# EFFECT OF THERMAL TREATMENT ON THE SURFACE CHARACTERISTICS AND HEMOLYTIC ACTIVITY OF RESPIRABLE SIZE SILICA PARTICLES

**B. L. RAZZABONI* • P. Bolsaitis* • W.E. Wallace, Jr.† • M.J. Keane†**

*Energy Laboratory, M.I.T., Cambridge, MA 02139
†NIOSH, Morgantown, WV 26505

## ABSTRACT

Thermal and chemical treatment of respirable size silica dusts samples is shown to induce marked changes in their hemolytic activity. The cytotoxicity of crystalline α-quartz (Min-U-Sil), and fumed silica (Cab-O-Sil) particles, as measured by a hemolytic activity protocol, is decreased by calcination and can be related to the dehydroxylation of the surface. The hemolytic activity of β-cristobalite particles of respirable dust size was also determined and found to be lower than that of α-quartz. The change in the surface structure resulting from thermal treatment is detectable by photo-acoustic infrared spectroscopy and zeta-potential measurements. The absorption band in the 3200–4000 $cm^{-1}$ frequency region of both Cab-O-Sil and Min-U-Sil disappears upon heat treatment while a sharp band, identified with single silanol groups, at 3750 $cm^{-1}$ increases in intensity. The zeta potential-pH profile, in the pH range of 4.0–7.0, of the calcined, siloxane surfaced particles is more negative than that of material with a silanol surface.

The cytotoxicity of the crystalline and fumed silica dusts was also found to be strongly dependent on particle size. Fumed silica of large surface area (small particle size) exhibits an initial increase in hemolytic activity upon calcination. This result confirms other experimental observations pointing to a particle size of maximum toxicity.

## INTRODUCTION

The toxicity of silica and other mineral particles, as manifested by their role in inducing pneumoconiosis, fibrosis, silicosis and other pulmonary disorders is largely traceable to the characteristics of particle surfaces and to particle morphology. The great wealth of data on the physical and chemical properties of silica is still insufficient for a comprehensive understanding of the specific parameters to be associated with fibrogenic activity and, correspondingly, with the optimum means for characterizing and quantifying the toxicity of dusts, and the design of possible preventive or therapeutic methods.

The great diversity of parameters that have been proposed and tested for correlation with cytotoxicity suggest that more than one mechanism may be involved in the fibrotic activity of silica particles. The crystalline structure of the material (more active tridymite versus less active crisobalite and passive stishovite), freshly formed surfaces and free radicals associated therewith, silicic acid adsorbed onto silica surfaces, the concentration of hydroxyl groups at silica surfaces, particle size and morphology, among other properties, have been investigated and found to correlate in tests of cytotoxicity.

The main thrust of research presented here is to seek more definitive correlations of silica particle properties with cytotoxic activity to identify the mechanism or mechanisms leading to cytotoxic activity and the most suitable instrumental methods for identifying the material properties associated with silicosis and other pathogenic properties of respirable dusts. In the present paper are presented results on the effect of dehydration and dehydroxylation of crystalline and amorphous silica particles on their cytotoxicity as measured by hemolytic activity. One of the prevalent theories on the mechanism of silicosis presumes that such activity is induced by "clean surfaces of crystalline silica, usually quartz,"[1] hence it is deemed of interest to assess the cytotoxic potential of the most common structures of such surfaces.

The main feature of normal, clean quartz surfaces is the degree of surface hydration. The normal, anhydrous surface terminates in -Si-O-Si- (siloxane) groups. Equilibration with water will first result in the formation of —Si—O—H (silanol) groups and, eventually, the physisorption of water onto the silanol groups.[1] Particularly well formed and clean surfaces of quartz may adsorb more than four molecular layers of water.[2] Another type of "clean" quartz surface, but not included in the present study, is that resulting from the fresh cleavage of quartz crystals. Such surface may present broken bonds in the form of free radicals which may exhibit particularly high chemical reactivity.[3] In the present study we compare the cytotoxic activities of quartz and amorphous silica dusts dehydroxylated at different temperatures and re-equilibrated with the atmosphere over prolonged time periods, thus comparing the relative cytotoxicities of surfaces of crystalline and amorphous silica particles covered by adsorbed water, silanol, and/or siloxane groups.

