THE EFFECT OF ALUMINIUM CITRATE ON ELECTROKINETIC POTENTIAL ON THE SURFACE OF QUARTZ AND TITANIUM DIOXIDE PARTICLES

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ABSTRACT

The electrophoretic mobility of quartz and titanium dioxide particles and the Al content and electrokinetic potential on their surface were measured. The effects of aluminium citrate (Al citrate) and AlCl3 on them were also examined. The results show that both these particles are negatively charged, but the surface charge density of quartz is higher than titanium dioxide. Moreover, it was found that a certain amount of Al can be firmly bound on quartz surface under the pretreatment with Al citrate or AlCl3 resulting in the decrease of their electrokinetic potential. In contrast, the significant changes of the Al content and electrokinetic potential on the surface of titanium dioxide particles pretreated by the same way could not be detected.

The present investigation provides further evidence for explaining the mechanisms of membrane damage caused by quartz and the antagonistic effect of Al citrate. Also, the probability of Al as a preventive measurement for silicosis in worksite was discussed in this paper.

INTRODUCTION

It has been demonstrated In Vitro that cytotoxic effect and membrane damage by quartz were much higher than by titanium dioxide under the same conditions of their dose and particle size. Al citrate can exert an antagonism against the toxicity of quartz by a possible mechanism of its action on the particle surface, but the effects of titanium dioxide on membranes were not affected by Al citrate, which may be attributed to the differences of both these particles it their surface structure and properties and the mechanisms involved in their interactions with cells.[1-4]

To evidence the hypothesis, the Al content, charge density and electrokinetic potential (−potential) on the surface of quartz and titanium dioxide particles were detected using the techniques of fluorescence and microelectrophoresis. The effects of Al citrate and AlCl3 on them were also examined in the present study.

MATERIALS AND METHODS

Chemicals and Preparation

Quartz (99% pure) was supplied by Hygiene Institute of Chinese Prophylactic Medical Center. Particle diameter was all less than 5 μm, among which 89.3% was less than 2 μm and its specific surface was 4.59 m^2/g. The suspension was prepared at the concentration of 1 mg quartz/ml with deionized water. Al citrate with Al of 9.26% was supplied by Pharmaceutical Factory of Beijing Medical University and its solution was prepared at the concentration of 1 mg Al/ml with deionized water. The suspension of quartz plus Al citrate was prepared by Al citrate with 1 mg Al dissolved in 1 ml quartz suspension mentioned above. Quartz particles were pretreated with a same Al amount contained in Al citrate or AlCl3 solution. So called pretreatment, quartz or titanium dioxide particles were mixed proportionally with a certain Al amount contained in Al citrate or AlCl3 solution. The suspension was centrifuged repeatedly, and washed with deionized water until Al in the last supernatant was not detected and then the precipitated particles were stoved.[1] Titanium dioxide with a similar pure and size was obtained from Beijing Chemical Factory and its preparation was also as same as quartz. Fluorescence probe morin was purchased from Merck, Germany. The concentration of its stocked solution and applied solution were 500 μm/ml ethanol and 50 μm/ml ethanol, respectively.

Instrument and Conditions

Viscosimeter Model E (Japan), 25°C, shear transformation velocity 100 S-1.

Cell electrophoresis Autotimer Model Sx-2 (China), 25°C, voltage 40 V, electrode distance 5 cm, e.g. electric field intensity 8 v/cm.

Formulation (5): particle electrophoretic mobility (V)=L/E

L: mobility distance (μm) of particle in unit time (sec);

E: electric field intensity

The charged property of colloidal particle is expressed usually by its surface −potential as following:

$$\zeta = 6\ \pi\eta\ L/DE.f(Kr) \quad (6)$$

D: dielectric constant of water, 78.54 at 25°C;
η: medium viscosity (P);
f(Kr): coeffient related to size and shape of the determined particle, taken 1 usually in small spherical particle

Statistical Methods

F test

RESULTS AND DISCUSSION

The Determinations of Surface-Bound Al of Quartz and Titanium Dioxide Particles.

