Solid-State ^{31}P NMR Study of Phosphonate Binding Sites in Guanidine-Functionalized, Molecular Imprinted Silica Xerogels

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Phosphonate binding sites in guanidine and ammonium surface-functionalized silica xerogels were prepared via the molecular imprinting technique and characterized using solid-state ^{31}P MAS NMR. One-point, two-point, and nonspecific host−guest interactions between phenylphosphonic acid (PPA) and the functionalized gels were distinguished by characteristic chemical shifts of the observed absorption peaks. Using solid-state as well as solution-phase NMR analyses, absorptions observed at 15.5 and 6.5 ppm were identified as resulting from the 1:1 (one-point) and 2:1 (two-point) guanidine to phosphonate interactions, respectively. Similar absorptions were observed with the ammonium functionalized gels. By examining the host−guest interactions within the gels, the efficiency of the molecular imprinting procedure with regard to the functional monomer-to-template interaction could be readily assessed. Template removal followed by substrate adsorption studies conducted on the guanidine-functionalized gels provided a method to evaluate the binding characteristics of the receptor sites to a phosphonate substrate. During these experiments, ^{29}Si and ^{31}P MAS NMR acted as diagnostic monitors to identify structural changes occurring in the gel matrix and at the receptor site from solvent-mediated processes.

Introduction

Over the past several decades molecular imprinting has been used to produce a large variety of materials with selective adsorption properties for specific molecules.[1] Organic polymers and metal-oxide gels have been imprinted with molecular memory for an assortment of compounds, which include alkyl orange dyes,[2−4] stereoisomers of drugs,[5] amino acids,[6] peptides,[7] saccharides,[8] nucleotides,[9] and proteins.[10,11] The imprinting molecule is used as a template to form a receptor site with complementary binding features with regard to the molecule's shape, structure, and spatial orientation of

the peripheral functionality. These imprinted materials have exhibited efficacy as sensor materials,[12−14] supports for chromatographic separations,[15,16] and materials with catalytic properties.[17]

The molecular selectivity observed with these materials, either in separations or catalytic activity, strongly suggests the formation of receptor sites in the solid matrix. This large body of work involving the molecular recognition phenomenon of imprinted materials, however, only indirectly characterizes the receptor site. Spectroscopic analysis of host−guest interactions in the imprinted materials could provide direct evidence on the formation of the receptor site upon the imprinting step and reveal how the site performs during adsorption and desorption of guest molecules. Such information would be useful in understanding the characteristics of the material and aid in further chemical modifications in pursuit of enhanced performance.

Reports on the spectroscopic characterization of the receptor sites in imprinted materials has been scarce due to the difficulty of identifying and isolating weak signals from low concentrations of receptor sites in the

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(1) For comprehensive reviews on molecular imprinting, see: (a) *Molecular and Ionic Recognition with Imprinted Polymers*, 703rd ed.; Bartsch, R. A., Maeda, M., Eds.; American Chemical Society: Washington, DC, 1998. (b) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166. (c) Steinke, J.; Sherrington, D. C.; Dunkin, I. R. *Adv. Polym. Sci.* **1995**, *123*, 81. (d) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812.
(2) Dickey, F. H. *J. Phys. Chem.* **1955**, *59*, 695.
(3) Reed, G. H.; Rogers, L. B. *Anal. Chem.* **1965**, *37*, 861.
(4) Majors, R. E.; Rogers, L. B. *Anal. Chem.* **1969**, *41*, 1058.
(5) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111.
(6) Sellergren, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853.
(7) Ranström, O.; Nicholls, I. A.; Mosbach, K. *Tetrahedron: Asymmetry* **1994**, *5*, 649.
(8) Wulff, G.; Kemmerer, R.; Vietmeier, J.; Poll, H.-G. *Nouv. J. Chem.* **1982**, *6*, 681.
(9) Spivak, D. A.; Shea, K. J. *Macromolecules* **1998**, *31*, 2160.
(10) Burow, M.; Minoura, N. *Biochem. Biophys. Res. Comm.* **1996**, *227*, 419.
(11) Glad, M.; Norrlöw, O.; Sellergren, B.; Siegbahn, N.; Mosbach, K. *J. Chromatogr.* **1985**, *347*, 11.

(12) Piletsky, S. A.; Piletskaya, E. V.; Panasyuk, T. L.; El'skaya, A. V.; Levi, R.; Karube, I.; Wulff, G. *Macromolecules* **1998**, *31*, 2137.
(13) Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. *Anal. Chem.* **1998**, *70*, 2025.
(14) Hedborg, E.; Winquist, F.; Lundström, I.; Andersson, L. I.; Mosbach, K. *Sensors Actuators A* **1993**, *37−38*, 796.
(15) Schweitz, L.; Andersson, L. I.; Nilsson, S. *Anal. Chem.* **1997**, *69*, 1179.
(16) Plunkett, S. D.; Arnold, F. H. *J. Chromatogr. A* **1995**, *708*, 19.
(17) Morihara, K.; Kurihara, S.; Suzuki, J. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3991.

materials. In one study, performed on imprinted cross-linked styrene/divinylbenzene copolymers, spectroscopic analysis of the binding of diketone templates to diol-functionalized receptor sites were examined with FTIR and ^{13}C CP/MAS NMR.[18] These kinds of spectroscopic measurements both identify and quantify specific host–guest interactions within the matrix. The low concentration of receptor sites (~1 mol %) necessitated ^{13}C labeling of the template and substrates to obtain good signal-to-noise ratios in the NMR experiment. Receptor sites with two-point and one-point binding could be readily distinguished and quantified. Such spectroscopic results demonstrate the importance of understanding various binding interactions that can occur in polymeric structures and the roles they play in the molecular recognition event. Additionally, the data can reveal structural changes that occur in the material during processing steps and their effects on the receptor site.

