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Surface and Bulk Infrared Modes of Crystalline and Amorphous Silica Particles: A Study of the Relation of Surface Structure to Cytotoxicity of Respirable Silica

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Surface IR (infrared) modes of crystalline and fumed (amorphous) silica particles, calcined at temperatures up to 1095°C, have been studied by Fourier transform infrared spectroscopy. The ability of these same particles to lyse cells has been measured by a hemolysis protocol. The untreated crystalline and amorphous materials differ by a factor of 40 in specific surface area, and the intensity per unit mass of the sharp surface silanol band near 3745 cm^{-1} in the amorphous material is an order of magnitude larger than in the crystalline material. A similar difference is observed in the lysing potential of the two materials. The intensity of the silanol band increases after calcination for both materials, reaching peak values near 500°C, followed by a dramatic drop at higher calcination temperatures, and reaching negligible values for materials calcined near 1100°C. The lysing potential data follow essentially the same pattern for both crystalline and fumed silica. These results are consistent with the hypothesis that the surface silanol groups are involved in cell lysis. Further experiments are suggested to evaluate the relationship between the surface structure of silica particles and their potential cytotoxicity.

Introduction

The characterization of the surface structure of various allotropic forms of silica is of interest because the cytotoxicity of silica particles of respirable size has been commonly related to the chemical interaction of functional groups on the surface of such particles with the lipoproteins contained in cell membranes (*1–3*). It has been found that the cytotoxic potential of silica particles varies greatly with their crystalline structure (*4,5*), particle size (*6–8*), surface composition (*9*), and molecular surface configuration (*1,10*). Studies of the infrared (IR) modes of various forms of silica have been reported by a number of researchers (*3,11–19*). Several books and reviews (*20–24*) have dealt with the identification of functional groups on silica surfaces and the effect of heat treatment on the surface structure. The nature of the surface is said to be critical in determining the biological activity of silica dusts (*3*).

Fourier transform infrared (FTIR) spectroscopy is a highly sensitive technique for identifying IR surface modes of fine particulates, and it was deemed of interest to examine the surface structure and its relation to lysing potential of two widely different types of respirable silica dust. The lysing potential of respirable dust particles has been considered a first-level test for potential cytotoxicity.

The materials selected for study were Min-U-Sil, a widely used crystalline dust of α-quartz structure, and Cab-O-Sil, an amorphous, fumed silica of a very large specific surface area. α-Quartz is the most common form of crystalline silica and is found in dusts generated in various mining operations. Fumed silica is a commonly used filler and additive but may may also be found in the atmosphere near glass blowing and other high-temperature processing operations that use siliceous materials. We have studied the hemolytic activity and

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the IR spectra of Min-U-Sil and Cab-O-Sil samples after different thermal treatments to determine the relationship between surface structure and the lysing potential of silica particles.

The IR spectra of the two sets of samples differ in that several bulk lattice modes and some of the surface modes that are present in the Min-U-Sil samples are missing in the Cab-O-Sil samples. Although Cab-O-Sil was found to be more cytotoxic than Min-U-Sil on a per unit weight basis and less toxic than Min-U-Sil on a per unit surface area basis, a positive correlation between the cytotoxicity and the intensity of the surface silanol band at 3745 cm^{-1} was observed for both materials. Details of these results and their interpretation are presented below.

Experimental Methods

Materials

The Min-U-Sil and Cab-O-Sil used in this study were obtained from U.S. Silica Inc. of Berkeley Springs, WV and from CABOT Corporation of Tuscola, IL, respectively. X-ray diffraction analysis of the two materials showed Min-U-Sil to be essentially pure α-quartz, while the Cab-O-Sil dust exhibited no detectable crystallinity. The particle size distribution of Min-U-Sil, determined by sedimentation, is shown in Figure 1. The specific surface areas of the Min-U-Sil and Cab-O-Sil dusts were measured by Branauer-Emmett-Teller (BET) adsorptometry as 5.0 m^2/g and 200 m^2/g, respectively. The particle morphology of the two materials is compared in the micrographs of Figure 2. These micrographs clearly show the crystalline nature (characteristic planes, sharp angles) of Min-U-Sil and the amorphous features of Cab-O-Sil.

The IR spectra of the dusts, as well as their hemolytic activity, were tested in the as-received condition and after calcination at several temperatures between 100°C and 1095°C. All materials were stored after calcination in a dessicator until the time of testing with erythrocytes. For the FTIR spectroscopy, thin discs (diameter = 12 mm) of both Cab-O-Sil and Min-U-Sil (using 14.9 mg of Cab-O-Sil and 34.0 mg of Min-U-Sil) were pressed at 30 tons/cm^2. Self-supporting discs of the above diameter could not be pressed from 14.9 mg of Min-U-Sil. These self-supported discs were then calcined in air for 12 hr at temperatures of 200, 400, 500, 600, 800, and 1095°C. After each calcination period the disc was removed from the furnace and immediately transferred to the IR cell, and its spectrum was measured at room temperature. Some of the sample disks were deliberately exposed to air for 10 hr after the 12-hr calcination period, and the IR spectra was redetermined. The same disc was used for experiments at progressively higher calcination temperatures.

Spectroscopy

The FTIR spectra were taken with a Mattson Instruments Cygnus 100 FTIR spectrometer. The sample cell was flushed thoroughly with dry nitrogen before each measurement. Usually 20 min of flushing was sufficient to remove any interference from water and carbon dioxide bands. During data collection, the flow rate of dry nitrogen was kept to a minimum. For most measurements, data were collected for 256 scans at a resolution of 1 cm^{-1}. The aperture of the beam was adjusted to match the diameter of the sample disc, allowing entire disc to be sampled during the measurements.

