FINAL REPORT

CHARACTERIZATION OF SURFACE PROPERTIES AFFECTING THE ACTIVITY OF THE "FREE SILICA" FRACTION OF RESPIRABLE DUSTS

Final Report for CAN 143

"Active Silica Content of Coal Mine Dust"

ABSTRACT

A. Scanning Electron Microscopy - Energy Dispersive X-Ray elemental analysis method for surface contaminants on silica particles:

SEM-EDX elemental analyses typically are performed using electron beam acceleration voltages on the order of 20 KeV. At this kinetic energy the electron beam penetrates around 3 micrometers into silicate or aluminosilicate materials. The resultant energy dispersive X-ray elemental spectra provide an average particle composition, averaged throughout the depth of the particle. However, if the electron beam accelerating voltage is lowered then the depth of penetration and depth of X-ray signal generation in the sample is lowered. At 5 KeV the depth of penetration is around 0.3 microns for silicates. A simple one-dimensional model of SEM-EDX spectra is developed based on a Beer's Law model of electron beam and X-ray diminution with passage through a particle with a two layer structure. This suggests the possibility of using varing voltage SEM-EDX to depth profile a particle which has the structure of a substrate with a thin sub-micron thick occlusion on it's surface, such as environmental respirable mixed dust samples contain quartz particles which are wholly or partly occluded with clays of other mineral material. Synthetic aggregate particles of small clay particles on larger quartz particles and 5 micron glass spheres, and asbestos fibers over spheres are analyzed by SEM-EDX at a series of accelerating voltages between 5 and 20 KeV to determine the technical feasibility of the proposed method for respirable sized particles. Qualitative agreement with the theory is observed.

B. Modification of respirable quartz particle cytotoxicity by surface treatment:

Respirable quartz cytotoxicity, as measured by erythrocyte hemolysis and pulmonary macrophage release of lactate dehydrogenase <u>in vitro</u>, is neutralized by boiling in water in glass test tubes for 10 to 40 minutes. The membranolytic potential is reduced to near zero by boiling up to 10 mg quartz per milliliter of water. For greater concentrations of quartz in water the hemolytic potential after 40 minutes of boiling approaches that of native quartz. Replacing the medium with fresh water midway through the boiling results in full detoxification through 20 mg quartz per ml water. Pre-boiling the medium with silica reduces the effect. Detoxification persists after mild drying at 110 C for 8 hours, and persists after three days of resuspension in water at room temperature.

4. CHARACTERIZATION OF SURFACE PROPERTIES AFFECTING THE ACTIVITY OF THE "FREE SILICA" FRACTION OF RESPIRABLE DUSTS

by
W. E. Wallace and M. J. Keane

4.1 SURFACE PROPERTIES AFFECTING QUARTZ CYTOTOXICITY

The mass percentage of quartz, sometimes referred to as the "free silica" content, is one respirable mine dust parameter measured to provide information for control of potentially hazardous exposures. The MSHA standard method for this is based on infrared spectroscopic methods. Other methods can also be used to measure the silica fraction of a collected, mixed composition respirable dust. For research purposes these frequently include X-ray diffraction or scanning electron microscopy in concert with energy dispersive X-ray analysis (SEM-EDX).

In the lung, respired mineral dust interaction with tissue involves the surface of the deposited particles. The cytotoxic potential in vitro and in vivo of a given mass of quartz dust in the general respirable size range may be dependent on the specific surface area of the dust and on the degree of contamination, occlusion, or modification of the dust surface.

In this project we have investigated two aspects of the role of dust surface properties in determining the disease-inducing potential of respirable quartz.

The first is the question of determining the degree of surface contamination of respirable quartz particles. That is, we investigated the feasibility of measuring low levels of surface associated contaminants of other than silicon elemental composition which might mask or alter in whole or in part the native quartz surface cytotoxic potential. We have modelled and performed limited testing of a modification of conventional SEM-EDX analysis to distinguish alumino-silicate and magnesium silicate surface occlusion from underlying silica particles. The second is an investigation of some treatments which can significantly alter the cytotoxic potential of quartz dust surface without changing the silicon elemental composition of the particle in a readily measurable way. These two studies indicate the possibility that compositional or structural features of dust surfaces may affect the cytotoxic potential of a given mass of respirable quartz dust. The treatment investigations additionally may be of some interest regarding mineral processing.

This chapter provides an analysis of the use of SEM-EDX at differing electron beam accelerating voltages to distinguish and semi-quantitate surface contaminants of aluminum or magnesium silicates on silica particles. The next chapter reviews our findings on the suppression of quartz cytotoxicity by relatively mild surface treatment procedures.

4.2 SCANNING ELECTRON MICROSCOPY - ENERGY DISPERSIVE X-RAY ELEMENTAL ANALYSIS METHODS FOR SURFACE CONTAMINANTS ON SILICA PARTICLES

4.2.1 THEORY

Typically, SEM-EDX analyses are performed using an electron beam accelerating voltage of 20 to 30 KeV. At such high voltages the electron beam penetrates the material being studied to a depth of more than a micrometer. The resultant energy dispersive X-ray spectra provide elemental composition data averaged throughout this depth.

However, if the accelerating voltage is lowered, then the electron beam penetrates less deeply into the material. The extinction of the electron beam intensity, E, with distance into the material, x, has been modelled to obey a Beer-Lambert form of behavior

$$dE = -k_e E\, dx$$

where k_e, the extinction coefficient, is approximately related to the electron beam accelerating voltage V as

$$k_e = A\, V^{-3/2}$$

between 5 and 15 KeV, and as

$$k_r = BV^{-2}$$

between 15 and 25 KeV (Cosslett and Thomas, Brit. J. Appl. Phys. 15 883, 1944).

The possibility thus exists to distinguish surface layer from substrate layer composition of a material with differing surface and bulk composition.

Our interest is in determining if there is such a surface versus bulk composition structure to respirable sized particles (micrometer diameter range) which are characterized in mixed dusts by IR or XRD to contain a certain percent quartz. The basic question is whether this mass percent composition faithfully and consistently measures or correlates with the available quartz surface area in the mixed dust, the toxicological properties of the dust being in large part dependent on the mineral surface area which is biologically accessible to pulmonary tissue. SEM-EDX at high voltages when performed on such dust often indicates the presence of aluminum in addition to silicon for an individual particle. Frequently such analysis defines a particle to be quartz if it is $\geq$ 95% silicon as determined by the ratio of intensity of the silicon line to those of the other lines present in the X-ray spectra. To look at the relationship of particle coating thickness, material properties, accelerating voltage, and consequent spectral line intensity ratios, consider a simplified one-dimensional model of X-ray emission induced by electron bombardment of a two phase material.

An electron beam of intensity E_o is incident on the surface of the coating (X=0, Z=L). The coating is of thickness T. Figure 9 is a schematic showing the sign conventions for the analysis.

