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# Investigation of the Contributions to the $^{29}$Si MAS NMR Line Widths of Zeolites and the Detection of Crystallographically Inequivalent Sites by the Study of Highly Siliceous Zeolites

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**Abstract:** The high-field, high-resolution, magic-angle-spinning (MAS) $^{29}$Si NMR spectra of various zeolites, their corresponding highly siliceous isomorphs, and a number of high-quality minerals have been studied. Factors that could possibly contribute to the $^{29}$Si NMR line widths are systematically discussed and their importance evaluated. It is concluded that the limiting $^{29}$Si NMR line widths are determined by long-range chemical shift effects resulting from the distribution of aluminum in second and further nearest neighbor coordination spheres with the additional line-broadening mechanism in many cases being a chemical-shift distribution due to crystallographic inequivalences. This implies that narrow $^{29}$Si resonances should be observed for *any* perfectly ordered system as is confirmed by the spectra of gem-quality minerals for which narrow resonances are observed even for silicon atoms with one or more aluminum atoms in the first coordination sphere. The multiple resonances observed in the $^{29}$Si MAS NMR spectra of highly dealuminated materials which are ascribed to crystallographically inequivalent silicon atoms in the unit cell are related directly to the structures previously proposed from XRD measurements.

In recent years, since the initial work of Lippmaa and Engelhardt,[1] there have been substantial efforts made in the use of MAS NMR techniques to obtain high-resolution $^{29}$Si solid-state NMR spectra of zeolites. These measurements, which have been reviewed,[2] relate to the detailed structures of these systems and are particularly important since X-ray diffraction studies do not distinguish between the Si and Al atoms in the lattice, although they provide the overall lattice structure. Lippmaa and Engelhardt[1] showed that, in general, the $^{29}$Si NMR spectra of these systems exhibited five resonances which fell within reasonably well-defined chemical shift ranges, a result confirmed by other workers.[2] The shift of each resonance is characteristic of the Si/Al distribution of the first nearest neighbors, i.e., the distribution of Si and Al atoms attached to the four oxygens at the corners of the $SiO_4$ tetrahedra. By convention these have been referred to as Si[4 Al], Si[1 Si, 3 Al], Si[2 Si, 2 Al], Si[3 Si, 1 Al], and Si[4 Si], the distribution being described within the brackets. Spectra of these compounds have been recorded at a variety of frequencies up to 400 MHz, and considerable literature data are available.[2] At higher frequencies, the five absorptions may be clearly resolved and the spectra deconvoluted in terms of Gaussian curves. However, there appears to be a substantial residual line broadening in the spectra. Thus, even at the highest fields, line widths are generally within the range of 2–7 ppm. For comparison, although the $^{13}$C CP/MAS NMR spectra of amorphous polymers are in this range, line widths of $\sim$1 ppm may generally be obtained for *crystalline* organic compounds.

The purpose of the present work is to attempt to identify the contributing factors to the $^{29}$Si line widths of the NMR spectra of zeolites to facilitate further work in this area and to detect and characterize crystallographically inequivalent sites within zeolite frameworks. To this end, the $^{29}$Si MAS NMR spectra of a number of highly siliceous zeolites and gem-quality minerals have been carefully examined.

## Experimental Section

High-resolution $^{29}$Si NMR spectra were recorded at 79.5 MHz on a Bruker WH-400 multinuclear spectrometer equipped with a narrow-bore Bruker Spectrospin/Oxford Instrument superconducting magnet and a homemade MAS probe which is described in detail elsewhere.[5]

The spinner assembly was of the Andrew-Beams type; the conical rotors were machined from Delrin and had an internal volume of approximately 0.5 cm$^3$. Rotors containing the finely powdered samples were spun at the magic angle (54.7°) at speeds of approximately 3.6 kHz with use of compressed air as the driving gas. Cross-polarization and proton decoupling were not used in these experiments.