In this report, we have also employed solid-state NMR to define receptor sites formed by a surface-imprinting procedure on silica sol–gel materials. The NMR data also was used to monitor changes that occur at the binding site due to alteration of material structure from template cleavage and substrate adsorption procedures. In contrast to the reference described above, the materials under consideration are inorganic metal oxides and the host–guest interactions are noncovalent. Furthermore, the template and substrate are phosphorus based, eliminating the need for isotopic labeling since ^{31}P is in 100% natural abundance. Surface-imprinted silica xerogels were prepared using phenylphosphonic acid (PPA) as a template to position guanidine-silanes on a silica matrix creating receptor sites for the selective binding of phosphates and phosphonates. In previous work[19] with these materials, enhanced selectivity was realized when compared to nonimprinted materials. The observed selectivity was believed to be due to the specific positioning of the guanidines that allowed efficient two-point binding between guanidines and PPA. Direct observation of the one-point vs two-point host–guest interactions in the gel should provide some insight into the efficiency of the imprinting process and performance of the receptor sites.

Experimental Section

General. Tetraethoxysilane (TEOS), 3-trimethoxysilylpropyl-1-trimethylammonium chloride, and 3-trimethoxysilylpropyl-1-amine were obtained from Gelest. Compound **1**, 1-trimethoxysilylpropy1–3-guanidinium chloride, was prepared as previously described[19] and stored in dry methanol. Phenylphosphonic acid (Aldrich) was recrystallized twice from hot 2% ethanol/benzene solution. Water was purified through a Barnstead Type D4700 NANOpure Analytical Deionization apparatus to a resistivity of 18 MΩ. All other solvents were of reagent grade from Fisher Scientific.

Phosphonate Salts. Sodium salts of phenylphosphonic acid were prepared through the addition of either 1 or 2 equiv of NaOH to produce Na–PPA and Na$_2$–PPA, respectively, followed by concentration of the solution, isolation of the crystalline salt, and recrystallizations in aqueous ethanol. For example, Na–PPA was formed by adding 0.5 mL of a 2.5 M

aqueous solution of NaOH to a pH 2.0 solution of phenylphosphonic acid (200 mg) in 20 mL of deionized water, yielding a resultant pH of 3.5. The solution was evaporated to dryness to yield a white powder that was then recrystallized thrice in hot 10% aqueous ethanol to yield colorless needles. Elemental analysis: Calcd for $C_6H_6O_3PNa$: C, 40.02; H, 3.36; Na, 12.77; P, 17.20. Found: C, 40.07; H, 3.40; Na, 12.77; P, 17.58. Na$_2$–PPA was prepared similarly using 1.0 mL of the 2.5 M NaOH solution to give a PPA solution pH of 7.8,[20] and recrystallization of the isolated solid with hot 5% aqueous ethanol. White, platelike crystals were isolated. Calcd for $C_6H_5O_3PNa_2$: C, 35.67; H, 2.49; Na, 22.76; P, 15.33. Found: C, 35.21; H, 2.63; Na, 21.15; P, 15.58.

Adsorption of the PPA sodium salts to blank xerogels was accomplished by suspending the xerogel particles (75–200 μm) in aqueous solution of the salts, followed by evaporation of the water then drying under vacuum at 60 °C overnight.

Molecular Imprinting in Xerogel.[19] An ethanol solution of TEOS (610 mL, 50% w/w) was stirred in a 1-L vessel followed by addition of water (24.5 mL) and 1 N aqueous HCl (1 mL). The mixture was heated to 60 °C for 1.5 h with stirring and then allowed to cool to room temperature, producing the homogeneous sol. Gelation of the sol was accomplished by adding 0.1 M aqueous NH$_4$OH (9.1 mL) and aging the closed container at 50 °C for 1 day. The resultant gel was crushed, washed twice with ethanol, collected by filtration, and then further aged in fresh ethanol at 50 °C overnight. The gel was collected and placed in a solution of 100 mL of ethanol containing PPA (4.0 mmol for the 2:1 gel, or 8.0 mmol for the 1:1 gel) and either **1** (8.0 mmol) or 3-trimethoxysilylpropyl-1-trimethylammonium chloride (8.0 mmol) with incubation for another day at 50 °C in a closed container. Following this time the container was opened and the solvent allowed to evaporate away at 50 °C over a period of 12 h. Nonfunctionalized (blank) and randomly functionalized xerogels were prepared identically, with the exclusion of functionalized silanes and PPA in the former and PPA in the latter. The finished xerogel was then crushed and sized to 75–200 μm size particles, washed with ethanol, and dried under vacuum at 60 °C for a day.

Phosphonate Adsorption Studies. Xerogel samples (200 mg) of imprinted and control materials (nonfunctionalized or randomly functionalized) were loaded with additional PPA by soaking the xerogels in ethanol solutions (2 mL) of PPA for 10 min, followed by evaporation of solvents under reduced pressure with a rotary evaporator at 40–50 °C.

Removal of the PPA template was achieved by washing the imprinted gels with acidic aqueous solution at room temperature using the following procedure: The imprinted material (200 mg) was stirred in aqueous 1 N HCl (30 mL) with an orbital shaker for 1 h. The gel was then collected on a glass frit Buchner funnel and washed once with aqueous 1 N HCl (30 mL), followed by water (30 mL). The procedure was repeated to effect complete removal of PPA. After drying in a vacuum (25 in Hg) at 60 °C overnight, the material was analyzed by NMR and carried onto the rebinding experiments.

Rebinding of PPA was conducted in an aqueous solution adjusted to pH 7 with dilute NaOH solution. The total molar quantity of substrate in solution was always maintained at greater than 10 times the nominal loading of guanidine on the gel. The solution was swirled overnight using an orbit shaker at room temperature and then collected in a Buchner funnel followed by drying overnight under vacuum at 60 °C.

