

Physicochemical properties of crystalline silica dusts and their possible implication in various biological responses

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The effect of grinding, heating, and etching was investigated on polymorphs of silicon dioxide exhibiting different biological responses. Diatomaceous earths were converted into cristobalite at 1000°C. Dusts obtained by grinding crystalline minerals exhibited different micromorphology and a propensity to originate surface radicals which decrease in the sequence cristobalite → quartz → coesite → stishovite. The production of surface radicals was suppressed by grinding in the presence of water. Thermal treatments selectively quenched the radicals and decreased surface hydrophilicity. Quartz treated with aluminum lactate exhibited higher surface acidity when compared with pure quartz, with a reduction in fibrogenicity. Etching by hydrofluoric acid smoothed the particles with loss of specific surface. Adsorption of water on three cristobalite dusts of different origin (ground mineral, ex-diatomite, heated quartz) indicated a loss in heated quartz (1300°C) that was relatable to the corresponding reduction in fibrogenicity.

Key terms cristobalite, diatomite, fibrogenicity, ground silica, hydrophilicity, membranolysis, particle size, quartz, stishovite, surface radicals.

In spite of extensive work since the early 1950s on the pathogenicity of silica-related diseases, the molecular basis for the mechanism of action of silica is still unknown. Two reasons may account for this: (i) experimental silicosis studies have often been carried out with ill-defined silica specimens, while epidemiology has been more concerned with the quantitative aspects of exposure than with the qualitative ones, such as the physicochemical characteristics of the inhaled dusts, and (ii) once inhaled, several particle-cell interactions take place whereby the silica surface elicits a specific biological response. It is well known that alveolar macrophages play a role in the particle ingestion cycle. Adsorption of endogenous matter, cell adhesion, oxidative burst, and membranolysis occur via different reactions. The molecular mechanism governing each step involves different particle surface functionalities so that there are likely to be multiple pathways influencing the overall pathogenicity.

The surface chemistry of crystalline silica dusts has been studied in detail so that each of the biological responses elicited in cells and tissues may be linked to a specific chemical feature of the particle. In an attempt to compare toxicities found with dusts having nominally the same chemical composition, the effect of heating, grinding, and etching has been studied for several crystalline polymorphs — quartz, cristobalite, coesite, stishovite — comparing pure samples with mineral and industrial ones.

Attention was focused on the following properties: (i) the hydrophilicity and propensity for hydrogen bonding, determined by the abundance and geometric arrangement of surface silanols (SiOH) and evaluated from the heat of adsorption of water vapor;

(ii) Lewis and Brønsted surface acidities, the former relatable to cation impurities and the latter to silanols acting as proton donors; (iii) strained siloxane and peroxy bridges, Si-(O)_n-Si, evaluated from their reactivity with water and ammonia; (iv) surface radicals and charges, originated by grinding and evaluated from the electron spin resonance (ESR) spectrum of the dusts. Although not strictly related to chronic fibrogenicity, surface charges may be implicated in acute cell damage, free radical release, and possibly also carcinogenicity.

The possible role played in vivo by the various surface sites is depicted in figure 1. Several factors — origin, crystallinity, thermal and mechanical history, and contaminants — determine the presence and abundance of the various surface sites, hence also the peculiarity of each silica specimen.

The aim of the present paper is to outline the influence of these factors on some surface properties that may be related to the biological response to silica.

Materials and methods

The following materials were used: pure quartz chips (99.999%, Atomergic) (QRZ-p), Madagascar quartz (QRZ-md), Min-U-Sil 5 and DQ12 (Pennsylvania, Glass Sand Co) (1); cristobalite: ground mineral (C&E Minerals, King of Prussia, PA) (2, 3) (CSR-m), pure quartz chips ground and heated (1300°C) (CSR-q), heated diatomaceous earth (CSR-d) (4); stishovite and coesite: purified dusts from Meteor Crater (Arizona) (5); and commercial amorphous silicas: biogenic “silica earth” and “celite 545” Merck amor-