## Results and Discussion

The most likely contributing factors to line broadening in the $^{29}$Si spectra of these systems are the following: (1) magnetic field inhomogeneity; (2) unaveraged chemical shift anisotropy due to missetting of the spinning axis; (3) amorphous character; (4) unaveraged dipolar interactions; and (5) distribution of chemical environments due to siting of silicon and aluminum atoms.

In the present experiments, which employed a superconducting magnet, field inhomogeneity may be ruled out as the magnetic field is homogeneous to approximately 0.1 ppm. A distinct improvement is observed in the spectra upon increasing the field from 60–90 to 200 MHz and above. At the lower fields, magnet inhomogeneity may contribute, as iron core electromagnets are used, but at the higher field strengths other factors must dominate the spectra. Likewise, unaveraged chemical shift anisotropy due to missetting of the spinning axis may be neglected due to careful experimental techniques, including the use of the $^{79}$Br resonance KBr added to the sample.[3]

In many organic systems, substantial line broadening may derive from amorphous character in the surrounding environment. However, truly amorphous effects, i.e., random orientation of atoms, may be ruled out in these systems, as X-ray diffraction measurements indicate that they are "completely" crystalline. That is, they show massive long-range three-dimensional order. However, as mentioned above, and as will be referred to again subsequently, the diffraction measurements are compromised by the fact that they do not distinguish between Si and Al atoms.

Dipolar interactions to protons are possible, but hydrogn nuclei in these systems are present as water of hydration and to a small degree as hydroxyl groups. However, they are very mobile and

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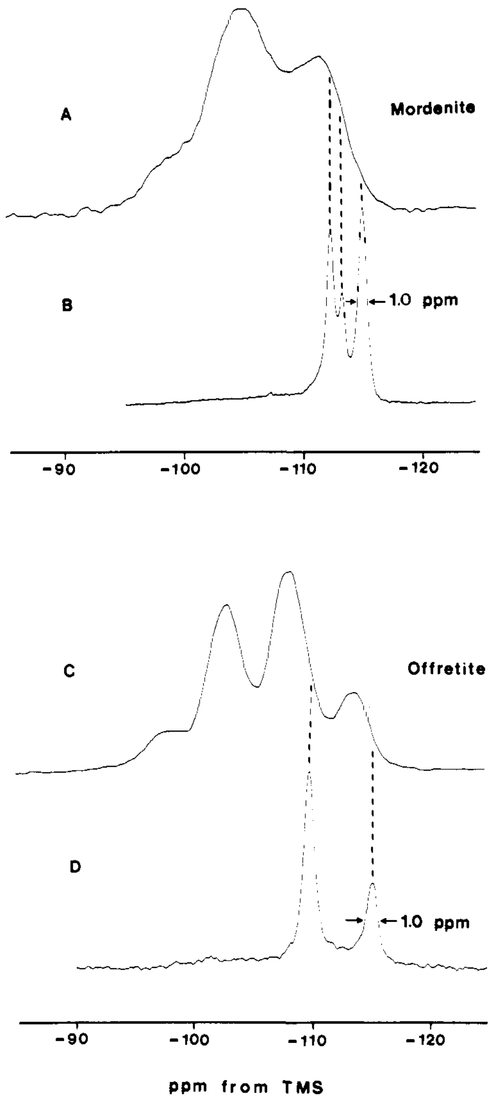

**Figure 1.** $^{29}$Si MAS NMR spectra obtained at 79.5 MHz; the indicated line widths are corrected for the line broadening applied as indicated below: (A) mordenite of low Si/Al ratio, 620 FIDs, 10-s delay and 35-Hz line broadening; (B) highly siliceous mordenite,[6] 1810 FIDs, 20-s delay and 2-Hz line broadening; (C) offretite of low Si/Al ratio, 2154 FIDs, 7-s delay and 20-Hz line broadening; (D) highly siliceous offretite,[6] 90 FIDs, 7-s delay and 10-Hz line broadening.