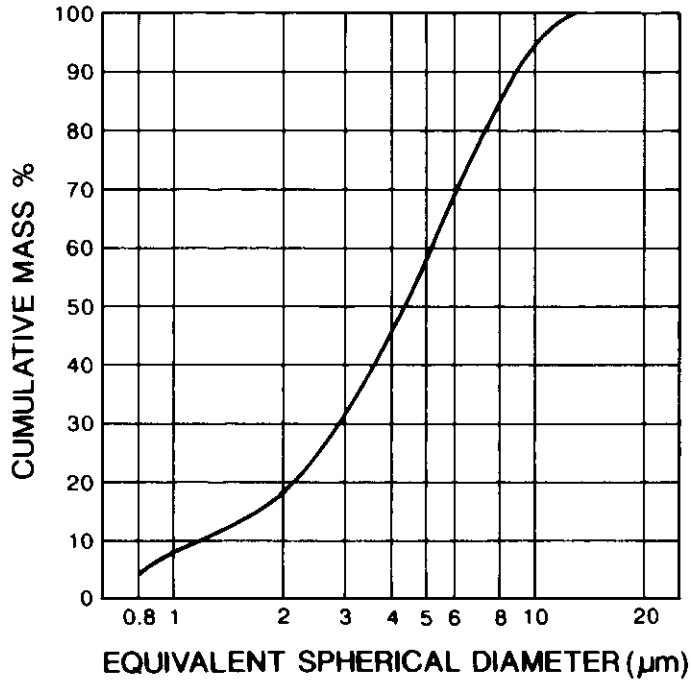


FIGURE 1. Particle size distribution of Min-U-Sil versus cumulative mass percentage (source: U.S. Silica, Berkeley Springs, WV). About 80% of the sample mass is contained in particle sizes between 2 and 10 μm.

Hemolysis Assay

The test protocol developed by Harington et al. (*9*) was used with slight modifications for the present investigation. Each test sample of dust was prepared in a stock concentration of 2 mg dust/mL of calcium- and magnesium-free Dulbecco's phosphate-buffered saline (PBS) obtained from Sigma Chemical Company (St. Louis, MO). The dust-saline mixture was stirred in a sonicator bath until the dust was fully dispersed and suspended in the liquid phase. The stock suspension was then diluted to make sample preparations, in duplicate, of 0.04 to 2.0 mg dust/mL PBS. Sheep blood erythrocytes, supplied by Scott Laboratories (Fiskeville, RI) were washed twice with PBS, centrifuged at 990*g* and diluted to a concentration of 2% by volume in PBS. Equal volumes of dust suspension and the 2% erythrocyte suspension were then mixed to obtain mixed suspensions of 1% by volume of erthyrocyte cells and dust concentrations in the range of 0.02 to 1.0 mg dust/mL.

A B

C D

FIGURE 2. Scanning electron micrographs of Min-U-Sil (*A,B*) and Cab-O-Sil (*C,D*) particles. Note the lack of crystallanity in the Cab-O-Sil samples.

These suspensions were subsequently incubated for 30 minutes at room temperature and agitated every 10 min, followed by centrifugation at 990*g* for 10 min. The amount of hemoglobin released, which is proportional to the number of ruptured blood cells, was used as the measure of the lysing potential of the dust particles. The hemoglobin concentrations were determined colorimetrically on a Bausch and Lomb Spectrometer (Spec 20) at a wavelength of 540 nm. Negative controls consisted of 2% suspensions of erythrocyte cells in PBS and positive controls of an equal volume mixture of water and the 2% by volume suspension of erythrocytes in PBS. Experiments were repeated two to seven times, and the standard deviations were determined by analysis of variance.

Results and Discussion

The FTIR spectra of the untreated Cab-O-Sil and Min-U-Sil discs in the range of 400 to 2500 cm^{-1}, are shown in Figure 3 and in the range of 2500 to 4000 cm^{-1} in Figure 4. Temperature-dependent runs were conducted on the self-supporting discs at 1 cm^{-1} resolution. A summary of the positions of major bands of untreated samples is presented in Table 1. A summary of the results follows.

Lattice Modes

The IR modes below 2000 cm^{-1} are usually associated with bulk lattice modes, different normal modes of the nonlinear O-Si-O configuration (*19–21*). The IR spectrum of the Min-U-Sil sample below 2000 cm^{-1} (Fig. 3) corresponds to that of crystalline α-quartz (*17–19*). In Cab-O-Sil (Fig. 3), on the other hand, two major bands at 469 and 693 cm^{-1} and the three minor bands at 1528, 1684 and 1794 cm^{-1} are absent, and some of the remaining ones appear to be blue-shifted. This suggests that the bulk symmetry of the Cab-O-Sil sample is different from that of α-quartz and that some bands are extinct because of the lowered symmetry.