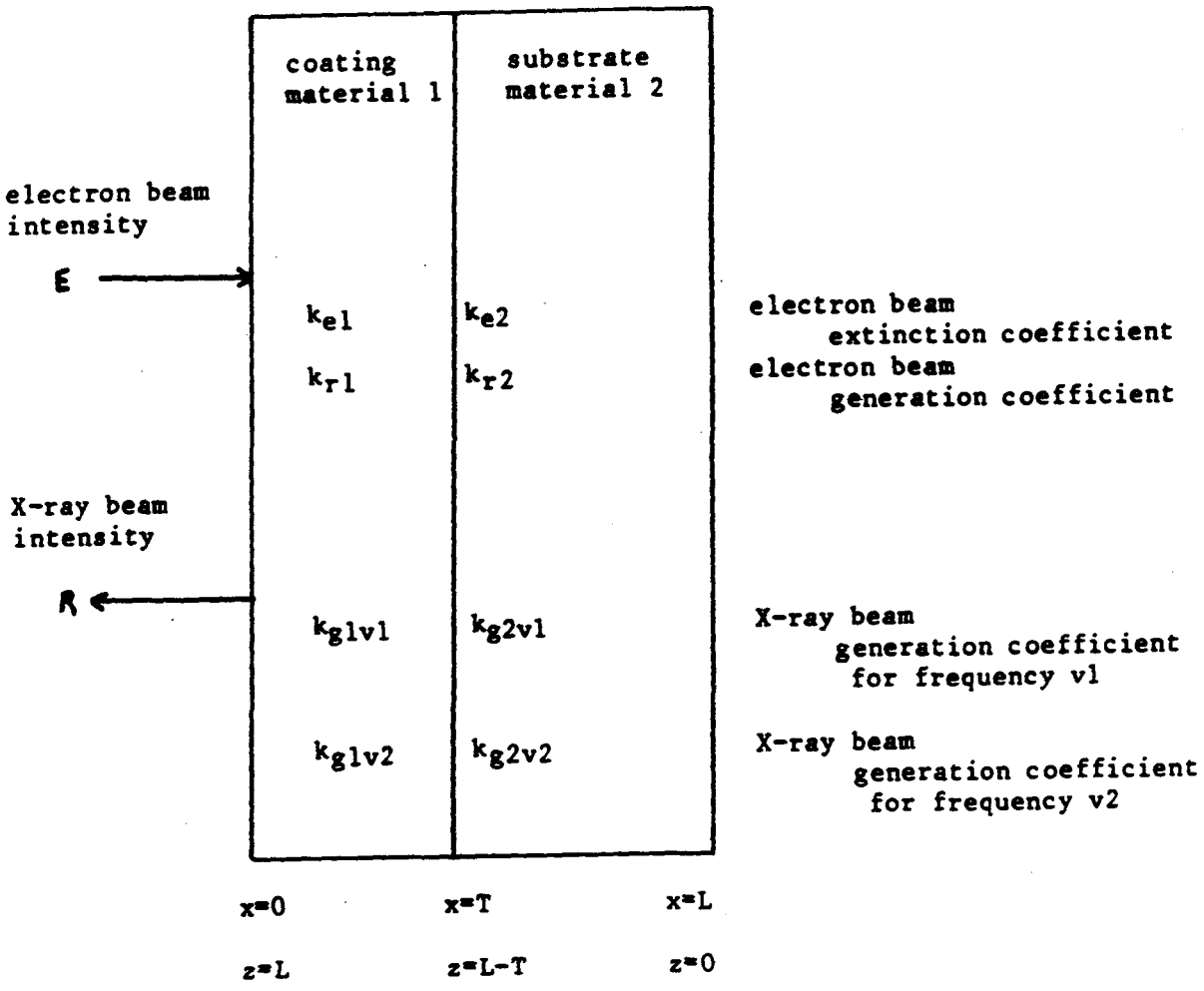

Fig. 9. Nomenclature and sign convention for one-dimensional analysis of SEM-EDX signal intensities from a coated particle.

In passing through a differential thickness of material 1, the coating, the electron beam intensity is diminished by

$$dE_1 = - k_{e1} E_1 \, dx$$

and in material 2, the substrate, by

$$dE_2 = - k_{e2} E_2 \, dx$$

The intensity of the X-ray beam at the wavelength of interest propagating in the positive z - direction is designated by R. The intensity of the X-ray beam as it exits the top of the coating, R_1 (x=0, z=L) is taken to be the recorded SEM-EDX signal.

As the X-rays pass through a differential thickness of material 1, they are extinguished by

$$dR_1 = - k_{r1} R_1 \, dz$$

But within that thickness the electron beam may also be generating X-rays at the monitored wavelength according to

$$dR_1 = + k_{g1} E_1 \, dz.$$

Thus, the X-ray intensity exiting the differential thickness in the positive Z-direction has changed from that entering by

$$dR_1 = - k_{r1} R_1 \, dz + k_{g1} E_1 \, dz.$$

Similarly for the substrate material

$$dR_2 = - k_{r2} R_2 \, dz + k_{g2} E_2 \, dz$$

The electron beam intensity at any depth x in the coating is found from

$$\int_0^x \frac{dE_1}{E_1} = \int_0^x - k_{e1} \, dx$$

to be

$$E_1 (x) = E_o e^{-k_{e1} x}$$

and, in particular, at the coating-substrate interface

$$E_1 (T) = E_o e^{-k_{e1} T}$$

The electron beam intensity at any depth x in the substrate is found from

$$\int_T^x \frac{dE_2}{E_2} = \int_T^x -k_{e2} dx$$

to be

$$E_2(x) = E_2(T)e^{-k_{e2}(x-T)}$$

But at the interface

$$E_1(T) = E_2(T)$$

so

$$E_2(X) = E_0e^{-k_{e1}T}\ e^{-k_{e2}(x-T)}$$

For convenience changing variables by $x = L - z$

$$E_1(z) = E_0e^{-k_{e1}(L-z)}$$

$$E_2(z) = E_0e^{-k_{e1}T}\ e^{-k_{e2}(L-z-T)}$$

$$= E_0e^{-(k_{e1}-k_{e2})T}e^{-k_{e2}L}e^{k_{e2}z}$$

To find the X-ray intensity in the positive Z-direction in material 1, combine the expressions for dR_1 and dE_1 to give

$$dR_1 = -k_{r1}R_1dz + k_{g1}E_0e^{-k_{e1}(L-z)}dz$$

This first order differential equation may be solved subject to the boundary condition that at the interface X-rays are entering from material 2 into material 1. Their intensity is not yet known so we designate it R_T; that is,

$$R_1(z=L-T) = R_2(z=L-T) = R_T$$

This differential equation for X-ray intensity in material 2 can be solved in terms of the unknown boundary value to give

$$R_1(z) = \frac{-k_{g1}E_0e^{k_{e1}L}\quad (e^{-k_{r1}z} - e^{k_{e1}z}) + R_Te^{-k_{r1}z}}{k_{r1} + k_{e1}}$$

To find the X-ray intensity in the substrate one solves

$$dR_2 = -k_{r2}R_2dz + k_{g2}E_0e^{-(k_{e1}-k_{e2})T}e^{-k_{e2}L}e^{k_{e2}z}dz$$

subject to the boundary condition that no X-rays are entering the bottom of the substrate, that is,

$$R_2(z=0) = 0$$

One obtains

$$R_2(z) = \frac{k_{g2}E_0e^{-(k_{e1}-k_{e2})T}e^{-k_{e2}L}[(e^{k_{e2}z} - e^{-k_{r2}z})]}{k_{r2} + k_{e2}}$$

Equating the expressions for R_1 (Z) and R_2 (Z) at the interface

$$R_1(L-T) = R_2(L-T)$$

provides an expression for R^T:

$$R_T = \frac{k_{g2}E_o e^{-(k_{e1}-k_{e2})T}\, e^{-k_{e2}L}\left(e^{+k_{e2}(L-T)} - e^{k_{r2}(L-T)}\right)}{k_{r2} + k_{e2}}$$

which can be used in the expression for R_1 (Z) to find the X-ray intensity in the coating. In particular, the X-ray intensity exiting the top of the coating, which is proportional to the observed signal, per unit incident electron beam intensity, is

$$\frac{R_1(x=0)}{Eo} = \frac{k_{g1}}{k_{r1} + k_{e1}}\left[1-e^{-(k_{e1}+k_{r1})T}\right]$$

$$+ \frac{k_{g2}}{k_{r2} + k_{e2}} e^{-(k_{e1}+k_{e2})T}\left[1-e^{+(k_{e2}+k_{r2})T} - e^{-(k_{e2}+k_{r2})L}\right]$$

Thus, if one measures the extinction coefficients k_{r1}, k_{r2}, k_{e1}, k_{e2} and the generation coefficients k_{g1}, k_{g2} on pure materials 1 and 2 then one can extract an approximation of the coating thickness from measurements of the relative X-ray intensities from coating and substrate signals versus accelerating voltages.