not directly incorporated into the zeolite lattice, and little improvement in the spectra is seen when proton dipolar decoupling is used. A second dipolar coupling which must be seriously considered is that between the silicon nuclei and the aluminum nuclei in the lattice. Since $^{27}$Al is a quadrupolar nucleus, the spins will not be quantized along the Zeeman field direction. Thus, dipolar couplings to quadrupolar nuclei are *not* averaged to zero by MAS as documented by several detailed investigations of the effect of MAS on dipolar interactions between $^{13}$C and $^{14}$N nuclei in directly bonded systems.[4] Thus, this interaction is particularly troublesome in the $^{29}$Si spectra of zeolite systems. It will be difficult to deal with by decoupling techniques since the energy levels of quadrupolar nuclei are very well separated, usually only the central $^{1}/_{2} \leftrightarrow {}^{-1}/_{2}$ transition (which is independent of the quadrupolar interaction to first order) being observed, the others being spread over too large a frequency range.

To clarify this problem, i.e., the nature of the involvement of the $^{27}$Al nuclei in the residual broadness of the $^{29}$Si resonances observed even at high fields, a variety of highly siliceous zeolites have been prepared. Figure 1 shows the $^{29}$Si MAS NMR spectra obtained at 79.5 MHz of low Si/Al ratio (<10:1) and the corresponding high Si/Al ratio forms (>100:1) of the zeolites mordenite and offretite. Within the pairs of spectra Figure 1A,B and Figure 1C,D comparisons may be made between the spectra of the low Si/Al ratio zeolites and those of the corresponding highly siliceous forms. A very marked narrowing of the resonances is observed in both cases, yielding spectra with line widths of 1 ppm or less. Line widths of this magnitude are characteristic of most highly siliceous systems investigated to data as shown in Table I. When no aluminum is present, the line broadening is removed. Further, when aluminum is present, but is in a very symmetrical environment (in which case the quadrupole moment is small) as in the case of zeolite A, Figure 2A, a very narrow line is again observed. *Clearly, the line-broadening mechanism involves the aluminum atoms in the lattice.*

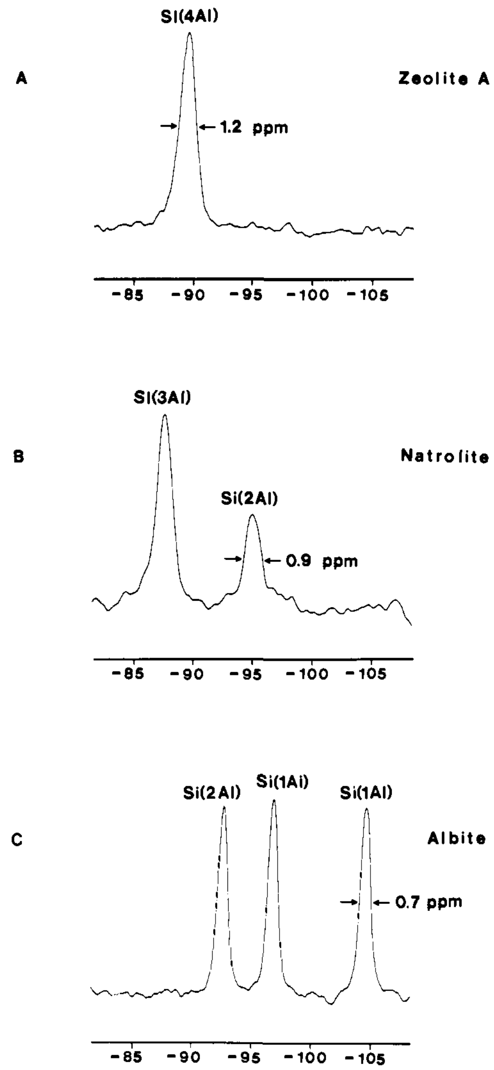

**Figure 2.** $^{29}$Si MAS NMR spectra obtained at 79.5 MHz; the indicated line widths are corrected for the applied line broadening: (A) zeolite A, 410 FIDs, 7-s delay and 25-Hz line broadening; (B) natrolite, 1600 FIDs, 15-s delay and 75-Hz line broadening; (C) albite, 1180 FIDs, 30-s delay and 25-Hz line bradening.