As shown in Figure 1 and 2, fluorescence excitation and emission peak position of Al citrate after the addition of morin shift all about 5 nm towards high frequency as compared to AlCl3. However, Figure 3 and 4 illustrate that the difference disappears under the pretreatment of quartz particles with Al citrate or AlCl3, and their excitation and emission are 440 nm and 515 nm, respectively, suggesting that ions such as Cl-, citrate radical which may interfere with the determination have been washed out after the pretreatment and only Al remains to be bound on the surface of quartz particles. While fluorescence intensity of titanium dioxide pretreated with Al citrate is not only very low, but also its excitation and emission peak position exhibit blue shift about 15 nm and 10 nm, respectively. On the other hand, no Al can be detected on the surface of quartz or titanium dioxide particles without the pretreatment, indicating a little of their inherent Al. Policard et al has determined the surface-bound Al of quartz by X-diffraction.[6] It is obvious that fluorescence label technique utilized in the present study is simple and sensitive and is used to do quantitative analysis.

The data listed in Table I show that: a) surface-bound Al of the pretreated quartz particles was increased with increasing Al dose to a certain extent, but not by proportion. It was also found that about 1:2 ratio of Al to quartz dose can prevent effectively the cytotoxicity or membrane damage from quartz in our other studies; b) the amount of surface-bound Al of quartz particles pretreated with a same Al dose is similar between Al citrate and AlCl3; c) the pretreated quartz under the washing with HCl led their surface-bound Al not to be detected, then the antagonistic effect of Al disappeared and the toxicity of quartz recovered;([1-2] d) the amount of surface-bound Al of titanium dioxide pretreated with a same Al dose of Al citrate is still very low, which is consistent with the finding that its effect on cells or membranes was unable to be affected by Al citrate.[2-4]

The Determinations of Surface 2 ζ −potential of Quartz and Titanium Dioxide Particles and the Effects of Al Citrate or AlCl3

We began with the examination of medium viscosity due to its effect on electrophoretic mobility and surface ζ −potential of particles. From Table II, viscosity of Al citrate solution and the addition of quartz or titanium dioxide is higher, but medium viscosity was not influenced by the pretreatment with Al citrate or AlCl3, suggesting that only free ions such as Al3+ presented in solution may affect medium viscosiy.

It is seen from Table III, both quartz and titanium dioxide particles charge negatively but surface charge density of quartz is much higher than titanium dioxide ($P<0.01$), resulting in its higher electrophoretic mobility and surface −potential, for instance, its ζ −potential value is 34.5% higher than titanium dioxide. Why quartz can interact with the choline groups charged positively in membranes leading

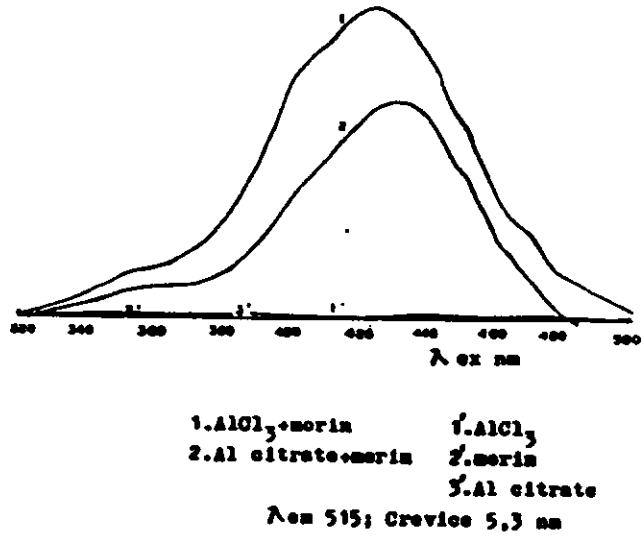

Figure 1. Fluorescence excitation spectra.

