogeneous surface can be deactivated by the addition of a polar solvent such as water, thus increasing the linear

Table 3.3. Retention Volume and Linear Capacity Data for Elution of Naphthalene by Pentane from Various Silicas Initially Activated at 195°C [a]

Pore size	Silica	Starting surface area, m^2 g^{-1}	Water added, wt%	V_r, ml g^{-1}	Linear, g g^{-1} $\times 10^{-4}$	Capacity, g m^{-2} $\times 10^{-7}$
Narrow	Davison Code 12	801	0	51.9	0.9	1.1
			2.0	25.1	7.5	
			7.5	6.3	29	
			16.0	1.84	26	
Medium	Davison MS	866	0	46.4	1.0	1.2
			2.1	18.0	2.5	
			7.9	5.0	15	
			16.8	2.16	25	
Wide	Davison Code 62	313	0	6.5	1.9	6.0
			0.8	4.42	2.9	
			2.9	2.74	11	
			6.2	1.77	4.2	

[a] Reprinted from Ref. 4 by courtesy of Marcel Dekker, Inc., New York, and L. R. Snyder.

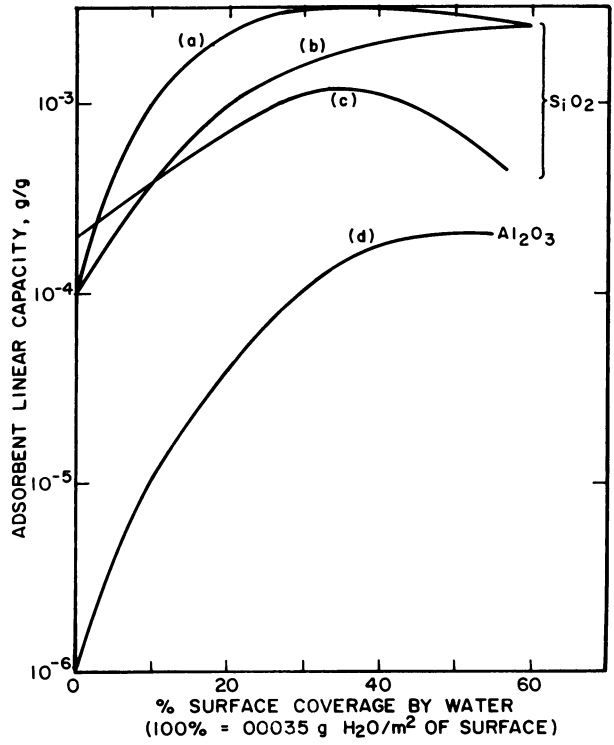


Fig. 3.4. Adsorbent linear capacity as a function of adsorbent type and relative deactivation by water. [Reproduced by courtesy of Marcel Dekker, Inc., and L. R. Snyder(2).]

capacity. Table 3.3 shows the effect of deactivation by water on the retention volume and the linear capacity of three silica gels differing in porosity and surface area.

The results are illustrated graphically in Fig. 3.4, which also includes data for a medium-pore alumina (Alcoa F-20) initially activated at 400°C. Wide-pore silica (Fig. 3.4c) has a relatively uniform surface and a low surface area. Since heterogeneity is a more dominant factor than surface area, the linear capacity of the activated adsorbent is higher than that of the medium-pore (Fig. 3.4b) or narrow-pore (Fig. 3.4a) silica. Because so few active sites are present in the wide-pore silica, the maximum linear capacity is reached after the addition of a relatively small amount of water. Addition of further water results in the coverage of the remaining uniform surface, resulting in a decrease of linear capacity. The narrow-pore silica, on the other hand, possesses a large surface area and a relatively high surface heterogeneity. Hence, the linear capacity of the activated adsorbent is low. A larger amount of

water is required to deactivate all the active sites, but when full deactivation has been achieved the linear capacity is greater than that of the wide-pore silica because of its greater surface area.

In general, we can say that the addition of 1–2% water per 100 $m^2 g^{-1}$ of surface of polar adsorbent such as alumina, silica, and other metal oxides (corresponding to 30–60% surface coverage) increases the linear capacity 5–100 fold. Where highly polar eluents or elevated temperatures are to be used, physically adsorbed water is likely to be lost, resulting in some reactivation and a reduction in linear capacity. In these circumstances deactivation is best achieved using glycol or glycerol[5]. Deactivation of adsorbents such as charcoal, which does not adsorb water, can be achieved with certain high-molecular-weight organic compounds such as cetyl alcohol or stearic acid[6].

The linear capacity of a heterogeneous adsorbent can also be increased by decreasing the value of the adsorption coefficient K. This can be achieved by: (i) increasing the temperature; however, polar adsorbents tend to lose adsorbed water on heating and become more activated, i.e., the surface would become even more heterogeneous; or (ii) increasing the polarity of the solvent; however, if K is reduced too much by this means, resolution is lost.

In summary, it is seen that the most useful way of increasing the capacity of a heterogeneous surface is to deactivate the active sites by the addition of a polar liquid.

(f) Linear Capacities in Columns and Thin Layers

In a column the solute band is in contact with only a fraction of the total adsorbent in the column at any instant. This means that the linear capacity of the column is related to the width of the solute band[2]. In an efficient column, solute bands are narrow and therefore the linear capacity is apparently low. But since efficient columns are generally used for analyzing complex mixtures, as the separation proceeds more solute bands are in contact with adsorbent at any instant. Therefore the linear capacity of an efficient column increases with increasing complexity of the mixture. Those components that are not adsorbed at all can be disregarded in calculating the linear capacity of the column.

In analytical scale TLC the sensitivity of the various detection methods used often enables less than 1 μg of a sample component to be located. The usual practice is to apply sample loadings within the range 10–50 μg to adsorbent layers of 250 μm thickness, which is the optimum layer thickness for maximum speed and resolution. Sample loadings within this range are well below the maximum linear capacity (75 μg), assuming it to be applied as a spot to 1 cm^2 silica gel, 250 μm thickness on a plate, and the chromatogram developed in the norm lamanner (see Chapter 6).

3.4.3. Adsorbent Standardization

The value of K° of a particular solute in a given mobile phase–adsorbent system depends upon the surface area and surface activity of the adsorbent. Variations arise because of differing manufacturing processes and subsequent thermal treatment and deactivation of the final product. It is highly desirable to be able to obtain repeatable and reproducible K° values so that experiments can be duplicated and so that R_F and V_r values can be accurately measured and used for identification purposes. The following equation shows how the adsorbent surface area and surface energy affect the value of K°[7]:

$$\log K^\circ = \log V_a + \alpha(S^\circ - A_s\varepsilon^\circ) \tag{3.1}$$

where V_a is the adsorbent surface volume, equal to the volume of a solvent monolayer (approximately 0.00035 times the surface area), α is an adsorbent energy function, proportional to the average surface energy of the adsorbent, and the parameter $(S^\circ - A_s\varepsilon^\circ)$ is a constant for a particular combination of solute, eluent, and adsorbent type. This equation has been experimentally verified for a number of systems[7].

Values of V_a and α for some of the common adsorbents deactivated by adding varying amounts of water have been tabulated[7]. The results show that V_a and α decrease regularly as the water content increases. Families of curves (Fig. 3.5) have been constructed relating V_r or R_F versus water content, each curve corresponding to a given value of $(S^\circ - A_s\varepsilon^\circ)$. It can be seen that decreasing the activity of the adsorbent increases the mobility of the solute.