While the general trends observed in the spectra and described above would appear to be in agreement with the broadening being due to the previously mentioned unaveraged dipolar interactions with the aluminum nuclei in the lattice, several other observations argue against this mechanism, at least at high magnetic field strengths. There is a substantial improvement in going from 60 to 200 MHz, and while dipolar effects may contribute at lower fields, there is no further marked improvement in going from 200 to 400 MHz. The dipolar coupling is proportional to the magnetogyric ratios and the reciprocal of the cube of the internuclear distance as indicated in eq 1 where $N$ is the number of aluminum atoms in the first coordination sphere. The magnetogyric ratios

$$\langle \Delta H^2 \rangle^{1/2} \propto \sum_{N=1}^{4} \frac{\gamma_{\mathrm{Si}} \gamma_{\mathrm{Al}}}{(r_{\mathrm{Si-Al}_N})^3} \qquad (1)$$

**Table I.** $^{29}$Si MAS NMR Line Widths of Highly Siliceous Zeolites and Related Materials Together with Those of the Si[4 Si] peaks of Corresponding Low Si/Al Ratio in Materials (Where Appropriate)[f]

| | $\Delta V_{1/2}$, ppm | $\Delta V_{1/2}$, Si[4 Si] (low ratio) | displacement, ppm | ref |
|---|---|---|---|---|
| offretite | 1.0 | 3.2 | 3.3, 3.4[c] | this work |
| mordenite | 1.0 | 4.6 | 1–4[c] | this work |
| faujasite | 1.0 | 2.1 | 2.2 | this work, 8 |
| silicalite[a–c] | 0.25 | 5.2[d] | 1–2 | this work, 9 |
| zeolite A | 1.2 | 3.2[e] | | this work, 10 |
| albite[c] | 0.6, 0.6, 0.7 | | | this work |
| natrolite[c] | 0.9, 0.9 | | | this work |
| microcline[c] | 0.9, 1.2, 1.2 | | | this work |

[a] For resolved peaks. The lower line width in this case may reflect the extremely high crystallinity and large crystal dimensions of this sample. [b] Upfield shift measured from ZSM-5 of Si/Al = 18 to the center of the multiplet of peaks in silicalite. [c] Multiple resonances. [d] ZSM-5, Si/Al = 18 taken as reference material. [e] $\Delta V_{1/2}$ of Si(4 Al) peak of Zeolite ZK-4, Si/Al = 1.65 taken as reference material.[11] [f] The approximate upfield shifts of the Si[4 Si] peak in the highly siliceous material are expressed in ppm from the Si[4 Si] peak maximum of the low Si/Al ratio materials. Spectra were obtained at 79.5 MHz on a high-resolution, narrow-bore spectrometer with a proton frequency of 400 MHz.

are low and the closest Si–Al approach is ~2.9 Å so the interaction will be small and even partial averaging will significantly reduce it further. Interactions to next and further nearest neighbor aluminum atoms will be very much smaller due to the $1/r^3$ distance dependence and can probably be neglected. The effects for the absorptions due to Si[3 Al, Si], Si[2 Al, 2 Si], Si[1 Al, 3 Si] should decrease down the series with none at all for Si[4 Si]. In general, although there is some differentiation between the absorptions, they are in all cases quite broad. As can be seen from Figure 1, the Si[4 Si] peak has a residual broadening of the same magnitude as the other peaks. This would suggest that although the aluminum atoms are, in general, responsible for the residual broadening in these systems, *it is by a less direct route* than by direct magnetic interactions which would depend mainly on the nearest neighbors. A further observation which tends to support this general conclusion is that the *very* substantial narrowing which occurs in the Si[4 Si] peak(s), as illustrated in Figure 1, occurs quite abruptly when Si/Al ratios of approximately 100 are reached, indicating that quite small concentrations of aluminum atoms can cause the effect and that it must therefore be quite long range in nature.

