Silicic-Acid Column Chromatography: Adsorption Mechanism and Solvent Systems

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Abstract

The relationship between chromatographic characteristics of silicic-acid columns and the electrophilicity-geometry of their absorbed solvent has been investigated. Data show that it is possible to prepare columns of almost identical separation characteristics from a variety of dissimilar solvents. Consideration must be given to the electron distribution and molecular geometry of the adsorbed solvent with which the sample components must compete during chromatography.

Introduction

Previous work from this Laboratory by Frankel et al. (1–3) has demonstrated the usefulness of silicic acid (SiO_2) and methanol-benzene as a chromatographic system for the separation of lipids and their oxidation products. Later investigation (4) dealt with parameters governing column efficiency offered by their system. This paper describes more recent efforts to evaluate other solvent systems and to gain further insight into the mechanism by which SiO_2 columns effect separations of lipid materials.

Experimental Section

Adsorption isotherms were plotted from data representing homologous series of alcohols, nitriles, and ketones. Each was constructed from the concentration of adsorbed and unadsorbed solute in a system containing SiO_2, solute, and benzene solvent. Of each solute 2, 4, 8, 16, and 25 g were equilibrated with 50 g of SiO_2 in the presence of 100 ml of benzene. Unadsorbed solute concentrations were determined refractometrically on the supernatant (4). Each concentration was converted to mole percentage from a calibration plot relating refractive index to the known mole percentage of solute. Solvents at these concentrations were used to elute their respective columns so that the column solvent composition would remain constant.

To test the applicability of new solvents to lipid analysis, a standard mixture of methyl esters of aliphatic, keto, and hydroxy fatty acids was chromatographed on columns prepared with each solvent. Details of column preparation and elution are described elsewhere (4).

A calibration plot from a previous paper (5), wherein the molecular cross-section of alcohols was related to the surface area of the adsorbent, allowed a prediction of the elution volumes of the standard mixture from knowledge of the area occupied by a given concentration of alcoholic solute.

Results and Discussion

Typical Langmuir isotherms in Figure 1 are representative of the L-2 designation of Giles et al. (6). Their flat plateau indicates that all alcohols, except methanol, are adsorbed on SiO_2 from benzene to a distinct monolayer concentration. At higher concentrations further adsorption is accompanied by simultaneous desorption that maintains the monolayer. Only methanol is small enough to allow the electrical field of the underlying adsorbent to extend its influence through the shielding alkyl residue to attract and hold more methanol molecules. For this reason the concentration corresponding to a methanol monolayer is graphically less distinct.

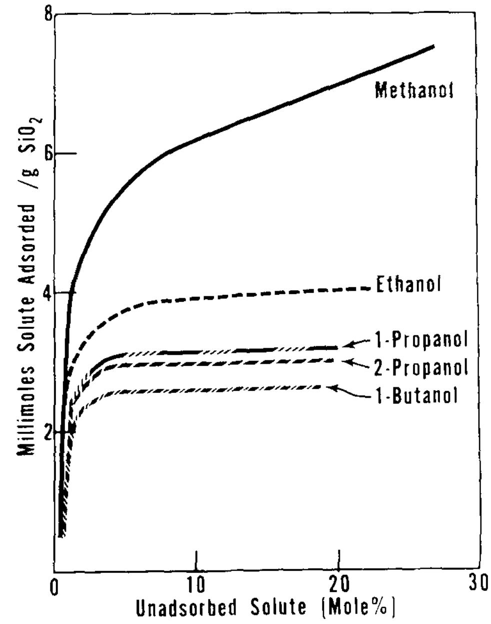

FIG. 1. Adsorption isotherms of aliphatic monohydric alcohols on silicic acid, SiO_2, from benzene.