NMR Analyses. Solutions of **1** (1.0 M) and PPA (10 mM) were prepared in dry ethanol. The high-resolution solution-phase NMR experiments were performed on a Bruker AMX 400 at a resonant frequency of 161.99 MHz using a 5 mm bb probe, with 1K to 8K scan averages with a 9 μs π/2 pulse and ^{1}H decoupling using a WALTZ-16 pulse sequence. The chemical shift was referenced to external phosphoric acid (δ = 0.0 ppm).

(18) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1991**, *113*, 4109.

(19) Sasaki, D. Y.; Rush, D. J.; Daitch, C. E.; Alam, T. M.; Assink, R. A.; Ashley, C. S.; Brinker, C. J.; Shea, K. J. *Molecular and Ionic Recognition with Imprinted Polymers*, 703rd ed.; Bartsch, R. A., Maeda, M., Eds.; American Chemical Society: Washington, DC, 1998; p 314.

(20) Nagarajan, K.; Shelly, K. P.; Perkins, R. R.; Stewart, R. *Can. J. Chem.* **1987**, *65*, 1729.

Solid-state magic angle spinning (MAS) ^{31}P NMR spectra of the prepared materials were obtained on a Bruker AMX 400 at a resonant frequency of 161.99 MHz using a 4 mm bb MAS probe and a 12.5 kHz spinning speed. The reference was set to external $(NH_4)H_2PO_4$ ($\delta = 0.8$ ppm with respect to external phosphoric acid). Single pulse Bloch decays with high power decoupling, 20-s recycle delays, and 128−1024 scan averages were employed. For materials where duplicate samples were prepared for an experiment, a ≤10% deviation in peak area was typically found. MAS peak intensities were obtained from full spectral simulation of the spinning sideband manifold resulting from the nonzero chemical shift anisotropy, including area of isotropic resonance and all associated spinning sidebands.
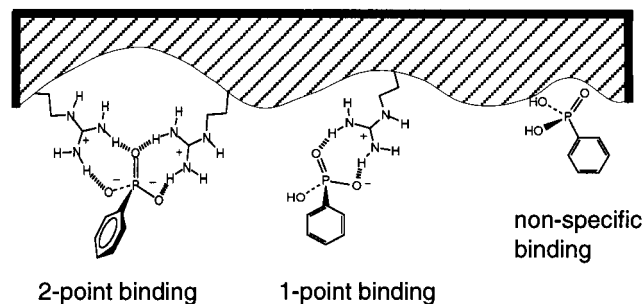
For the variable-temperature solid-state ^{31}P NMR experiments, spin−lattice relaxation times (T_{1Z}) were evaluated using a standard inversion−recovery pulse sequence, with 40 time increments, spinning at 10 kHz. The ^{31}P spin−lattice relaxation (T_{1Z}) behaviors of the 15.5 and 6.5 ppm resonances in the 2:1 **1**: PPA gel and of PPA in a nonimprinted silica gel are reported in Table 1S (Supporting Information). From the temperature dependence of T_{1Z} an apparent energy of activation (E_a) for the motional process responsible was also evaluated and is given in Table 1S.

Solid-state ^{29}Si MAS NMR spectra were obtained on a Bruker AMX 400 at 79.5 MHz using a 7 mm MAS BB probe spinning at 4 Hz. The Q_n distribution[33] were obtained using a single pulse decay, with 240-s recycle delay and 64−256 scan averages. MAS was performed under air to reduce the spin−lattice relaxation of the [29]Si nucleus.

Results

Molecular imprinting is the efficient assembly, or coordination, of functionalized monomers in a specific pattern about a template and the fixation of these functionality in two- or three-dimensional space. The number of interaction points between the template and the formed receptor site can be numerous, although in most cases just a few are considered dominant. In the present study, the dominant host−guest interaction exists between the cationic, hydrogen bond donating guanidine group and the anionic, hydrogen bond accepting phosphonate. This interaction is reminiscent of the guanidine−phosphate interaction found in many biological systems.[21−23]

^{31}P NMR has proven to be a valuable diagnostic tool to monitor the protonation state and coordination of phosphates and phosphonates in solution and solid-phase systems. In studies of extracellular pH of rabbit bladder, phenylphosphonic acid (PPA) was shown to be

(21) Crane, B. R.; Siegel, L. M.; Getzoff, E. D. *Science* **1995**, *270*, 59.

(22) Sprang, S. R.; Withers, S. G.; Goldsmith, E. J.; Fletterick, R. J.; Madsen, N. B. *Science* **1991**, *254*, 1367.

(23) Cho, Y.; Gorina, S.; Jeffrey, P. D.; Pavletich, N. P. *Science* **1994**, *265*, 346.

(24) Fisher, M. J.; Dillon, P. F. *Circ. Res.* **1987**, *60*, 472.

(25) Chu, F.; Flatt, L. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 4194.

(26) Kneeland, D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042.

(27) Král, V.; Sessler, J. L.; Furuta, H. *J. Am. Chem. Soc.* **1992**, *114*, 8704.

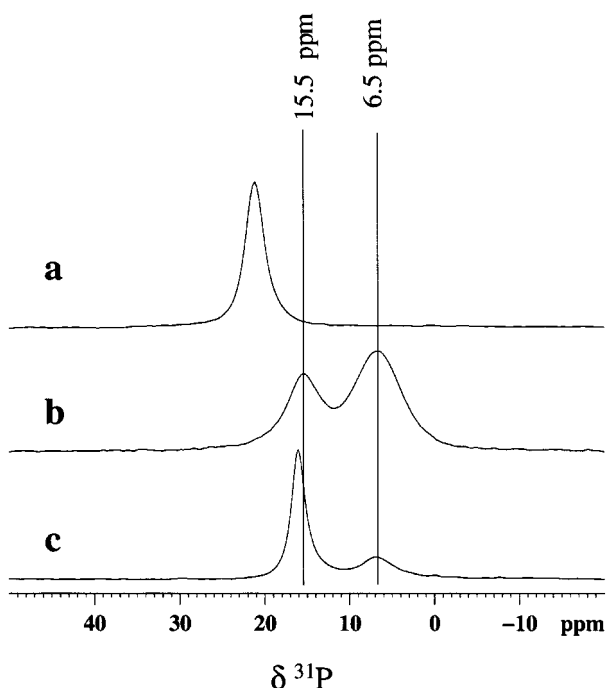(28) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896.