The *alternative mechanism* by which the aluminum atoms may affect the $^{29}$Si NMR of these systems is by creating a large number of silicon environments for each of the different first coordination sphere absorptions. This occurs because a structure is created in general which is disordered in terms of the distribution of aluminum atoms within the second and further nearest neighbors. This effect may be both by proximity of different atoms and also by distortions of bond angles and bond lengths. Classically, diffraction measurements have indicated that zeolites are "crystalline" in terms of their overall three-dimensional structure. However, these measurements do not distinguish between the placing of individual silicon and aluminum atoms, and *in terms of local ordering*, the systems can have a considerable degree of disorder or "amorphous" character. Experimental proof that the $^{29}$Si chemical shifts are indeed sensitive to second and further nearest neighbor interactions is seen in Figure 1. There, substantial upfield shifts occur for the different $^{29}$Si resonances in zeolites when the Si/Al ratio increases. The effect is general[6,7] but maximized for the Si[4 Si] resonance as this absorption can be observed in its extreme limiting value in the completely siliceous materials. The upfield shifts may be used as a rough estimate of the magnitude of the effect (Table I). Namely, they are, as measured from the center of the resonance of the low Si/Al materials for the Si[4 Si] absorption, 3.3, 3.4 ppm for offretite, 1–4 ppm for mordenite, and 2.2 ppm for Faujasite. For low Si/Al ratio zeolites, the distribution of such environments may be large and it is unlikely that increasing magnetic fields will resolve them completely. This mechanism involving general environmental effects of next and further nearest neighbors would also explain why narrowing is only observed during dealumination to *very* high Si/Al ratios as the placing of a single aluminum atom may affect many silicon atoms and final narrowing will be achieved only when it is essentially completely removed.

An implication of the above arguments which suggests the line broadening is due to disordering of second and further nearest neighbor interactions is that narrow $^{29}$Si resonances should be observed for any perfectly ordered system, whatever the first coordination sphere environment. Thus, the sharp resonance observed for zeolite A shown in Figure 2A is considered to be due to ordering being perfect, not particularly that the ordering is Si[4 Al]. Figure 2B,C presents spectra of gem-quality natrolite and albite obtained to further test this hypothesis. As can be seen from the figure, the resonances are all extremely sharp (~1 ppm, Table I) and independent of the nature of the first coordination sphere.

An additional complication which for some zeolites will reduce the resolution and which again illustrates the sensitivity of the $^{29}$Si resonances to environmental effects is the existence of multiple absorptions due to crystallographic inequivalence. This can be seen clearly in Figure 1, parts B and D, and has been previously observed in silicalite.[8] The shift dispersion may be quite large (up to 7–8 ppm). In fact, in the case of offretite this effect is as large as the shift dispersion due to the first coordination effect and two separate absorptions are observed for the Si[4 Si] peak at lower Si/Al ratios even though they are considerably broadened due to the other effects discussed above. In face, due to peak overlap, the highest field peak in the low Si/Al system is due to Si[4 Si] but the next resonance has contributions from Si[4 Si] and Si[Al, 3 Si]. The complete assignment of all of the resonances of offretite is complex, and a full description will be reported separately.

To summarize, the $^{29}$Si MAS high-field NMR spectra of zeolites are considered to a first approximation to be split into five resonances as previously described due to the placing of first nearest neighbor aluminum atoms. With second and further nearest neighbor interactions in all cases, and additionally crystallographic inequivalence in some cases, many different environments are created and substantial line broadening results. (In extreme cases, e.g., offretite, the crystallographic inequivalence may be comparable in magnitude to the effect of the first nearest neighbors giving overlap of the peaks and a deceptively simple spectrum.)

In the case of the very highly siliceous zeolites, the resolution of chemical shifts for the Si[4 Si] peaks makes it possible to compare directly the NMR spectra with the gross lattice structures found by X-ray diffraction measurements. Thus, in the case of offretite, two absorptions are observed of relative intensities 2:1, corresponding to the 12 and 6 tetrahedra in the ring systems, respectively.[11] Similar spectra showing two peaks of relative intensities 2:1 but with different chemical shift values are also observed for the zeolites erionite and omega which are structurally similar, again in agreement with the XRD-determined structures. In mordenite, three lines are observed of relative intensities 2:1:3 compared to the predicted 2:1:1:2 from the crystal structure.[12]