Hydrogen bonding between solute and silanol groups present on the silica surface can occur only to the extent that unshared electron pairs on the solute are electrically and sterically unhindered. Among the alcohols studied, all were aliphatic. Since electron-donating tendencies of saturated alkyl chains do not differ widely, each alcohol is adsorbed with an avidity

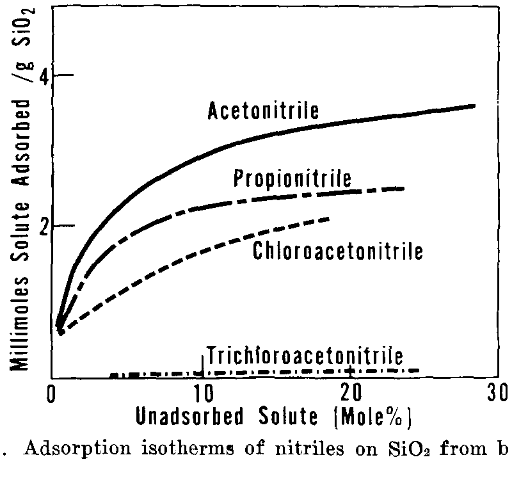

FIG. 2. Adsorption isotherms of nitriles on SiO_2 from benzene.

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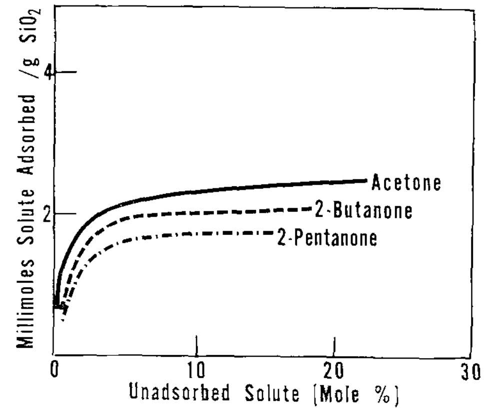


FIG. 3. Adsorption isotherms of homologous methyl-ketones on SiO_2 from benzene.

that is proportional to the steric availability of the two pairs of unshared electrons on its hydroxylic oxygen atom (5).

Figure 2 includes isotherms of two aliphatic nitriles. In the unchlorinated series, geometric factors predominate because the electrical influences are almost equal; both solutes have one unshared pair of electrons and neither bears a substituent group. Propionitrile is larger than acetonitrile and can therefore occupy more of the adsorbent surface. Consequently it is less adsorbed than is its lower molecular weight homolog.

Within the series of ketones (Figure 3) the physically smaller molecule is adsorbed to the greatest extent.

The solute series in Figure 4 demonstrates how both steric availability and the number of unshared electrons can influence adsorption. Although three solutes have the same carbon content, 1-propanol adsorbs most readily because it has two pairs of unshared electrons to which silanol hydrogens may bond. Acetone exhibits an intermediate affinity for SiO_2 because its carbonyl bond restricts electron pair orientation and permits adsorption only in an attitude of greatest spatial bulk. Propionitrile adsorbs the least of the three because it has but one pair of unshared electrons.

Steric influences can be effectively illustrated with molecular models and scaled coordinate paper. Within any adsorbate series the longer-chain homolog can "occupy-by-influence" more of the adsorbent surface because its hydrocarbon chain can be rotated about the hydrogen-bonded oxygen atom. For example, the adsorption volume of 2-propanol arises from molecular width rather than from molecular-chain length. When measured along the carbon trio perpendicular to its C–OH bond, 2-propanol has a greater molecular width than has 1-propanol. Since a normal alkyl chain can extend farther from a hydrogen-bonded alcoholic oxygen than can a branched homolog, the straight-chain isomer has the larger "sphere of influence."

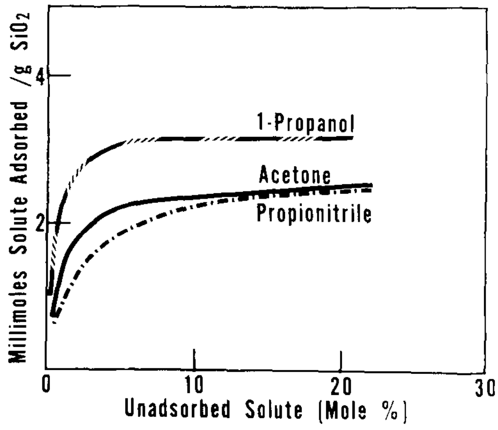


FIG. 4. Adsorption isotherms of a three-carbon aliphatic monohydric alcohol, methyl-ketone, and aliphatic nitrile on SiO_2 from benzene.

