

However, a realistic treatment of the delocalized π electrons in conjugated molecules requires a more rigorous approach. The equilibrium torsional angle and torsional barrier of biphenyl have been calculated by ab initio molecular orbital theory,²⁷ but substituted anthracenes represent a significant increase in complexity for those calculations. Semiempirical theories such as CNDO and INDO fail to give qualitatively correct potential functions for rotation about nominally single bonds in conjugated molecules. Improved results are obtained by inclusion of adequate geometry optimization or by retaining more overlap.²⁸ More accurate theories have been used recently which are hybrids of the empirical and semiempirical methods.²⁹

Of the substituted anthracenes we have studied, S₀ 9PA is the only one for which an equilibrium torsional angle has been calculated experimentally.³⁰ The value obtained was 67° instead of the experimentally derived value of 90°. Equilibrium torsional angles and torsional barriers have been calculated for other biaryls,^{18,19,20,27,31} but usually good experimental information for

comparison is lacking. We believe that our LIF experiments on substituted anthracenes, along with a limited number of other LIF examples (e.g., tolane^{10c}), now yield sufficiently accurate torsional potentials for biaryls to provide a fresh stimulus and points of reference for future work in this field.

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Supplementary Material Available: Tables of assignments of the 9(2N)A, 2PA, and 9Va fluorescence excitation spectra (8 pages). Ordering information is given on any current masthead page.

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³¹P NMR Study of Trialkylphosphine Probes Adsorbed on Silica-Alumina

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Abstract: Three trialkylphosphines have been investigated as probe molecules for the study of acidic sites on amorphous silica-alumina. These molecules incorporate many favorable NMR features, such as high sensitivity of the ³¹P nuclide, high natural abundance, and short relaxation times, which allow convenient quantitative studies of acidic sites on surfaces. Phosphines bound at Brønsted and Lewis sites have been distinguished by chemical shift analogies to model systems. Unlike some other spectroscopic methods that typically measure both surface and bulk sites, the analysis of amorphous materials by probe molecules such as phosphines results in the detection of acidic sites that are strictly on the surface and thus catalytically accessible. Variation of the surface phosphine concentration yields a titration of acidic sites. At low phosphine concentrations only phosphines bound to Brønsted sites are observed. As the phosphine concentration is increased, phosphines bound to Lewis sites and physisorbed phosphines appear simultaneously. Because of the relatively large chemical shift separation of Brønsted bound phosphines from all other phosphine chemical species, it is possible to assay the absolute numbers of surface Brønsted sites directly. Different phosphines count different numbers of Brønsted sites, demonstrating the desirability of using more than one type of probe molecule to characterize surfaces with specific binding sites. It has not been possible to quantitate the number of Lewis sites on silica-alumina because of small differences in chemical shifts and binding constants of Lewis-complexed and physisorbed phosphines. Variable-temperature studies have shown phosphine surface species to be in slow chemical exchange with one another, in contrast to the case of pyridine as a probe molecule previously studied by ¹⁵N and ¹³C NMR.

The catalytic activities of aluminosilicates (e.g., zeolites and amorphous silica-alumina) have inspired a wealth of studies designed to characterize the active sites. The charge-compensating protons (Brønsted sites) adjacent to the tetrahedral aluminate anionic centers in the lattice and tricoordinate neutral aluminum centers (Lewis sites) are believed to be important catalytic sites of aluminosilicates.¹⁻⁴ Numerous chemical methods have been developed to determine the numbers and types of surface active sites; these methods include aqueous titrations, nonaqueous ti-

trations, calorimetric measurements, and model cracking reactions. Spectroscopic methods that have been applied in the study of these materials include X-ray fluorescence, infrared, and nuclear magnetic resonance.¹⁻⁴ With new developments in high-resolution multinuclear solid-state NMR, the number of NMR investigations of amorphous materials has steadily increased, with a particular emphasis on the study of surface-related phenomena of such materials.^{5,6}

Various NMR approaches have been used in attempts to characterize acidic sites in aluminosilicates. There have been many

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reports of ²⁷Al magic-angle spinning (MAS) NMR studies of zeolites⁷⁻²⁹ and amorphous aluminosilicates.³⁰⁻³² One problem with MAS ²⁷Al NMR is the great difficulty of detecting ²⁷Al signals from low-symmetry aluminum atoms,³³ such as Lewis acid sites on silica-alumina. Furthermore, ²⁷Al MAS NMR has not been able to distinguish between those aluminum sites on the surface that are accessible to reactant molecules and, therefore, catalytically active and aluminum sites that would otherwise qualify as potentially catalytically active in the sense of local structure but are buried within the bulk structure and not accessible to reactant molecules.