To look at the underlying functional dependence of the ratio of substrate to coating signal, consider the expression for $R_1(L)/E_o$ for X-ray emissions from different elements in the substrate and coating. Let the electromagnetic signal from the substrate have frequency ν_2, and from the coating have frequency ν_1. In general, the extinction coefficients for electron beam and X-ray adsorption and for x-ray generation will be different. To examine the simplest case assume x-rays of frequency ν_1 are generated only by the coating, and ν_2 only by the substrate:

$$k_{g1,\nu1}=g_1 \quad , \quad k_{g2,\nu1}=0$$

and

$$k_{g1,\nu2}=0 \quad , \quad k_{g2,\nu2}=g_2.$$

Assume electron beam and x-ray extinction coefficients are the same for each medium:

$$k_{e1}=k_{e2}=k_e,$$

$$k_{r1,\nu1}=k_{r1,\nu2}=k_{r2,\nu1}=k_{r2,\nu2}=k_r.$$

Also assume the substrate is "thick" compared to T and compared to electron and X-ray diminution characteristic depths. Then the signal expressions become

$$\frac{R_1(x=0)}{E_o}\Big|_{v2} = \frac{g_2}{k_r+k_e}[e^{-(k_e+k_r)T}]$$

and

$$\frac{R_1(x=0)}{E_o}\Big|_{v1} = -\frac{g_1}{k_r+k_e}[\,1-e^{-(k_e+k_r)L}]$$

In the simplest case assume equally effective x-ray signal production by the two media, that is,

$$g_1=g_2=g.$$

Then the fraction of substrate signal to substrate and coating signal is then

$$\frac{R_1(x=0)/E_o|_{v2}}{R_1(x=0)/E_o|_{v2}+R_1(x=0)/E_o|_{v1}} = e^{-(k_r+k_e)T} = e^{-k_rT}EXP[-[AT(V^{-3/2})]$$

Fig. 10 shows this simplified functional dependence for

$$Y = EXP[-AT(V^{-3/2})]$$

for several values of "AT". To apply the full formula requires pure material measurements to obtain extinction and generation coefficients.

4.2.2 EXPERIMENTAL RESULTS

We here present some data on the relationship of measured fraction of SEM-EDX signal coming from the substrate to coating thickness and electron beam accelerating voltage. The resolution degradation with decreasing accelerating voltage for the SEM available to us limits us to 5 to 10 KEV as the lowest workable voltage. The status of newer SEM technology might make it possible to work at lower voltages - essentially, the lower the voltage the thinner the coating which one can distinguish. The limit is the accelerating voltage needed to provide sufficient electron beam energy to excite the primary X-ray line specific for the element of interest. In the data which follow, voltages down to 5 kV are used. This is still high compared to the K lines of silicon, aluminum, and magnesium, so coatings of less than 1 micrometer thickness can be resolved from bulk contaminant composition. However intensities for other elements shown are diminished.

Table 24 provides data for an 0.25 micron diameter crocidolite fiber on a six micron silica sphere. An increase of aluminum-to-silicon with decreasing voltage demonstrates the effect of voltage on surface-to-substrate contribution to the spectra.

Table 24

Relative intensities of X-ray emission lines versus electron beam accelerating voltage for Scanning Electron Microscopy - Energy Dispersive X-Ray (SEM-EDX) analyses of an 0.25 micrometer diameter asbestos fiber on an 6.0 micrometer diameter silica sphere on a tin sample plate. The Si, Al, and Mg lines are all below 5 kV and their ratios can be used to distinguish surface from bulk composition.

	20 Kev	10 KeV	5 Kev
Mg%	2.92	3.64	7.70
Al%	2.12	2.46	4.24
Si%	77.49	86.04	88.06
Sn%	17.47	7.85	0

Fig. 11 shows the percent substrate (Titanium) for silica particles of 0.5, 1, and 3 micrometers sitting on a Titanium metal block. That is, the SEM-EDX elemental composition percentage for Titanium, %Ti, and for silicon, %Si, are used to compute

$$\% \text{ substrate (Ti)} = \frac{\%Ti}{\%Ti + \%Si} \times 100$$

as a function of electron beam accelerating voltage V (in kilovolts) for different particle diameters, D. As is seen, at a given voltage the percent substrate signal increases for decreasing particle size. That is, the smaller the particle the lesser the fraction of the signal originating from the particle. And as the accelerating voltage is dropped, the contribution to the signal from the coating becomes dominant. Note that the 5 kV induced values cannot be used for Ti.

Because of low resolution at low voltages with the SEM scope used, it was difficult to locate sub-micrometer diameter particles at 10 Kev and below, and to center the beam on the particle. Therefore, we worked in part with asbestos fibers on a substrate block, and crossing the surface of an underlying particle. It is easier to locate and center the beam on a fiber. And the circular cross-section of the fiber provides a better estimate of the thickness of the particle than for a not-necessarily-spherical clay particle.

Table 25 shows the silicon and aluminum lines of an agglomerate particle of kaolin of unknown but approximately one micron thickness on a larger silica substrate particle about seven microns across. At 20 keV the particle would be defined typically as a silica particle with some aluminum contamination observed in the spectra. At five keV the particle would be defined to be a clay particle.

Table 25

Relative intensities of SEM-EDX lines versus accelerating voltage for a kaolin chip of 0.7 micrometer lateral dimension on an 5 micron silica sphere on a tin plate.

	20 Kev	10 KeV	5 Key
Al%	6.37	20.02	36.27
Si%	55.02	57.18	63.73
Sn%	38.61	22.80	0

Fig. 12 shows signal ratios from crocidolite asbestos fibers on a Tin substrate block. An 0.5 micrometer diameter fiber could be seen down to 5 KeV, but the excitation voltage is insufficient for Tin there.

It appears that with this system coatings down to about 0.1 micrometer can be "resolved", and perhaps lower, for elements with K lines below 2 or 3 kV. This is fortunate for silicon, aluminum, and magnesium, but not for iron or other elements of interest. Scanning Auger spectroscopy looks at the composition to a depth on the order of 10 angstroms, or .001 micrometers. It may be possible to use SEM-EDX in concert with Scanning Auger, leaving a one-to-two order of magnitude "gap" between perhaps 0.1 and .001 micrometers, to semi-quantitate surface versus bulk composition of respirable particles. The use of other X-ray lines, L lines, stimulated from an element by the electron beam might help to overcome two restrictions. Requiring lower excitation energies, those lines could provide signals at lower electron beam accelerating voltages, thus resolving thinner coatings. And elements whose K lines are at energies which are not excited at 5 kV might be analyzed at that voltage using the lower energy spectral lines. Tests using SEM with wavelength dispersive X-ray detection provide evidence for such a feasibility.

4.3 MODIFICATION OF RESPIRABLE QUARTZ PARTICLE CYTOTOXICITY BY SURFACE TREATMENT

4.3.1 SUPPRESSION OF QUARTZ TOXICITY TO PULMONARY MACROPHAGES

Research underway to determine interactions of quartz and other mineral dust surfaces with pulmonary fluids and alveolar macrophages in culture found that when dusts were autoclaved in aqueous suspension, their cytotoxic effects on macrophages were suppressed, in some cases fully and even after several days incubation with the cells. (Hill, C. A., Wallace, W. E., Keane, M. J., Page, S. J., Bolsaitis, P., Razzaboni, B. L., Vallyathan, V. and Mike, P.; "Alteration of Respirable Quartz Particle Cytotoxicity by Thermal Treatment in Aqueous Media"; Proceedings: VII International Pneumoconiosis Conference, Pittsburgh, Pa., 1988; In Press.) This finding was in direct contradiction to

earlier results from both short term macrophage lysosomal enzyme release assays, as well as longer term cytotoxicity assays from macrophages in culture; in those studies, dusts were steam autoclaved at 121°C with no liquid water present.

4.3.2. TREATMENT METHODS AND ASSAY METHODS

It was decided to use the hemolysis assay to further investigate these findings, because of the sensitivity, simplicity and cost. Briefly, dusts suspended in buffer are mixed with an equal volume of 4% sheep red blood cells, incubated 60 min. at 37°C with periodic mixing, the cells spun down, and the absorbance of the released hemoglobin from any lysed cells read at 540 nm. Absorbance values are compared to positive controls (100% lysed cells) and negative controls (cells in buffer only).

Initial experiments involved bringing deionized water to a boil, adding to the dry dust (12 mg)in glass tubes, vortexing, and boiling for periods up to 60 min., without stirring. After the boiling period was completed, sample tubes were spun down for 60 sec., the supernatant discarded, and the dust resuspended in PBS buffer and run in the hemolysis assay. Results indicated that the toxicity was reduced almost to zero at 1 mg/ml, and increased in a roughly linear fashion to approximately full (native dust) toxicity at 20 mg/ml dust concentration during boiling (Fig. 13). When individual magnetic stirrers were used in each sample, results were similar, except the toxicity was reduced to virtually zero at concentrations to 5 mg/ml, and then increased in a linear fashion. The treatment was also applied to kaolin and alumina dusts; kaolin was unaffected, but alumina was similarly detoxified.