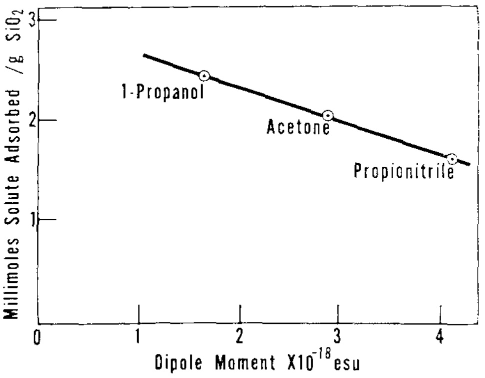


FIG. 5. Relationship between the extent of adsorption and dipole moment for 1-propanol, acetone, and propionitrile.

Lowen and Broge (7) experimented with the adsorption of methyl red on a silica that had some of its silanol hydrogens replaced with butyl groups. Even though active –OH groups remained (it was still possible to incorporate more butoxy groups chemically), methyl red was unable to penetrate the alkyl residues to reach the underlying hydroxyls. Butoxy groups therefore are capable of excluding methyl red from considerably more area than they actually cover at their adsorption site. These steric effects account for the relative adsorption characteristics of normal and branched alcohols on SiO_2.

Dipole moment is a measure of electrical dissymmetry, and the linear relationship in Figure 5 indicates unshared electron availability and spatial orientation within the solute significantly influences its affinity for an adsorbent. This effect is exemplified by the chlorinated nitriles shown in Figure 2. Since chlorine is powerfully electronegative, it pulls the nitrile-nitrogen pair of unshared electrons nearer to the nitrogen atom. The degree to which this pair is unavailable depends on the number of chlorine atoms present. It will be noted that trichloroacetonitrile exhibits a low affinity for SiO_2. Molecular models fail to provide evidence of a significant steric effect caused by the substitution of chlorines for hydrogen. The lack of a well-defined plateau on the isotherms is evidence that the monolayer concentration is less distinct. Apparently, terminal chlorines on the nitrile offer sites to which additional nitrile molecules may be attracted.

Experiments with trifluoroethanol show it to have less affinity for SiO_2 than does ethanol. Steric effects can be discounted because the fluorine substituents are not much larger than the hydrogen atoms that they replace. Furthermore intermolecular forces that cause trifluoroethanol to be more dense than ethanol compensate for any bulk contributions arising from the replacement of hydrogen with fluorine.

When equal weights of each of six alcoholic solutes are adsorbed on SiO_2 from benzene, a linear relationship exists between the logarithm of the moles represented by this weight of solute and the molar volume of each adsorbate (Figure 6). If adsorption is sterically controlled, as it is in the unsubstituted alcohol series, the increased molar volume of trifluoroethanol

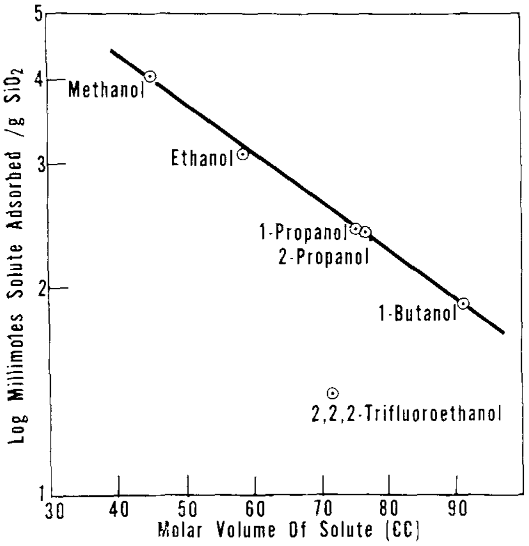
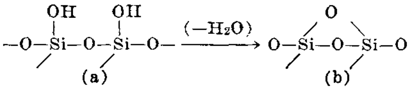


FIG. 6. Relationship between the molar volume of alcoholic adsorbate and the log of the moles adsorbed when 1.4×10^{-3} g of solute is adsorbed per g of SiO_2.

would reduce its adsorption to a value similar to that for the propanols. The data point for trifluoroethanol should then lie on the line in the figure. Since this point deviates appreciably from the expected linear relationship, the steric factor must be minimal. Models of trifluoroethanol also indicate the lack of a significant steric influence.