An alternative approach utilizes small basic molecules as probes of acid sites on surfaces. This allows one to study specifically sites on the surface of catalytic materials. Probe molecules such as ammonia and pyridine have been used in infrared studies to characterize Brønsted and Lewis sites.⁴ NMR investigations of amines on catalytic surfaces have utilized ¹³C and ¹⁵N results.³⁴⁻⁴⁰

Table I. ³¹P Chemical Shifts^a

species	state	TMP	TEP	TBP
Model Compounds				
R ₃ P	liquid	-62	-19	-32
R ₃ P	physisorbed to silica		-19	
R ₃ P	physisorbed to γ-alumina	-55 to -60		
R ₃ P-AlCl ₃	solution		-17	-25
R ₃ P-AlEt ₃ ^b	solution	-48	-19	-27
R ₃ PH ⁺	polystyrene sulfonic acid resin	-1	23	
R ₃ PH ⁺	solutn	-3 ^c	19	13
Bound to Silica-Alumina				
R ₃ P	physisorbed	-62	-21	-32
R ₃ P-Al	Lewis	-49 to -60	-14 to -18	-30 to -32
R ₃ PH ⁺	Brønsted	-4	18	8

^a ppm from external 85% H₃PO₄; see text. ^b Reference 51. ^c Reference 49.

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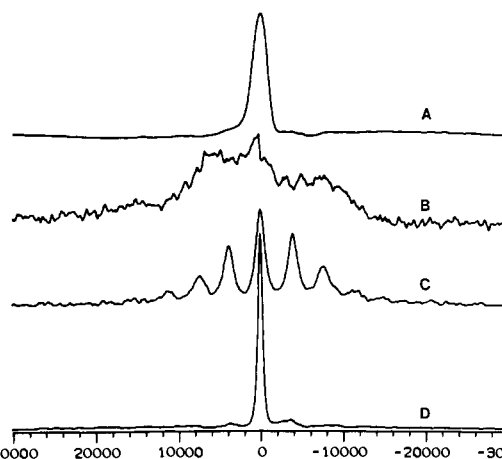


Figure 1. ³¹P MAS NMR spectra (60.7-MHz) of triethylphosphine on polystyrene sulfonic acid resin: (A) static ¹H decoupled, (B) static ¹H coupled, (C) MAS ¹H coupled, (D) MAS ¹H decoupled.

The disadvantage of ¹³C is the relatively small chemical shift changes found for the bound species. The disadvantage of ¹⁵N NMR is intrinsic to the ¹⁵N nucleus: a low magnetogyric ratio that produces low sensitivity even with isotopically enriched compounds. To overcome these problems we have investigated trimethylphosphine (TMP), triethylphosphine (TEP), and tri-*n*-butylphosphine (TBP) as probe molecules of surface acid sites.⁴¹ TMP has recently been studied by ³¹P NMR^{42,43} and by infrared spectroscopy^{43,44} in the investigation of acid sites in zeolites. The ³¹P spectra in those studies^{42,43} showed not only distinct peaks for Brønsted-complexed and Lewis-complexed (CH₃)₃P but also distinct peaks assigned to different local environments of the complexes. An amorphous surface is more likely to exhibit a continuous range of local environments for each complex, but three chemically distinct species types may still be resolved as relatively

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broad manifolds. The high magnetogyric ratio and 100% natural abundance of the ^{31}P isotope eliminate severe sensitivity problems encountered in the earlier ^{15}N studies, permitting experiments over a wide range of surface coverage.

Experimental Section

Solid-state ^{31}P MAS NMR spectra were obtained on a modified Nicolet NT-360 spectrometer at a frequency of 145 MHz, a modified Nicolet NT-150 spectrometer at a frequency of 60.7 MHz, and a Chemagnetics M-100S' spectrometer at a frequency of 36.6 MHz. The ^1H and ^{31}P rf fields were 9.4 and 23 G, respectively (40 kHz). The ^{31}P peak widths broadened only approximately twofold in the absence of decoupling. Therefore, the resolution is not significantly limited by decoupling power. Variable-temperature solid-state ^{31}P spectra were obtained at 36.6 MHz by using a Chemagnetics variable-temperature probe and accessories. Chemical shifts were obtained relative to an external secondary ^{31}P chemical shift reference of (-)-(2S,3S)-bis(diphenylphosphino)butane (chiraphos), for which the higher shielding resonance was assigned a chemical shift of -14.0 ppm relative to external 85% phosphoric acid at 0 ppm. Spectra were obtained in both the cross-polarization and single-pulse/acquire/delay modes. Solution ^{31}P NMR spectra were obtained at 80.9 MHz on a IBM WP-200 spectrometer and were referenced to external phosphoric acid.

The silica-alumina (from American Cyanamid) was formally 25% $\text{Al}_2\text{O}_3/75\%$ SiO_2 by weight, with a surface area of 485 m^2/g , as determined by the nitrogen BET method. It was dried for 12-16 h at 10^{-4} torr and 160-170 $^\circ\text{C}$ prior to reaction with the phosphines.

The air sensitivity of phosphine/silica-alumina samples required the use of conventional vacuum line techniques and dry (N_2) chamber techniques for sample preparations. Solid-state NMR samples were spun in the spectrometers at 3 to 4 KHz, by using dry nitrogen as the drive gas.