## EXPERIMENTAL METHOD

Materials: The materials used in this study were Min-U-Sil crystalline silica dust from two different batches obtained from U.S. Silica Inc. of Pittsburgh, PA, Cab-O-Sil, a fumed silica dust provided by the CABOT Corporation, and respirable size β-cristobalite dust, a standard reference material of National Bureau of Standards. X-ray diffraction analysis showed Min-U-Sil to be essentially pure α-quartz, while the Cab-O-Sil dust exhibited no detectable crystallinity. The Min-U-Sil particle size ranged from 0.4 to 10.0 microns with 98.2% of the particles below 4.7 μm and 85.2% below 1.1 μm; its BET specific surface area was determined as 5.2 $m^2/g$. The β-cristobalite particle size was in the 2–5 μm range with a specific surface area of 2.5 $m^2/g$. The Cab-O-Sil material consists of 2 to 40 μm diameter aggregates formed by primary particles of 0.1 to 0.2 μm diameter. The BET specific surface of the M-5 grade Cab-O-Sil used in most of the experiments reported was measured to be 195.4 $m^2/g$. Other grades of Cab-O-Sil tested had specific surface areas of 100, 255, and 380 $m^2/g$. The thermal treatment of the materials were conducted in air at temperatures ranging between 100 C and 1095 C for time periods of 48 and 72 hours.

Hemolysis assay: The test protocol developed by Harington et al.[4] was used, with slight modifications (Wallace et al. (4a)), for the present investigation. Dusts were made up to a stock concentration of 2 mg dust per ml of calcium- and magnesium-free Dulbecco's phosphate buffered saline (PBS) obtained from Sigma Corp of 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 0.04–2.0 mg dust/ml PBS. Sheep blood erythrocytes, supplied by Scott Laboratories of Fiskeville, RI, were washed twice with PBS, centrifuged at 990g and diluted to a 2% by volume suspension in PBS. Equal volumes of dust suspension and the 2% erythrocyte suspension were then mixed to obtain mixed suspensions of 1% by volume of erythrocyte cells and dust concentrations in the range of 0.02 to 1.0 mg dust/ml. These suspensions were subsequently incubated for 30 minutes at room temperature with agitation every 10 minutes and then centrifuged at 990g. The amount of hemoglobin released was determined colorimetrically on a Bausch and Lomb Spectrometer (Spec 20) at a wavelength of 540 nm. Negative controls consisted of 1% suspensions of erythrocyte cells in PBS and positive controls of an equal volume mixture of water and 2% by volume suspension of erythrocytes in PBS.

## EXPERIMENTAL RESULTS

The hemolytic activity of untreated Min-U-Sil, β-cristobalite, and Cab-O-Sil, as function of dust concentration, are compared in Figure 1. These results show that on a per unit weight basis the Cab-O-Sil material is about an order of magnitude more active than the crystalline Min-U-Sil. On a surface area basis, however, Min-U-Sil is more toxic than the amorphous material by, approximately a factor of 3 to 4, since the specific surface area of Cab-O-Sil was found to be over 30 times larger than that of Min-U-Sil. The hemolytic activities of Cab-O-Sil and β-cristobalite are, on the other hand, comparable on a unit area basis. This is in general agreement with other findings on the relative biological activity of various allotropic forms of silica.[1,5,6] However, a test of hemolytic activity conducted on Cab-O-Sil material of different specific surface areas (Figure 2) shows a decrease in activity with increasing specific surface area (or decreasing primary particle size) suggesting that a different mechanism may underlie the toxicity of the fumed silica particles. This latter finding is in qualitative agreement with the particle size dependence of colloidal silica reported by Harley and Margolis.[7]