Surface Modes

The untreated Cab-O-Sil sample shows a broad band in the range of 3000 to 3700 cm^{-1} with a weak shoulder

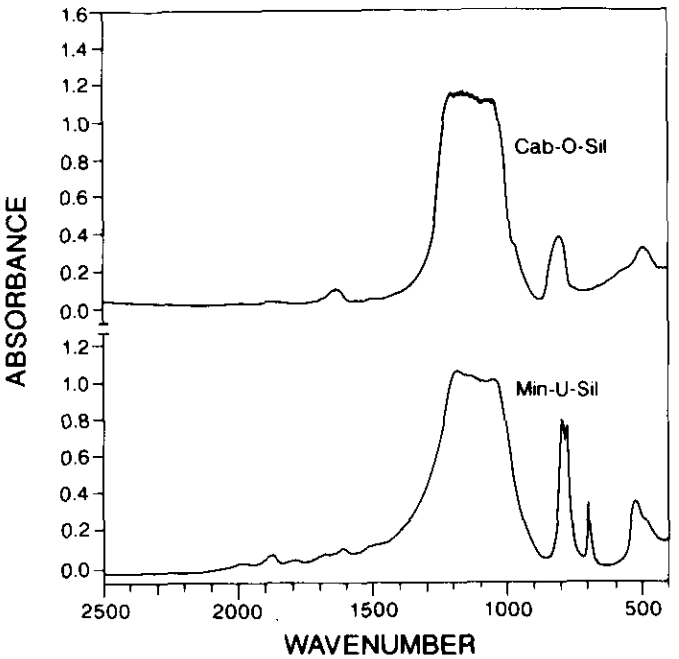


FIGURE 3. FTIR spectra of Cab-O-Sil and Min-U-Sil samples in the range of 400 to 2500 cm^{-1}. Note the missing bands in Cab-O-Sil (see Table 1 also).

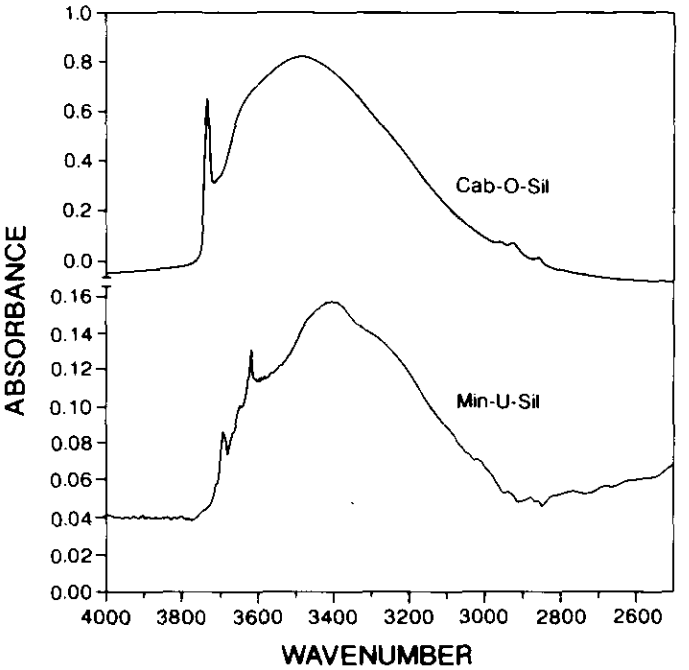


FIGURE 4. FTIR absorbance of Cab-O-Sil and Min-U-Sil samples in the range of 2500 to 4000 cm^{-1}. Note the different ordinate scales for the two samples.

Table 1. Comparison of the position of various bands (in cm^{-1}) in Min-U-Sil and Cab-O-Sil; note several missing bands in Cab-O-Sil due to lack of crystallanity.

Untreated Min-U-Sil	Untreated Cab-O-Sil	Comments
469	Absent	
522	521	
693	Absent	
797, 784 } doublet	812	
1080	1130	
Absent	988 (Shoulder)	(*23,24*)
1528	Absent	
1609	1626	
1684	Absent	
1869	1864	
1998	2002	
2900 group of bands	Present	Aliphatic impurities
3700–3000 broad band	Present	H-bonded impurities
3745 band, low intensity	Very sharp and high intensity	(OH) of Si-(OH)
3695	Absent	H-bonded species (*1*)
3619	Absent	
3258	Absent	
Absent	3637 shoulder	

around 3637 cm^{-1} and a sharp band at 3745 cm^{-1} (Fig. 4). These two bands have been observed by a number of workers in earlier studies (*3,11–16,21–25*). The broad band is identified with hydrogen-bonded OH surface groups, physisorbed water, and other impurities on the silica surface. The sharp band at 3745 cm^{-1} has been assigned to the free surface OH group attached to Si as Si-O-H (*14–16,25–27*). The weak kink appearing at 988 cm^{-1} (Fig. 3) is also assigned to the A-type mode of the Si-O-H group. This assignment is based on the studies of deuterated samples (*23,24*) and on the close relationship between the intensity of this kink and that of the 3745 cm^{-1} band. When the material is calcined at temperatures below 500°C, the intensities of both the 988 cm^{-1} and the 3745 cm^{-1} bands increase with calcination temperatures. This observation is in agreement with other studies that have shown a growth of the free surface OH band after heating at temperatures below 500 to 600°C (*16,22,25*).

The untreated Min-U-Sil also shows a broad band in the range of 3000 to 3700 cm^{-1} and a weak band at 3745 cm^{-1}. On closer examination, peaks are also found at 3695 cm^{-1} and 3619 cm^{-1} with a small shoulder at 3258 cm^{-1} (Fig. 4). The bands at 3695 cm^{-1} and 3619 cm^{-1} have also been reported by Kriegseis et al. (*1*).