(29) Lukes, I.; Borbaruah, M.; Quin, L. D. *J. Am. Chem. Soc.* **1994**, *116*, 1737.

(30) Carlino, S.; Hudson, M. J.; Husain, S. W.; Knowles, J. A. *Solid State Ionics* **1996**, *84*, 117.

(31) Hall, N. F.; Sprinkle, M. R. *J. Am. Chem. Soc.* **1932**, *54*, 3469.

(32) Cotton, F. A.; Day, V. W.; Hazen Jr., E. E.; Larsen, S.; Wong, S. T. K. *J. Am. Chem. Soc.* **1974**, *96*, 4471.

(33) $Q_n = Si(OSi)_n(OR)_{4-n}$, where R = H, alkyl, aryl.

Figure 1. Schematic of the various binding modes of PPA to a guanidine functionalized xerogel surface.

an excellent pH-sensitive probe exhibiting a chemical shift from 13.6 to 12.0 ppm with changing pH from 6.3 to 7.8.[24] Binding of adenosine triphosphate (ATP) and dibenzyl phosphate guests to synthetic amine and guanidine functionalized hosts in solution phase have also been monitored with ^{31}P NMR solution-phase studies.[25−28] Host−guest complexation typically produces an upfield shift of a few parts per million. On solid surfaces, PPA has been reported to covalently link to silica gel.[29] The surface bound PPA has a ^{31}P NMR chemical shift of 10.4 ppm. On alumina, PPA can be found in multiple states coordinated with the matrix with distinct chemical shifts.[30]

Phosphonate substrate binding to imprinted gels can occur through two-point and one-point binding interactions with the guanidine functionality. Nonspecific adsorption could occur as well with the gel surface. Figure 1 illustrates these various binding interactions of PPA to the functionalized gel. Under the pH conditions of these experiments the guanidine groups will remain as guanidinium cations ($pK_a = 13.6$).[31] Ionic interactions will foster long-range attraction between PPA and the guanidine groups. Shorter range hydrogen-bonding interactions may contribute to more specific phosphonate−guanidine pairing.[32] Two-point binding of PPA to the gel should occur through the dianionic form of the phosphonate, whereas for the one-point mode the monoanionic form of PPA will be bound. Nonspecifically adsorbed PPA could be bound in the fully protonated form or as various salt species depending on its environment on the gel matrix.

In the following sections, we will first describe the preparation of imprinted silica sol−gel materials and the solid-state ^{31}P NMR of those gels in their initial state, after template removal, and following uptake of PPA ligand. We will then attempt to associate the spectral peaks with specific binding modes of PPA to the imprinted gels through solid-state and solution-phase NMR experiments.

Imprinted Gels: PPA Removal and Rebinding. The molecularly imprinted TEOS xerogels were prepared as described previously.[19] The functionalized monomers, 3-trimethoxysilylpropyl-1-guanidinium chloride (**1**) and 3-trimethoxysilylpropyl-1-trimethylammonium chloride (**2**), were covalently bound to a silica xerogel matrix in the presence of the imprinting molecule phenylphosphonic acid. The molar loading of guanidine or ammonium monomer on the silica xerogel was 0.6 mmol/g.

Figure 2 shows a display of stacked solid-state ^{31}P NMR spectra of crystalline PPA (spectrum a) and silica

Figure 2. Solid-state ^{31}P MAS NMR of crystalline PPA (a) and silica xerogels imprinted with a 2:1 (b) and 1:1 (c) ratios of **1** and PPA.

Table 1. Relative Solid-State ^{31}P NMR Peak Areas from Guanidine-Functionalized Xerogels Imprinted with PPA

Imprint Ratio **1**:PPA	relative % peak areas[a]	
	15.5 ppm	6.5 ppm
2:1	30	70
1:1	69	31

[a] Separately prepared samples varied in peak area by ≤ 10%.

xerogels functionalized with **1** and templated with PPA at a 2:1 (spectrum b) and 1:1 (spectrum c) molar ratio. Crystalline PPA has one resonance at 21.0 ppm, identical to that found in the literature.[30] For the imprinted gels, PPA yields two peaks at the chemical shifts of 15.5 and 6.5 ppm. At a 2:1 ratio of **1** to PPA, the integration of these peaks shows a relative area of 70% for the peak at 6.5 ppm and 30% for the other at 15.5 ppm (Table 1). At a 1:1 ratio, the peak at 15.5 ppm becomes dominant with a relative percentage of 69% and 31% for the adsorption at 6.5 ppm.

Affinity studies with PPA were performed on the gels imprinted with a 2:1 ratio of **1** to PPA. The gels were characterized by solid-state ^{31}P NMR prior to and after removal of PPA and then following PPA readsorption. Removal of the PPA template was achieved by soaking the imprinted gels in a 1 N aqueous HCl solution at room temperature for 1 h. Approximately 93% of the template was extracted in this first washing as determined by corroborating evidence from Bartlett phosphate analysis, UV spectroscopy, and solid-state ^{31}P NMR.[19] The remaining PPA gave well-resolved absorption peaks with the same chemical shifts of 15.5 and 6.5 ppm, similar to spectra in Figure 2, with the peak area ratio favoring the complex associated with the 6.5 ppm peak (Table 2).

As a comparison, imprinted gels were prepared using the trimethylammonium monomer **2** to examine PPA complexation behavior through ionic interaction alone.

Table 2. Solid-State ^{31}P NMR Analysis of PPA Imprinted Guanidine and Ammonium-Functionalized Gels before and after Template Removal[a]

	rel. % peak area		rel. % bound PPA
	16−15 ppm	8−6 ppm	
	Guanidine Gels		
initial[b]	36	64	100
after wash	18	82	7
	Ammonium Gels		
initial	74	26	100
after wash	52	48	3

[a] After first acid wash in aqueous 1 N HCl, 1 h, room temperature. [b] Prepared separately from that reported in Table 1 and 4.