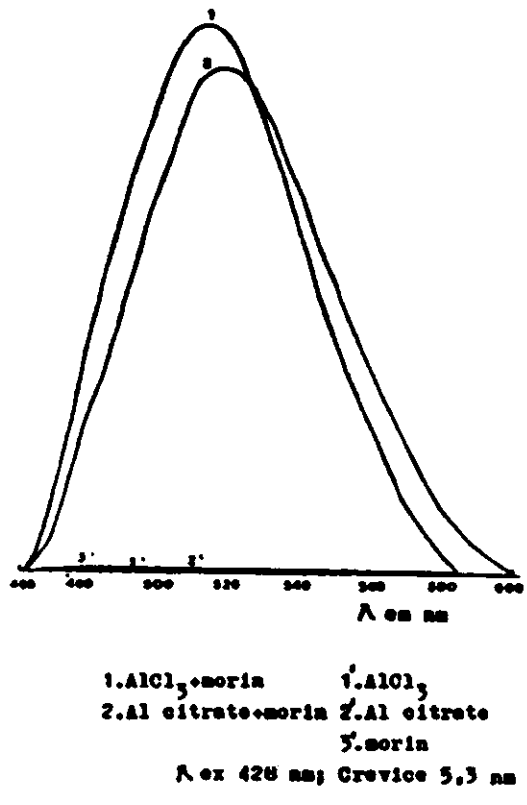

Figure 2. Fluorescence emission spectra.

Table I

Surface-bound Al of Quartz and Titanium Dioxide Particles Pretreated with Al Citrate or $AlCl_3$

Al dose	quartz		titanium	HCl washing
(μg)	μgAl/mg	nM Al/cm	μg Al/mg	
31.3	1.82	1.47	----	----
62.5	2.58	2.08	----	----
125.0	3.22	2.60	0.32	undetected
125.0*	2.99	2.41	----	undetected
250.0	3.12	2.52	----	----
500.0	3.16	2.55	----	----

The doses of quartz and titanium are all 300 μg. *$AlCl_3$

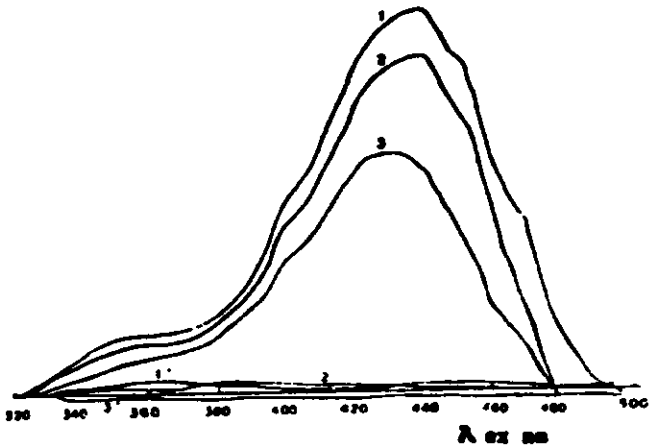

Figure 3. Fluorescence excitation spectra.

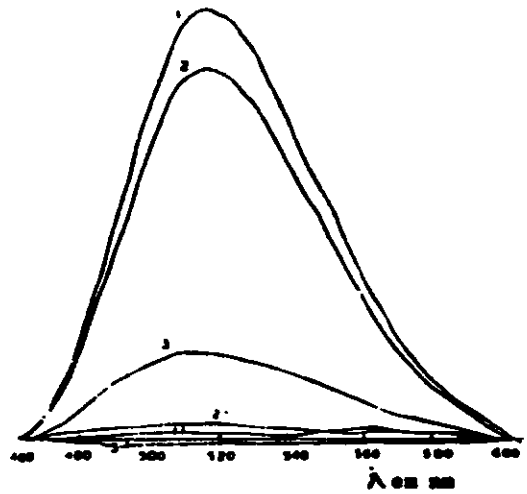

Figure 4. Fluorescence emission spectra.