The intensity of the 3745-cm^{-1} band is about 18 times larger for the uncalcined Cab-O-Sil sample than for the similarly uncalcined Min-U-Sil sample of equal weight. However, the specific surface area of Cab-O-Sil is about 40 times larger than that of Min-U-Sil. It follows that for samples of equal surface area, Min-U-Sil would show a larger peak intensity of this band.

Change of the Surface IR Modes with Calcination Temperature

The change of the surface IR modes between 2500 cm^{-1} and 4000 cm^{-1} for Cab-O-Sil calcined at several temperatures is show in Figure 5. The intensity of the broad band between 3000 cm^{-1} and 3700 cm^{-1} was computed by measuring first the area under both the narrow and broad band in the range of 3000 cm^{-1} and

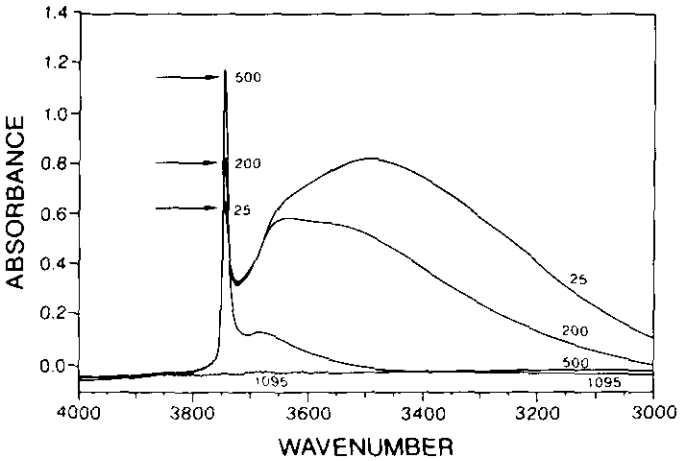


FIGURE 5. The FTIR spectra of the Cab-O-Sil disc for calcination temperatures of 25, 200, 500, and 1095°C in the range of 3000 to 4000 cm^{-1}. Arrows point to the peak position of the narrow band at different temperatures. For samples calcined at 1095°C, no bands are observed in this range.

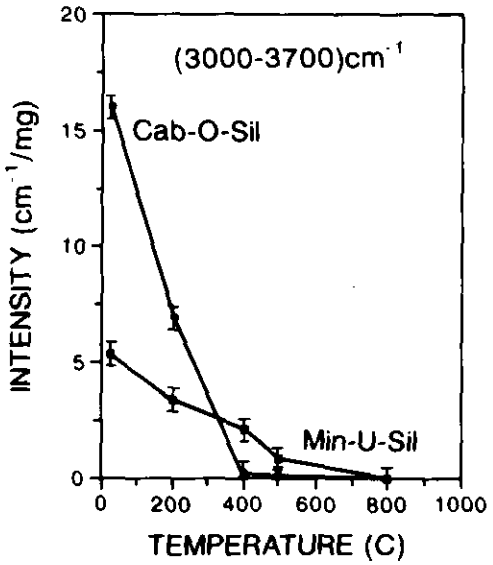


FIGURE 6. Variation of the intensity of the broad band (3000–3700 cm^{-1}) per mg of the samples with calcination temperature. Intensity refers to the area under the band in cm^{-1} (see text for the procedure of calculating intensity). Lines connecting the points are drawn for visual aid.

3760 cm^{-1} and then subtracting from it the area of the narrow band. This procedure allowed us to use a nearly horizontal base line for computing area of the broad band.

The change in the intensity of the broad band with calcination temperatures for Cab-O-Sil and Min-U-Sil samples is shown in Figure 6. As noted earlier, this broad band is attributed to H-bonded OH groups, physisorbed water, and other impurities on the surface of silica. On a per unit mass basis, the intensity of this band is about three times larger in Cab-O-Sil than in Min-U-Sil in the noncalcined condition. Considering that the surface area of Cab-O-Sil is 40 times larger than Min-U-Sil, this still indicates more extensive hydrogen bonding among the silanol groups or a larger amount of absorbed water on the surface of the crystalline material. The latter observation is consistent with the finding of multiple absorbed water layers on α-quartz surfaces reported by Baumann (*28*). According to the results shown in Figure 6, the intensity of this band decreases sharply with increasing calcination temperatures for both materials. The temperature at which the intensity approaches zero is about 500°C for Cab-O-Sil and about 800°C for Min-U-Sil. The small shoulders to the band at 3619 cm^{-1} and 3695 cm^{-1} found in the uncalcined materials are not observed after calcination at temperatures above 500°C.

The shape of the narrow band at 3745 cm^{-1} after calcination at several temperatures for the Cab-O-Sil is shown in Figure 7. In Figure 8 the intensity of this band is plotted against calcination temperature for both Cab-O-Sil and Min-U-Sil. Both samples show peak magnitudes of this band near 500°C followed by a decrease to almost zero at 1095°C. The upper set of points in Figure 8 represent the data obtained when the disc samples were heated for 12 hr, removed from the furnace, and immediately placed into the IR cell. The lower set of points represent data obtained for Cab-O-Sil when the disks were partly rehydroxylated by exposure to ambient air for 10 hr after calcination. These results show that the time required for hydroxylation decreases with increasing calcination temperature and that beyond some temperature, the surface becomes essentially inert to rehydroxylation. This is in agreement with previously reported observations on the kinetics of rehydroxylation