To prepare a standardized adsorbent it is safest to assume that the commercial adsorbent, as received, has an unknown water content. In TLC, standardization of the plate is achieved by equilibrating it in a tank containing an atmosphere of known relative humidity. Details on how this is done are described in Chapter 6. In column chromatography the adsorbent is first heated in air for periods of 4–16 hr at some specified temperature to remove

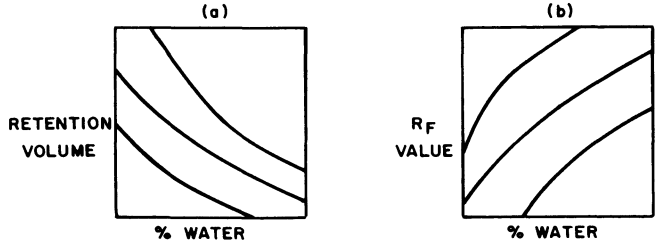


Fig. 3.5. Effect of water deactivation on (a) retention volume and (b)R_F value.

adsorbed water (see the section on individual adsorbents). This produces an adsorbent of definite (even if unknown) activity and water content. Deactivation is then carried out by the addition of a measured volume of water (or other deactivator) to a weighed amount of activated adsorbent contained in a stoppered flask. After a short period of vigorous shaking to disperse lumps the adsorbent is left to stand for 24 hr with occasional shaking, during which time the added deactivator distributes itself evenly over the whole adsorbent surface. Adsorbents prepared in this manner are stable indefinitely provided they are stored in tightly closed containers.

After deactivation the activity of the adsorbent should be checked by a standard procedure. In TLC a test mixture of the three dyes indophenol blue, *p*-dimethylaminoazobenzene, and Sudan red G (50 mg of each dissolved in 50 ml benzene) is developed in benzene using the standard procedure given in Chapter 6. The R_F values are measured and any slight adjustment in activity that may be necessary is achieved by changing the relative humidity of the storage chamber in which the plates are stored.

In column chromatography the same test dye mixture is eluted with benzene and the retention volumes are measured. Any slight adjustments are achieved by adding a small amount of water or dry (activated) adsorbent and allowing to equilibrate a further 24 hr. The use of naphthalene, eluted by *n*-pentane, is now gaining popularity as a reference standard since this system is more amenable to the wide range of sensitive detectors now coming into use.

Deviations from Equation (3.1) have been known to occur, for example, with solvents that are sufficiently polar to displace water from the adsorbent surface, with Florisil containing less than 1% water([2]), and in cases where extremely-fine-pore adsorbents have been used with solvents of large molecular diameter. Such a case is solute elution from fine-pore silica (average pore diameter 20 Å) with carbon tetrachloride.

3.4.4. Choice of Mobile Phase

Having chosen the adsorbent type and standardized its activity commensurate with a maximum linear capacity, the most important method of adjusting K° to give an optimum value of R_F or V_r is to choose a mobile phase with the correct eluent strength. The effect of the mobile phase upon K° is given by the expression $(S^\circ - A_s\varepsilon^\circ)$ in Eq. (3.1), where S° is the sample adsorption energy on an adsorbent of standard activity ($a = 1$), A_s is proportional to the molecular area of the solute molecule, and ε° is defined as the eluent strength. The greater the eluent strength, the smaler is the value fo K° for a given solute and adsorbent.

Values of ε° (relative to *n*-pentane, for which ε° is defined as zero) for numerous solvents have been determined on alumina([2]). Placing the solvents

Table 3.4. Eluotropic Series for Polar Adsorbents [a]

Solvent	$\varepsilon_o(Al_2O_3)$	Viscosity, cP, 20°	RI	UV cutoff, nm	Boiling point,°C
Fluoroalkanes	−0.25	—	1.25	—	—
n-Pentane	0.00	0.23	1.358	210	36
i-Octane	0.01	0.54	1.404	210	118
n-Heptane	0.01	0.41	1.388	210	98.4
n-Decane	0.04	0.92	1.412	210	174
Cyclohexane	0.04	1.00	1.427	210	81
Cyclopentane	0.05	0.47	1.406	210	49.3
Carbon disulfide	0.15	0.37	1.626	380	45
Carbon tetrachloride	0.18	0 97	1.466	265	76.7
Amyl chloride	0.26	0.43	1.413	225	108.2
i-Propyl ether	0.28	0.37	1.368	220	69
i-Propyl chloride	0.29	0.33	1.378	225	34.8
Toluene	0.29	0.59	1.496	285	110.6
n-Propyl chloride	0.30	0.35	1.389	225	46.6
Chlorobenzene	0.30	0.80	1.525	280	132
Benzene	0.32	0.65	1.501	280	80.1
Ethyl bromide	0.37	0.41	1.424	225	38.4
Ethyl ether	0.38	0.23	1.353	220	34.6
Chloroform	0.40	0.57	1.443	245	61.2
Methylene chloride	0.42	0.44	1.424	245	41
Tetrahydrofurane	0.45	0.55	1.408	220	65
Ethylene dichloride	0.49	0.79	1.445	230	84
Methylethylketone	0.51	0.43	1.381	330	79.6
Acetone	0.56	0.32	1.359	330	56.2
Dioxane	0.56	1.54	1.422	220	104
Ethyl acetate	0.58	0.45	1.370	260	77.1
Methyl acetate	0.60	0.37	1.362	260	57
Amyl alcohol	0.61	4.1	1.410	210	137.3
Dimethyl sulfoxide	0.62	2.24	1.478	270	190
Aniline	0.62	4.4	1.586	325	184
Nitromethane	0.64	0.67	1.394	380	100.8
Acetonitrile	0.65	0.37	1.344	210	80.1
Pyridine	0.71	0.94	1.510	305	115.5
i-Propanol	0.82	2.3	1.38	210	82.4
Ethanol	0.88	1.20	1.361	210	78.5
Methanol	0.95	0.60	1.329	210	65.0
Ethylene glycol	1.11	19.9	1.427	210	198
Acetic acid	Large	1.26	1.372	251	118.5

[a] Data taken partly from Ref. 2.

in order of increasing eluent strength gives rise to the so-called eluotropic series (Table 3.4). The order is similar for all polar adsorbents, but is reversed for graphitized carbon (Table 3.5) because nonpolar molecules are preferentially adsorbed on this adsorbent.

Table 3.5. Eluotropic Series for Graphitized Carbon [a]

Water
Methanol
Ethanol
Acetone
Propanol
Ethyl ether
Butanol
Ethyl acetate
n-Hexane
Benzene

[a] Eluent strength increases downward.

In practice, the best way of choosing the correct eluent is by carrying out trial TLC on microscope slides. A pair of microscope slides, back-to-back, is dipped into a slurry of adsorbent (e.g., silica gel suspended in chloroform–methanol mixtures). The slides are separated and allowed to dry quickly. Then, 2 μl of a 1% solution of the sample to be separated is applied to the plate and developed in a solvent of low eluent strength. This is usually achieved in 2–3 min. The plates are then allowed to dry and the chromatogram revealed by placing the plate in an iodine chamber. The experiment is repeated using solvents of increasing strength, using binary mixtures if necessary, until the R_F values of the components of interest lie between 0.3 and 0.8.