the increase of the negative charge density on the surface of macrophages may be just due to its larger inherent negative charge density and its higher affinity for $-N+(CH3)3$. As pointed out by Nolan, the more the negative charge is imparted by ionized silanol groups on quartz surface the stronger its haemolysis is.[7]

It is interesting to find the amount of surface-bound Al of quartz particles pretreated with Al citrate or AlCl3 were not only increased significantly, but also their electrophoretic mobility and surface ζ −potential were lowered markedly. Although the decrease of ζ −potential is less in the pretreatment than in quartz plus Al citrate without the washing, surface-bound Al of the pretreated particles is very firm, and plays an important role in pharmacology. The combination of quartz with Al by Si-0- Al bond can block the direct in

Table II
Medium Viscosity (η)

medium	η (cp)
Water	1.008
Solution of Al citrate	1.089
Suspension of quartz	0.996
Suspension of titanium dioxide	0.990
Suspension of quartz+Al citrate	1.071
Suspension of titanium dioxide+Al citrate	1.056
Suspension of quartz pretreated with Al citrate	1.002
Suspension of titanium dioxide pretreated with Al citrate	0.990

Table III
Electrophoretic Mobility (V) and Surface Electro Kinetic Potential (ζ −potential) of Quartz and Titanium Dioxide Particles and the Effects of Al Citrate on Them

Groups	V (μm/sec/ v/ cm)	ζ-potential (mv)	
	$\bar{X}$ ± SE	$\bar{X}$±SE	%
Quartz	4.051±0.075	87.5±1.6	100.0
Titanium dioxide	2.654±0.081	57.3±1.8	65.5
Quartz pretreated with $AlCl_3$	3.426±0.112	74.0±2.4	84.6
Quartz pretreated with Al citrate	3.381±0.118	73.1±2.5	83.5
Titanium dioxide pretreated with Al citrate	2.568±0.032	55.5±0.7	63.4
Quartz+Al citrate	2.296±0.062	54.0±1.5	61.7
	----	(49.6±1.4)	(56.7)
Titanium dioxide+Al citrate	2.173±0.048	51.4±1.1	58.7
	----	(46.9±1.1)	(53.8)

The values in brackets are theoretical values calculated except the effect of medium viscosity.

teraction of quartz with choline groups of membranes, so that the order structure and stability of membranes were maintained.

As compared with quartz, no significant decrease of surface ζ −potential of the particles was found after the addition of Al citrate to titanium dioxide suspension, a difference of ζ −potential occurred only between the theoretical value calculated except the effect of medium viscosity and the determined value of titanium dioxide itself ($P < 0.01$). However, there is no significant difference in ζ −potential between under the pretreatment with Al citrate and without the pretreatment ($P > 0.05$) (Table III). This fact indicates that surface-bound Al of titanium dioxide is very little and is unable to change the structure and property of the particles and explains why its effect on cells or membranes can not be affected by Al citrate.

In deed, surface-bound cations like A13+ of quartz will increase its nonpoisonous surface area resulting in the reduction of its toxicity, which will provide a clue for screening new drugs for silicosis. In addition, according to theory of electric double layer, whether collosol is stable in a certain condition will depend on the gravitational force and electrostatic repulsion among these particles. They will become easy to coacervate and sedimentate due to the reduction of their repellent potential energy and stability, if ζ −potential and charge of the colloidal particles are decreased. The

coacervation and sedimentation of these particles are not only beneficial in discharging from lung, but also in precipitation from the air. Therefore, it will be more efficient to prevent silicosis through the way changes the physical and chemical properties of the particles, so that their toxicity themselves will be lowered and their fall will be increased. It should be considered that a potential role of such cations as A13+ plays in wet clearing dust, for example wet drilling.