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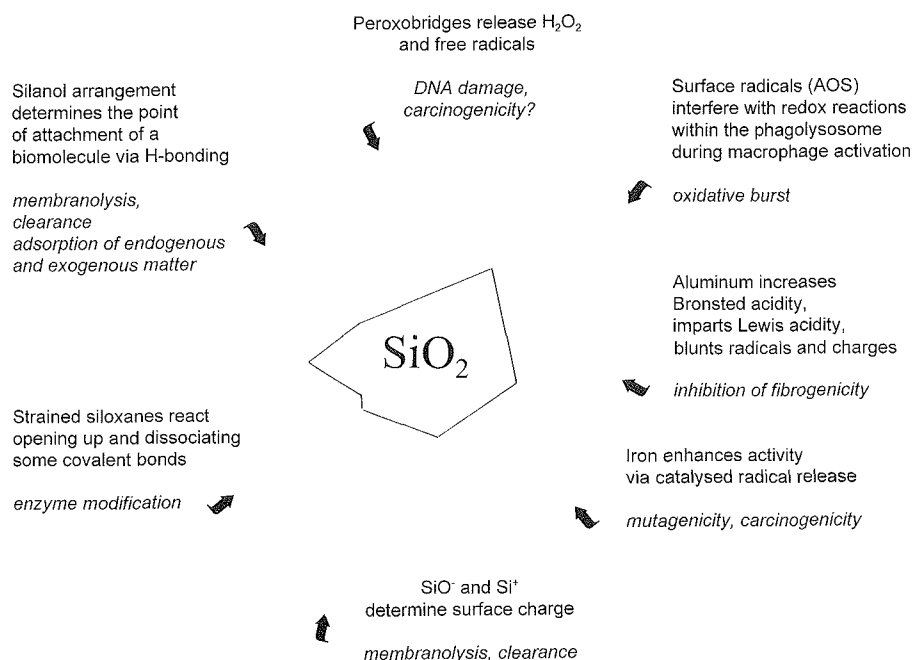


Figure 1. Possible role in the pathogenicity of chemical functionalities at the silica surface.

phous Aerosil 50, A-50, Degussa, an amorphous diatomaceous earth sample.

The set of samples was chosen to compare well-established pathogenic silicas (quartz and cristobalite) with less or nonpathogenic polymorphs (coesite, stishovite and amorphous silica); to investigate surface properties of three cristobalites of different origin: ground cristobalite mineral (CSR-m), quartz dust transformed into cristobalite by heating above 1300°C (CSR-q), and calcined diatomaceous earth (CSR-d); and to contrast samples largely used as standard dusts for in vivo and in vitro tests (Min-U-Sil and DQ12).

The crystal structures were investigated by X-ray diffraction (Philips diffractometer, $\text{Co } K_\alpha$ radiation) and (micro)morphology by transmission electron microscopy (TEM) (Jeol JEM 2000EX). Particle-size distributions were evaluated by means of a Coulter LS 130 with a fluid module.

Surface areas were measured with the BET method (nitrogen adsorption at -196°C , "Quantasorb," Quantachrome).

Hydrophilicity, propensity for hydrogen bonding, surface acidity, and the reactivity of strained bridges were all evaluated from the heat of adsorption of appropriate probe molecules (water, ammonia) using a Tian-Calvet microcalorimeter (Setaram) connected to a volumetric apparatus, according to previously described techniques (6–8). Surface radicals and charges were measured by electron spin resonance spectroscopy by means of a Varian E 109 spectrometer as previously described (9, 10).

Results and discussion

The interaction of a mineral particle with living matter depends upon its form and its surface reactivity. The term "form" comprises the crystal structure, size, shape, and micromorphology of the crystallites, while "surface reactivity" is any surface arrangement or site liable to react at the liquid-solid or air-solid interface.

Form

All crystalline samples of mineral origin exhibited the expected XRD pattern. TEM images (omitted from this paper but available from the authors) of some of them revealed substantial differences that may be ascribed to the different crystal structure and to the origin of the mineral. The heated diatomaceous earth CRS-d was fully crystalline, retaining the diatom morphology, and it exhibited only the cristobalite crystalline structure. It is noteworthy that these products are sold under commercial names that do not indicate their crystalline nature. They have been prepared from amorphous diatomaceous earths calcined below 1000°C (ie, in a temperature range in which cristobalite is thermodynamically unstable with respect to quartz). Such a low-temperature material should be regarded with great concern, being in the respirable size range and in the most toxic crystalline form. The transition temperature of any lot of biogenic silica to cristobalite will depend on its particle size and impurities. It is possible, however, to set up ad hoc chemical treatments for displacing the transition at higher temperatures.