In addition to choosing a mobile phase of the correct eluent strength, the following factors have to be taken into consideration: (a) volatility, (b) method of detection of solute, (c) viscosity, (d) solvent demixing, (e) solubility of solute, and (f) effect on adsorbent.

In TLC the mobile phase must be sufficiently volatile so that it can be removed from the adsorbent to allow detection of the separated solutes to take place. This is particularly important when the mobile phase reacts with the revealing agent; e.g., most organic substances react with the dichromate–sulfuric acid reagent. With high-boiling polar mobile phases prolonged heating of the plate in an oven may be necessary before the last traces of eluent are removed. The mobile phase must also be free from nonvolatile impurities in cases where recovery of the separated solute is required. The removal of large amounts of high-boiling solvent can lead to substantial loss or decomposition of the solute.

In column chromatography the choice of mobile phase must be compatible with the choice of detector. Transport ionization detectors, for example, require separation of mobile phase from the solute by evaporation before the latter is carried forward into the detector. Where an ultraviolet spectrophotometer is used as a detector, the mobile phase must be transparent over the wavelength range where the solute is to be measured. Sol-

vents that are particularly useful are saturated hydrocarbons, halogenated hydrocarbons, ethers, acetonitrile, and alcohols. Ethers must be free from peroxides and oxidation inhibitors. They should therefore be distilled immediately before use, utilizing only the first 75 vol % collected. With differential refractometers maximum sensitivity will occur when a solvent is chosen such that the difference in refractive index between solvent and solute is maximized.

As discussed in an earlier chapter, the viscosity of the solvent must be as low as possible to achieve acceptable efficiencies in the shortest times. Solvents which are particularly suitable from this point of view include *n*-pentane, carbon disulfide, 1- and 2-chloropropane, diethyl ether, acetone, acetonitrile, and methanol.

Solvent demixing often occurs when using binary or more complex mixtures and is readily observable in TLC. It occurs when the strongest solvent is preferentially adsorbed, leaving the advancing solvent front richer in the least polar component. Such demixing is generally undesirable since two or more components may travel unresolved with the secondary solvent front. It is more likely to occur when two solvents of widely different polarity are mixed, e.g., chloroform–methanol. If binary mixtures must be used, therefore, it is better to mix two solvents of similar polarity. As an example, 5% diethyl ether in heptane is an excellent mobile phase for the separation of polynuclear aromatics on alumina. On the other hand, 1% ethanol in heptane, while of similar eluent strength, is not a good mobile phase for this separation, since demixing occurs.

The solvent must be able to dissolve the solute at the working temperature of the separation. Even in difficult cases this can usually be achieved by selecting suitable solvent mixtures of the correct eluent strength. For example, a suitable solvent system for the chromatography of a substance containing a long hydrocarbon chain attached to a strongly polar group may require a solvent system containing a hydrocarbon such as toluene to provide the solvent power and ammonia to provide the correct eluent strength, together with a "bridging" solvent such as isopropanol to provide a homogeneous mobile phase.

When using deactivated adsorbents care must be taken that the mobile phase does not reactivate the adsorbent. Water-miscible solvents such as alcohols will strip the adsorbent of surface-adsorbed water, while even solvents as weak as *n*-pentane will gradually reactivate magnesia, silica, and alumina. To prevent this, a small amount, usually 0.1% or less, of water must be added to the eluent to maintain the adsorbent's activity. Regular standardization of the column as described in the previous section will determine whether the water content of the eluent should be decreased or increased. With water-miscible solvents addition of the requisite amount of

water is all that is required. With water-immiscible solvents such as pentane shaking with water is unsatisfactory because dissolution of water is very slow and water droplets form in the solvent. A satisfactory method is to percolate it through a column (100 × 4 cm is satisfactory) packed with silica gel impregnated with about 30% by weight with water.

3.5. VARIATION OF ADSORPTION COEFFICIENT K° WITH SOLUTE STRUCTURE

The most important parameter affecting the value of $K°$ is the molecular structure of the solute. The effect of molecular structure upon the value of $K°$ in Eq. (3.1) is described by $S°$, the solute adsorption energy. This is approximately equal to the sum of the adsorption energies $Q_i°$ of the individual functional groups within the molecule(2).

Group adsorption energies of a number of functional groups have been determined for different adsorbents, some values on alumina and silica being given in Table 3.6. The values for Florisil are usually identical to those of silica. The higher the value of the group adsorption energy, the more strongly is the molecule adsorbed.

A consequence of the low value for the methylene group is that members of a homologous series have nearly identical adsorption energies. In adsorption chromatography, therefore, in contrast to partition chromatography, combounds are separated by type, but not by molecular weight.

The values of group adsorption energies may be modified by intramolecular electronic effects, steric effects (e.g., planar molecules are more easily adsorbed than nonplanar ones, *trans* isomers more than *cis*; *ortho*-substituted aromatic rings less strongly than *meta* or *para* ones), and chemical interaction between adjacent functional groups (e.g., hydrogen bonding reduces adsorption energies).

For a detailed discussion of the effect of sample structure on $K°$ values the reader is referred to Ref. 2.

3.6. INDIVIDUAL ADSORBENTS

3.6.1. Silica Gel

Adsorbents with the general formula SiO_2–xH_2O have been described as silica, silica gel, or silicic acid. Silica gel is probably the most commonly used chromatographic adsorbent at the present time. This is a consequence of its high sample capacity, inertness to most labile solutes, and commercial availability. There is, therefore, a large amount of literature on the applications of silica gel as a chromatographic adsorbent. Unfortunately, because

Table 3.6. Adsorption Energies $Q°$, of Various Sample Groups [a]

Group	Alumina			Silica			Florisil[b]		
	X,Y =Ar	X=Al Y=Ar	X,Y =Al	X,Y =Ar	X=Al Y=Ar	X,Y =Al	X,Y =Ar	X=Al Y=Ar	X,Y =Al
X—CH_3 methyl	0.06	—	—0.03	0.11	—	0.07	0.10	—	—0.01
X—CH_2—Y methylene	0.12	0.07	0.02	0.07	0.01	—0.05	0.19	0.10	0.01
X—Cl chloro	0.20	—	1.82	—0.20	—	1.32	—0.20	—	1.74
X—F fluoro	0.11	—	1.64	—0.15	—	1.30	—0.15	—	1.54
X—Br bromo	0.33	—	2.00	—0.17	—	1.32	—0.17	—	1.94
X—I iodo	0.51	—	2.00	—0.15	—	1.28	—0.15	—	1.94
X—SH mercapto	8.70	—	2.80	0.67	—	1.70	0.67	—	1.70
X—S—S—Y disulfide	?	~1.1	2.70	?	0.94	1.90	?	0.94	1.90
X—S—Y sulfide	0.76	1.32	2.65	0.48	1.29	2.94	?	1.30	2.94
X—O—Y ether	1.04	1.77	3.50	0.87	1.83	3.61	0.87	1.81	3.61
X—N—Y tertiary amine	?	2.48	4.40	?	2.52	~5.8	?	?	?
X—CHO aldehyde	3.35	—	4.73	3.48	—	4.97	3.35	—	4.97
X—NO^2 nitro	2.75	—	5.40	2.77	—	5.71	3.07	—	5.71
X—C≡nitrile	3.25	—	5.00	3.33	—	5.27	3.33	—	5.27
X—CO_2 —Y ester	4.02	3.40	5.00	4.18	3.45	5.27	4.18	3.45	5.27
X—CO—Y keto	4.36	3.74	5.00	4.56	4.69	5.27	4.56	4.32	5.27
X—OH hydroxyl	7.40	—	6.50	4.20	—	5.60	4.20	—	5.60
X—C=N—Y imine	4.14	4.46	6.00	?	?	?	?	?	?
X—NH^2 amino	4.41	—	6.24	5.10	—	8.00	?	?	?
X—SO—Y sulfoxide	?	4.0	6.70	?	4.2	7.2	?	4.2	7.2
X—COOH carboxylic acid	19	—	21	6.1	—	7.6	6.1	—	7.6
X—$CONH^2$ amide	6.2	—	8.9	6.6	—	9.6	6.6	—	9.6
C=aromatic carbon	0.31	0.31	0.31	0.25	0.25	0.25	0.18	0.18	0.18