TBP and TEP were used as received from Aldrich and Strem. Because of the rather low vapor pressures of these phosphines, they were absorbed on the silica-alumina from solution. The desired amount of phosphine was added to approximately 30 cm^3 of pentane which had been distilled from sodium/benzophenone. The resulting solution was then added to a weighed approximately 1-g portion of dried silica-alumina under nitrogen atmosphere and stirred for 15 min. The pentane was then evaporated under vacuum. The amount of TEP or TBP remaining on the surface was determined by elemental analysis, which agreed quite well with the amount of phosphorous predicted from the details of preparation. TMP was generated by thermal decomposition of the silver iodide complex (from Aldrich) at 150 $^\circ\text{C}$ under vacuum. The amount of TMP on the surface was determined by measuring the vapor pressure change after condensation onto silica-alumina and calculating the number of moles from the known volume of the vacuum line. The amount of TMP on the surface was determined also by phosphorus elemental analysis and at low loading levels agreed well with the phosphorus content predicted from the details of preparation; at higher levels the elemental analyses were systematically low, suggesting increasing loss of the volatile TMP during handling. For TEP, parallel experiments of vapor deposition and solution-phase deposition showed no difference in the ^{31}P NMR spectra of corresponding samples prepared by the two techniques. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

Polystyrene sulfonic acid resin was obtained from Rohm and Haas and has 4.8 mequiv of Brønsted acid sites per g. The resin was dried at 110 $^\circ\text{C}$ overnight at 10^{-4} torr to remove adsorbed water. TMP or TEP was adsorbed at its vapor pressure onto the resin at room temperature.

Triphenylphosphonium chloride was prepared from the reaction of dry HCl gas with triphenylphosphine in ether. Solvent was removed under a stream of N_2 and HCl at room temperature.

Complexes (1:1) of AlCl_3 with TEP or TBP in solution were prepared by the addition of the phosphine to a suspension of freshly sublimed aluminum chloride in dichloromethane (dried and distilled from P_2O_5) under nitrogen atmosphere at room temperature.⁴⁵⁻⁴⁹ Solution ^{31}P NMR spectra were obtained on the filtered reaction mixtures.

Experimental Data on Model Systems. Our initial efforts were devoted to characterizing the ^{31}P NMR parameters of chemical analogues of the

R_3P species expected on the silica-alumina surface. Solid-state Brønsted acid-base complexes were modeled by the reaction products of phosphines with polystyrene sulfonic acid resin. Figure 1 shows solid-state ^{31}P NMR spectra of TEP on polystyrene sulfonic acid resin under a variety of NMR experimental conditions. Reaction of TMP with polystyrene sulfonic acid results in analogous spectra. The isotropic chemical shifts are collected in Table I. Protonated phosphines are well-resolved in chemical shifts from other phosphine chemical species. The observed line width in Figure 1A (nonspinning, ^1H decoupled) includes contributions from both chemical shift anisotropy (CSA) and isotropic chemical shift dispersion arising from a variety of local environments. Thus, the width at base line provides a generous overestimate of the upper limit to the effective CSA. Since the CSA thereby estimated is less than 70 ppm, it is not surprising that only very small spinning side bands are observed in Figure 1D (spinning, ^1H decoupled). The ^{31}P NMR spectrum of resin-bound phosphine, with no proton decoupling (Figure 1C), displays a strong ^1H - ^{31}P interaction, behaving as one would expect for an isolated spin pair that gives rise to a pattern of dipolar-modulation spinning sidebands under MAS conditions. The residual line width in the isotropic average ^{31}P peak includes the effect that long-range homogeneous ^1H - ^{31}P dipolar interactions have on the MAS averaging of ^1H - ^{31}P dipolar interactions. Similar behaviors were observed for TMP in HY zeolite.^{42,43} In contrast, the ^{31}P NMR spectrum (not given here) of the pure triphenylphosphonium salt (C_6H_5) $_3\text{P}^+\text{Cl}^-$ shows a very broad signal with no apparent dipolar spinning sidebands without decoupling but a narrow peak with ^1H decoupling; this indicates that the acidic hydrogen in (C_6H_5) $_3\text{P}^+$ is not effectively isolated by rapid motion from spin diffusion with the protons in the phenyl groups. Thus, in the pure salt the ^{31}P - ^1H pair does not behave as an isolated spin system at this spinning rate.

In addition to ^{31}P - ^1H dipolar interactions, we have been able to observe for TMP on silica-alumina a ^1H - ^{31}P J coupling of 490 Hz. In solution this coupling constant has been reported as 495-515 Hz⁵⁰ and in HY zeolite TMP showed a splitting of 525-550 Hz, depending on the calcination temperature.^{42,43}

Complexes (1:1) of TEP and TBP with AlCl_3 and AlEt_3 in solution were used as model systems for Lewis complexes on surfaces (Table I). In noncoordinating solvents aluminum trichloride exists as a dimer with two bridging chlorines. In the presence of electron-donating ligands such as phosphines, amines, or ethers, the aluminum dimer is easily cleaved, resulting in a 1:1 Lewis acid-base complex.⁴⁶ A 1:1 complex of a phosphine with aluminum isopropoxide would have provided a closer model of surface aluminum by having oxygen atoms in the first coordination sphere of the aluminum, but attempts to synthesize such a species failed. Apparently the phosphine Lewis basicity is too weak to cause dissociation of the tetramer.⁵¹⁻⁵³

The identification of the phosphine-aluminum trichloride complexes in solution was supported by the observation of ^{27}Al - ^{31}P J coupling. In toluene solution the ^{31}P resonances of the 1:1 triethylphosphine-aluminum trichloride complex appears as a sextuplet with a ^{27}Al - ^{31}P coupling of 250 Hz. Others have observed phosphine-aluminum trichloride complexes in solution via ^{27}Al NMR and have observed the aluminum resonance as a doublet due to J coupling to the ^{31}P , with coupling constants similar to the value we have observed.⁴⁵ In HY zeolite and an AlCl_3 /TMP model system several R_3P ^{31}P peaks attributed to Lewis complexes have been observed between -58 and -32 ppm.⁴³

Results for Phosphines on Amorphous Materials

Spectroscopic studies of surface adsorption customarily include calculations of fractional surface coverage. Our samples include a surface-coverage range of 5-150%, estimated on the basis of the diameters of molecular models. These estimates can vary considerably, depending on the assumed molecular shapes, but our conclusions regarding the assay of specific sites do not depend on this coverage calculation.