Experiments involving various boiling times showed a weak dependence of detoxification with time, except at 1 mg/ml, where the effect seemed significant (Fig. 14). Samples were also vacuum dried after boiling, and assayed the following day; fully detoxified samples remained the same, and partially detoxified samples had less toxicity than replicate samples promptly assayed (Fig. 15).

Samples were also left standing for extended periods after boiling, in the supernatant from the boiling water. Fully detoxified samples (1 mg/ml) remained at zero toxicity after 4 days; samples at higher concentrations showed some increase with time, as shown in Fig. 16. Samples were also boiled for specific periods, centrifuged, and the medium changed to fresh water. The toxicity was reduced to very low levels, as shown in Fig. 17.

Certain samples in the assay yielded consistently anomolous results, and were difficult to reconcile with any simple physical model; specifically, samples boiled at 0.5 mg/ml were not detoxified. The only experimental difference in these samples was that they were boiled in plastic (polycarbonate) centrifuge tubes, since glass tubes were not available in an appropriate size. When quartz was boiled in polycarbonate and Tefzel tubes, only partial detoxification was seen (Fig. 18). When boiled in additionally purified and filtered water that had been boiled in polycarbonate, no detoxification was seen at any concentration.

An additional anomolous result occurred when there was a catastrophic failure in the reverse osmosis water purification cartridge in the NIOSH/ALOSH building distilled water system, resulting in a higher impurity level than that present in tap water. Toxicity was partially supressed in all samples, even those boiled in polycarbonate. When the system was restored to proper operation, the results agreed with previous findings.

Since the effect seemed clearly to be an effect of the glass containers, additional experiments were done to clarify the finding. Quartz dusts were boiled in polycarbonate-boiled water with varying amounts of 3 mm soda-lime glass beads. Results showed a dependence of detoxification on the number of glass beads, and thus the glass surface area present. The converse of this hypothesis, that polycarbonate somehow supressed the detoxification of quartz, was tested by boiling quartz in flint glass tubes with polycarbonate pieces in suspension; no significant effect of the polycarbonate was seen.

Quartz samples were also boiled with sodium and calcium chloride solutions of several different concentrations; the effects were weak, slightly lessening the detoxification.

4.3.3 SUMMARY RESULTS

At this point, several conclusions may be stated, and a working hypothesis formulated, namely:

-Quartz boiled in flint glass for times greater than ten minutes at concentrations between 1 and 10 mg/ml is partially to fully detoxified in the Hemolysis assay.

-The effect is strongly concentration dependent between 10 and 30 mg/ml

-If a change is made to fresh boiling water midway in the process, then full detoxification occurs across the entire concentration range

-The effect is present only in samples boiled in flint or borosilicate glass tubes; inert plastic tubes do not show the effect.

-The effect is only moderately time-dependent; at most concentrations, the effect seems nearly complete at 10-15 minutes or less.

-The effect seems to persist on mild drying (overnight vacuum drying)

-Fully detoxified samples appear to show little or no cytotoxicity after soaking in the supernatant from boiling for periods up to 5 days; partially detoxified samples show a gradual increase with time.

-The detoxification seems approximately proportional to available surface area of glass present during boiling.

4.3.4 RESEARCH HYPOTHESIS

Further investigation is needed to more fully clarify the mechanism of silica detoxification, but a partial hypothesis can be stated:

Boiling water releases a soluble or partially soluble factor, probably sodium and/or calcium silicates or hydroxides, which react or are physically adsorbed on the quartz surface, which fully or partially detoxifies the mineral surfaces, as shown in in-vitro cellular toxicity assays.

Suggested strategies for clarifying this would include ESCA and/or Auger surface analysis to determine the surface elemental composition and chemical state(s). If acid-base reactions are involved, the pH dependence of detoxification should be looked at in detail; preliminary data indicate that this is an important variable. Diffuse Reflectance Fourier transform Infrared Spectrophotometry may also yield some information on the surface chemistry of the native and surface-modified quartz.

Current directions in this project are the investigation of the effect of pH and soluble silicates on the detoxification, and physical methods to investigate surface chemical composition.

The prime questions here are whether quartz can be surface modified so that it becomes biologically inert (non-fibrogenic). The first question is:

What physical and chemical conditions are necessary and sufficient to passivate the quartz surface? This project has partially answered this question since; 1) the process proceeds in aqueous solution; 2) the concentration of (less than 5 micron) quartz in suspension must be less than 10 mg/ml; 3) soda-lime or borosilicate glass of sufficient surface area must be present; and 4) the temperature must be close to 100^{o}C.

The second question is whether the passivation effect persists. The effect would have to be verified in long term experiments involving physical and/or chemical methods, as well as <u>in vitro</u> assays, and possibly <u>in vivo</u> bioassays.

Question three is whether this approach is feasible as a prevention strategy. With some additional research on question one, the kinetics and chemical engineering practical approaches would seem feasible to study. The major unknown here is still question two; development of long-term predictive assays for silicosis-inducing minerals is still an area fertile for research. No single assay is without some criticism or controversy.

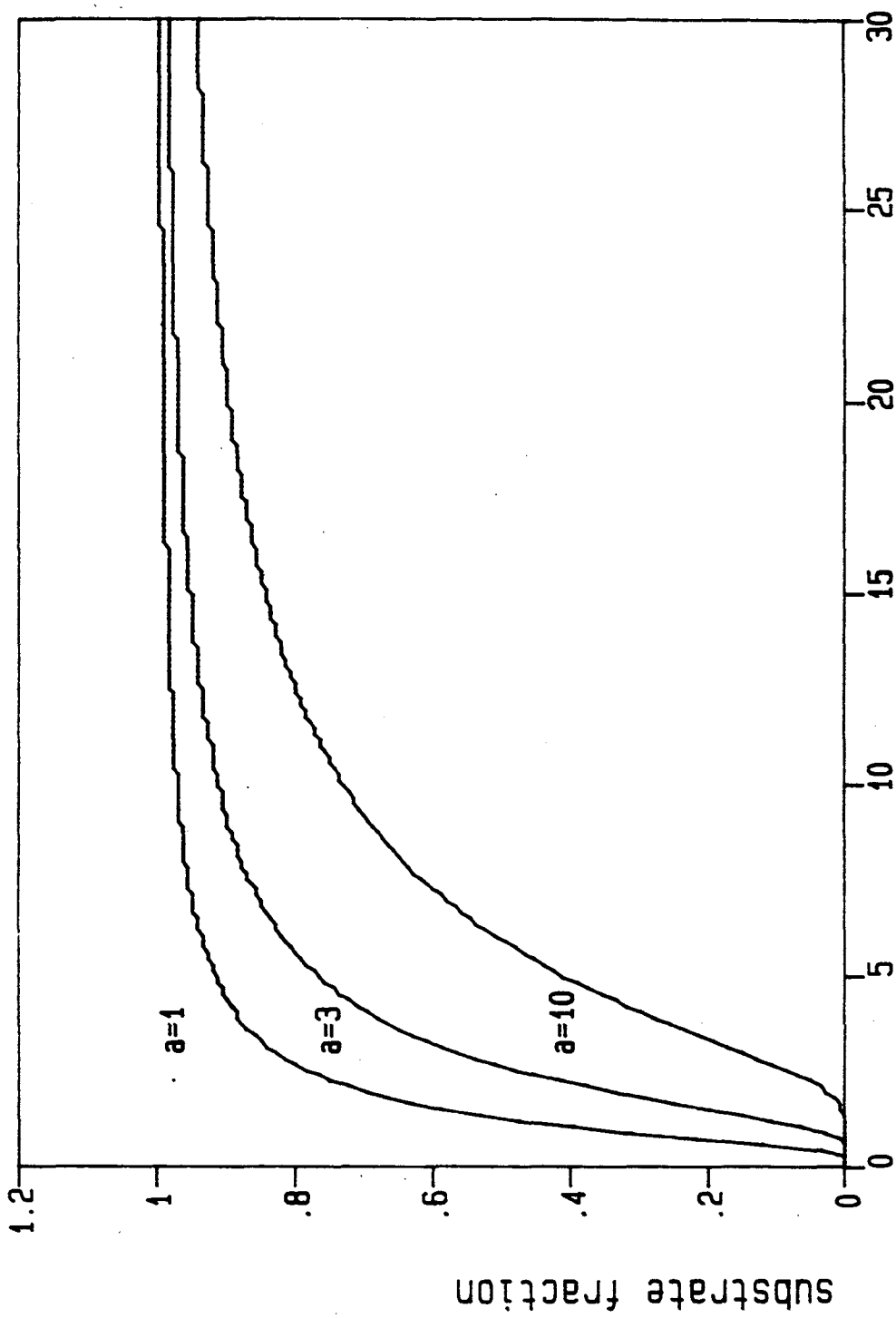

Fig. 10. Theoretical ratio of substrate to substrate plus coating SEM-EDX line intensities versus electron beam accelerating voltage for various coating thicknesses.