[a] Reproduced from Ref. 8, by courtesy of Elsevier Publishing Co. and L. R. Snyder.
[b] Assumes $\alpha = 1.00$ for 1% H_2O–Florisil.

of the many commercial sources, wide variations in properties exist, e.g., surface *p*H, pore-size distribution, and surface area.

Silicas are usually prepared either by acid precipitation from metal silicate solutions, especially sodium silicate, or by the hydrolysis of silicon compounds, such as silicon tetrachloride, in the liquid or vapor phase. The pore size, surface area, and nature of the surface vary according to the method of preparation. Variation of the solution *p*H during the acid gelation of sodium silicate, for example, produces silicas with surface area varying from about 200 $m^2\ g^{-1}$ (*p*H about 10) to 800 $m^2\ g^{-1}$ (*p*H ≤ 4). Most chromatographic silicas, especially those used for TLC, have surface areas of 300–600 $m^2\ g^{-1}$ and pore diameters of 100–250 Å, and are classed as large-pore silicas. They have a semicrystalline structure and a relatively uniform surface covered predominantly with free hydroxyl groups (4–5 hydroxyls per 100 $Å^2$ of surface). A number of small-pore silicas are now commercially available whose average pore diameters are less than 100 Å and whose surface areas are in excess of 500 $m^2\ g^{-1}$. They have an irregular, amorphous structure, their surfaces being covered predominantly with reactive and bound hydroxyl groups (see below). Silicas are also available in a series of controlled pore sizes covering the range 100–2500 Å and are useful for separating polymers by the molecular exclusion principle. These are further discussed in Chapter 5.

As has already been indicated, a silica surface that has not been heated for long periods in excess of 400 °C is covered, to a greater or less extent, by hydroxyl groups. It is the presence of these surface hydroxyl groups that is responsible for the selective adsorption properties of silica. Thus silica adsorbs unsaturated, aromatic, or polar molecules by hydrogen bonding, the solute functioning as an electron donor. Carbon–carbon double bonds contribute somewhat less to sample adsorption energy on silica compared with other polar adsorbents. Aromatic hydrocarbons and compounds differing only in their relative degree of unsaturation are better separated on other polar adsorbents such as alumina.

The silica surface is weakly acidic (*p*H 3–5) and therefore there is a tendency toward preferential adsorption of strongly basic compounds ($pK_B < 5$) relative to their adsorption on neutral or basic adsorbents. Sometimes the silica surface is found to be strongly acidic. This is due to contamination by acids left over from the gelation step. Such silicas should be cleaned by repeated washing with distilled water; otherwise chemisorption of bases or reaction of acid-sensitive solutes will occur.

The nature of the silica surface has been studied and reviewed by a number of workers (e.g., see Ref. 35). Conflicting views on the relationships between silica processing, surface structure, and chromatographic properties still exist, but many discrepancies now seem to have been resolved, mainly

due to the efforts of Snyder and his coworkers (see Ref. 2). A greatly simplified summary of their conclusions is given below.

An air-dried silica surface that has not undergone previous heat treatment contains physically adsorbed water. Heating at temperatures between 150 and 200 °C drives off most of the adsorbed water leaving a surface containing hydroxyl groups. The hydroxyl groups can be divided into three types as shown in Fig. 3.6. The surface of large-pore silicas consists predominantly of free hydroxyls (Fig. 3.6a), while that of small-pore silicas contains reactive (Fig. 3.6c) and bound (Fig. 3.6b, c) hydroxyls. The activity of the different types of site increases in the order: bound < free < reactive hydroxyls. This means that the surface activity of small-pore silicas is greater than that of wide-pore silicas because of the greater concentration of reactive hydroxyls in the small-pore silica. However, the effect of adding water to an activated silica surface is to deactivate the reactive hydroxyls of a small-pore silica first, leaving a surface of bound hydroxyls. Corresponding deactivation of a large-pore silica leaves a surface of free hydroxyls. Consequently, the surface activity of a heavily deactivated small-pore silica is less than a similarly deactivated large-pore silica. The effect of water deactivation on the linear capacity of both large- and small-pore silicas has already been discussed (Fig. 3.4).

To classify the surface structure of a silica gel we need to know the ratio of reactive to total hydroxyls (S_r/S_t) and the surface area. A method for determining these has been proposed by Snyder and Ward (see Ref. 2). Reactive hydroxyls are determined by selective silanization with trimethylchlorosilane, and total hydroxyls are determined by complete silanization

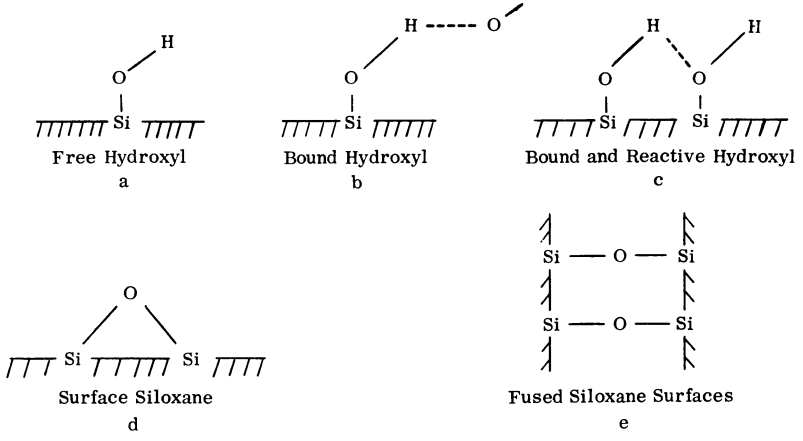


Fig. 3.6. The nature of the silica surface. [Reproduced by courtesy of Marcel Dekker, Inc., and L. R. Snyder(2).]