Calcined diatomaceous earth represents one of the few cases in which a crystalline form of silica is directly obtained in a finely divided form. Usually quartz and other crystalline dusts are obtained by grinding bulk pieces of the mineral. In such cases fresh reactive surfaces are created during the grinding process (11). The single silica particles exhibit sharp and protruding edges but are absent in stishovite. The particles tend to stick together, the smaller ones covering the larger ones, because of surface charges. The latter have been supposed to play a role in particle-tissue interaction (12, 13). This stickiness may somehow distort particle size measurements. (See the following discussion.)

In the evaluation of size distribution, the question of parameter arises. Figure 2 illustrates data obtained with a quartz dust finely ground for biological tests. In the number or diameter representation few bigger particles escape detection, even if they represent a

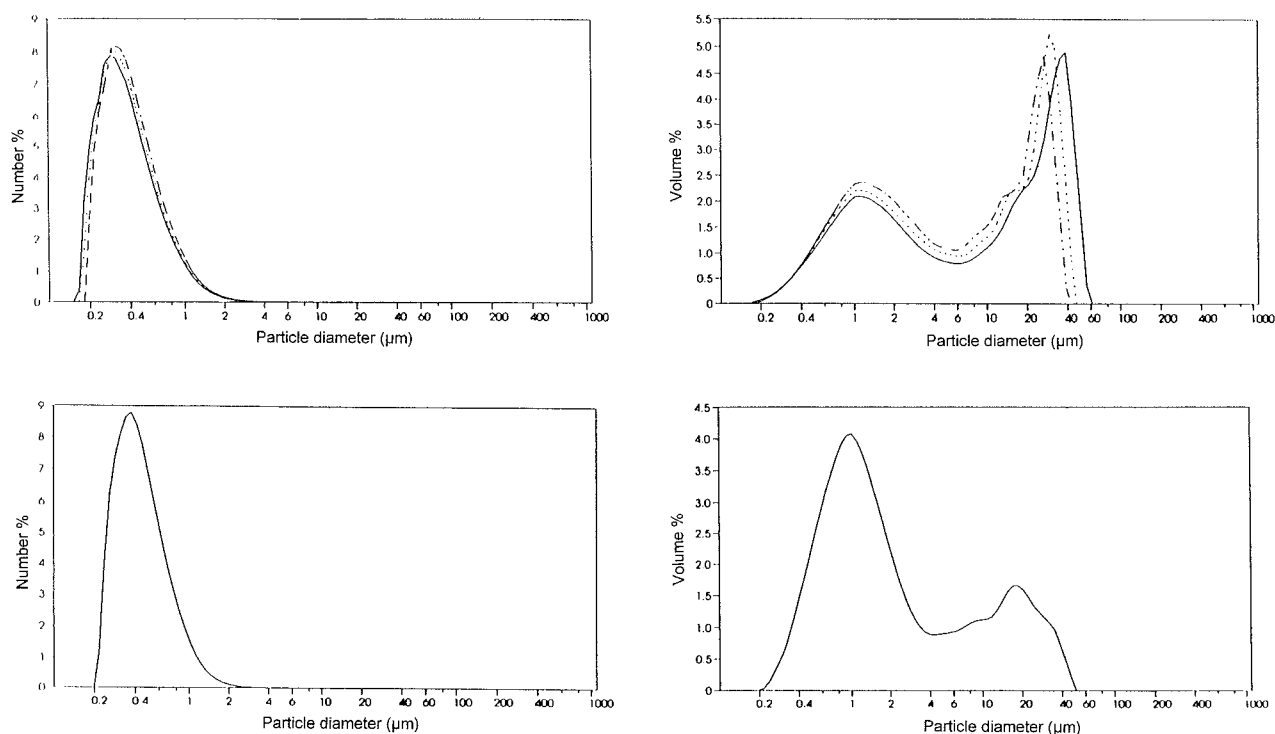


Figure 2. Evaluation of particle size distribution. (left side: number (%) of particles as a function of particle diameter; right side: volume (%) of particles as a function of particle diameter; upper graphs: bold lines, no sonication; dotted and dashed lines, sonication of particle suspensions with ultrasounds from the Coulter LS Fluid Module applied for 2 and 5 min, respectively, before the measurement; lower graphs: ultrasounds from a stronger external bath applied for 5 min before the measurement)