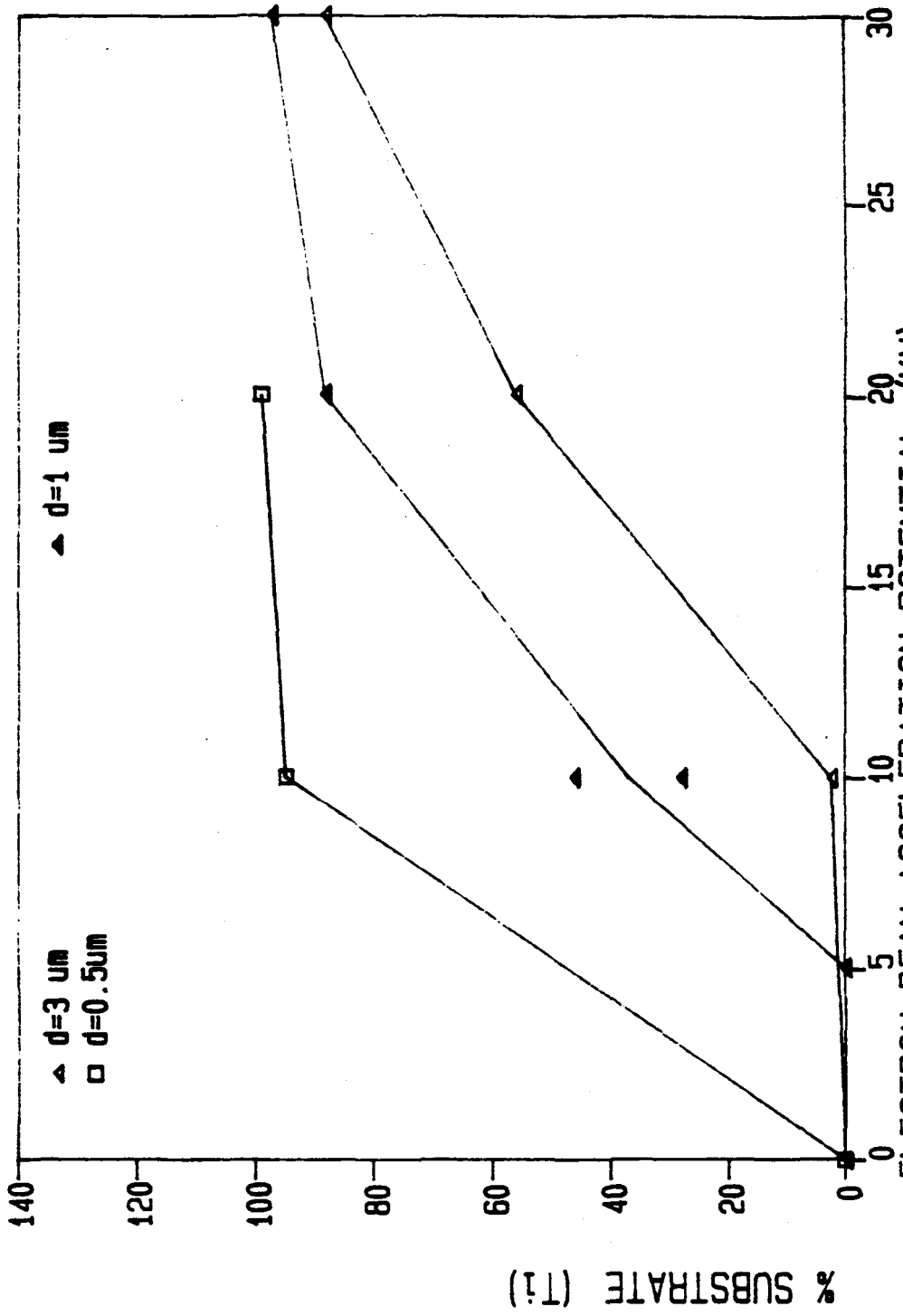

Fig. 11. Experimental ratio of silicon to silicon and titanium SEM-EDX line intensities for silica spheres of three different diameters on a titanium block.

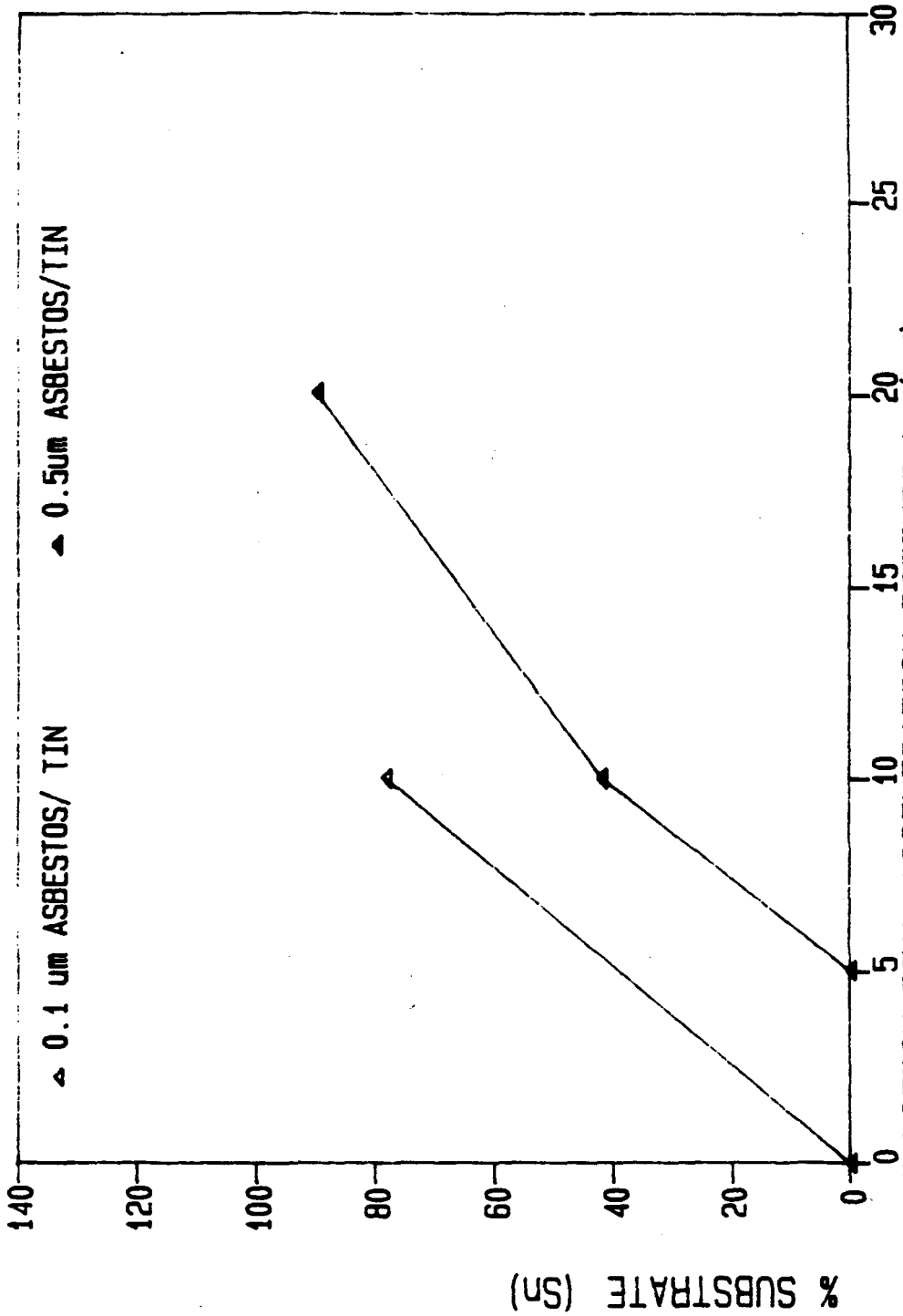

Fig. 12. Experimental ratio of silicon to silicon and tin SEM-EDX line intensities for crocidolite fibers of two different diameters on a tin block.