Table 3.7. Differences Between Small- and Large-Pore Silicas [a]

Property	Small-pore silicas	Large-pore silicas
Average pore diameter	< 100 Å	> 150 Å
Specific surface area	> 500 m^2 g^{-1}	< 600 m^2 g^{-1}
Surface structure	Irregular, amorphous, heterogeneous	Semicrystalline, uniform
Predominant hydroxyl types	Reactive plus bound	Free
Surface activity α		
Activated silica	> 1.00	< 0.9
Deactivated silica[b]	~ 0.6	~ 0.7
Variation in α with increase in silica activation temperature (150–400°)	Decreases	Little change
Linear capacity		
Activated silica	$\sim 10^{-4} \times$ g g^{-1}	$\sim 2 \times 10^{-4}$ g g^{-1}
Deactivated silica	25×10^{-4} g g^{-1}	4×10^{-4} g g^{-1}

[a] Reproduced in part from [2] by courtesy of Marcel Dekker, Inc., New York and L.R. Snyder.
[b] 60% Coverage of surface by water (2.1% water per 100 m^2 g^{-1} of surface).

with hexamethyldisilazane. The silica surface area can be calculated from an experimental value of S_t. The differences in properties between small- and large-pore silicas are summarized in Table 3.7.

If activated silicas are heated at temperatures between 200 and 400 °C reactive hydroxyl groups condense to liberate water to form surface siloxane groups (Fig. 3.6d). Similarly, free hydroxyl groups begin to migrate about the surface, form transient reactive groups, and then decompose to form surface siloxane groups. The rehydration of such siloxane groups is difficult and requires prolonged heating at 95 °C with water. Heating silica surfaces at temperatures above 400 °C causes condensation between adjacent planes (Fig. 3.6e), resulting in decrease in surface area and loss of selectivity.

The conclusions to be drawn from this account are that the chromatographic properties of silica can be expected to vary widely according to the method of manufacture and subsequent thermal treatment. To obtain reproducible results, it is therefore necessary to have a knowledge of the pore diameter and surface properties, first, to ensure that adsorbents with similar selectivities are being used, and second, to give a guide to the further treatment required to produce a standardized adsorbent of optimized linear capacity.

3.6.2. Alumina

After silica, alumina is the most popular adsorbent in use today and is readily available commercially. It is a polar adsorbent like silica, and the order of elution of solutes on the two adsorbents is generally similar. There are three important practical differences from silica:

(a) The adsorption energy of a solute molecule containing isolated and conjugated carbon–carbon double bonds is greater on alumina. Consequently, alumina is preferred for the separation of mixtures of aromatic hydrocarbons, because their adsorption coefficients cover a wider range.

(b) Alumina contains a number of strongly basic sites and therefore shows a preferential adsorption of acidic samples. Strong acids ($pK_a \leq 5$) are chemisorbed, but weaker acids can be separated in order of their pK_a values, especially when basic eluents are used.

(c) Activated alumina cannot be used for some separations because certain solute types undergo chemical reaction at the reactive sites[9]. Examples are: salt formation with acids, saponification of esters and anhydrides, condensation reactions with aldehydes and ketones, elimination reactions with loss of hydrogen halides, isomerization and polymerization of olefins, oxidation reactions, and complex formation.

Alumina can exist in a number of crystalline forms, depending upon the method of preparation and thermal history. Most commercial aluminas prepared for chromatography probably consist mainly of the γ-form, but also contain small amounts of other crystalline forms, e.g., η, χ, ϱ. All these forms have similar chromatographic properties. On heating to 900–1000 °C these are converted to other crystalline modifications[1], (θ, δ, K). On heating to temperatures higher than 1100 °C all aluminas are converted to α-alumina, which is chromatographically inactive, presumably on account of its low surface area and different lattice structure. The ideal crystal of γ-alumina consists of layers of large oxide ions (O^{2-}) with small aluminum ions (Al^{3+}) occupying three out of every four holes between the oxide ions. At room temperature up to a monolayer of water is readily adsorbed onto the alumina surface, each water molecule being bound to two surface oxide ions. On heating a hydrated alumina to 300–400 °C most of the adsorbed water is driven off, with the remainder of the water reacting with the surface to form hydroxyl groups, up to six hydroxyl groups per 100 Å^2 of surface being formed. This is the form in which alumina for chromatography is generally used. The linear capacity is increased by the addition of water corresponding to 50% coverage, although many workers have deactivated their adsorbents with greater amounts of water.

Activated alumina has been observed by electron microscopy[10] to consist of a system of regular, cylindrical micropores of 27 Å diameter arranged hexagonally, in addition to two types of random macropore, those within the particles of alumina that make up the granules, and those between such particles. The surface area of a typical chromatographic alumina lies in the range 100–200 m^2 g^{-1}.

Various proposals have been made regarding the nature of the active sites and the corresponding adsorption interactions on the alumina surface.

On heating a hydrated alumina to more than 400°C, hydroxyl groups are gradually removed, but surface hydroxyl groups are not completely eliminated even by heating under vacuum at 800–1000°C. Nevertheless, chromatographic activity increases with increasing temperature of activation up to 1100°C. This is taken as evidence that unlike the case for silica, surface hydroxyl groups do not play an important role in the adsorption of solutes onto alumina. Snyder[2] recognizes three distinct types of adsorption site:

(a) Acidic or electrophilic field sites interact with solutes possessing regions of high electron density. This is the most common adsorption mechanism encountered, showing that the alumina surface behaves as an acid toward most solute types.

(b) Basic or nucleophilic sites (probably oxide ions) are responsible for the preferential adsorption of acids relative to other adsorbents.

(c) Electron-acceptor (charge-transfer) sites form complexes with easily polarized aromatic molecules like naphthalene. The exact nature of these sites is not yet known.

3.6.3. Magnesium Silicates

These adsorbents are coprecipitates of silica and magnesia. A well-known commercial product is Florisil (Floridin Co., Pittsburgh, Pa.), which is a white material containing 84% of silica. Reviews of its use and chromatographic properties are given in Refs. 11 and 12.

The product, as received, has an average pore diameter of 62 Å and a surface area of 300 $m^2\ g^{-1}$. Activated Florisil, obtained by heating to 400° for 16 hr, possesses strongly acidic sites on its surface, and in addition to chemisorbing organic nitrogen bases, also partially but irreversibly adsorbs other compound types such as esters and aromatic hydrocarbons. Deactivation by the addition of up to 1% water preferentially covers these acidic sites. Further deactivation produces an adsorbent whose chromatographic properties are intermediate between those of silica and alumina.

3.6.4. Magnesia

Magnesia (magnesium hydroxide or oxide) is a polar, basic adsorbent which has recently been evaluated by Snyder[13]. It is available as a very fine white powder suitable for both TLC and high-efficiency chromatography.

It is believed that surface hydroxyl groups play an important part in the adsorption mechanism. On heating to 150°C, varying amounts of physically adsorbed water are lost. On heating to 350°C the activity of magnesium hydroxide sharply increases due to loss of surface hydroxyls and formation of oxide groups. On further heating the activity of the magnesia surface is reduced and above 1000°C becomes completely inactive. Activated magnesia

chemisorbs aromatic compounds. To avoid this, a satisfactory adsorbent may be prepared by activation at 500°C for 16 hr followed by deactivation with 3–7% water. The linear capacity of 3% H_2O–MgO was found to be approximately 2×10^{-5} g g^{-1}[13].

Deactivated magnesia is readily reactivated by dry solvents such as pentane. Water-saturated solvents must therefore always be used if chemisorption is to be avoided. The selectivity of deactivated magnesia is similar to that of silica or alumina. However, compounds containing carbon–carbon unsaturation are much more strongly held on magnesia than on alumina. Magnesia is therefore a valuable adsorbent for the separation of compound classes differing only by degree of unsaturation, e.g., olefins from diolefins, polynuclear aromatics, etc. On account of its high surface *p*H, acids are chemisorbed.