The effect of calcination on the hemolytic activity of Min-U-Sil and Cab-O-Sil and the change in the specific surface area of the materials on calcination is illustrated in Figures 3 and 4. It is evident from these results that the percent decrease in hemolytic activity exceeds the percent decrease in surface area by sintering, indicating a net decrease in activity on a per unit of surface basis. This decrease in activity coincides with the dehydroxylation of the silica surface reported for various types of silica.[1] The dehydration/dehydroxylation process was followed by photo-acoustic spectra of surface modes of the calcined materials and is illustrated in Figure 5. The untreated sample exhibits a broad absorption band in the 3000–3700 $cm^{-1}$ range which is associated with hydrogen bonded silanol groups and adsorbed water molecules, and the sharp 3747 $cm^{-1}$ band characteristic of free silanol groups. Calcination leads first to the disappearance of the broad band and an increase in the intensity of the free silanol group concentration at temperatures below 800°C and, subsequently, to the disappearance of this band at temperatures above 800°C. It has been found by previous investigators of the dehydroxylation of silica surfaces that at temperatures below approximately 400–450°C less than half of the hydroxyl groups have been removed and thus there is an appreciable concentration of adjacent hydroxyl groups which facilitate rapid rehydroxylation of the surface. As the calcination temperature is increased beyond this range the dehydroxylation process becomes more irreversible until, at around 1100°C, a fully dehydrated, hydrophobic siloxane surface is attained.[8,9] This process appears remarkably well reflected in the observed hemolytic activity of calcined silica particles shown in Figures 3 and 4, which leads to the conclusion that the hemolytic activity of the siloxane surface is much lower than that of the normal, hydroxylated surface, regardless of the type of crystal structure (α-quartz or amorphous) under consideration. It was also observed that the calcined materials, maintained under normal desiccator conditions, recovered their cytotoxicity with time. For fumed dusts calcined at 800–950°C this recovery occurs over a period of 10 to 20 days, as is illustrated in Figure 6. For crystalline materials and calcination temperatures where a fully siloxinated surface is generated, these times were found to be considerably longer. For example, a sample of Min-U-Sil calcined at 1100°C recovered only about 30% of its precalcination activity after a period of 180 days. This observation is in general agreement with hydroxylation rates reported by other researchers.[1,9] The recovery of cytotoxicity by the calcined dusts is accompanied by the reappearance of the I.R. absorption bands characteristic of the hydroxylated surface.

Another method for monitoring the surface structure changes of the silica particles is by the measurement of the electrophoretic mobility or "zeta-potential." This potential measures, if other variables are held constant, the magnitude of the electric charge of the surface double-layer of the particles. It is thus a convenient method for detecting changes in the surface structure of particles. Figures 7a and 7b compare zeta-potential-pH profiles for untreated and calcined Min-U-Sil, and for β-cristobalite and α-quartz (Min-U-Sil), respectively. For the calcined samples it is observed that the zeta potential decreases for calcination temperatures up to 500°C which may be associated with desorption of water from the particle surface and a corresponding increase in the double-layer potential. Further dehydroxylation to form surface silane bonds results in a reduced electrical double-layer potential and, consequently, a more negative zeta potential. Cristobalite particles have a lower zeta-potential than quartz particles and a lower hemolytic activity as well.

The effect of degree of surface hydroxylation was also tested by treating Min-U-Sil samples with alkaline and acidic solutions. It has been reported that the rehydroxylation of partly dehydroxylated silica surfaces is catalyzed by alkali.[10] Min-U-Sil samples calcined for 48 hours at 800°C and which had lost 50% of their hemolytic activity by this treatment, recovered their full hemolytic potential after immersion in a stirred 5% solution of NaOH in water. Similar treatment of non-calcined Min-U-Sil dust resulted in a slight increase in hemolytic activity while exposure to acid solutions (10% HCl) had no significant effect on hemolytic activity, as is shown in Figure 8a. However, the enhancement of hemolytic activity resulting from the alkaline solution treatment was found not to be permanent and the particles so treated were observed to return to the initial cytotoxic levels over a period of 40–80 days (Figure 8b).

## CONCLUSIONS

A test of the effect of dehydration and dehydroxylation on the cytotoxicity of respirable silica dust particles, as measured by their hemolytic activity, suggests that such activity can be correlated with the total concentration of surface silanol groups in the sample. Although this may not be the only mechanism for cytotoxicity of silica, the finding confirms the results of Nash et al. of some years ago.[11] This correlation appears, however, to be independent of the structure of the underlying silica (crystalline or amorphous), contrary to other findings on this issue. The concentration of OH groups on hydroxylated silica surfaces is not a readily definable parameter and may vary with particle size, porosity, degree and type of crystallinity, thermal history, etc.[12,13] which may explain the great variability in cytotoxicity resulting from various surface treatments of the material. In the study reported here, the elimination of hydroxyl groups at calcination temperatures above 500°C coincides well with the observed decrease in cytotoxicity.