consistent part of the weight of the sample. Depending on the kind of test (eg, inhalation, injection, cytotoxicity, or cell transformation) big particles may be involved. In experiments in which results are compared by the weight of dust, this effect may be a source of error. The dotted lines in the figures show the effect of sonicating the dust suspensions, a process which somehow detaches particles held together by surface charges within the aggregates. Clearly, when tightly held particle aggregates such as quartz are being dealt with, the intensity of the ultrasound influences the results.

Surface reactivity

Grinding. Freshly ground silicas have shown a higher degree of toxicity ascribed to the peculiar reactivity of newly created surfaces. Grinding cleaves the silicon-oxygen bond via an homolytic or an heterolytic path (figure 3), with consequent formation respectively of dangling bonds ($\text{Si}^\bullet$, $\text{SiO}^\bullet$: reactive surface radicals with an impaired electron in a p orbital) and surface charges (Si^+ , SiO^-). Once formed, these very reactive species tend either to recombine with the formation of strained reactive bridges $[\text{Si}-\text{O}-\text{Si}]$; $[\text{Si}-(\text{O})_n-\text{Si}]$ or to react with atmospheric components, yielding active oxygen species at the surface and in subsurface layers. The ESR spectra of the dusts, largely reported in previous papers (9, 10, 11, 14), revealed the identity and abundance of the various radical forms. The potential role of radicals in silica toxicity has been previously reported (9, 11, 14, 15). Some radicals underwent a relatively rapid decay in the first hours after grinding (11, 16–18), and this decay was related to acute damage in workers involved in mining, drilling, or sandblasting and exposed to freshly ground dusts in the respirable

range. The most stable surface radicals, also visible in aged dusts (14), did not seem to be involved in the pathogenic process; still they appeared to be a good marker of reactivity and hence also of the potential toxicity of a given dust. The ESR spectrum of the solid revealed the amount and the chemical features of the radicals formed. We compared the spectra obtained for quartz, coesite, and stishovite before and after a very mild grinding in a ball mill. It is noteworthy that with coesite, whose pathogenicity in experimental silicosis is still under debate (19, 20), a structured spectrum similar to quartz appeared after grinding. By contrast, stishovite was nonpathogenic (5) and did not develop any radical when ground. In comparison with previous results the overall number of radicals decreases in the series cristobalite $\rightarrow$ quartz $\rightarrow$ coesite $\rightarrow$ stishovite (ie, upon increasing density of the SiO_2 polymorph).

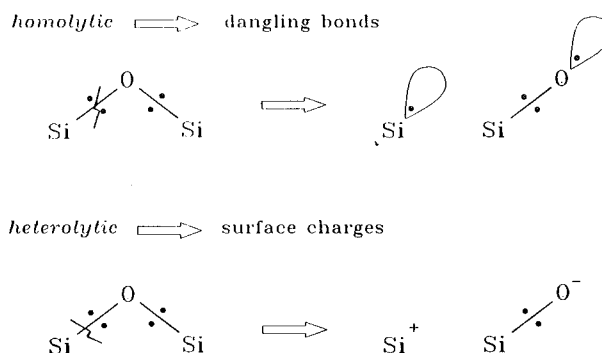
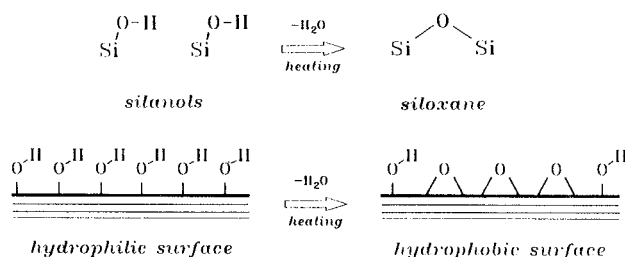


Figure 3. Cleavage of the silicon-oxygen bond after mechanical grinding.