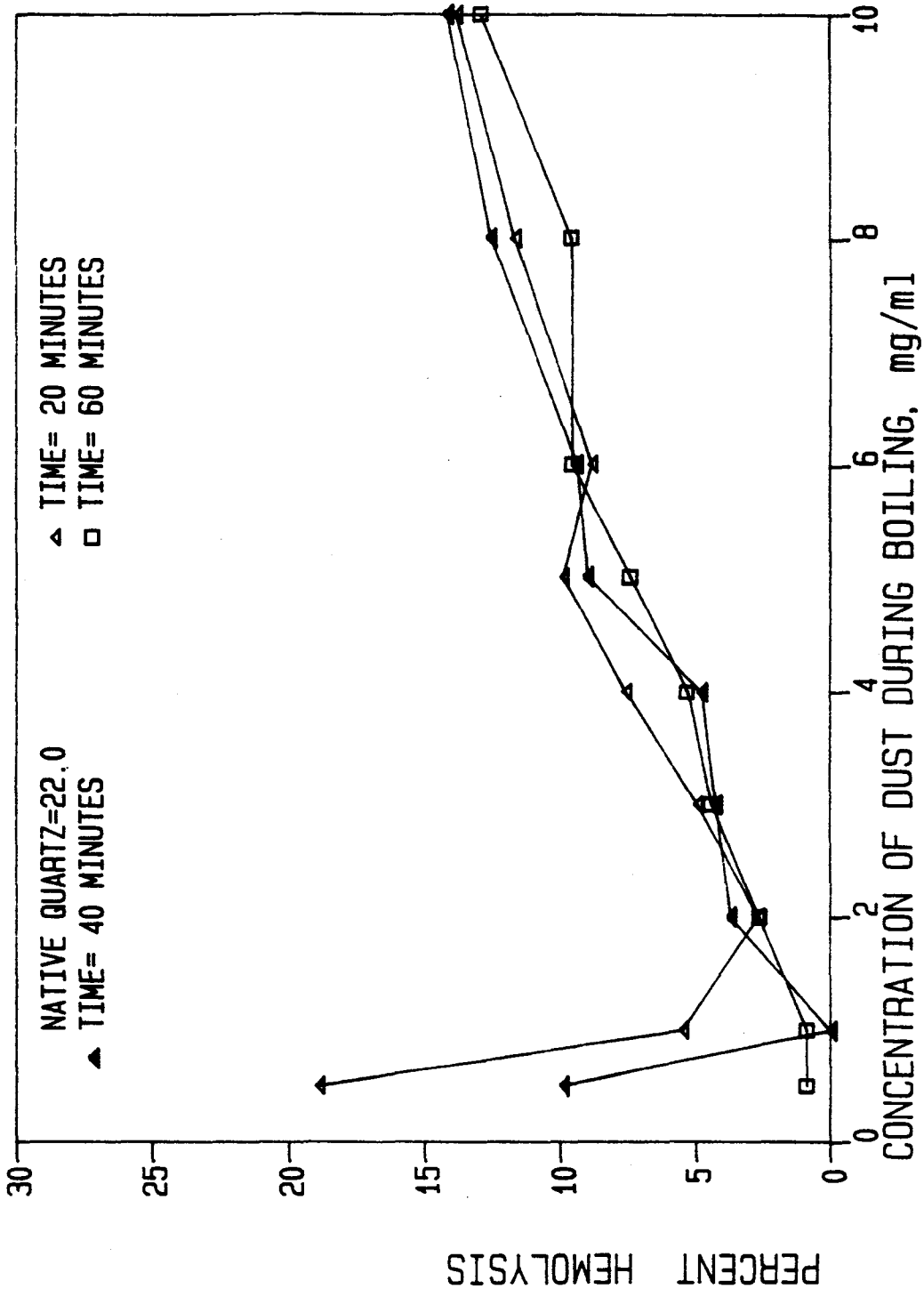

Fig. 13. Hemolytic potential of respirable quartz dust versus dust concentration in distilled water during pretreatment boiling, for various boiling times.

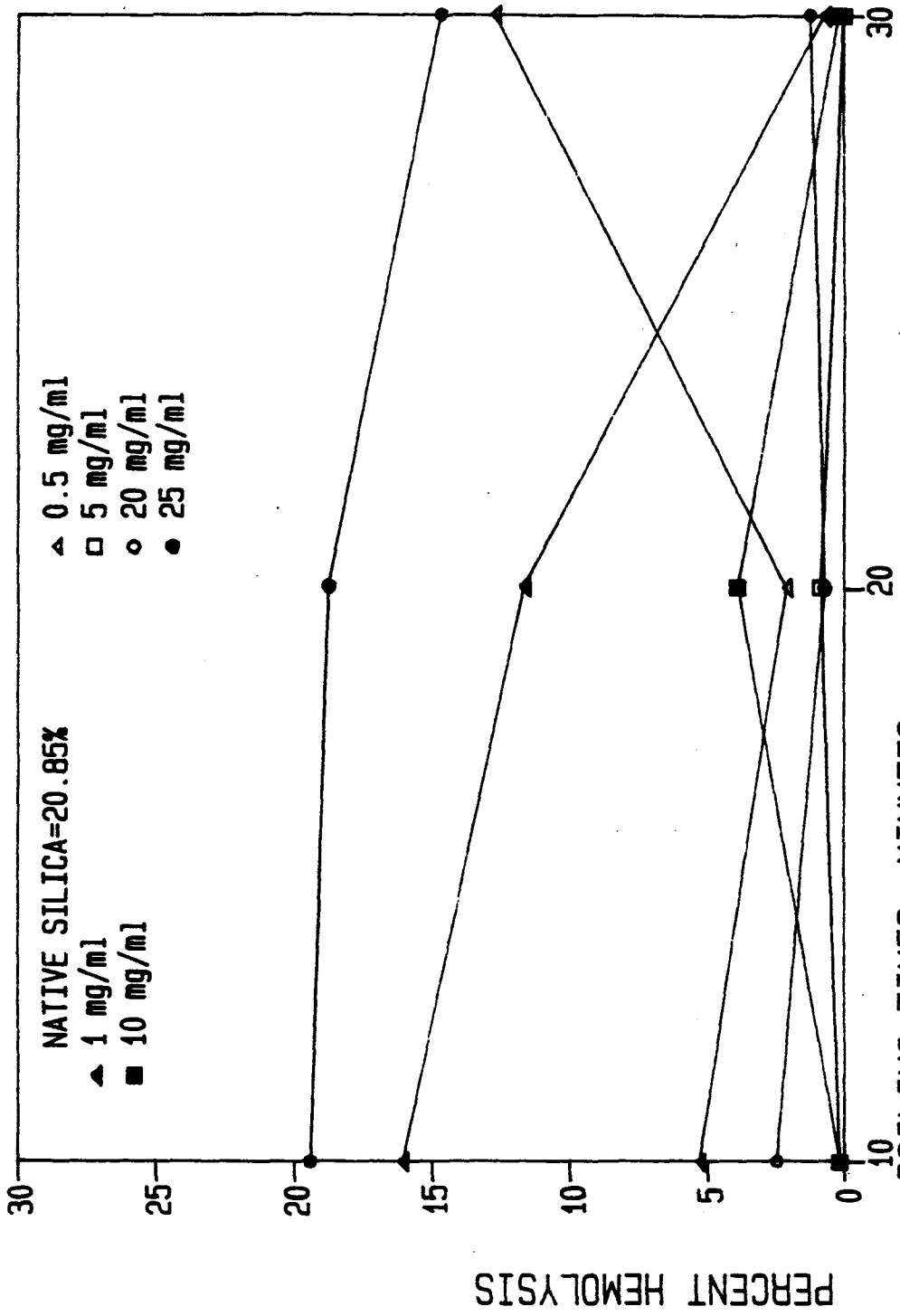

Fig. 14. Hemolytic potential of native and boiled respirable quartz dust versus boiling time for various concentrations of quartz-to-water during boiling.