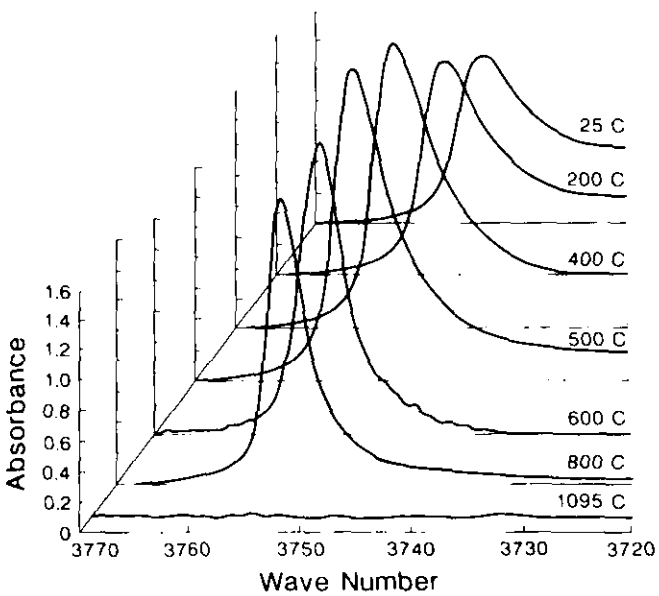


FIGURE 7. Profiles of the narrow silanol band near 3745 cm^{-1} in Cab-O-Sil at different calcination temperatures.

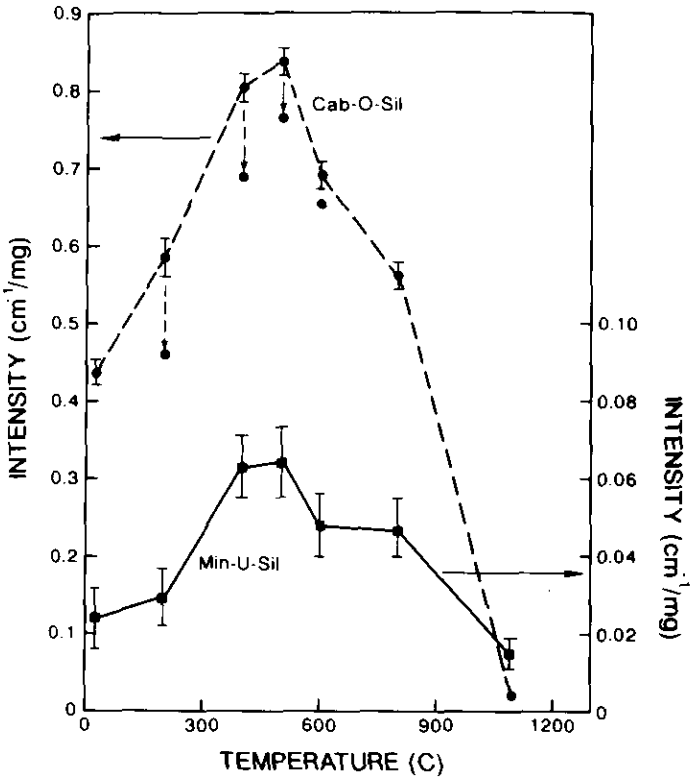


FIGURE 8. Intensity of the narrow silanol band near 3745 cm^{-1} at different calcination temperatures in Cab-O-Sil and Min-U-Sil. The lower set of points for Cab-O-Sil are obtained after the sample is allowed to rehydroxylate in air for 10 hr. Note the different scales for the intensity for Cab-O-Sil and Min-U-Sil. Lines connecting the points are drawn for visual aid.

of silica surfaces (*29,30*). Also, the upper points should be close to the high temperature equilibrium values, since no significant change in the intensity of the band is expected in the few minutes needed to transfer the sample from the furnace to the IR cell.

The position of the narrow band is also observed to shift to higher frequencies with increasing calcination temperatures in both Cab-O-Sil and Min-U-Sil as is shown in Figure 9. Similar results were reported by Hoffman et al. (*16*). Changes in $\Delta\nu$ (half-width at half-maximum) of this band with calcination temperatures are shown in Figure 10. Both the line position and $\Delta\nu$ are temperature dependent below 500°C, whereas above 500°C, there are only minor changes in their magnitudes. From Figures 6 and 8 through 10, it is apparent that as the intensity of the broad band (3000–3700 cm^{-1}) decreases with calcination temperatures to 500°C, the intensity of the narrow band increases, and its line position and line width are also affected. Note also that above 500°C, the line positions of the narrow band for Cab-O-Sil and Min-U-Sil coincide and there are no significant changes in $\Delta\nu$. We believe that changes observed in the narrow band below 500°C are primarily due to pertubation from the broad band.

The effect of calcination on the particle morphology is as follows: X-ray powder patterns of the calcined Cab-O-Sil showed no detectable crystallanity. However, appreciable sintering of the material was evidenced by a decrease in the specific surface area from 200 m^2/g for the untreated material to 186 ($\pm$ 5) m^2/g for material calcined at 800°C and 71 ($\pm$ 5) m^2/g for material calcined at 1095°C. No change in specific surface area of Min-U-Sil was observed under the calcination conditions em-

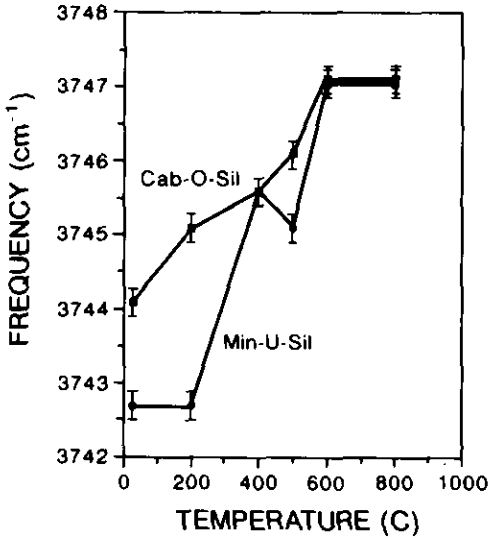


FIGURE 9. Variation of the frequency (line position) of the silanol band in Cab-O-Sil and Min-U-Sil with calcination temperature. Lines connecting the points are drawn for visual aid.