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RELATIVE TOXICITIES OF PHLOGOPITE, BARITE AND QUARTZ

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ABSTRACT

Phlogopite is a silicate mineral belonging to the biotite mica group. Apatite mine deposits of eastern Finland contain 65% phlogopite, 14% calcite, 4% dolomite, 10% apatite, and 5% richterite. Phlogopite is used in many industrial applications including paints and fillers. Although mica is considered as a non-toxic mineral, recent toxicity studies in our laboratories and sporadic case reports have indicated a potential for lung damage. This investigation evaluated the *in vitro* effects of respirable phlogopite using conventional toxicity bioassays. The results were then compared with positive (quartz) and negative (barite) controls to assess relative toxicities. Cytotoxicity of minerals were compared by measuring their effects on sheep erythrocyte hemolysis, lactate dehydrogenase, β-glucuronidase, β-N-acetyl glucosaminidase release from alveolar macrophages (AM) and hydrogen peroxide and superoxide secretions from AM. Results of hemolysis studies indicated that phlogopite mineral is non-toxic to erythrocyte cell membranes at a dust concentration as high as 10 mg/ml. Data on the AM enzyme studies have, however, shown considerably greater levels of enzyme release and secretions of superoxide and hydrogen peroxide from AM by phlogopite. These results suggest that phlogopite mineral in respirable fraction is cytotoxic, and further studies are warranted to evaluate the fibrogenic potential.

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EFFECTS OF MINERAL DUSTS ON ULTRASTRUCTURE AND FUNCTION OF ALVEOLAR MACROPHAGES

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ABSTRACT

The effects of mineral dust on biomembrane and organelle in alveolar machrophages in vitro were studied. The results suggest:

1. When the mineral dusts were concentrated at the secondary lysosome in the alveolar macrophages in vitro it indicates that the structure of cytoplasma is always normal. Swelling and degenerative mitochondria, abnormal structure cytoplasma and separated karyolemma were observed when silicon dust spread in cytoplasma.
2. The investigation of the effects of dusts of quartz, asbestos, graphite, TiO_2, Be, Sb etc. on biomembrane of alveolar macrophages in vitro indicated that they differed greatly at the same concentration.
3. It is postulated that stress should be put on choice of drugs with protection effect on cytomembrane. Such as VitE, SOD, PVPNO and piperaquie in order to prevent cell damage.
4. The study on effects of the four kinds of mineral dusts on protein synthesis in alveolar macrophages was carried out by incorporation of ^{5}H-Leu.

INTRODUCTION

Alveolar macrophages play an important role in the onset and development of the diseases in the lung, especially during the process of pulmonary fibrosis in pneumoconiosis.[1-2]

Scientists in various country have paid special attention to the investigation of the relation between macrophages and the factors which cause diseases. In this investigation the electron microscopy had been applied to study the effects of different dusts on the morphorological changes of cell membrane and organellae and to observe the protective effects of several kinds of drugs to cell membrane. Radioactive isotope tracer technique has been used to determine the synthesis of protein to illustrate the toxicity of alternative way.

MATERIAL AND METHOD

Dusts

The content of free SiO_2 in quartz is 99%. All asbestos are UICC product produced in Germany. The Particles of Sb_2O_3 dust are less than 1 μm. No free SiO_2 was found in it. The purity of TiO_2 was more than 99%. All the dust particles were less than 5 μm. the particles of graphite and Be dust were less than 5 μm. There was no SiO_2 to be found. All of the dust was steriled by autoclaving and a 1 mg/ml solution was prepared with medium. Before using mixer. It was stirred thoroughly with a magnetic.

^{5}H-Leucine

The activity of the solution was 3.7×10^5. Bq/ml(100μ Ci/ml)

Drug

VitE was in capsules. SOD was obtained from the laboratory of Suzhou Medical College. Piperaquine and P_{204} were unprocessed powder.