Vastly different intensities of the ^{31}P NMR signals from phosphine-derived chemical species on surfaces can be observed by using different NMR pulse modes for generating transverse ^{31}P magnetization. Figure 2 shows the cross polarization and single-pulse spectra for two surface concentrations of TMP on γ -alumina. The cross polarization spectrum reveals the presence

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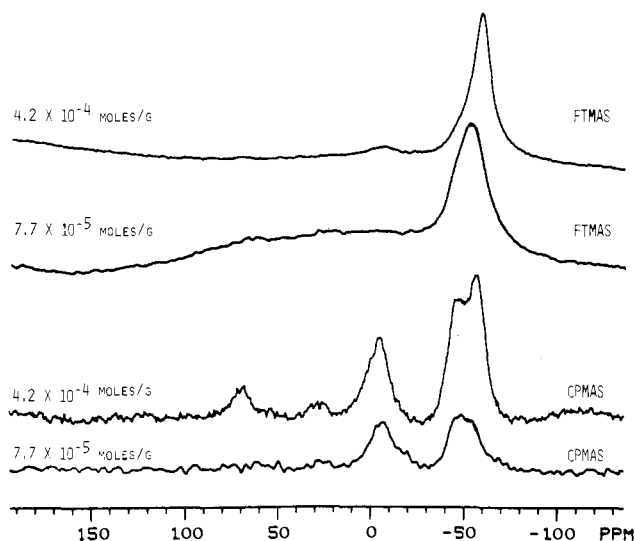


Figure 2. ³¹P MAS single-pulse (FTMAS) and cross polarization (CPMAS) spectra (60.7-MHz) of trimethylphosphine on γ -alumina at different surface coverages (as indicated).

Table II. ³¹P T_1 Values for P(C₂H₅)₃ on Silica-Alumina

site	chem shift (ppm)	T_1^a (s)
Low Coverage, 0.67 mmol·g ⁻¹		
Brønsted	20	0.026
Lewis	-17	0.014
physisorbed	-21	0.023
High Coverage, 4.9 mmol·g ⁻¹		
Brønsted	18	0.096
Lewis	not resolved	
physisorbed	-21	0.064

^a Estimated error, $\pm 10\%$.

of Brønsted (-4 ppm) and Lewis (-48 ppm) complexes in addition to physisorbed phosphine (-58 ppm). At short (<1 ms) contact time the cross polarization experiment discriminates strongly against the physisorbed species relative to the more tightly bound acid-base complexes. The physisorbed phosphine peak grows relative to the other peaks at longer contact times (up to 10 ms) but does not achieve the full relative intensity seen in the single-pulse spectra. Lunsford et al.⁴³ examined this system only in the single-pulse mode and, therefore, detected only the large physisorbed phosphine signal. Clearly the spectra obtained from single-pulse experiments should reflect more straightforwardly the true populations of the different species on the surface if proper attention is paid to spin-lattice relaxation considerations.

The results of ³¹P T_1 measurements obtained from inversion recovery experiments⁵⁴ for high and low surface coverages of TEP are shown in Table II. The longest T_1 value measured is approximately 0.1 s. Hence, for quantitative analysis, a 0.5–1 s repetition time is an excellent choice for efficient data averaging. The efficiency of the ³¹P spin-lattice relaxation of a phosphine on this surface is probably due to paramagnetic impurities in the silica-alumina sample. The phosphorus T_1 value measured for TEP on polystyrene sulfonic acid resin is 1.3 s, more than 10-fold longer than for the silica-alumina surface species. For TEP dissolved in chloroform the ³¹P T_1 value is 10 s.⁵⁵ Typical ³¹P spectra for TEP adsorbed on silica-alumina are presented in Figure 3. The Brønsted-bound phosphines on silica-alumina have been identified by chemical shift analogy to model systems. Furthermore, a spectrum identical with that shown in Figure 1C for a proton-coupled single-pulse spectrum can be obtained for all phosphines studied; this kind of pattern indicates an isolated ¹H-³¹P spin pair, as observed for resin-bound phosphines. TMP