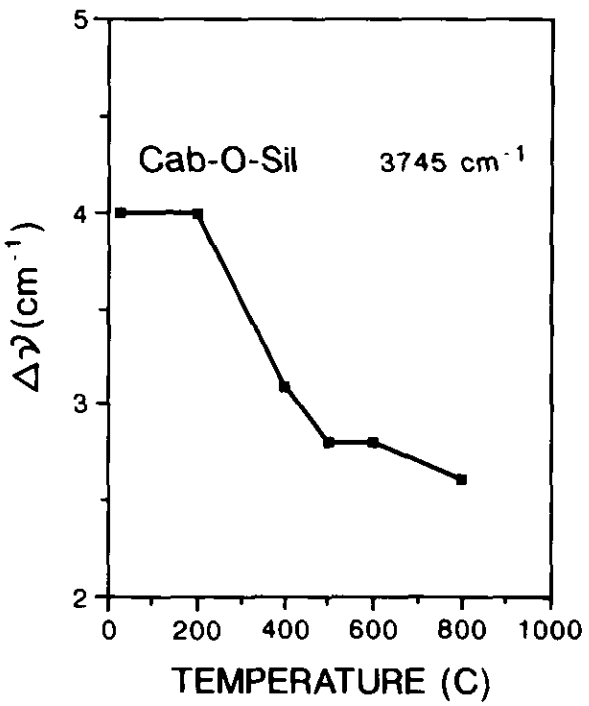


FIGURE 10. Variation of $\Delta\nu$ (half-width at half maximum) of the silanol band near 3745 cm^{-1} in the Cab-O-Sil sample with calcination temperature. Lines connecting the points are drawn for visual aid. For Min-U-Sil, intensity of the band is too low to accurately measure $\Delta\nu$.

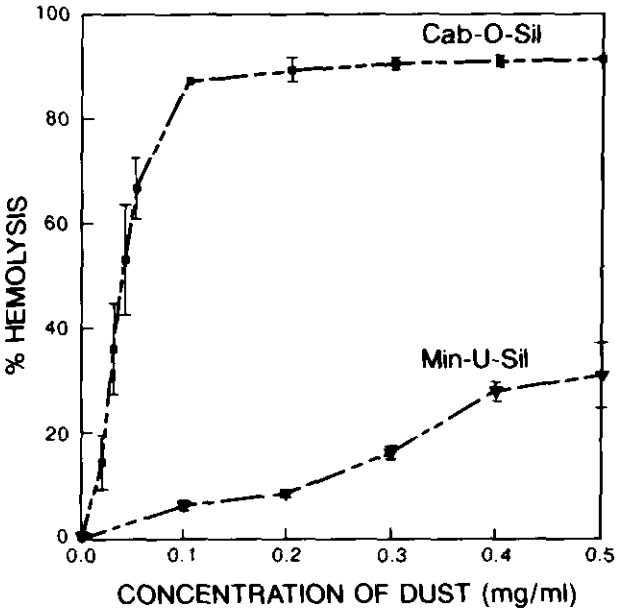


FIGURE 11. Hemolytic activity of uncalcined (untreated) samples versus silica dust concentration. At low concentration Cab-O-Sil is about an order of magnitude more cytotoxic than Min-U-Sil on a per unit mass basis. Data are median values ± SD, based on three to seven duplicate samples for Cab-O-Sil and four to five duplicate samples of Min-U-Sil. Lines are drawn through the data points for visual aid.

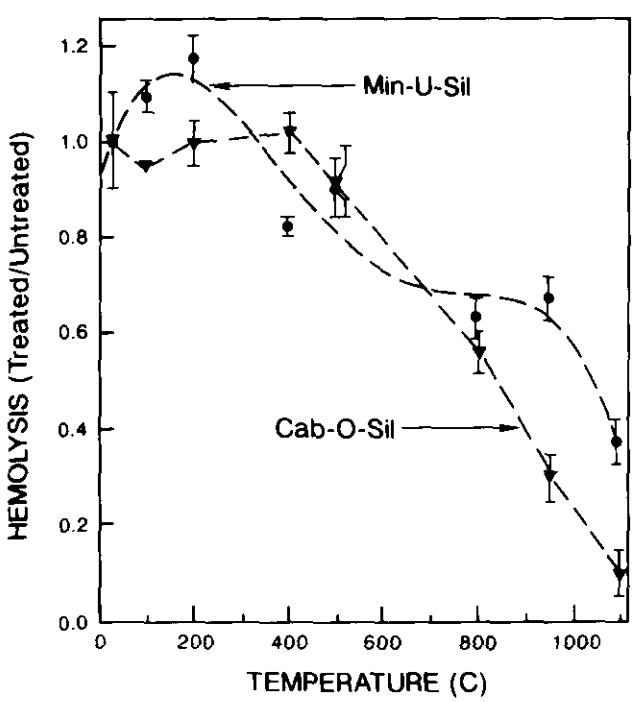


FIGURE 12. Effect of calcination temperature on hemolytic activity of Cab-O-Sil and Min-U-Sil samples at dust concentration of 0.05 mg/mL. The data are normalized to the hemolytic activity of untreated (uncalcined) sample and represent median values ± SD based on three to four duplicate samples. Lines are drawn through the data points for visual aid.

ployed. The FTIR spectra of calcined Cab-O-Sil also showed no new bulk modes which would be evidence of change in crystallinity.