3.6.5. Modified Adsorbents

The properties of silica gel or of other polar adsorbents may be modified by incorporating a complexing agent into the adsorbent. For example, the separation of olefinic from saturated hydrocarbons is much better if silica gel is first impregnated with silver nitrate solution. Further examples of modifiers are given in Table 3.8. In general, 1–10% solutions of the complexing agent in water or acetone are slurried with the adsorbent. The slurries are either directly spread over the plates in the usual manner of else dried at 110°C in an oven before being packed into columns.

3.6.6. Porous Layer Beads

Rigid nonporous supports such as glass beads covered by a thin porous layer of adsorbent (and on which a liquid stationary phase can be deposited—

Table 3.8. Modified Adsorbents

Complexing agent	Selective for	References
0.1–0.5 *N* acids or bases	*p*H-sensitive compounds	(14)
Silver nitrate	Olefinic or acetylenic materials	(15-17)
Boric acid, sodium borate, sodium arsenite, basic lead acetate, sodium metavanadate, sodium molybdate	Polyhydroxyl compounds	(18)
Caffeine, 2,4,7-trinitrofluorene, picric acid, trinitrobenzene	Polynuclear aromatic hydrocarbons	(19-22)
Sodium bisulfite	Aldehydes	(23)
Ferric chloride	Oxines	(24)
Copper sulfate	Amines	(25)
Zinc ferrocyanide	Sulfonamides	(26)

see Chapter 4) are currently being developed for use in high-efficiency columns. A wider range than that shown in Table 3.2 will probably be commercially available by the time this book is published.

The materials are hard, free-flowing, and have regular dimensions so that they can easily be dry-packed to give columns with efficiencies of the order of 5000 plates per meter. The flatness of their HETP/mobile phase velocity curves (see Fig. 4.6) indicates that such packings can be used at high flow rates giving fast analyses with little sacrifice in column efficiency. This is made possible because they can withstand pressures up to at least 500 atm, thus enabling columns to be operated at high flow rates.

Horvath *et al.*[27,28] coated glass beads with a thin skin (pellicule) of ion-exchange resin by copolymerizing styrene–divinyl benzene directly on the beads, followed by chemical conversion to the appropriate ionic form.

Kirkland[29,30] introduced his "controlled-surface-porosity" supports, which consist of glass beads with a porous surface of controlled thickness and pore size (Zipax*). A support of this type with an average pore diameter of about 1000 A has a surface area of 0.65 $m^2\ g^{-1}$. They are very weak adsorbents with a low capacity and are therefore more useful as support materials for partition chromatography (see Chapter 4). A packing with cation-exchange properties has also been prepared in which the porous layer consists of a fluoropolymer containing free sulfonic acid groups. The packing can be used at elevated temperatures with a variety of mobile phases and can withstand column inlet pressures of greater than 200 atm. The cation-exchange capacity was 3.5 μeq g^{-1}. Similarly, a strongly basic, controlled-surface-porosity anion-exchange packing has been prepared containing tetraalkylammonium groups. The capacity of this particular packing was found to be 12 μeq g^{-1}.

Corasil (Waters Associates) is an adsorbent consisting of glass beads coated with either a single or double layer of porous silica (Corasil I and II, respectively). They are activated by heating overnight at 110°C and then deactivated by adding up to 0.5% by weight of water. Heating above 300°C produces a permanent reduction of surface activity. The linear capacity of Corasil II (surface area 14 $m^2\ g^{-1}$) is an order of magnitude less than that of conventional silica gel. A recent evaluation[31] illustrates high-speed analyses of phenolic and amine antioxidants.

3.6.7. Carbon

Carbon is an excellent adsorbent but, on account of its ill-defined properties and its color, which makes visual detection of solute bands

*Registered trademark, E. I. du Pont de Nemours & Co., Inc.

difficult, it has so far found limited use in adsorption chromatography. It strongly adsorbs aromatics and high-molecular-weight compounds and it has therefore been used as a "cleaning-up" agent, e.g., for removing high-molcular-weight compounds from complex mixtures to enable low-molecular-weight materials to be more easily analyzed. An example is the removal of high-molecular-weight carbohydrate material from plant residues in the search for insecticides.

The color of carbon has almost completely precluded its use in TLC because of detection difficulties. However, with the types of column and detection systems described in this book the field is wide open for a reappraisal of the use of certain forms of activated carbon as adsorbent. The evaluation of previously qublished work using active carbon as an adsorbent is often difficult because of the ill-defined nature of the carbon used. However, the nature of the carbon surface is now more fully understood (for recent reviews see the list of further reading) and carbons with more precisely defined properties are now available. Examples are Graphon, a completely nonpolar charcoal with a hydrophobic surface, and the more polar Spheron 6. Both are available from Cabot Corporation. It is the nonpolar and hydrophobic nature of active carbons which distinguishes them from the metal oxides and makes them particularly attractive as adsorbents.

Carbon occurs naturally in two allotropic forms—diamond, which need not concern us any further, and graphite. Graphite is a well-defined crystalline form of elemental carbon consisting of layers 3.35 Å apart of carbon atoms joined by sp^2 covalent bonds in a fused hexagonal ring system. The layers are held together by the relatively weak van der Waals forces.

Adsorbent carbons can be divided into two main categories—charcoal and carbon black. Charcoals are made by the destructive distillation of organic matter such as wood or bone. Activation is achieved by slow oxidation at elevated temperatures with air, steam, carbon dioxide, or chlorine, or by impregnation by salts, acids, or alkalis followed by calcination. Charcoals so produced possess heterogeneous surfaces containing inorganic atoms in addition to organic functional groups, making adsorption data difficult, if not impossible, to interpret. The adsorbent properties of charcoals from different suppliers will therefore differ, and variations in the properties from a given supplier can also be expected from batch to batch.

Carbon blacks are formed by the incomplete combustion of hydrocarbons. They are microcrystalline materials consisting of graphite-like layers 3.6 Å apart stacked in packets of 3–30 layers about 10–100 Å thick. However, the layers often contain tetrahedrally bonded carbon atoms giving rise to cross-linking and causing lattice defects. The unsatisfied bonds at the edges of the graphitic layer planes are very reactive and during manufacture combine with foreign atoms or groups. For example, active carbons formed

by low-temperature oxidation usually possess acidic oxide surfaces due to the presence of carboxyl, carbonyl, and phenolic groups. Basic groups have also been observed.

In general, the surface of carbon black is hydrophobic, nonpolar, and shows poor specificity toward functional groups. However, owing to the presence of inorganic substances and polar functional groups, the surface also contains hydrophilic sites. It is due to the presence of these sites that many carbon blacks possess adsorption properties resembling those of the metal oxides. Adsorption on such sites involves electrostatic forces and hydrogen bonding. Ion-exchange properties are also exhibited by such carbons, e.g., they adsorb certain organic salts with the liberation of acids or bases.

On heating a carbon black such as Spheron 6 to a temperature of 3000°C under inert conditions, hydrophilic functional groups are lost, while the graphitic layer planes undergo a reorientation to form a more crystalline material. The resulting surface has a more hydrophobic, nonpolar character and specificity toward functional groups is absent. In contrast to the metal oxides, the main contribution to the adsorption energy between solutes and this material (Graphon) is due to dispersion forces.