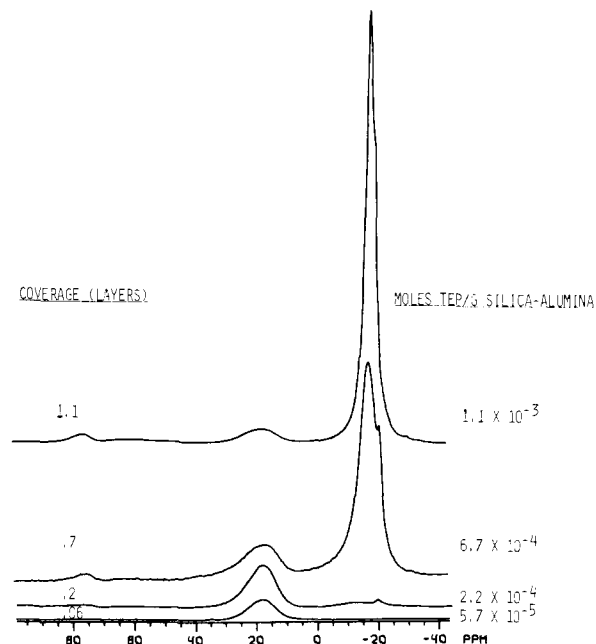


Figure 3. ³¹P single-pulse MAS NMR spectra (60.7-MHz) of triethylphosphine on silica-alumina at different surface coverages (as indicated).

on silica-alumina additionally shows ³¹P-¹H J coupling of 490 Hz.

At higher TEP concentration, two additional resonances are observed, which differ from each other in chemical shift by a few ppm (Figure 3 and Table I). The lower shielding of these two resonances is assigned to Lewis complexed phosphines, since its chemical shift corresponds most closely to the Lewis acid-base phosphine complex observed in model compound studies. The higher shielding resonance is assigned to physisorbed phosphines. The chemical shift of this peak agrees with the chemical shift reported for solution studies of TEP. We studied TEP adsorbed on silica gel and found that only one resonance was observed, with the same chemical shift reported for TEP in solution. This confirms our expectation that phosphines adsorbed on silica gel exist only as physisorbed species.

Because the ³¹P chemical shift differences observed between the Lewis bound and free phosphines for model compounds and for phosphines on silica-alumina were relatively small, additional assignment approaches were required. We found no evidence for ²⁷Al-³¹P J coupling of phosphines on silica-alumina by ³¹P NMR, presumably because of the very short ²⁷Al T_1 (<1 ms). The 94-MHz ²⁷Al MAS spectra for silica-alumina samples on which phosphines are adsorbed (not given here) show no additional resonances for Lewis acid-base adducts in addition to the normally observed resonances of tetrahedral and octahedral aluminum sites. We studied the field dependence of the ³¹P resonances tentatively assigned to Lewis bound phosphines. Because of the small ²⁷Al-³¹P internuclear distance associated with the direct aluminum-phosphorus covalent bond of Lewis site adducts, the quadrupole moment of the ²⁷Al can be expected to interfere with the efficiency of MAS averaging of the ³¹P-²⁷Al dipolar interaction and, therefore, influence the ³¹P MAS resonance. This effect has been well-studied in ¹⁴N-¹³C systems^{56–70} and should diminish with

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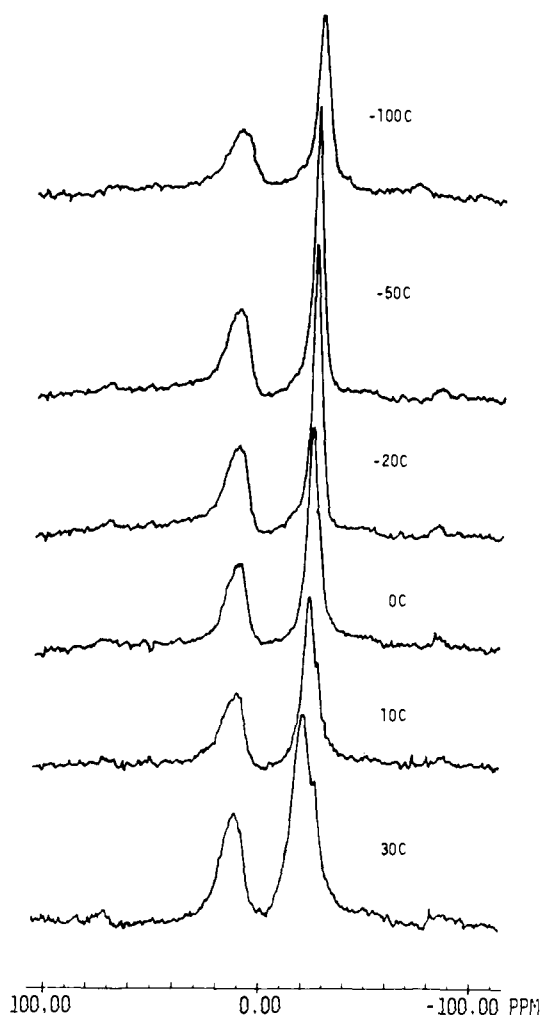


Figure 4. Variable-temperature single-pulse ^{31}P MAS NMR spectra (36.5-MHz) of triethylphosphine on silica-alumina.

increasing magnetic field strength, because the Zeeman interaction in the ^{27}Al spin system dominates more strongly the quadrupole interaction at higher field. This should lead to a decrease in ^{31}P line width (in ppm) at higher static field.