Trifluoroethanol has an electrically induced acidity about equal to that of phenol (pH of $CF_3CH_2OH:H_2O$ [1:1 v/v] is approximately 5), but the lability of the hydroxylic proton seems to have no enhancing effect whatever on the affinity of this alcohol for SiO_2. In fact, an isotherm plot indicates that fluorinated substituents actually reduce the adsorption of ethanol on SiO_2. This behavior provides evidence that the hydrogen bond which joins the solute and adsorbent is formed between the silanol hydrogen and the hydroxylic oxygen of the solute rather than between silanol oxygen and hydroxylic hydrogen. The trifluoro substituent withdraws electrons from the hydrogen bonded oxygen atom, thereby weakening its hold on the -OH proton. If bonding occurred between this labile proton and a silanol oxygen, trifluoroethanol should adsorb more readily than does ethanol. Since it does not, the silanol-hydrogen to hydroxylic-oxygen bonding scheme described above seems plausible. Relative to unsubstituted alcohols, the adsorption of trifluoroethanol on SiO_2 can be explained by the same electron availability argument cited for the halogenated nitriles.

Kiselev (8) and Hockey (9) have stated that the adsorption of solutes on particulate silica depends on the degree of surface hydration. Removal of water from vicinal hydroxyls converts the free silanol form, (a), to a much less adsorptive siloxane-bridge form, (b).



They further indicate that lattice impurities, such as magnesia and tervalent metal oxides (particularly alumina), greatly increase the acidity of silanol hydrogens and increase the adsorptivity of the SiO_2. Their views on the adsorption influences of surface hydration and impurities are in complete accord with evidence in this Laboratory that hydrogen bonds between solute and adsorbent are formed from silanol-hydrogen atoms.

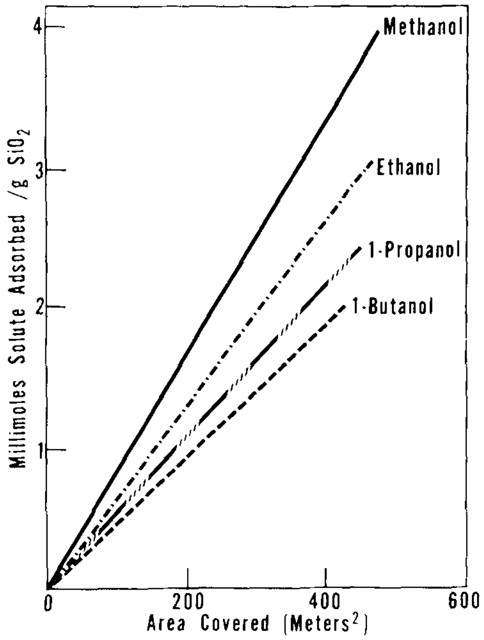


FIG. 7. Relationship between the amount of alcohol adsorbed and the area represented by that number of alcohol molecules.

Even though Figures 2–6 show that electron availability in the solute is influenced by both steric and electrical factors, the SiO_2-alcohol-benzene system is apparently governed primarily by steric effects. The actual amount of surface covered by the solute determines the efficiency of chromatography, and though the electrophilicity of substituent groups on the solute may influence its affinity for SiO_2, surface coverage seems to be the over-riding consideration in the chromatographic process.

Figure 7 shows the relationship between the surface area of adsorbent covered and the different concentrations of each of four aliphatic alcohols. From this plot it is possible to choose concentrations of different alcohols which represent equal surface coverage. For example, by reading upward from the abscissa through the lines representing butanol to methanol, it is possible to determine the millimoles of alcohol per gram of SiO_2 which are necessary to cover any specified area. When millimoles are converted to grams, Table I or a plot from its data allows determination of the weight of alcohol which must be added to 50 g of SiO_2 and 100 ml of benzene to cover the desired area. Table I also illustrates the mass relationship which governs alcohol adsorption on SiO_2 from benzene. At any solute concentration (first column) the weight of different alcohols adsorbed is remarkably constant. Only when the adsorbent is inundated by solute (16 and 25 g/50 g SiO_2), do the masses of alcohol adsorbed deviate from constancy. Constancy in the weights of