The differences in properties between Spheron 6 and Graphon have been demonstrated by a number of workers. On the more polar Spheron 6, methanol is adsorbed more strongly than benzene, while in the case of Graphon the reverse is true. On Spheron 6 adsorption is by hydrogen bonding between the polar group of the adsorbent and the hydroxyl group of the solute, the hydrocarbon chain of the alcohol extending vertically outward into the solution. On Graphon the alcohol lies flat along the surface of the adsorbent. It has also been observed that aliphatic acids are adsorbed on Graphon in the dimeric form and lie with their long axes parallel to the surface. Similar orientations have been observed with alkyl benzenes and methyl esters of monocarboxylic acids.

Many commercial carbons have properties that fall between the two extremes, i.e., both surface oxygenated groups and the graphite structure are present. The adsorption properties of the oxygenated carbons resemble those of the metal oxides, but, due to the graphite structure, aromatic compounds are adsorbed more strongly than the corresponding aliphatic derivatives. This gives rise to a different eluotropic series than that of the metal oxides, an example of which is given in Table 3.5. It will be noted that the solvent strength increases with increasing size of solvent molecule. Aromatic solvents will also be seen to be stronger than the corresponding aliphatic solvents.

Active carbons are spherical, porous particles, their surface areas and pore diameters depending upon the method of preparation and subsequent

Table 3.9

Pore type	Pore diameter, Å	Surface area, $m^2\ g^{-1}$
Macropores	2000–1,000,000	0.5–2
Transitional pores	30–2000	80–400
Micropores	<30	>400

activation treatment. In general, three pore types can be recognized, macropores, transitional pores, and micropores, the characteristics of each being given in Table 3.9.

The low surface area of the macropores indicates that such pores play a negligible role in adsorption. However, they do provide access channels to the more important transitional pores and micropores.

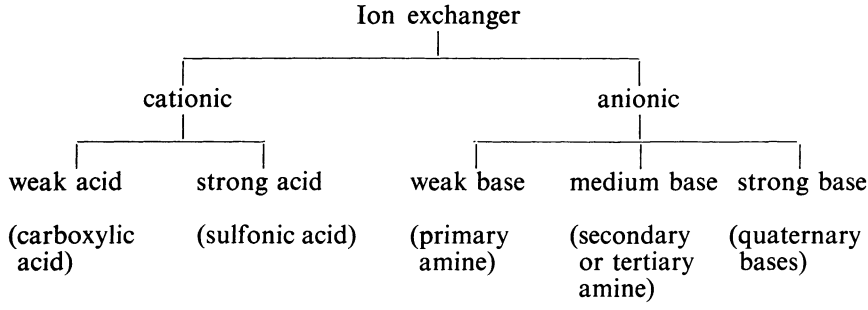
The surface area and pore diameter of transitional pores compare with those of silica gel or alumina, and carbons of this type have been widely used for removing large molecules or colloidal material from solutions containing smaller molecules, e.g., in the cleaning up of biological residues. Molecules are adsorbed on the surface to form a monolayer.

The pore diameter of microporous carbons is of the same order as the size of many organic molecules. Adsorption energies are relatively high, giving rise to slow mass-transfer rates. This in turn can lead to poor separations.

3.6.8. Ion Exchangers

Ion exchangers are naturally occurring or synthetic, insoluble polyelectrolytes having a porous structure which can take up positive or negative ions from an electrolyte solution in contact with them in exchange for an equivalent amount of its own ions which are liberated into solution. Extensive use has been made of this property for the removal, concentration, exchange, or analysis of inorganic ions in aqueous solutions. These aspects, which are beyond the scope of this book, are adequately discussed elsewhere (see the list of further reading). Of more concern to us is the property of ion exchangers to act as acidic or basic adsorbents in nonionic organic media([32]).

The most common type of ion exchanger in use today consists of an irregular, macromolecular, three-dimensional network of hydrocarbon chains bearing ionizable groups. The matrix is a copolymer of styrene and divinyl benzene into which various functional groups are introduced either before or after polymerization to give the resin its ion-exchange properties. The products are classified according to the type of active group as follows:



The so-called "macroreticular" ion-exchange resins have a rigid, macroporous structure with pore diameters up to 800 Å superimposed upon the normal gel structure. Their physical structure is therefore similar to silica or alumina. Otherwise, they are chemically similar to the conventional polystyrene–divinyl benzene based resins generally used in aqueous solution. As a consequence of their macroporous structure they can be used in solvents in which resins do not swell (i.e., nonpolar organic solvents) and for the adsorption of large organic molecules or ions.

A number of macroreticular resins are now commercially available. Their properties (Table 3.10) can be compared with a commonly used conventional resin. As a typical example([33]), Amberlyst A-15, a sulfonic acid cation exchanger, has a specific surface area of 42.5 $m^2\ g^{-1}$ compared with under 0.1 $m^2\ g^{-1}$ for the conventional resin Amberlite IR-120. Examination by electron microscopy revealed no internal pore structure for the conventional resin, but a definite porous structure for A-15, pores of 400–800 Å diameter being evident. Ion-exchange equilibria and kinetic studies showed

Table 3.10. Some Properties of Macroreticular Resins

	Amberlyst resins[a]					Amberlite[a]
Designation	A-15	XN1005	A-29	A-27	A-21	IR-120
Type	Cationic	Cationic	Anionic	Anionic	Anionic	Cationic
Ionic form	Hydrogen	Hydrogen	Chloride	Chloride	Free base	Hydrogen
Active group	$-SO_3H$	$-SO_3H$	Quaternary ammonium	Quaternary ammonium	Tertiary amine	$-SO_3H$
Capacity, meq g^{-1}	4.9	3.5	2.7	2.6	4.8	4.5
Capacity, meq ml^{-1}	2.9	—	1.0	0.7	1.6	1.7
Pore diameter, Å	200–600	—	200–600	400–800	700–1200	<5
Surface area, $m^2\ g^{-1}$	40–50	122	40–50	60–70	20–30	<0.1

[a] Manufactured by Rohm and Haas Co., Philadelphia, Pa.

the macroreticular resin to be much less sensitive to the nature of the solvent than the conventional resin. They are able to withstand alternate wetting and drying without degrading in particle size, showing their superior physical stability over conventional resins. Furthermore, they show no marked deterioration when subjected to repeated changes from an aqueous to a non-aqueous environment.

Ion exchangers behave as acidic or basic adsorbents in organic media. Both physical and chemical adsorption are known to occur. For example, the acetate form of the anion exchanger Amberlyst A-29 physically adsorbs polar compounds such as pyrroles and phenols from hydrocarbon solutions. These solutes can be eluted from the resin with polar solvents such as pyridine or methanol. Acids, on the other hand, are chemisorbed on this resin and acidic solvents are required to elute them. In a similar fashion, the strong acid cation-exchange resin Amberlyst A-15 chemisorbs nitrogen bases from hydrocarbon solutions; they can be desorbed with basic solvents.