Hemolytic Activity

The hemolytic activities of untreated Min-U-Sil and Cab-O-Sil as a function of dust concentration are compared in Figure 11. These results show that on a per unit weight basis Cab-O-Sil is about an order of magnitude more hemolytic than Min-U-Sil. The opposite is the case, however, if the hemolytic activity is measured per unit surface area, since the specific surface area of Cab-O-Sil is approximately 40 times larger than that of Min-U-Sil.

The effects of calcination on the relative hemolytic activity (treated sample/untreated) of Cab-O-Sil and Min-U-Sil are depicted in Figure 12. The activity increased slightly after calcination at temperatures below 500°C for Min-U-Sil. Both materials exhibit dramatic decreases in hemolytic behavior when calcined at higher temperatures. For samples calcined at 1095°C, the ability to lyse erythrocytes is reduced to about 10% of the untreated value for Cab-O-Sil and to about 30% of the untreated value for Min-U-Sil. Considering that calcination at 1095°C reduces the specific surface area of Cab-O-Sil to only one-third of its original value, it may be inferred that the reduction of hemolytic activity by effects other than the reduction of surface areas is similar for both materials.

The effects of rehydroxylation of the silica surface observed by FTIR spectroscopy were also seen in hemolysis assay results. Min-U-Sil and Cab-O-Sil calcined at 1095°C showed only a slight increase in hemolytic activity after several months of storage, as shown in Figure 13. The same dusts calcined at lower temperatures returned values of hemolytic activity of untreated material within 10 to 20 days, as illustrated in Figure 14.

Discussion

The parallel results of hemolytic activity and surface-free OH group concentration suggests that silanol groups may be directly involved in the lysing process. If so, then the relative changes in the intensity of the silanol groups measured by the FTIR band at 3745 cm^{-1} may be used to determine the hemolytic potential and, possibly, the cytotoxicity of respirable silica particles. This hypothesis is based on the following positive correlations: *a*) The intensity of the silanol band at 3745 cm^{-1} after calcination at different temperatures (Fig. 8) and the relative hemolytic activities (Fig. 12) show a similar dependence on calcination temperature, namely, an increase at lower temperatures, reaching maximum value near 500°C, followed by drastic decreases at higher temperatures. In contrast to the free silanol band at 3745 cm^{-1}, the intensity of the broad band at 3000 to 3700 cm^{-1} that is attributed to various hydrogen bonded OH groups, physisorbed water, and other impurities decreases monotonically with calcination temperature (Fig. 8). *b*) The ratio of the intensity of the free silanol

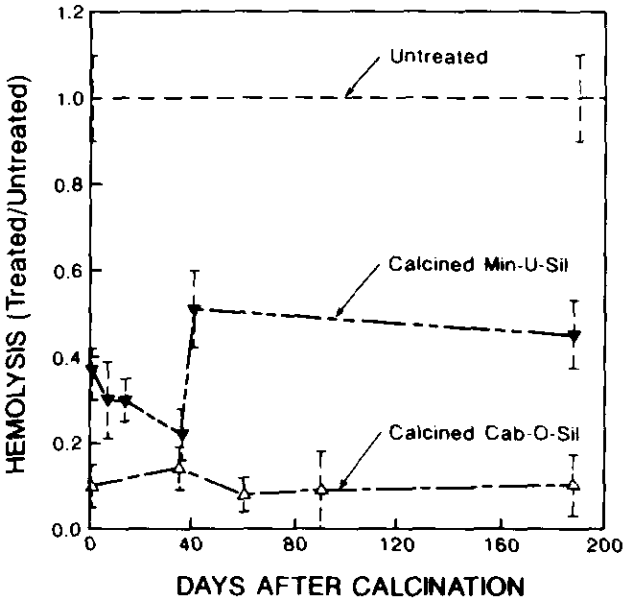


FIGURE 13. Data on the recovery of the hemolysis of the Cab-O-Sil and Min-U-Sil calcined at 1095°C and tested at dust concentration of 0.5 mg/mL, with exposure in air in days. The untreated (uncalcined) sample shows no change in hemolysis with time. Lines through the data points are for visual aid.

band in Cab-O-Sil to that in Min-U-Sil (≃ 20 in Fig. 8) is about the same as the ratio of their hemolytic activities (Fig. 11). The lack of one-to-one correlation between hemolysis and intensity of the silinol band (Figs. 8 and 12), particularly below 500°C, may be due to other factors. For example, the broad band may have some effect on hemolytic activity, and the relationship between the surface structure of particles and their hemolytic activity may be complex and dependent on several variables. However the correspondence observed here between the intensity of the silanol band and hemolytic activity, particularly above 500°C, is more consistent than any other relationship that has been proposed for quantification of hemolytic activity (e.g., quartz content, crystallinity, etc).