Alveolar Macrophage (AM)

AM were obtained by pulmonary lavage of guinea pigs using RPMR-1640 at sterile condition. Lhe cell suspensions were cultured with quartz and TiO_2 dust at different concentrations. Control cultures were treated similarly except that the dusts were omitted. After 6 and 15 hr incubation respectively the AM were collected by centrifugation. The precipitates were fixed. The samples were examined in transmission EM. 0.5 ml cell suspension was transferred into culture bottle with a sterile cover glass slip. After 2 hr incubation the cover glass slips were taken out. Then the cover glass slips were placed in the bottles with 199 medium 100 μg of quartz, asbestos, Sb, Be, graphite and TiO_2 dust were added respectively with the exception of the control. After incubation, the samples were taken out at different intervals.

The experiment of protein synthesis in vitro was carried out

in Hank's solution.[3] One milliliter of AM suspension at a concentration of 1×10^6/ml was put in centrifuge tubes and the dust of quartz, asbestos, graphite or TiO_2 was added to make the final concentration at 100 mg/ml. All tubes and the control were incubated at 37°C for 2 hr. After incubation 7.4×10^4 BQ(2 μCi) labeled leucine was added to each aliquot and incubated for another 3 hr. The same amount of labeled leucine was added to the control after incubation. The protein was collected and the activity was counted by liquid scintillation counter.

RESULTS

Effect of SiO_2 on Damage of Organella

Under the TEM one can find that pseudopodia disappeared, lysome disrupted Si particles existed in cytoplasm freely, mitochondria expanded pyknosis, necrosis of some cells appeared losing normal cytostracture with vacuolar changes of matrix, presence of spare cytoplasm, nuclei were expanded to become round or oval, matrix vacuolar change in nuclei, heterochromatin condensed under the nuclear envelope, expending or nuclear envelope disrupting were observed. In some cases the content of nuclei became homogeneous and showed a medium electron density. It was completely impossible to distinguish euchromatin, heterochromatin and nucleolus. The boundary of cells became indistinct. Cells were disrupted finally.

Damage to cells caused by TiO_2 was less than that caused by SiO_2. In this case the majority of cells are in stress shown by expanding of cell volume, increasing of pseudopodia, phagosome and rough surfaced endoplasmic reticulum. No necrosis cells were observed in the control.

The Effect of Quartz and Other Dusts on Cell Membrane

The investigation of the effects of quartz, asbestos, graphite, TiO_2, Sb, Be on the biological membranes has demonstrated that AM be different in response to different kinds of dusts. Among them the damage effect of quartz to membrane is the most obvious one. No abnormal changes of cell membrane were observed in the control in which cells had been cultured for different periods of time. The majority of these cultured adhesive AM cells in vitro coming from healthy rabbits were round, oval and astroid. There were evenly spreading ruffles at the cell membrane with irregular margin. Long or short filopodia, finger-like and pseudopodia were observed at cell membrane (Figure 1). The static pseudopodia were less and the active pseudopodia were more. When the pseudopodia accept a stimulating information it stretched itself to the foreign body and the cell at the opposite side then the cell moved to the foreign body (Figure 2).

The response of AM cultured in medium to Si dust was active. The change of membrane was characterized by the following features: (1) uneven ruffles were present first then disappear gradually and the cell membrane becomes homogeneous and smooth (Figure 3); (2) vacuoles were present at the surface of cell (Figure 4); (3) various size of holes were present at cell membrane (Figure 5); (4) pseudopodia and microvilli disappeared.

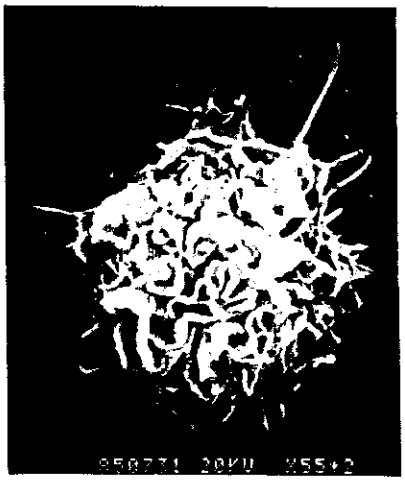


Figure 1. Control M ×5500.