The sample used in these two-field experiments contained 6.0×10^{-4} mol TEP/g silica-alumina (Figure 3). The widths of the two high shielding, partially overlapping ^{31}P signals that we tentatively assigned to Lewis site phosphines and physisorbed phosphine species were extracted by using the Nicolet program "NTCCAP" by optimizing the visual fit to the experimental spectrum. A mixture of Lorentzian and Gaussian character provided a marginally better fit to the spectrum than did either pure line shape, but the presence of chemical shift dispersion and the quadrupole perturbation makes the detailed line shape unpredictable. The results were confirmed by resolving the overlapping peaks with pen and ruler on an expanded plot and measuring the

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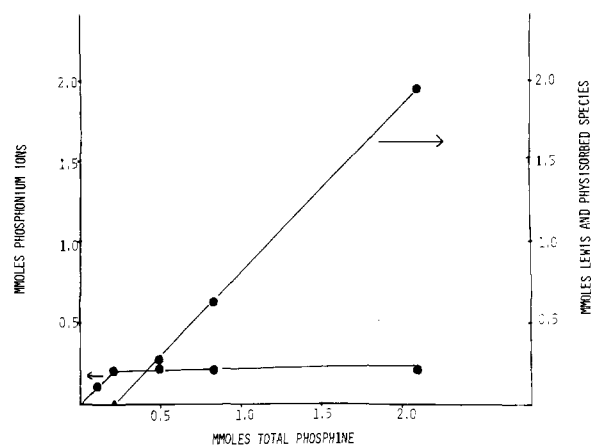


Figure 5. Experimental phosphine titration curve of trimethylphosphine on silica-alumina.

widths directly. The results of this analysis show that the two peaks tentatively assigned to the Brønsted complex and physisorbed species (least shielded and most shielded, respectively) in Figure 3 have line widths that remain essentially constant at 11.2 and 1.1 ppm, respectively, suggesting that these line widths are determined by chemical shift dispersion (chemical structure heterogeneity). The resonance assigned tentatively to the Lewis acid-base adduct narrows from a width of 7.6 ppm at 3.52 T to 5.2 ppm at 8.45 T, indicating that the line width in this case is determined by a combination of chemical shift dispersion and the ^{27}Al quadrupolar perturbation of MAS averaging of the ^{27}Al - ^{31}P dipolar interaction.

Chemical shift heterogeneity of sites has been confirmed by T_2 measurements, carried out by using a Hahn spin-echo method synchronized with the sample spinning.⁷¹ The ^{31}P T_2 values measured for a 10^{-4} mol TMP/g silica-alumina are 5 ms for the Brønsted acid site and 0.7 ms for the Lewis acid site. Based on the T_2 measurement for the Brønsted site, a 60-Hz line width would be predicted, but the observed line width is 300 Hz (5 ppm). For the Lewis site a 500-Hz line width is predicted, but a line width of 900 Hz (15 ppm) is observed.

Figure 4 shows the variable-temperature ^{31}P NMR spectra of a 6.0×10^{-4} mol TEP/g silica-alumina sample over a temperature range from 30 to -100 °C. The middle resonance (-15 ppm) gradually narrows and moves to higher shielding as the temperature decreases, but at the lowest temperature the peak broadens significantly. The peak due to the Brønsted complex in such spectra reveals no significant temperature dependence of width or position. There is no evidence for exchange broadening of the peaks at room temperature.

Discussion

The solution-phase $\text{p}K_a$'s for the three trialkylphosphine conjugate acids range in value from 8.69 to 8.43.⁷² These phosphines are an order of magnitude less basic than trimethylamine and two orders of magnitude less basic than monoalkyl amines toward H_3O^+ (aqueous) but roughly three orders of magnitude stronger bases than pyridine. The similarity of the $\text{p}K_a$ values is a significant characteristic of the three phosphines employed in the present study. The absence of dramatic intramolecular steric or electronic effects of the alkyl groups on the solution-phase basicities of these phosphines has allowed us to study differences among these probe molecules in Brønsted basicity toward silica-alumina arising from differences in steric interactions of the alkyl groups with the local surface topography or in high-coverage samples with adjacent probe molecules.

Plots of the populations of the various phosphine environments (i.e., Brønsted or Lewis acid-base complex or van der Waals "complex") as a function of total phosphine content yield a set of "titration" curves. Depending on the relative thermodynamics

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of the phosphines' interaction with the various surface sites on silica-alumina, three limiting types of "titration" curves can be envisioned. In the most informative case, the equilibrium constants of the phosphine protonation reaction, the reaction of the phosphine with surface Lewis sites and the van der Waals interaction of the phosphine on the surface are substantially different from one another and allow the observation of titration "break points" for Brønsted bound and Lewis bound phosphines as each type of site becomes saturated. In a less straightforward case, the binding constants corresponding to various weakly bound R₃P species are comparable and ³¹P peaks from these species will appear simultaneously after the stronger binding sites are saturated. This results in a breakpoint only for the most strongly bound (in this case, Brønsted) species. If all binding constants are comparable, all three species will be present at all R₃P concentrations, and the surface binding sites cannot be assayed by inspection of the titration curve.