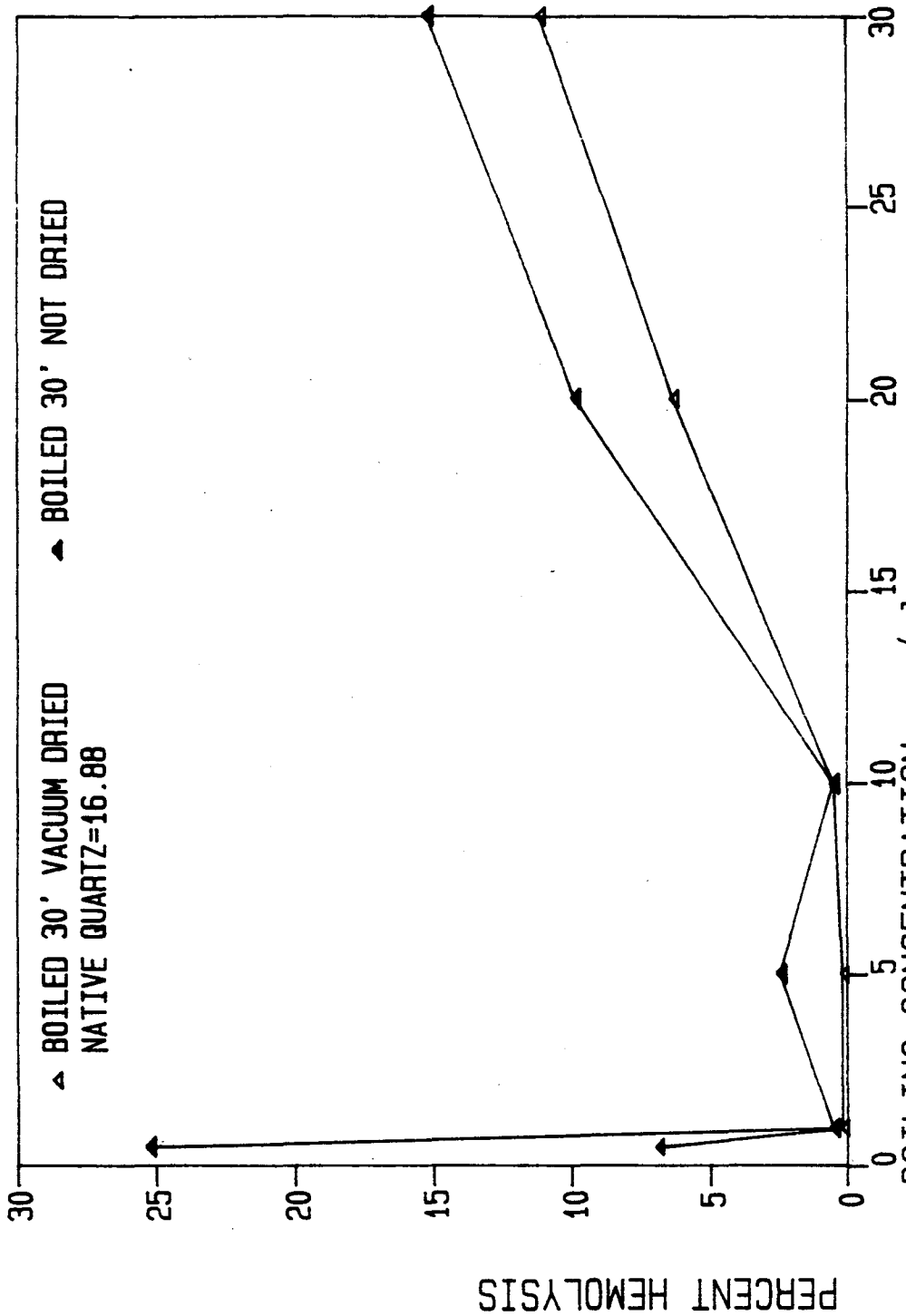

Fig 15. Hemolytic potential of native and boiled respirable quartz dust versus dust-to-water concentration during boiling for samples decanted and promptly assayed, and for samples decanted and vacuum dried before assay.

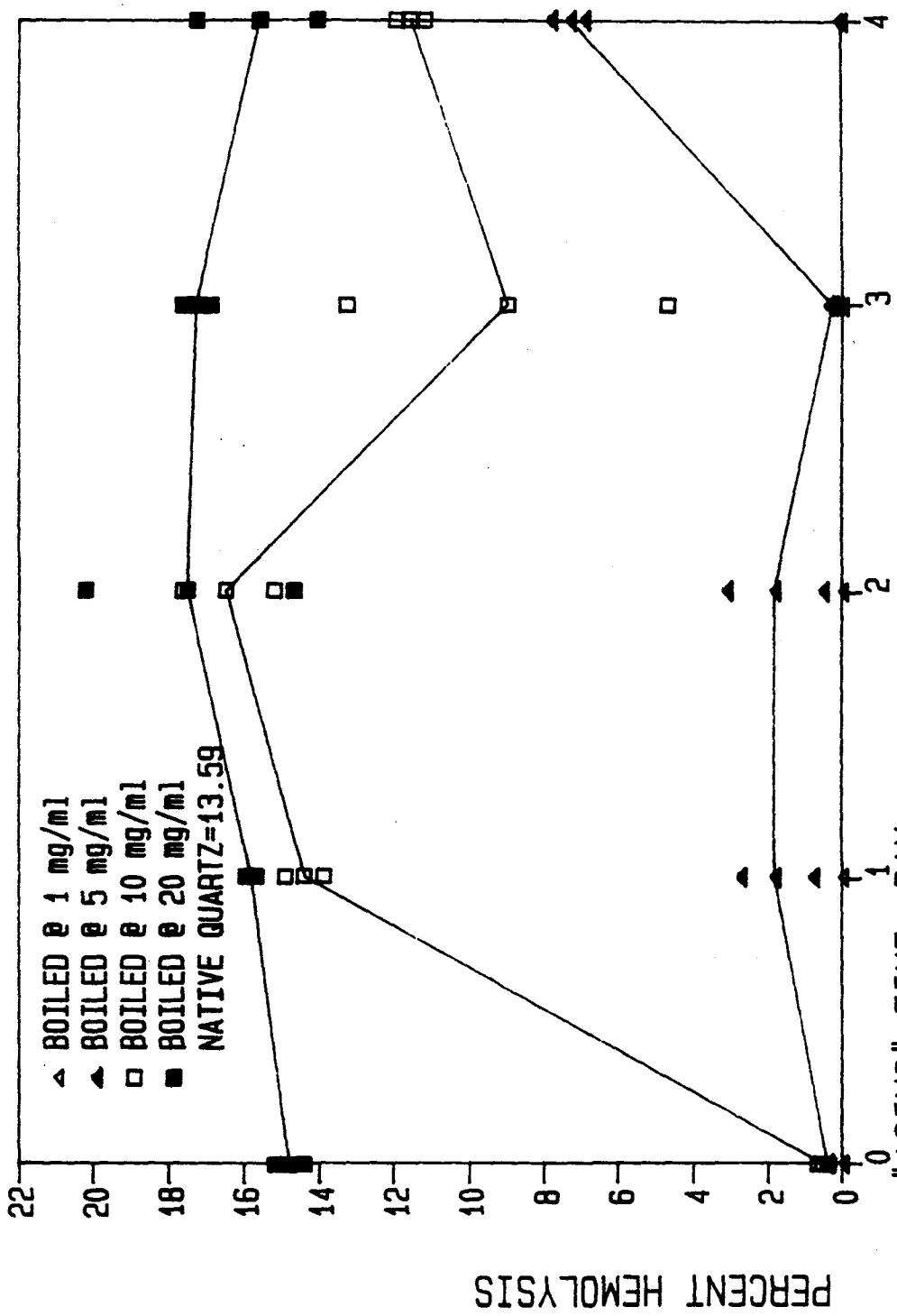

Fig. 16. Hemolytic potential of boiled respirable quartz versus delay between boiling and assay for various dust-to-water concentrations during boiling.