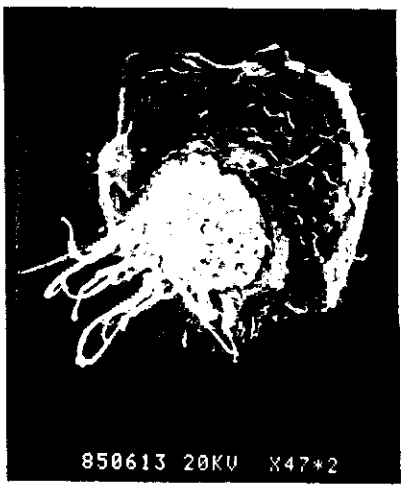
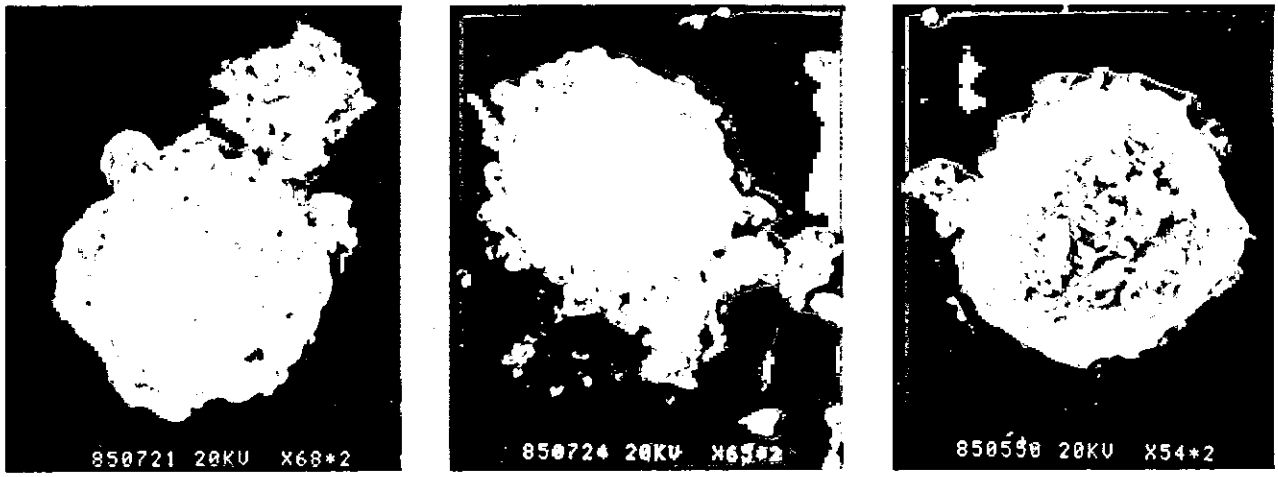


Figure 2. Pseudopodia and skirt margin ×4700.

In the AM cultured with asbestos fibers attachment was observed in the samples taken at the 5 min after the adding of the dust. The response of AM to different length of asbestos fibers was different. The short asbestos fibers were absorbed locally and were phagocytosed in situ. In this case the response was not obvious and the change of cell membrane was less. As the long asbestos fibers were concerned, the cells were phagocytosed in a sleeve-like fashion or phagocytosed the dust from the near end of the asbestos fibers by stretching numerous small pseudopodia at the surface of the cells. Some macrophages could phagocytose a large amount of asbestos (Figure 6). Such kinds of cell were more often to be observed at the interval of 18 hr and 24 hr samples cultured and structure of cell membrane and its morphology were normal. The phaocytosis in the cells cultured with Si dust was different from that in the cells cultured with



Figures 3–5. After adding SiO_2 and incubated at 37°C for 30 min. Ruffles are uneven and pseudopodia disappear, ×5400. The vacuole structure is present at the surface of membrane. The membrane is even, ×6500. Various sizes of holes are present at the pyknosis membrame, ×6800.