Table 1. Uptake and molar heat of adsorption [H_2O = water, NH_3 = ammonia, n = number of moles adsorbed, q = molar heat of adsorption (H_2O at $p = 5$ torr; NH_3 at $p = 40$ torr)]

	Reversible		Irreversible	
	n ($\mu\text{mol} \cdot \text{m}^{-2}$)	q ($\text{kJ} \cdot \text{mol}^{-1}$)	n ($\mu\text{mol} \cdot \text{m}^{-2}$)	q ($\text{kJ} \cdot \text{mol}^{-1}$)
H_2O adsorption				
Aged quartz (unheated Min-U-Sil)	8.50	52	0.00	0
Freshly ground quartz (unheated QRZ-p)	7.20	57	1.00	50
NH_3 adsorption				
Untreated quartz (QRZ-md heated at 500°C)	9.50	68	1.50	20
Aluminum-treated quartz (QRZ-md heated at 500°C)	8.00	56	3.00	67

Comparisons, however, are not straightforward, as the number of radicals formed depends not only upon the grinding procedure (time, weight of the balls), but also upon the environment. A dry

**Figure 4.** Progressive conversion, upon heating, of a hydrophilic surface into a hydrophobic one, after the condensation of silanols into siloxanes.

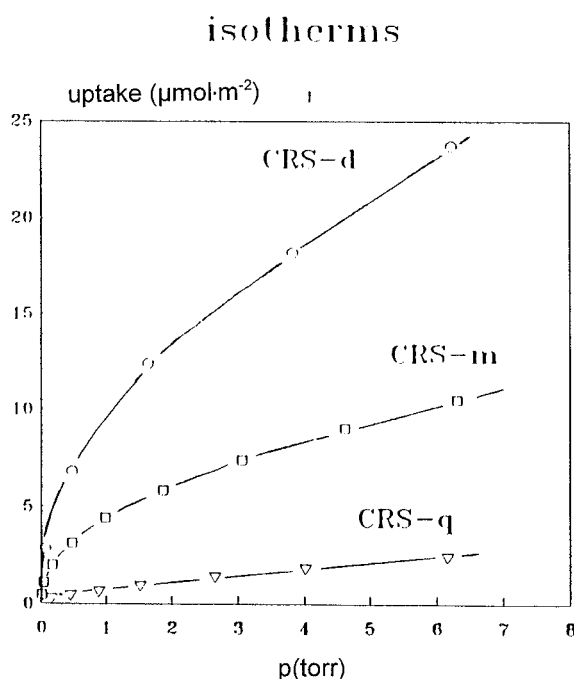
atmosphere favored the formation of radicals and distorted bridges, while a wet one inhibited radicals because water assists surface reconstruction (21). When mineral is ground wet, the dust loses its capability to originate radicals when compared with dust from mineral ground while dry, even if previously heated. Differences in the pathogenic potential of dusts are a function of their mechanical history, and they govern the release of active oxygen species involved in an oxidative burst and in lipid peroxidation.

Other manifestations of the reactivity of freshly ground dusts include (i) oxygen being absorbed at -196°C and yielding an "ozonide" radical O_3^- whose abundance parallels the fibrogenic potential (10), (ii) only freshly ground dusts release H_2O_2 , following the hydrolysis of the surface peroxide bridges ($\text{Si}-\text{O}-\text{O}-\text{Si}$) reported by Kolbanov et al (22), and (iii) irreversible uptakes of water (table 1) on freshly ground QRZ-p revealing strained siloxane bridges ($\text{Si}-\text{O}-\text{Si}$), absent on Min-U-Sil, which readily dissociate the molecule and form a couple of vicinal silanols.

Heating. When silica is heated, surface radicals are healed and disappear (9), while silanols are condensed into siloxanes (figure 4) and the hydrophilic surface is progressively converted into a relatively hydrophobic one (8). This change apparently does not modify the ultimate fibrogenicity of the dust, as long as some hydrophilic patches persist on the surface, but it does markedly lower the membranolytic potential of the dust and affect transport properties. More heated than unheated cristobalite particles are found in cells in bronchoalveolar lavage and are transported to lymph nodes in exposed rats (3, 23). Hydrophilicity thus determines the fate of inhaled particles.