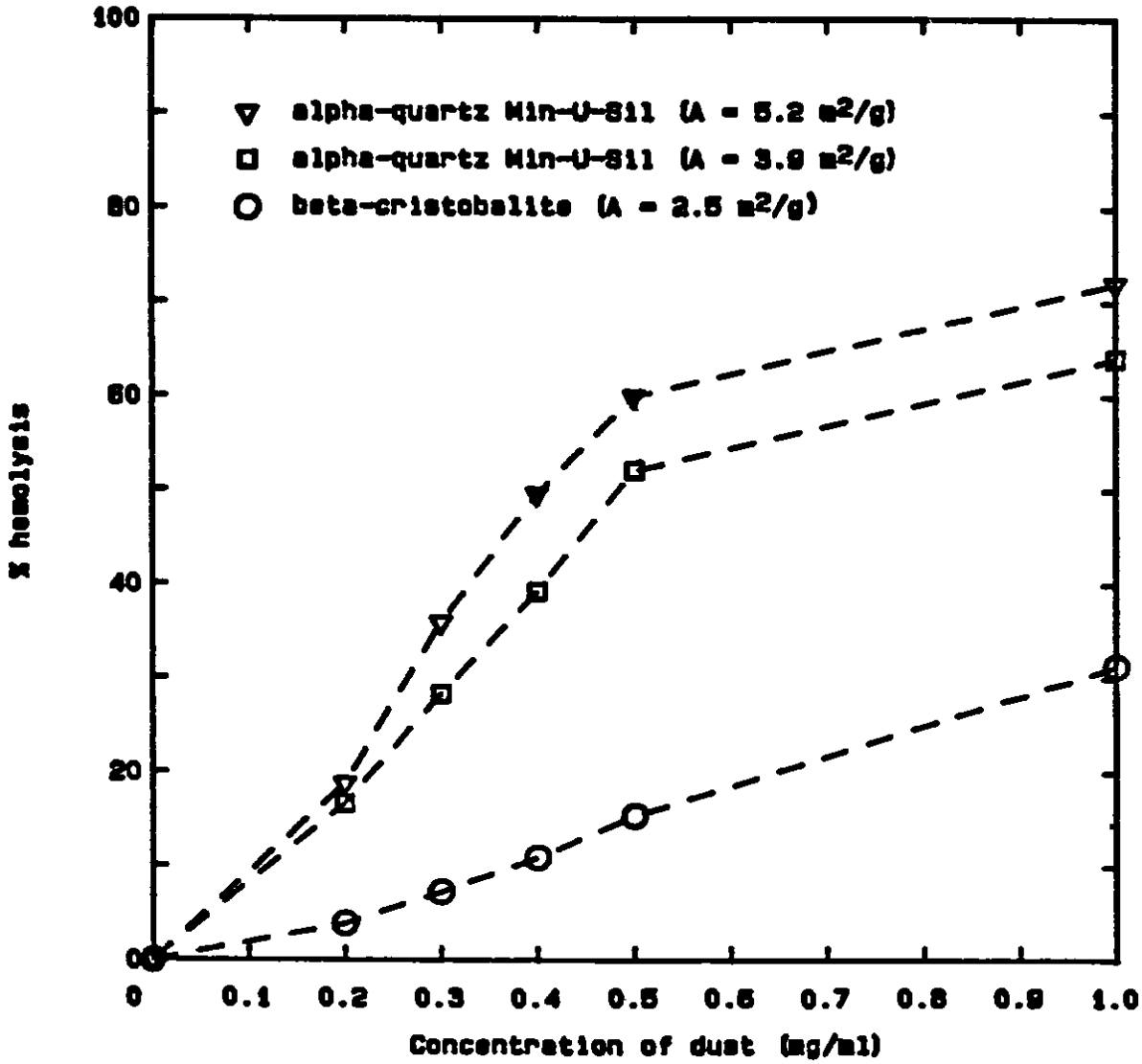



Figure 1a. Cytotoxicity as function of dust concentration for quartz and cristobalite.