Table 3. Readsorption Studies of PPA to Guanidine-Functionalized Imprinted Gels

[PPA]	rel. % peak area		rel. % bound PPA
	12 ppm	5 ppm	
10^{-4} M	82	18	11
10^{-3} M	87	13	35
10^{-2} M	65	35	47

On a gel loaded with a 2:1 ratio of **2** to PPA, solid-state ^{31}P NMR detected resonances at 15.9 and 7.8 ppm (Table 2). The ammonium-functionalized gel gave a peak area ratio with a strong preference for the 15.9 ppm resonance (74:26). The chemical shifts of these peaks are similar to those observed with the guanidine-functionalized gels; however, the peak area distribution favors the peak at higher field for the latter gels. Interestingly, acid washing of the ammonium-functionalized gel produces a similar result to the guanidine-functionalized gel. That is, there is a distinct preference for the PPA complex associated with the higher field 7.8 ppm resonance to remain bound to the gel. Repeating the acid washing procedure for both guanidine and ammonium functionalized gels effects complete removal of the template.

Following template removal, rebinding studies were performed on the guanidine-functionalized gels to determine the binding mode of PPA substrate molecules to the receptor site. The xerogels were soaked overnight in an aqueous solution of PPA at pH 7, at concentrations greater than 10-fold in excess to the nominal number of functional groups on the gel. The solution concentrations of PPA that were used are listed in Table 3 along with the relative solid-state ^{31}P NMR peak areas of the bound PPA. Blank gels subjected to the same rebinding conditions did not show any adsorption of PPA as determined by solid-state ^{31}P NMR of the gel and UV analysis of the solution phase.

The solid-state ^{31}P NMR spectra of the imprinted gels rebound with PPA are shown in Figure 3. The peaks are significantly broader than those observed with the gels in their initial state and after washing with acid solution. Curve fitting analysis showed that the spectra in Figure 3 can be fit to two absorptions with chemical shifts of ~12 and 5 ppm. The significant change in chemical shift of the bound PPA relative to the initial gel, particularly at lower field, and the broadening of the peaks indicate some alteration of the silica matrix and the receptor site.

In light of the findings above, a solid-state ^{29}Si MAS NMR analysis of the gels in the initial state, after PPA removal with acid washing, and after the rebinding
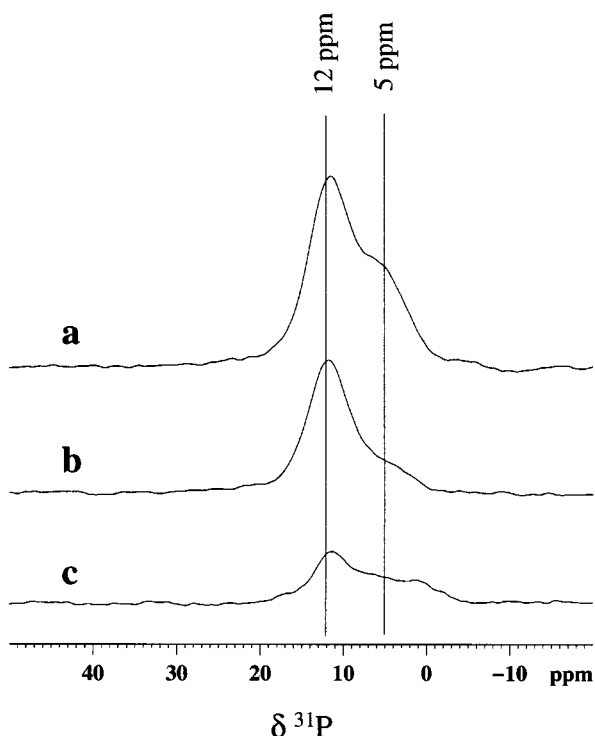
Figure 3. Solid-state ^{31}P MAS NMR of an imprinted gel (2:1 ratio of **1** and PPA) completely removed of template then equilibrated with 10 mM (a), 1.0 mM (b), and 0.1 mM (c) concentrations of PPA in aqueous solutions at pH 7.0.
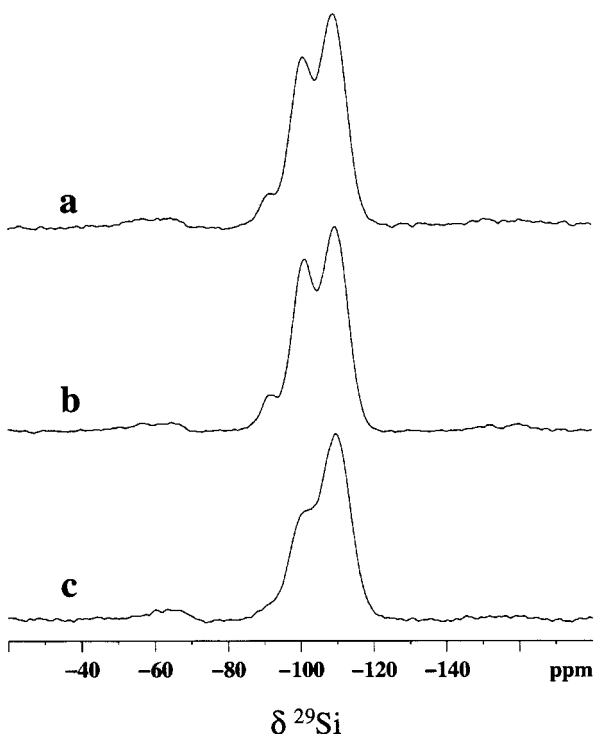
Figure 4. Solid-state ^{29}Si MAS NMR of a 2:1 **1** to PPA imprinted gel prior to (a) and after (b) washing with aqueous 1 N HCl solution for 1 h, and following overnight equilibration with an aqueous solution of 1 mM PPA at pH 7.0 (c).

experiment with PPA were conducted to determine the extent of structural change of the matrix following each step. The spectra are displayed in Figure 4. The Q_n distribution[33] of the silicon species is identical between the initial gel and the gel following acid washing (Q_4 54%, Q_3 37%, Q_2 6%, T from monomer **1** 3−4%).