TABLE I
Adsorption of Alcohol Solutes on Silicic Acid from Benzene

Total grams solute added to 50 g SiO_2 and 100 ml benzene	Grams solute adsorbed/50 g SiO_2				
	MeOH	EtOH	1-PrOH	2-PrOH	1-BuOH
2	0.039	0.038	0.039	0.038	0.038
4	0.078	0.076	0.076	0.077	0.076
8	0.145	0.144	0.145	0.146	0.147
16	0.206	0.182	0.192	0.179	0.193
25	0.233	0.186	0.192	0.180	0.190

TABLE II

Relationship Between Surface Area of SiO_2, Covered by Various Alcohols, and the Elution Volume of a Three-Component Mixture

Solute	Alcohol added to SiO_2 (% by weight)	Millimoles solute adsorbed per g SiO_2	Approximate area SiO_2 covered by moles adsorbed m^2	Elution volume of fatty esters[a] (ml)		
				Aliphatic	Keto	Hydroxy
Methanol	13.0	3.8	450	74	107	259
Ethanol	15.1	3.1	455	74	103	189
1-Propanol	16.0	2.4	450	74	107	164

[a] Aliphatic: Methyl octadeca-9,11,13-trienoate.
Keto: methyl 4-keto octadeca-9,11,13-trienoate.
Hydroxy: methyl 12-hydroxy octadeca-9-enoate.

adsorbed alcohols indicates that equal surface coverage depends on molecular size. In any given mass, more molecules of solute are available from the alcohol of lowest molecular weight. Because the smaller molecules cover less area individually, more are required to offer the same amount of SiO_2 coverage collectively.

Hereinafter the addition of monolayer concentrations of alcohol to SiO_2 shall be referred to as deactivation with a competing phase. Table II shows data pertinent to the chromatography of the sandard lipid sample on SiO_2 with various alcohols as a competing phase. The reproducibility of elution volumes may be noted even though the weight percentage of each alcohol differs widely. Columns prepared either with 13.0% methanol, 15.1% ethanol, or 16.0% 1-propanol by weight of SiO_2 all have about the same elution characteristics. For alcoholic SiO_2 deactivators, surface coverage is the most significant factor in predicting elution volumes. Constant elution volumes can be obtained, regardless of the alcoholic deactivator, so long as equal areas, or the number of active SiO_2 sites, are deactivated. Elution volumes must therefore be controlled by the site availability when the adsorbent is nearly or completely covered with a monolayer of adsorbed alcohol.

The foregoing indicates that the standard SiO_2-methanol-benzene system empirically developed more than six years ago (1–3) is indeed based on conditions at which alcohol is adsorbed at or near monolayer concentration. Under these conditions lipid materials receive the most efficient competition from methanol for active sites on the SiO_2 surface. Separations occur from the differential exchange of sample and alcohol on adsorbent sites. Experimental evidence further points out that the most useful of the original SiO_2-methanol-benzene systems is one that is governed primarily by an adsorption phenomenon occurring at monolayer conditions rather than by partition. Observed chromatographic characteristics of the system cannot be fully explained by a partitioning multilayer. Eluted components must be adsorbed on active adsorbent sites in direct competition with the alcohol molecules already present. Lipid materials which are easily chromatographed on adsorption monolayer columns are not unusually soluble in pure alcohol. Therefore, if the retentive mechanism is that of partition chromatography, they should not be appreciably soluble in a partitioning multilayer. In the absence of adsorptive retardation, components would elute quickly and would be poorly resolved. Since they elute neither quickly nor with poor separation, some adsorptive mechanism is indicated.

The importance of a competitive phase in near monolayer concentration is evident since high molecular weight materials, in the total absence of an alcoholic competitor, would be strongly adsorbed on bare SiO_2. Too much adsorbed alcohol displaces the separating medium toward partition chromatography whereas too little permits unrestrained sample adsorption that causes uselessly large retention volumes and peak tailing. The best and most efficient chromatography occurs when the sample components meet active competition from a continuously renewed alcoholic deactivating phase. This phenomenon appears to be the discriminating factor that establishes different retention volumes and makes component separation possible.

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