Figure 5 shows the ³¹P NMR titration curve for TMP adsorbed on silica-alumina, by using populations derived from relative peak areas and total phosphorus content determined by elemental analysis. Since there was no loading level at which peaks arising from only Brønsted and Lewis complexes were observed, the data (Figure 5) fit the second case discussed above, allowing an assay only of Brønsted acid sites accessible to each probe. The endpoints occur at 0.23, 0.19, and 0.15 mmol H⁺ per g of silica-alumina for TMP, TEP, and TBP, respectively, and may be compared with the ¹⁵N NMR results from a study of pyridine/*n*-butylamine competition for Brønsted sites on the same silica-alumina used in this study, namely 0.17 mmol H⁺/g.⁴⁰ Some combination of several mechanisms could account for this observed variation. It is possible that some of the Brønsted sites may be situated at the bottom of crevices and are accessible only to small probe molecules. Such topographically determined differences could also contribute to the chemical shift heterogeneity in both Lewis and Brønsted complexes, as demonstrated by T₂ experiments. Secondly, the Brønsted sites may be clustered on flat surfaces in such a way that larger probe molecules interfere with one another, preventing a complete assay of sites. A third possibility is that the three probes differ sufficiently in basicity toward surface Brønsted sites that the "apparent" Brønsted titration end-points, namely the appearance of peaks due to Lewis complex and physisorbed species, are not at the true equivalence points, and thus represent only a lower limit to the concentrations of Brønsted sites. This explanation is not supported by the solution-phase pK_a values, which are quite similar to each other; but these solution-phase numbers may not faithfully reflect the relative base strengths in surface systems. The present data cannot distinguish among these possibilities; and since they are not mutually exclusive, it may be impossible to isolate such effects for an amorphous material. In any case, the apparent discrepancy in assaying Brønsted sites highlights the need for using more than one type of probe molecule to characterize surfaces with specific binding sites.

Only TMP on silica-alumina exhibits resolved J_{PH} coupling. J_{PH} splitting will appear only when both chemical exchange and spin diffusion are much slower than about 300 μs (J_{PH}⁻¹). The spin diffusion rate between the acidic proton and the alkyl group protons is sensitive both to the number of short H-H internuclear vectors and to motional averaging of the dipolar coupling. Therefore, rapid methyl rotation may result in slower H-H spin diffusion in TMPH⁺ than in TEPH⁺ and TBPH⁺. In the case of resin bound phosphine the J_{PH} may be averaged either by chemical exchange or by spin diffusion between the acidic proton and the hydrogen atoms of the polystyrene framework.

A straightforward ³¹P NMR titration of Lewis acid sites with a phosphine probe is not possible because of the insufficient resolution of the chemical shifts and binding constants of Lewis acid-base complexes and the physisorbed species. The limitation of chemical shift resolution might be overcome by suitable resolution enhancement techniques or by choosing a different probe molecule, but in the present examples the similarity in binding strengths at the two types of sites ensures that there is no spectral event that signals the saturation of Lewis acid sites. Since there

is a dispersion in ³¹P chemical shifts within the Lewis acid-base manifold, there is almost certainly a range of binding constants for the various environments of Lewis sites. These considerations appear to preclude a quantitative analysis of the Lewis sites by the present method.

For quantitative analysis of surface sites by probe molecules it is necessary to confirm that exchange processes do not affect the integration of the spectrum. Our initial observation of three distinct chemical shifts of phosphines on silica-alumina indicated that the surface-bound phosphine species are not in fast exchange with one another. Earlier studies of pyridine on silica-alumina have shown that various pyridine species at the surface (Brønsted, Lewis, physisorbed) are in fast exchange with one another on the NMR time scale (determined by ¹³C and ¹⁵N chemical shifts) at room temperature,³⁹ while separate peaks corresponding to individual surface species can be observed at low temperatures.⁷³ From the study of the behavior of the ³¹P peaks of phosphines as a function of temperature (Figure 4), it is clear that the surface bound molecules are in slow exchange with one another. The only change observed as a function of temperature is the gradual narrowing and movement to higher shielding of the Lewis bound phosphine as the temperature is lowered.

The effects of changing temperature on the spectrum of TEP adsorbed on silica-alumina (Figure 4) may arise from two distinct sources. The T₂ experiment clearly indicates a distribution in chemical shifts of Lewis bound phosphines in various local environments. Presumably these various sites have different binding strengths, and ΔH° for the process of transferring a phosphine from one binding site to another with a different chemical shift will not in general be zero. The population distribution within the Lewis complex manifold thus depends on the temperature. The temperature effect in Figure 4 could arise if the transfer of phosphine from a Lewis site with low ³¹P shielding to one with higher ³¹P shielding were associated with a negative ΔH°.

A more reasonable explanation is based on assuming a temperature dependence of the ³¹P chemical shifts of the individual sites. If the strongest Lewis acid-base complexes from a thermodynamic view have the greatest increase in shielding as the temperature decreases, then the manifold corresponding to the Lewis complex would narrow as the shifts of the most tightly bound phosphines approach those of the most weakly bound. At sufficiently low temperatures the ³¹P of the most tightly bound complexes would be more shielded than for the most weakly bound, and the peak would broaden. Figure 4 illustrates just this type of pattern. It is plausible that the Lewis complex would exhibit the largest temperature dependence of the ³¹P chemical shift of all the phosphine species on the surface, because a weak bond between two fairly massive nuclei should give a set of closely spaced vibrational energy levels, with Boltzmann populations that vary significantly over the temperature range covered. This explanation must be considered speculation in the absence of variable-temperature NMR data on well-defined model compounds in the solid state. A population change may be superimposed upon the temperature dependence of the chemical shifts, but our present results do not permit a separation of these effects.