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In the structurally similar zeolite ferrierite, where 4 peaks of relative intensities 8:8:4:16 are to be expected, two peaks of relative intensities 4:5 (16:20) are observed due to overlap. For highly siliceous faujasite, a single line has previously been reported[7] corresponding to the single unique environment identified by X-ray diffraction.[13] It is probable that the chemical shifts are related to the "tetrahedral" bonding around the silicon atom in terms of deviations from perfect tetrahedral symmetry and/or Si–O–Si angles in these highly siliceous systems, and it may be possible in the future to make more quantitative comparisons between the results of the NMR and X-ray diffraction techniques. Substantial efforts are currently being made in this area[14] to refine the XRD data for the completely siliceous systems to provide detailed structures with accurate bond lengths and angles (XRD data on low Si/Al ratio zeolites will be inaccurate due to averaging over the Si/Al distribution of the various sites) and to compare these with the $^{29}$Si chemical shift data found in this work. It is probable that such a correlation will be found and may be used to investigate zeolites of unknown structures if highly siliceous forms can be prepared. In addition, the well-resolved spectra of highly siliceous systems and their characteristic chemical shifts may make possible the study of mixed zeolites and intergrowths by NMR techniques.

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**Registry No.** $^{29}$Si, 14304-87-1; albite, 12244-10-9; natrolite, 1318-95-2; microcline, 12251-43-3.

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# Proton NMR Studies of the Electronic and Molecular Structure of Ferric Low-Spin Horseradish Peroxidase Complexes

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**Abstract:** High-field proton NMR studies of native and reconstituted horseradish peroxidase (HRP) were used to investigate the electronic and physical structure of the heme pocket in three ferric low-spin forms of the protein. Reconstitution of selectively deuterium labeled hemes into apo-HRP led to the definitive assignment of many hyperfine shifted peaks. Consideration of line width, pH titration data, and the effect of heme peripheral substituents has permitted the assignment of additional heme as well as proximal histidine peaks. Variable temperature data present further evidence of tight heme–apoprotein contacts in HRP. It is shown that the transition of the cyanide complex of HRP at high pH to a new cyanide-ligated alkaline form is consistent with a change in axial magnetic anisotropy. At high pH resting state HRP undergoes a more drastic change in spectrum and structure that is attributed to coordination of a distal histidine in a configuration with its imidazole deprotonated and its $\pi$ plane oriented essentially perpendicular to that of the proximal histidyl imidazole.

Horseradish peroxidase, HRP (E.C. 1.11.1.7), is a heme b protein obtainable from horseradish roots whose biological function, in common with other peroxidases, is the reduction of hydrogen peroxide produced in vivo as a byproduct of enzymatic processes.[1,2] HRP is known to be a monomeric glycoprotein (42 500 daltons) containing a single heme, iron(III) protoporphyrin IX. The carbohydrate is covalently bound and constitutes 18% of the mass of the protein.[1] The amino acid composition[3] and sequence[4] of HRP have been determined, and thus the primary structure of HRP is known, but little information is available on the secondary or tertiary structure of the protein. Glycoproteins such as HRP are difficult to crystallize, and the lack of satisfactory crystals has prevented a detailed X-ray structure of HRP from being obtained.[5] In the absence of X-ray data a variety of physicochemical methods, including ESR,[6] MCD,[7] resonance Raman,[8] as well as NMR spectroscopy,[9-12] have been used to characterize the heme pocket of HRP.

Previous NMR studies have yielded a wealth of information on the electronic/physical structure of the heme environment for both the ferric (largely high-spin) resting state of HRP[9] and the enzymatic intermediate ferryl compound I of HRP.[10] Less thoroughly studied have been the low-spin forms of ferric HRP. Three separate forms of low-spin ferric HRP have been identified.[11] In common with other heme proteins, HRP will coordinate added cyanide to form a ferric low-spin species; this form is designated HRPCN. Above pH 10.9, HRP, largely ferric high-spin, will convert to a ferric low-spin form; this form is

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