The extent of hydrophilicity was measured from the amounts of adsorbed water vapor and its energy of interaction: the higher these values, the more hydrophilic the surface. The results of the adsorption of water on the three cristobalite samples of different origin, CSR-m, CSR-q, CSR-d, are shown in figure 5. Both the adsorption isotherms and the heat of adsorption reveal remarkable differences between the three samples. CSR-m and CSR-d are very hydrophilic, whereas CSR-q is fully hydrophobic. The figure clearly demonstrates how the surface reactivity may differ, even within a set of samples of similar size and identical crystal structure. CSR-m has been shown to be highly pathogenic in inhalation studies (2), while, in the same intratracheal injection study, CSR-d provoked a typical silicotic response and CSR-q only elicited alveolar lipoproteinosis (4, 24). A remarkable reduction in fibrogenicity upon heating quartz and cristobalite at a high temperature has been also found by Więcek (25).

A possible explanation for the heat effect is that unheated ground dusts or mildly heated ones still retain the active surface sites prerequisite to determining the biological responses leading

**Figure 5.** Adsorption of water vapor on three cristobalites of different origin: CSR-d ($45 \text{ kJ} \cdot \text{mol}^{-1}$) = calcined diatomaceous earth; CSR-m ($58 \text{ kJ} \cdot \text{mol}^{-1}$) = ground cristobalite mineral crystal; CSR-q ($25 \text{ kJ} \cdot \text{mol}^{-1}$) = pure quartz dust converted into cristobalite by heating up to 1300°C . Adsorption isotherms (uptakes versus pressure) and average molar heat. (molar heat at $p = 3$ Torr)

to silicosis, whereas, when dusts are heated at a very high temperature, surface irregularities, radicals, and charges are healed, and silanols are wholly eliminated to form stable unreactive siloxanes, with consequent loss of the pathogenic potential. The higher hydrophilicity of CSR-d compared with that of CRS-m may be simply due to metal oxide contaminants.

Etching. Crystalline silicas were etched by three chemical agents, hydrofluoric acid, alkaline hydroxides and catechol. By attacking the silica framework, these chemicals destroyed the external surface layers and progressively eliminated surface radicals. However silanols and charges were maintained or reformed after etching (10). With hydrofluoric acid the external surface is smoothed, while the specific surface decreases. As a consequence of the dissolution of smaller particles the size distribution reveals a higher proportion of bigger particles.

Etching also eliminates impurities which modulate silica toxicity and to some extent substitute fluoride for hydroxyl, with consequent variations in the overall hydrophilicity. It has been reported that membranolytic activity was decreased (26), but in some cases fibrogenicity increased (27).

Impurities. Silica particulates obtained from mineral ores may contain some impurities that impart their chemical properties to the dust and may modify the pathogenic responses either by enhancing or decreasing intensity. The results obtained from the simple adsorption of water on two of the quartz dusts primarily used in animal and cell experiments, Min-U-Sil and DQ12, are shown in figure 6. Both the adsorbed amounts and the heat of interaction are higher for DQ12, for which 5% of the dust is not silica and the adsorption kinetics reveal a very slow reaction on DQ12 that does not take place with Min-U-Sil. If these surface properties have something to do with the molecular mechanisms predictive of the pathogenic process, the biological tests performed with these dusts may yield different results. It is noteworthy that Muhle et al (28) found the steepest cancer potency slope with DQ12, whereas Dagle et al (29), with Min-U-Sil, found the shallowest. The two tests are not fully comparable however because the maximum tolerated dose was exceeded by Dagle et al and different rat strains were used. Surface properties may, however, also account for some of the differences in carcinogenicity.

The metal ion contaminants most commonly found in silica specimens are iron and aluminum. They both decrease membranolytic activity (26, 30, 31), and, while aluminum may also inhibit the development of silicosis (32), iron impurities may act as a potential source of active oxygen species (33), causing DNA damage (34) with an increase in the carcinogenic potential of the dust.