However, following the rebinding experiment the Q_n distribution changed to Q_4 59%, Q_3 38%, Q_2 0%, and T 3−4%, indicating significant changes to the gel structure effected by soaking in pH 7 buffer solution. The changes are likely due to extended condensation of the silica matrix as is observed with sol−gel materials in neutral to basic solutions.[34] Additionally, development of surface silanols from prolonged exposure of the TEOS gel to aqueous solutions can also alter the PPA receptor sites with regard to hydrophilicity, hydrogen bonding interactions, and polarity of the surface. The ^{29}Si NMR data coincide with the ^{31}P NMR spectral observations and scenarios described above.

Through a series of controlled complexation studies, attempts were made to correlate the NMR absorptions observed above with particular PPA binding modes. The subsequent sections will describe those experiments and results.

Induced Adsorption of PPA to Imprinted Gels. Although PPA does not adsorb onto nonfunctionalized (blank) gels at concentrations below 1.0 M in water or ethanol, it is possible to induce adsorption through solvent evaporation. To identify the solid-state NMR absorptions associated with nonspecific adsorption, PPA was adsorbed onto blank gel from ethanolic solution using the solvent evaporation procedure. The gel gave peaks at 17.6 and 8.2 ppm (Figure 5b). The peak at 17.6 ppm is similar to that found in ref 30, which identifies this peak as "ionized", or monoanionic PPA. The peak at 8.2 ppm, however, does not correlate with any form of PPA found in the literature. It is possible that this peak is associated with the dianionic form of PPA interacting with the gel.[35] None of the materials characterized in these studies exhibited absorptions associated with covalently bound PPA to silica gel (10−11 ppm), as observed by Lukes et al.[29]

Additional quantities of PPA were also adsorbed onto imprinted gels by solvent evaporation from ethanolic solution to evaluate the binding modes associated with the peaks at 15.5 and 6.5 ppm. By increasing the PPA loading on the imprinted gel the two-point binding mode should give way to the one-point binding mode. Figure 5 shows solid-state ^{31}P NMR spectra of the imprinted gel (2:1 ratio of **1** to PPA, spectrum c) and the same gel with increased loading of PPA (spectra d and e). Table 4 lists the relative peak areas observed. As a reference, the spectrum of crystalline PPA is shown as spectrum a. With the addition of 1 mol equiv of PPA to the imprinted gel, a total guanidine to PPA molar ratio of 1:1 is produced. The relative peak ratio of this gel is 63:37 in favor of the resonance at 6.5 ppm (Figure 5d), only slightly different than the initial imprinted gel. At higher loadings of 1:3 guanidine to PPA, the intensity of the resonance at 15.5 ppm becomes dominant (51%) relative to the peak at 6.5 ppm (43%) (Figure 5e). In

(34) Brinker, C. J.; Scherer, G. W. *Sol−Gel Science*; Academic Press: San Diego, 1990.

(35) The assignment of monoanionic and dianionic forms of PPA on the nonimprinted gel to the 17.6 and 8.2 ppm resonances, respectively, is supported by the relaxation data in Table 1S. The site for dianionic PPA binding shows a longer relaxation time, corresponding to a slower reorientational time constant. Compared to the imprinted gels the differences in the spin relaxation between the mono- and dianionic binding sites is significantly smaller (~2−4). The nonimprinted gel also shows smaller apparent energies of activation in comparison to the imprinted material.
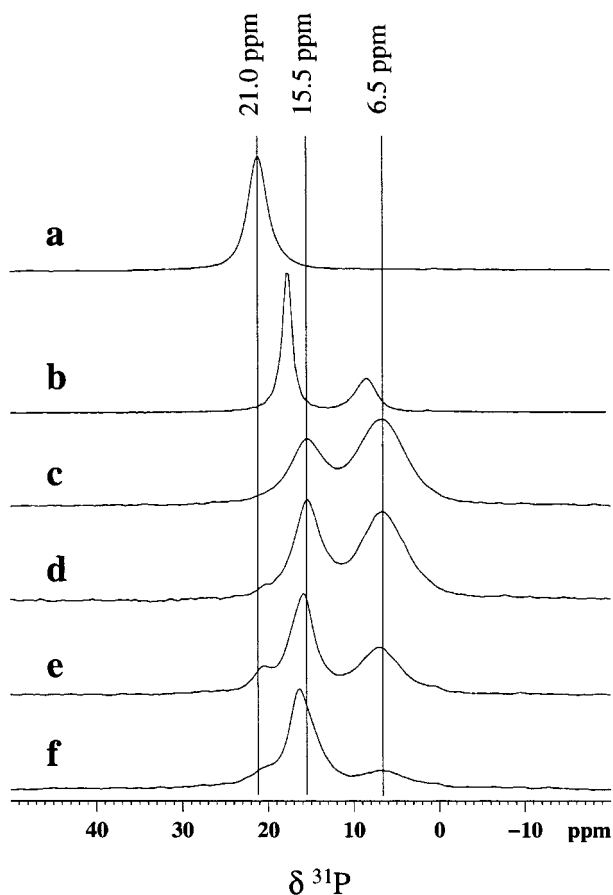
Figure 5. Solid-state ^{31}P MAS NMR of a silica xerogel imprinted with a 2:1 ratio of **1** and PPA (c) with increased loadings of PPA to 1:1 (d) and 1:3 (e) total molar ratios of **1** to PPA. Spectra of crystalline PPA (a), PPA adsorbed on blank gel (b), and a randomly functionalized guanidine gel adsorbed with a 1:3 guanidine to PPA loading (f) are included as references.