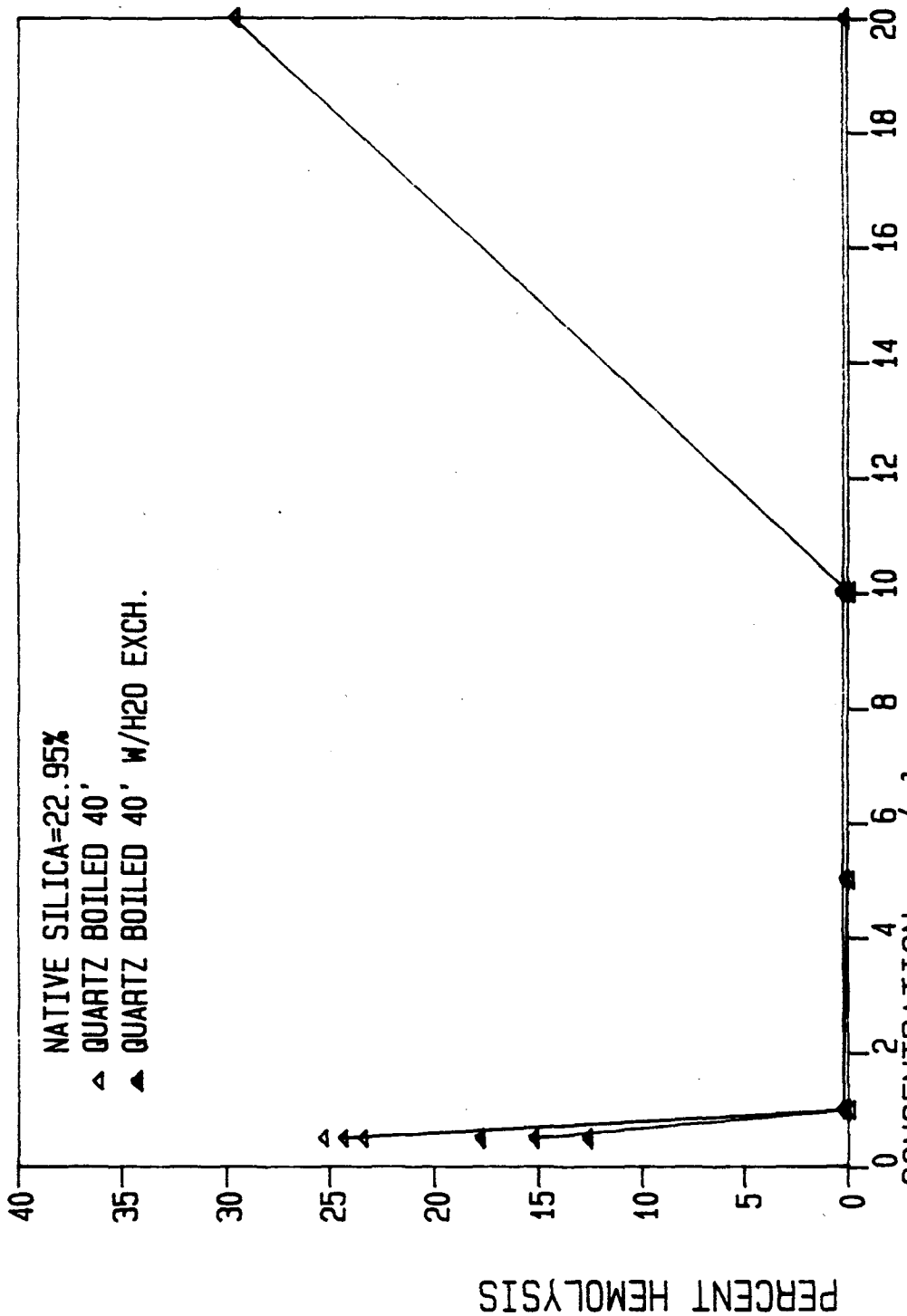

Fig. 17. Hemolytic potential of boiled respirable quartz dust versus dust-to-water concentration during boiling for samples boiled 40 minutes and for samples boiled 20 minutes, the medium changed to fresh water, and then boiled 20 minutes more.

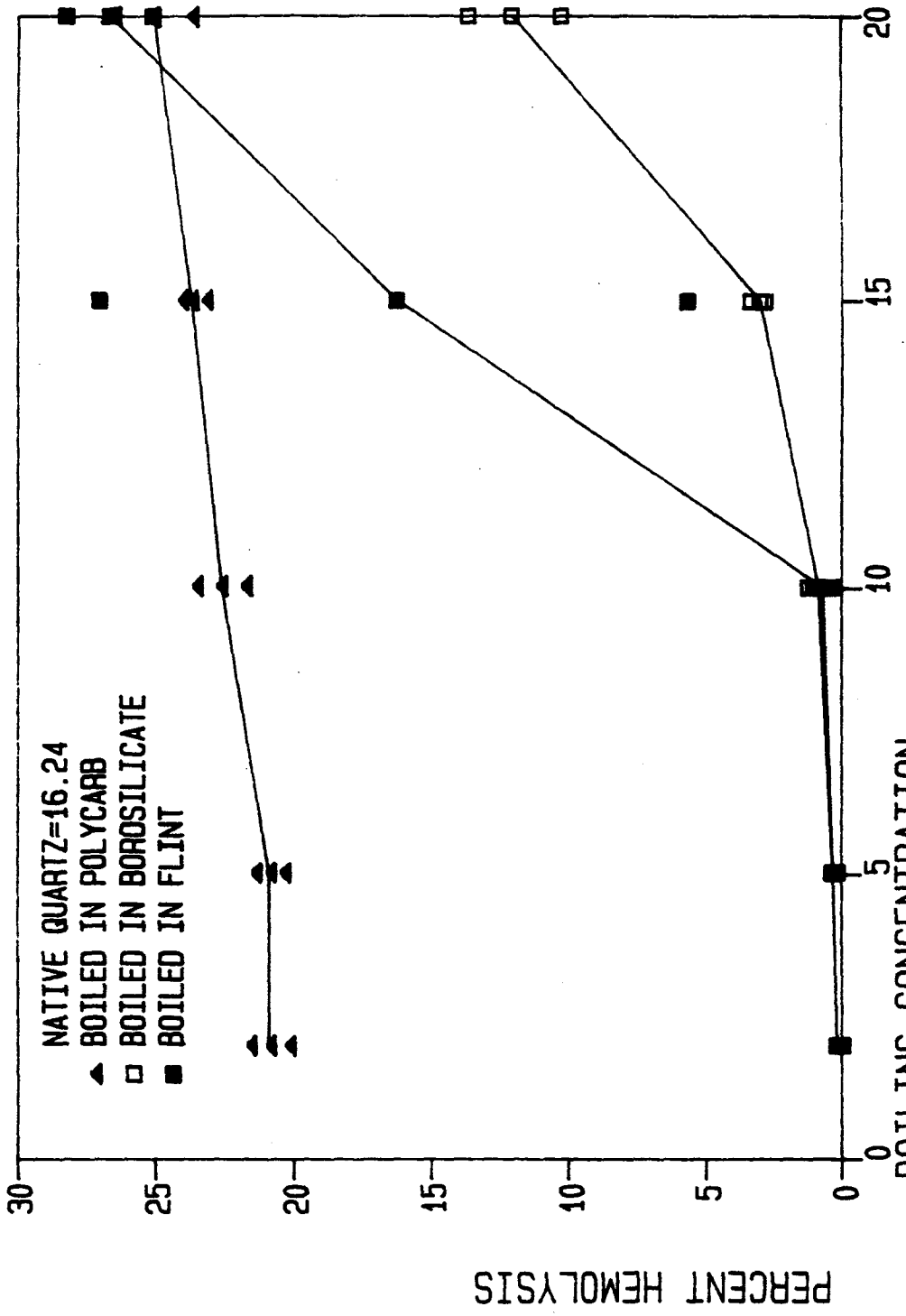

Fig. 18. Hemolytic potential of boiled respirable quartz dust versus dust-to-water concentration during boiling for different boiling tube materials: flint glass, borosilicate glass, polycarbonate.