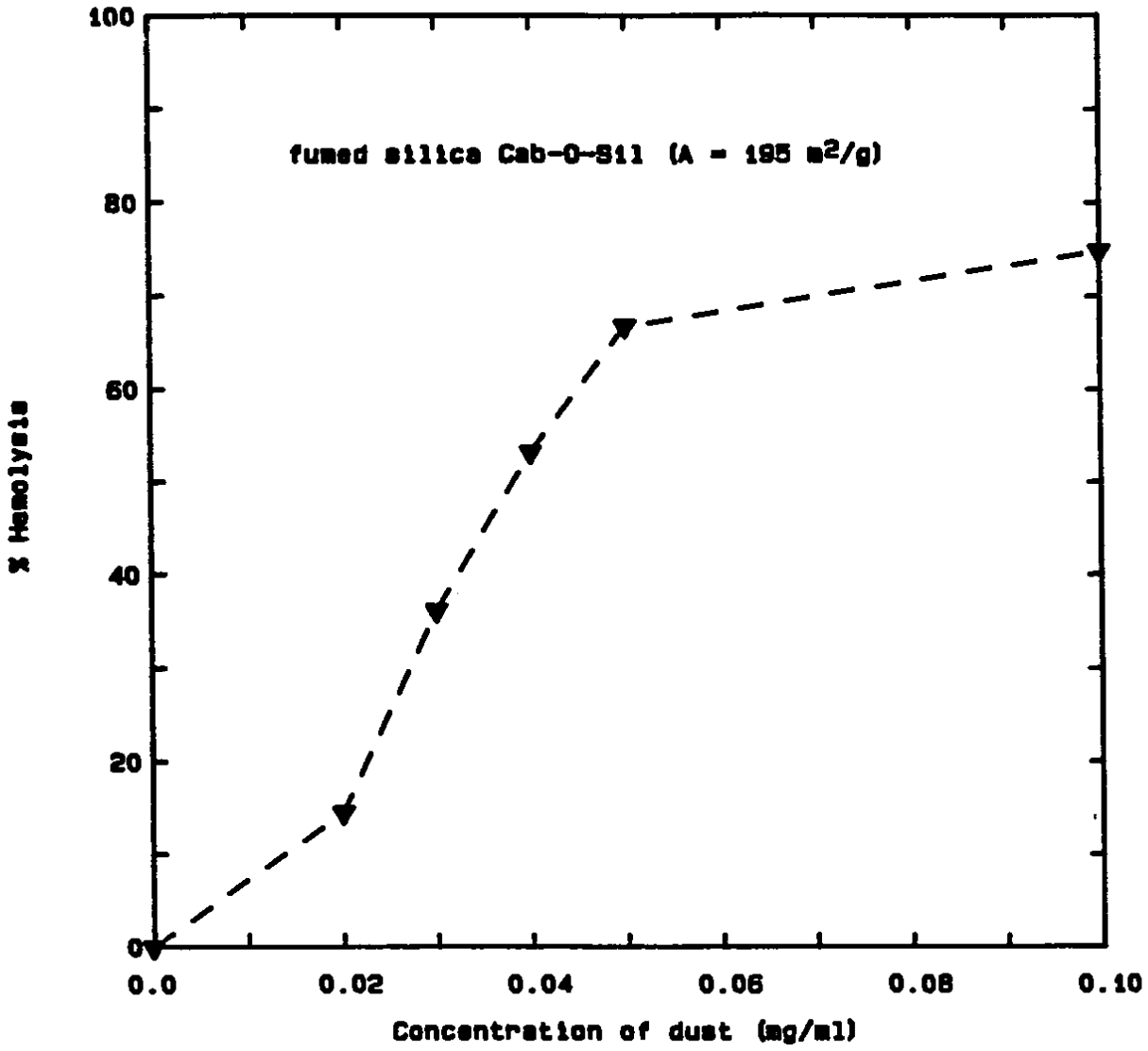



Figure 1b. Cytotoxicity as function of dust concentration for fumed silica (Cab-O-Sil).