Table 4. Relative Solid-State ^{31}P NMR Peak Areas of PPA Adsorbed on Functionalized Xerogels

spectrum[b]	**1**:PPA	relative peak area[a]		
		21.0 ppm	15.5 ppm	6.5 ppm
5c	2:1	0	30	70
5d	1:1	0	37	63
5e	1:3	6	51	43
5f	1:3[c]	4	76	20

[a] Relative intensity includes integration of spinning sideband manifold associated with each reported chemical shift. [b] From Figure 5. [c] Nonimprinted gel functionalized with **1**.

addition, another peak emerges at 21.0 ppm, identical to the chemical shift of crystalline PPA. Figure 5f shows a spectrum of a randomly functionalized gel also with a 1:3 ratio of **1** to PPA loading. This material, although identical in surface density of guanidine functionalization as the imprinted gels, displays a comparatively smaller peak area from the absorption at 6.5 ppm (20%) relative to the peak at 15.5 ppm (76%).

From these results a trend emerges linking the absorption at 15.5 ppm to the one-point binding interaction of PPA to guanidine and that of the 6.5 ppm peak to the two-point binding mode. The initial 2:1 **1** to PPA imprinted gel, by stoichiometry, should favor the two-point binding mode, which is consistent with the dominance of the absorption at 6.5 ppm. By loading increasing quantities of PPA to the gel the two-point, interac-
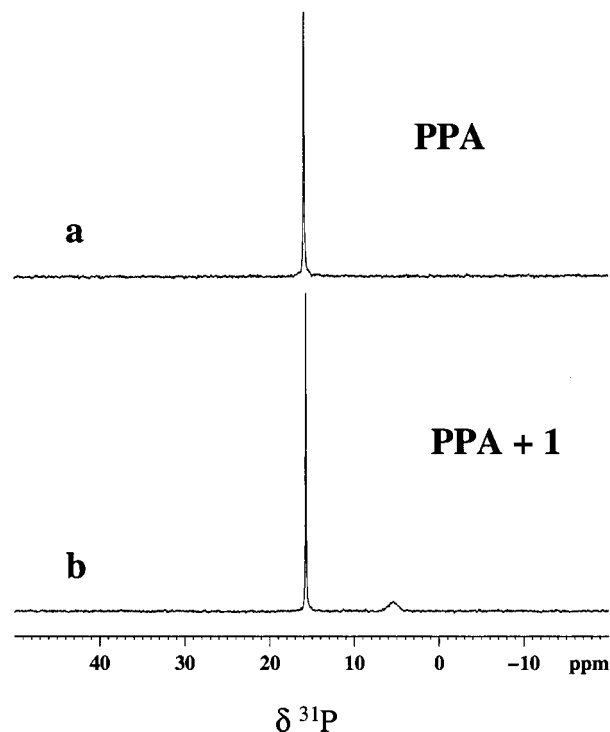
Figure 6. High-resolution ^{31}P NMR solution spectra of 10 mM ethanol solutions of PPA alone (a) and with **1** at 1 M concentration (b).

tions were forced into single-point interactions. Thus, the peak at 15.5 ppm grows in relative area.

In this induced PPA adsorption study, nonspecific binding plays a role. By the stoichiometry of the loading amounts, a significant portion of PPA should be nonspecifically bound, some in crystalline form. The slight shifts of the peaks to lower field at higher loadings (Parts e and f of Figure 5) are consistent with contributions from nonspecific binding (Figure 5b). Similar arguments can be made for the 1:1 imprinted gel (Figure 2c). Quantification of the data is beyond the scope of this report, and we limit our discussions to qualitative assessment of peak areas and binding modes.

The assignment of the 15.5 and 6.5 ppm resonances corresponding to the one-point and two-point binding environments, respectively, is also further supported by the ^{31}P T_{1Z} relaxation behavior observed for these two resonances (Supporting Information). The ^{31}P T_{1Z} temperature dependence demonstrates that for the 2:1 xerogel the relaxation of both resonances corresponds to the slow correlation time region of the spin−lattice relaxation curve. The resonance at 6.5 ppm (two-point) has a significantly longer T_{1Z} (∼10−20-fold) than the resonance at 15.5 ppm (one-point) as would be expected for a phosphorus environment having a longer correlation time (or a reduced amplitude of motional fluctuations). This observation also provides evidence against the argument that both the 15.5 and 6.5 ppm ^{31}P resonances represent one-point binding interactions in two different sites. If both resonances were indeed the result of one-point binding interactions, one would expect that the T_{1Z} relaxation times would be similar, a situation not observed experimentally.

Attempts to match the NMR absorptions with specific ionic states of the PPA on the gel were made by preparing samples of mono- and disodium salts of PPA.

Although the samples were pure as determined by elemental analysis, their NMR spectra in the crystalline and silica gel adsorbed states were difficult to interpret. In the crystalline state both mono- and disodium salts gave peaks at 15 and 13 ppm of near equal intensity. As adsorbed onto blank xerogel, variable results were obtained. In most cases, two peaks emerged at 15 and 7 ppm; however, the peak areas did not show any correlation with the ionic state of PPA. The variability of the NMR spectra with these samples may be affected by the water content of the sample. More rigorous analyses of these samples are warranted and will be pursued.

Solution-Phase PPA-1 Complexation. A ^{31}P NMR solution-phase study of the guanidine monomer **1** with PPA was also performed in an effort to establish a correlation with the solid-phase data. In the presence of **1** at 10 mM concentration in ethanol, PPA gave rise to two peaks at 15.5 (sharp) and 5.5 (broad) ppm, slightly different from that of PPA alone at 15.9 ppm. The narrow peak at 15.5 shows a chemical shift that changes with temperature and begins to broaden at −90 °C. This resonance probably represents an average chemical shift of free PPA and bound PPA, but these two species could not be resolved by NMR in the temperature range investigated (−90 to 50 °C). The broad resonance at 5.5 ppm shows no variations with temperature and represents a stable, nonexchanging complex on the time scale probed by the ^{31}P NMR. This peak's characteristics are consistent with a slower on/off rate of formation for the 2:1 guanidine to PPA complex. The solution-phase data correlate well with the solid-phase data adding further weight to the interpretation of the one-point and two-point interactions with PPA belonging to the ^{31}P NMR peaks at 15.5 and 6.5 ppm, respectively, in the solid phase.