The selectivity of the resin can be made highly specific by making use of a form that will form a complex (ligand) with the solute([36]). For example, cation exchangers in either the Ag^+, Cu^{2+}, or Ni^{2+} forms have been used for the separation of amines and carboxylic acids; the Ag^+ form has also been used for isolating compounds with olefinic double bonds. Elution development is carried out with an agent which complexes less strongly than the substances to be separated; displacement development is carried out with an agent which complexes more strongly.

REFERENCES

1. Giles, C. H., MacEwan, T. H., Nakhwa, S. N., and Smith, D., *J, Chem. Soc.* 3973 (1960).
2. Snyder, L. R., *Principles of Adsorption Chromatography,* Dekker, New York (1968).
3. Snyder, L. R., *J. Chromatog.* 5, 430 (1961).
4. Snyder, L. R., *Separation Sci.* **1,** 191 (1966).
5. Hesse, G., and Roscher, G., *Z. Anal. Chem.* **200,** 3 (1964).
6. Hagdahl, L., and Holman, R. T., *J. Am. Chem. Soc.* **72,** 701 (1950).
7. Snyder, L. R., *Advan. Anal. Chem. Inst.* **3,** 251 (1964).
8. Snyder, L. R., *J. Chromatog.* **23,** 388 (1966).
9. Hesse, G., *Z. Anal. Chem.* **211,** 5 (1965).
10. Bowen, J. N., Bowrey, R., and Malin, A. S., *J. Catalysis* **1,** 209 (1967).
11. Snyder, L. R., *J. Chromatog.* **12,** 488 (1963).
12. *Floridin Technical Data and Product Specifications,* Floridin Co., Tallahassee, Fla.
13. Snyder, L. R., *J. Chromatog.* **28,** 300 (1967).
14. Stahl, E., and Dumont, E., *J. Chromatog. Sci.* **7,** 517 (1969)
15. Urbach, G., *J. Chromatog.* **12,** 196 (1963).
16. Gupta, A. S., and Dev. S., *J. Chromatog.* **12,** 189 (1963).
17. Chapman L. R., and Kuemmel, D. F., *Anal. Chem.* **37,** 1598 (1965).
18. Morris, L. J., *J. Chromatog.* **12,** 321 (1963).
19. Berg, A., and Lam, J., *J. Chromatog.* **16,** 157 (1964).
20. Klemm, L. H., Reed, D., and Lind, C. D., *J. Org. Chem.* **22,** 739 (1957).
21. Harvey, R. G., and Halonen, M., *J. Chromatog.* **25,** 294 (1966).
22. Kessler, H., and Müller, E., *J. Chromatog.* **24,** 469 (1966).

23. Adachi, S., *J. Chromatog.* **17,** 295 (1965).
24. Cawthorne, M. A., *J. Chromatog.* **25,** 164 (1966).
25. Brockmann, H., *Disc. Faraday Soc.*, **7,** 58 (1949).
26. Fogg, A. G., and Wood, R., *J. Chromatog.* **20,** 613 (1965).
27. Horvath, C. G., and Lipsky, S. R., *J. Chromatog. Sci.* **7,** 109 (1969).
28. Horvath, C. G., Preiss, B. A., and Lipsky, S. R., *Anal Chim.* **39,** 1422 (1967).
29. Kirkland, J. J., *Anal. Chem.* **41,** 218 (1969).
30. Kirkland, J. J., *J. Chromatog. Sci.* **7,** 361 (1969).
31. Majors, R. E., *J. Chromatog. Sci.* **8,** 338 (1970).
32. Webster, P. V., Wilson, J. N., and Franks, M. C., *Anal. Chim. Acta* **38,** 193 (1967).
33. Kunin, R., Meitzner, E. F., Oline, J. A., Fisher, S. A., and Frisch, N., *Ind. Eng. Chem. Prod. Res. Dev.* **1,** 140 (1962).
34. Heftmann, E., *Chromatography,* Reinhold, New York (1967).
35. Hockey, J. E., *Chem. Ind. (London)* 57 (1965).
36. Helfferich, F., Ion-exchange chromatography, *Advan. Chromatog.* **1,** 3 (1966).

FURTHER READING

Giles, C. H., and Easton, I. A., Adsorption chromatography, *Advan. Chromatog.* **3,** 70 (1966). (A discussion of isotherm shape and the nature of adsorption forces on silica, alumina, and carbon.)

Young, D. M., and Crowell, A. D., *Physical Adsorption of Gases*, Butterworths, London (1962). (A comprehensive review which gives an insight into the nature of physical adsorption.)

Snyder, L. R., *Principles of Adsorption Chromatography,* Dekker, New York (1968). (A comprehensive account of separation processes and basic principles of liquid–solid adsorption chromatography together with a systematic generalization of available data on the separation of nonionic organic compounds.)

Hayward, D. O., and Trapnell, B. M. W., *Chemisorption,* Butterworths, London (1964). (A comprehensive review which gives an insight into the nature of chemisorption.)

Snyder, L. R., R_F values in thin-layer chromatography on alumina and silica, *Advan. Chromatog.* **4,** 3 (1967). (A general theory for the correlation and prediction of R_F values in TLC. The theory is tested and confirmed by applying it to literature data.)

Everett, D. H., and Stone, F. S. (eds.), *The Structure and Properties of Porous Materials*, Butterworths, London (1958). (Contains chapters on the surface characteristics of carbons and silicas.)

Hockey, J. A., The surface properties of silica powders, *Chem. Ind. (London)* **1965,** 57;
Mitchell, S. A., surface properties of amorphous silicas, *Chem. Ind. (London)* **1966,** 924. (Accounts of the effects of different methods of preparation and subsequent thermal or chemical treatment on the nature and properties of the silica surface.)

Heftmann, E., *Chromatography,* Reinhold, New York (1967). (A general review of chromatography.)

Coughlin, R. W., Carbon as adsorbent and catalyst, *Ind. Eng. Chem. Prod. Res. Dev.* **8,** 12 (1969). (The structure and surface chemistry of carbon is related to its catalytic and adsorptive behavior.)

Dubinin, M. M., *Porous structure and adsorption properties of active carbons,* in *Chemistry and Physics of Carbon,* Walker, P. L., ed., Dekker, New York (1966), vol. 2, p. 51. (Basic studies.)

Zettlemoyer, A. C., and Narayan, K. S., Adsorption from solution by graphite surfaces, *Ibid.*, p. 197. (Some basic studies including a comparison of Graphon and Spheron 6.)

Amphlett, C. B., *Inorganic Ion Exchangers,* Elsevier, London (1964). (Covers the recently developed inorganic ion exchangers useful for high-temperature work. Mainly inorganic separations, though possibly applicable to nonaqueous work.)

Helfferich, F., *Inorganic Ion Exchangers,* McGraw-Hill, New York (1962). (A comprehensive account covering basic theory of ion exchangers.)

Inczédy, J., *Analytical Applications of Ion Exchangers,* Pergammon Press, Oxford (1966); Samuelson, C., *Ion Exchange Separations in Analytical Chemistry,* Wiley, New York (1963). (Useful practical books covering elementary theory and basic techniques of ion-exchange chromatography, including separations of organic mixtures.)

Helfferich, F., Ion exchange chromatography, *Advan. Chromatog.* **1,** 3 (1966). (A review of advances in ion-exchange chromatography during 1960–1965. Includes examples of organic separations in nonaqueous media and of ligand-exchange chromatography.)