In order to detect any surface modification brought about by aluminum, we measured the adsorption of ammonia, a Lewis and Brønsted base, on a quartz dust (QRZ-md) before and after treatment with aluminum lactate, according to the protocol used by Bégin and his associates (32), to prevent silicosis. The most remarkable differences between the two samples were found in the irreversible interaction with ammonia in the partially dehydrated samples (500°C), which evidenced a much higher surface acidity on the treated than on the untreated sample (table 1). Aluminum in a silica framework substitutes for silicon in a tetrahedral position, acts as a Lewis acid (electron acceptor), and also enhances Brønsted acidity (proton donor) by facilitating hydrogen ion donation from nearby silanols. A higher acidity may affect surface affinity for biomolecules, membranolytic potential, and the overall reactivity of the surface.

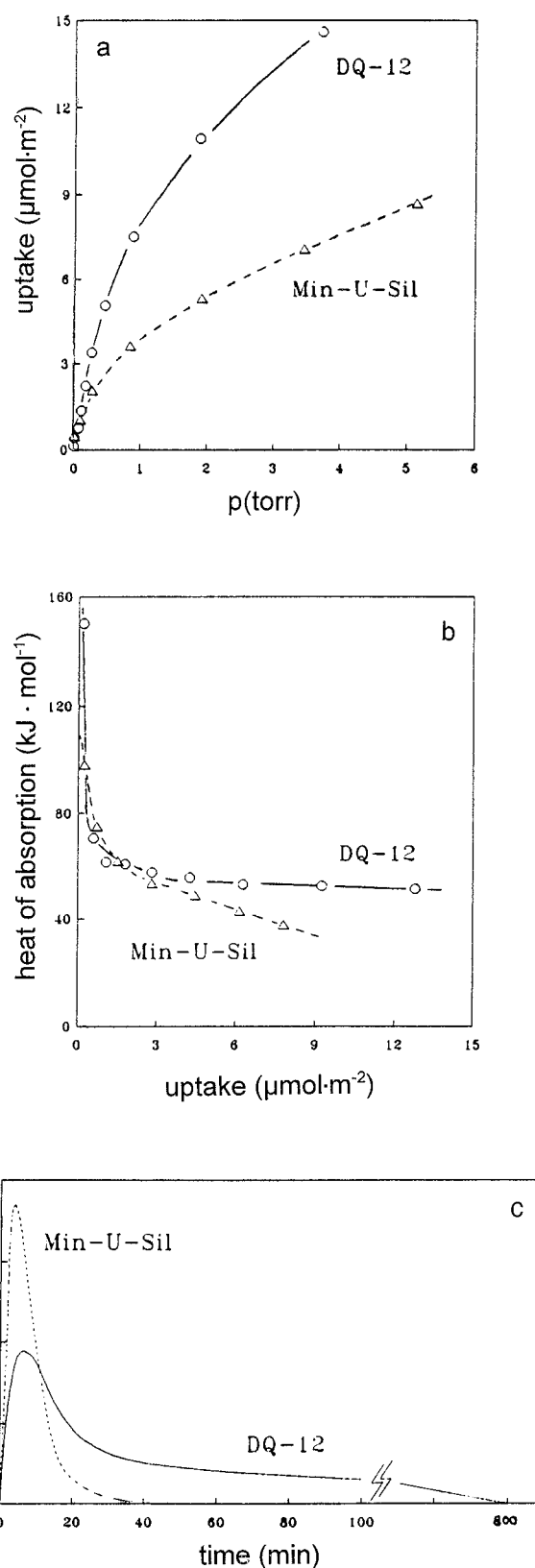


Figure 6. Adsorption of water vapor on two types of quartz, Min-U-Sil and DQ12, widely used in biological tests: a: adsorption isotherms (uptake versus pressure); b: heat of adsorption as a function of coverage; c: kinetics of the heat emission of the first adsorbed dose of water, heat emission (au), versus time.

Concluding remarks

Grinding, heating, and etching strongly influence surface properties of silica dusts. Furthermore, contaminants and the presence of water and other ions alter surface properties. Our data strongly suggest the need to expand comparisons to explain biological differences in the toxicity and carcinogenicity of silica particulates.

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