Discussion

With the PPA binding modes now assigned to specific NMR absorptions, an evaluation of the host−guest complexation behavior of the functionalized gels can be made. In the 1:1 and 2:1 guanidine to PPA imprinted gels (Table 1), the desired template to functional monomer stoichiometry in the receptor is favorably expressed in the gel. That is, the two-point PPA binding mode is favored in the 2:1 gel, whereas the one-point mode is favored in the 1:1 gel. In comparing PPA binding onto imprinted versus nonimprinted gels (Table 4), the single-point binding mode was largely preferred in the nonimprinted gels. In the absence of an imprinting molecule, functionalization of the gel surface becomes random. In this case, the coincidence of having two guanidines in the correct proximity and orientation for a two-point interaction is relatively low compared to an imprinted site.

In an interesting comparison of guanidine versus ammonium functionalized gels, identical imprint loadings of 2:1 functional monomer to PPA gave almost opposite results. If we may equate the binding modes interpreted for the guanidine with the similar absorption peaks from the ammonium functionalized gel, then the guanidine gels produced a predominance of two-point interactions whereas the ammonium gels yielded mostly single-point interactions (Table 2). This result

is similar to other studies that compare the binding interactions of guanidine and ammonium functionalized hosts with phosphates.[36,37] The guanidine functionality, with its hydrogen bond donating properties and delocalized cationic charge, is more amenable to intimate host−guest interactions compared to the tetraalkylammonium cation, resulting in superior molecularly imprinted sites.

Removal and readsorption of the PPA template also gave some insight into the binding energies and kinetics of PPA to the functionalized gels. Table 2 summarizes these results. On template removal, the guanidine functionalized gels released 93% of the PPA template. Of the 7% PPA that remains bound, 82% was in the two-point binding mode and 18% as the one-point mode. There is a distinct preference for the two-point binders to remain on the gel. This result is consistent for a higher binding constant and/or slower k_{off} rate for two-point interactions relative to the one-point interaction. A similar result was also observed for the ammonium-functionalized gels.

In the PPA readsorption studies, the data reported are consistent with previous results regarding the trend of PPA affinity to these gels with increasing pH. [19] That is, the apparent PPA affinity at pH 7, as estimated from Table 3 ($K_a \sim 10^2$ M^{-1}), is lower than that at pH 5 ($K_a = 3 \times 10^3$ M^{-1}). In the present studies the adsorption data was insufficient for an accurate determination of a binding constant. The spectral data, however, could distinguish different host−guest binding interactions. Two absorptions at 12 and 5 ppm are observed. Changes in the silica matrix are believed to have shifted the absorptions of the two-point (15.5 ppm) and one-point (6.5 ppm) interactions upfield to 12 and 5 ppm, respectively. With this assumption some assessment of the PPA readsorption can be made. At 0.1 mM concentration of PPA, 11% of the capacity (based on nominal guanidine loading) was bound. The majority of the bound PPA reside in the one-point binding mode (82%) with the remainder in the two-point mode. Raising the PPA concentration to 1.0 mM increases the amount of PPA bound to 35% of capacity with only slight changes in the one-point to two-point binding distribution. And, at 10.0 mM concentration, 47% of capacity is occupied and a shift in the binding mode distribution is observed tending toward the two-point binding interaction.

The results suggests either that the one-point interaction has a larger binding constant than the two-point interaction, considered unlikely, or that the one-point mode has faster binding kinetics. Similar results were obtained in previous rebinding studies with divinylbenzene/styrene cross-linked polymers where the one-point binding interaction dominated over the two-point interaction in the initial phase of substrate binding.[18] Molecular selectivity was still observed, as in the present study, revealing that the host−guest interaction is not solely dependent on the strong interactions (e.g., ionic, covalent), but rather is likely dictated by subtle forces (e.g., van der Waals) that determine shape selectivity.

(36) Sasaki, D. Y.; Kurihara, K.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 9685.

(37) Dietrich, B.; Fyles, D. L.; Fyles, T. M.; Lehn, J.-M. *Helv. Chim. Acta* **1979**, *62*, 2763.

Conclusion

Solid-state ^{31}P NMR has been shown to be an excellent spectroscopic technique to probe the modes of guest complexation in molecularly imprinted materials. Considering most materials do not incorporate phosphorus species and a growing interest in preparing molecular recognition materials with biomolecule affinity, many of which are phosphorylated, this technique should prove an important tool to characterize and develop imprinted materials with separation and sensor applications. In our results we were able to identify nonspecific, one-point, and two-point guanidine to phenylphosphonic acid interactions on imprinted silica xerogels. This allowed a qualitative determination of the types of sites prepared by our molecular imprinting technique as well as an assessment of the imprinted sites following template removal and subsequent complexation with phosphonate guests. The affinity experiments on the template-removed gels were complicated by structural changes in the metal-oxide gel that occurred during overnight exposure to aqueous solution at pH 7, as determined by solid-state ^{29}Si NMR. We are currently examining methods to postfunctionalize the gel surface to produce metal-oxide imprinted gels with recognition properties that are stable and functional under a large range of aqueous conditions for sensing and separations applications.

Supporting Information Available: The solid-state ^{31}P spin–lattice relaxation (T_{1Z}) behaviors of the 15.5 and 6.5 ppm resonances in the 2:1 **1**:PPA gel and of PPA in a nonimprinted silica gel. This material is available free of charge via the Internet at http://pubs.acs.org.

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