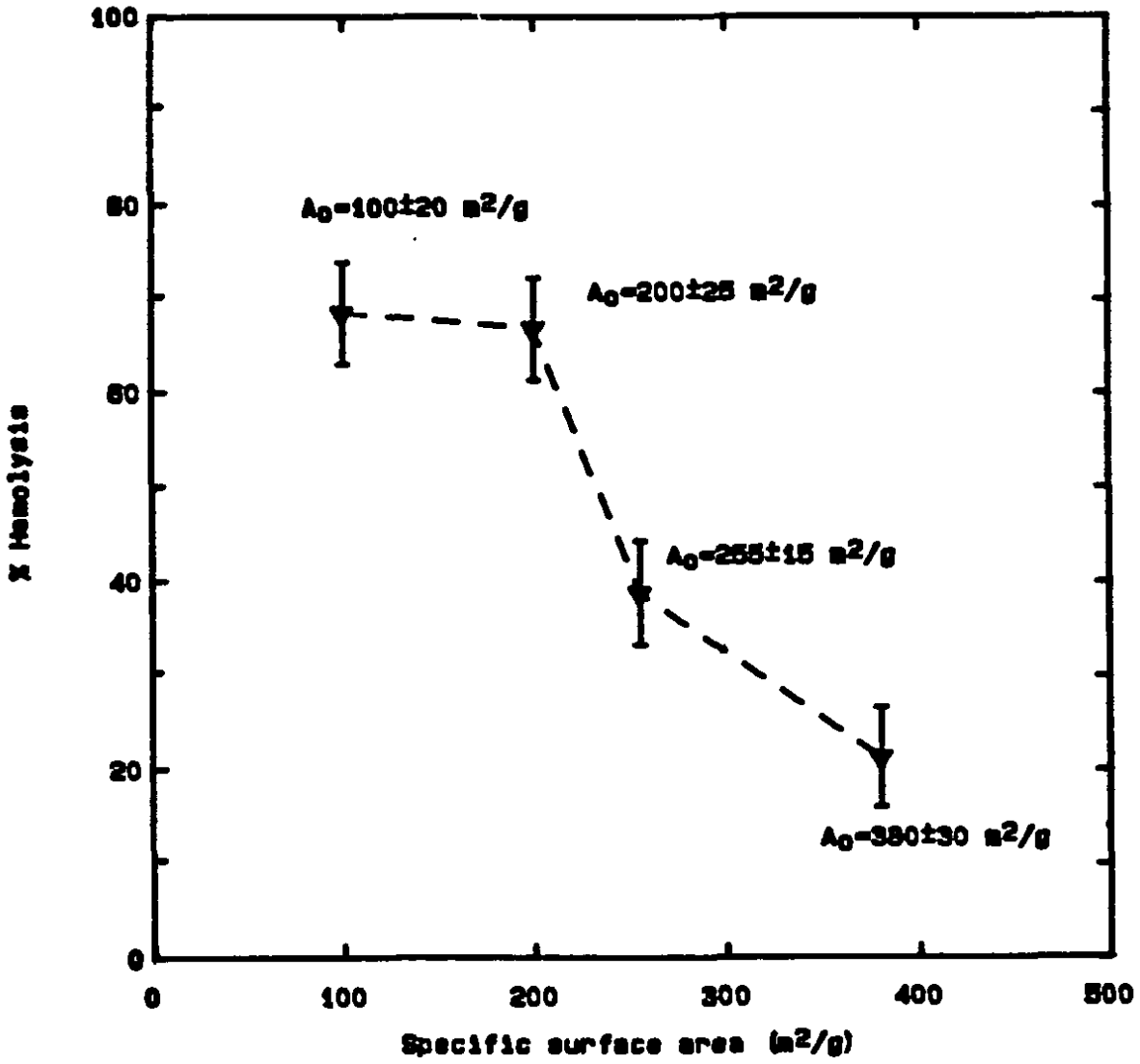
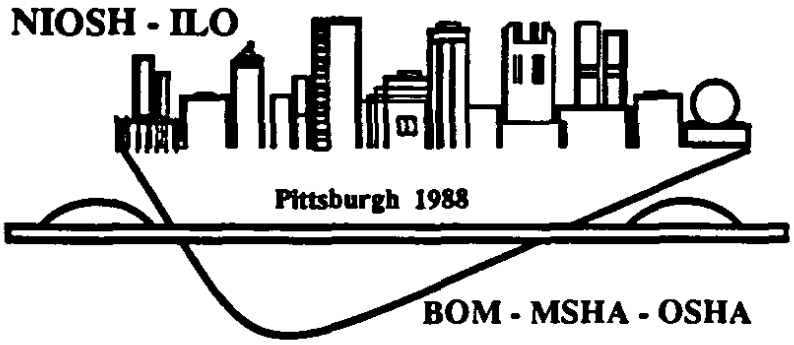



Figure 2. Hemolysis by fumed silicas of different surface areas (tested at dust concentration of 0.05 mg/ml).

*Proceedings of the VIIth International Pneumoconioses Conference* | *Part*
*Transactions de la VIIe Conférence Internationale sur les Pneumoconioses* | *Tome*
*Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis* | *Parte*

# I




Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
Pittsburgh, Pennsylvania EE. UU—23–26 de agosto de 1988

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

CDC
CENTERS FOR DISEASE CONTROL

## Sponsors

International Labour Office (ILO)
National Institute for Occupational Safety and Health (NIOSH)
Mine Safety and Health Administration (MSHA)
Occupational Safety and Health Administration (OSHA)
Bureau of Mines (BOM)

September 1990

## DISCLAIMER

Sponsorship of this conference and these proceedings by the sponsoring organizations does not constitute endorsement of the views expressed or recommendation for the use of any commercial product, commodity, or service mentioned.

The opinions and conclusions expressed herein are those of the authors and not the sponsoring organizations.

**DHHS (NIOSH) Publication No. 90-108 Part I**