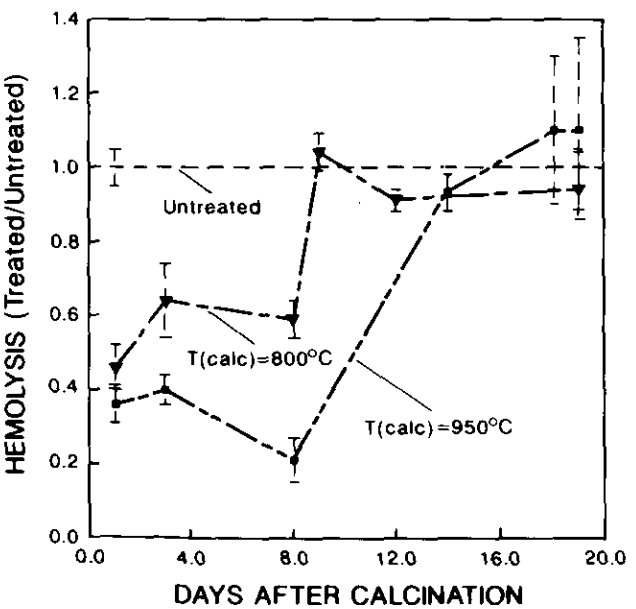
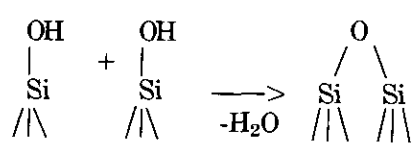


FIGURE 14. Evidence for the recovery of hemolytic activity of Cab-O-Sil calcined at 800°C and 950°C, by exposure to air. The sample calcined at lower temperature recovers its hemolytic activity sooner. Data represent median values ± SD with three duplicate samples for 800°C and four duplicate samples for 950°C. Data points are connected by lines for visual aid.

Previously reported experiments (*2–10*) have indicated a possible correlation between cytotoxicity and the concentration of surface silanol groups on silica particles. However, the results presented here on the variations with calcination temperatures and the comparison between Min-U-Sil and Cab-O-Sil are new and, in our view, provide a more convincing case for the correlation between the hemolytic activity and the concentration of the surface silanol groups as measured by the intensity of the 3745 cm^{-1} FTIR band.

A possible explanation for the observed relationship between the intensity of the silanol band and the calcination temperature shown in Figure 8 is considered next. The initial increase in the intensity of this band for calcination temperatures below 500°C may be related to the accompanying decrease in the intensity of the broad band (Fig. 6). As the calcination temperature increases, the absorbed water that is hydrogen bonded to the silanol groups is desorbed. The intensity of the narrow band of the free-surface OH groups increases. Most OH groups on the silica surface [namely, vicinal and linearly bonded OH groups (*16*)] are mutually bonded; when the H-bonds between these species are broken by calcination, the intensity of the free silanol band is further increased. After calcination at about 500°C, the broad band nearly disappears (Fig. 6), signaling the removal of H-bonded impurities and surface groups. Calcination above 600°C causes neighboring free silanol groups to combine, liberate water, and form the stable siloxane bonds:



The position and the half-width of the sharp band then become constant (Figs. 9 and 10), whereas its intensity continues to decrease. After calcination temperatures near 1100°C, the silica surface approaches a fully siloxinated configuration. The siloxane surface is hydrophobic, and the rehydroxylation of this material is extremely slow. The lysing or hemolytic potential of silica particles with a siloxane surface structure is also very low.

The theory that the toxicity of silica is related to its surface silanol groups was first advanced by Nash et al. (*31*). The theory is based on evidence that proteins denature in the presence of proton-donating compounds,

e.g., Si-O-H, and this is proposed as the mechanism underlying membrane damage and hemolysis caused by respirable silica particles. The more recent paper by Summerton et al. (*32*) suggests that surface hydrogen of silica bonds to protein components of the membrane and subsequently abstracts these proteins from the membrane. The research by Light and Wei (*33*) on the hemolytic activity of abestos particles linked membrolytic activity to the negative charge on particle surfaces (i.e., ionized silanol groups). More recently Langer and Nolan (*10*) proposed that both functionalities of the surface silanol are involved in the lysing of red blood cells. Also the possible role of free radicals of crystalline silica to its cytotoxicity has been discussed by Vallyathan et al. (*34*) in a recent paper.

It has also been shown that various blocking and/or neutralizing processes can reduce or eliminate the hemolytic activity of silica particles. The hydrogen bonding of certain molecules such as polyvinyl pyridine-N-oxide and pyridine-N-oxide to the silanol groups on a silica surface effectively inhibit the hemolytic activity of silica particles (*35,36*). Similar effects have been obtained by the absorption of surfactants, aluminum compounds, and treatment with hydrofluoric acid (*8,20*). Recent experiments by Razzaboni and Bolsaitis (*37*) have shown that the addition of exogenous catalase to erythrocytes markedly reduces the hemolysis caused by silica particles.

The present experiments show a very direct relationship between free silanol group intensity and cell lysis and suggest that the FTIR spectroscopy may be useful for monitoring the cytotoxicity of silica particles. Hemolytic activity is not, however, definitive proof of fibrogenicity. Fibrogenicity and cytotoxicity must be corroborated by macrophage tests and *in vivo* assays. We are currently conducting such experiments with variously treated silica particles, and we will apply FTIR spectroscopy to monitor the surface functional groups of the materials tested.

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