As the total TEP concentration is increased, the room temperature ³¹P peak assigned to the Lewis acid-base complex gradually narrows and moves to increased shielding (Figure 3). This behavior may arise from chemical exchange between binding sites or from changing populations within the possible manifold of Lewis complex and the physisorbed species. Similar behaviors were observed for TMP and TBP adsorbed on silica-alumina over a wide range of surface coverages. In an effort to resolve these effects we studied TMP on γ-alumina, which is believed to have a large number of Lewis acid sites on the surface. The CP and single-pulse spectra for two surface concentrations of TMP on γ-alumina are presented in Figure 2. In the single-pulse spectra the broad peak composed of resonances of both Lewis acid-base and physisorbed species behaves as the corresponding peak in the silica-alumina spectra. The CP spectra, in contrast, demonstrate

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the absence of rapid chemical exchange between Lewis acid-base complexed and physisorbed TMP at both loading levels; i.e., distinct peaks are observed, especially for the higher loading sample. Hence, the change in the position of the peak maximum and the apparent peak width in the single-pulse spectra of TMP on γ -alumina must originate in both the distribution of chemical shifts within the Lewis complex manifold and the changing ratio of Lewis complexed to physisorbed phosphines in this system.

Only the Brønsted acid sites of silica-alumina can be assayed quantitatively in the present study, and this result of the Brønsted site assay is dependent upon the choice of probe molecule. A more extensive array of more rigid probes might allow a mapping of the steric constraints of these sites. This would require detailed consideration of the variation in Brønsted binding constants arising from the changes in phosphine substituents and the range of topographical environments on the surface. A more direct approach to characterizing both Brønsted and Lewis sites would be

to search for a probe with greater differences in chemical shift and binding constants among the three types of surface species. Preliminary studies indicate that phosphine oxides may offer such advantages.⁷⁴

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Registry No. TMP, 594-09-2; TEP, 554-70-1; TBP, 998-40-3; SiO₂, 7631-86-9; Al₂O₃, 1344-28-1.

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Orientation and Mobility of a Copper Square-Planar Complex in a Lipid Bilayer[†]

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Abstract: The copper complex 3-ethoxy-2-oxobutylaldehyde bis(*N*⁴,*N*⁴-dimethylthiosemicarbazonato)copper(II), CuKTSM2, a derivative of a potent antitumor drug, has been found to partition favorably into dimyristoylphosphatidylcholine (DMPC) vesicles. An indirect electron spin resonance (ESR) method to study partitioning was developed; by using the spin-label TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl), which partitions similarly between the hydrophobic and hydrophilic phases, it is found that CuKTSM2 broadens only the TEMPO hydrophobic signal thereby establishing the partition of the complex. ESR spectra of oriented membranes show that the complex is well-oriented with the plane of the complex normal to the bilayer surface. At 1 mol %, CuKTSM2 shifts the fluid/gel phase transition temperature by 1.4 °C. By using saturation recovery ESR techniques to measure the effect of bimolecular collisions between Cu complexes and stearic acid spin labels on the spin-lattice relaxation time of the nitroxide moiety, it was found that the translational diffusion constant of the complex lies between 6.0 and 9.5 × 10⁻⁷ cm²/s in the fluid phase. This value is ten times greater than that of the lipids. The saturation recovery method shows that the complex tends to be distributed throughout the bilayer.

The chemical basis for the pharmacological activity of metallo drugs has been a subject of increasing research in recent years.^{2,3} Bis(thiosemicarbazonato)copper complexes constitute one group of copper complexes for which the relationship between structure and biological activity has been examined. They form tetradentate chelates with Cu²⁺ involving two nitrogen and two sulfur donor atoms (Figure 1). The copper complex of 3-ethoxy-2-oxobutylaldehyde bis(thiosemicarbazone) (R₃, R₄ = H), CuKTS, has potent antitumor properties in animals.⁴ The substitution of methyl groups for hydrogens at R₃ and R₄ greatly decreases the toxicity of the complex toward cells.⁵ This is thought to be due to the decrease in rate and extent of reductive dissociation of CuKTSM2 relative to CuKTS which occurs when these structures react with cells.⁵⁻⁷ Replacement of hydrogens by methyl groups lowers the *E*_{1/2} of the Cu(II) complexes from -178 to -283 mV and results in a decrease in the first-order rate constant for re-

ductive dissociation of the bis(thiosemicarbazonato)copper(II) from 8 × 10⁻³ s⁻¹ for CuKTS to 3 × 10⁻⁵ s⁻¹ for CuKTSM2. The methylated structure also has a much larger 1-octanol/H₂O partition coefficient.⁷

The relative stability of CuKTSM2 in Ehrlich tumor cells is seen in the retention of a red hue by the cells, characteristic of

[†] Abbreviations used: DMPC, L- α -dimyristoylphosphatidylcholine; DPPC, L- α -dipalmitoylphosphatidylcholine; DSPC, L- α -distearoylphosphatidylcholine; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; 5-SASL, 5-doxy stearic acid spin label; 16-SASL, 16-doxy stearic acid spin label; CuKTSM2, [3-ethoxy-2-oxobutylaldehyde bis(*N*⁴,*N*⁴-dimethylthiosemicarbazonato)]copper(II); CuKTS, [3-ethoxy-2-oxobutylaldehyde bis(thiosemicarbazonato)]copper(II).

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