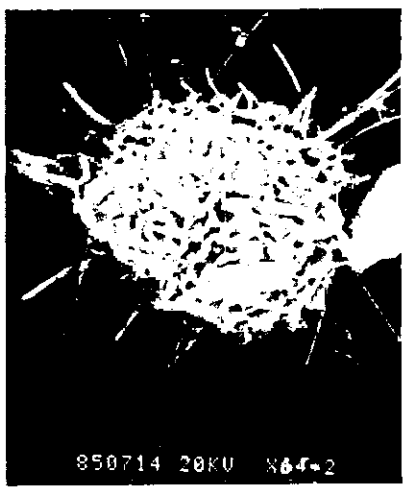


Figure 6. After adding asbestos fibers and incubating at 37°C for 10 hrs randomly fibers are present. ×6400

asbestos. In the latter case the cells aggregation should be observed on the cover glass slips and more cells phagocytosed a bundle of asbestos in common. As the culture time prolonged the dying cells increased gradually. The cell membrane interrupts before dying, the membrane ruffles disappeared and membrane dissolved locally. Comparing with that in Si group the dying of cell delays.

When Sb, Be, Graphite and TiO_2 dusts were added into the medium with AM and incubated for 5 hr the difference of cell membrane was very significant. In the group of Sb dust filopodia stretched themselves to various directions, and disorder of ruffles observed. The reaction of cells were very strong (Figure 7). In the group of Be dust straw-hat-like changes were observed in the majority of cells (Figure 8). In the group of graphite no obvious changes in the cell membrane were observed. In the group of TiO_2, large amount of absorbed TiO_2 particles which were phagocytosed in situ were observed. Some dust particles were taken by the flattened pseudopodia and there were partially morphorological changes of cell membrane. Ruffles at cell membrane could still be observed. It seems that TiO_2 has less effect on membrane structure.

Protection Effect of Drugs on AM Cell Membrane

SiO_2 was used as a cell damage agent. Cells were cultured in vitro and SiO_2, VitE, SOD, 4% P_{204}, and piperaquine were added to the cell suspension to make the final concentration to be 100 μg/ml, 40 μl/ml, 40 μl/ml, 100 μg/ml respectively. The samples for SEM were prepared at the 5 hr and 10 hr interval after the addition of drugs. All of the 4 drugs have protective effect on cell membrane. After the addition of these drugs AM were very active and the activity of pseudopodia was very frequent. Among them the effect of Vit E and SOD were more obvious.[4] Incorporation of H-Leucine in Cultured Cells

The effect of 4 kinds of dusts on protein synthesis in AM were shown that suppressive effects on protein synthesis the asbestos dust is the most significant one. The ratio to control counterparts for protein synthesis was 54.6%. The effect of graphite was the minimum.[4] (Table I)

DISCUSSION

AM is one of the most effective cells for phagocytosis. It plays an important role in removing foreign bodies in lung.

Figure 7. After adding Sb dust and incubating at 37°C for 5 hrs. filopodia are present, ×3300.

Figure 8. After adding Sb dust and incubating at 37°C for 5 hrs straw-hat like margin is present, ×4900.

Table I
Effects of 4 Kinds of Dusts on Protein Synthesis in AM

Sample	No	Viability %	Counts cpm/10^5 AM	Correcting to living cells	Ratio to the control
Control	7	96	26346	27443	100
Quartz	13	85	15838	18629	67.9
Asbestos	9	90	13478	14975	54.6
Graphite	8	89	22313	24792	91.2
TiO_2	10	87	17825	19375	74.7
*Blank			1221		

*^{3}H-Leu was addid at the end of incu bation in blank.

Recent investigations demonstrate that the function of AM have close relation with various pulmonary diseases e.g., infection, tumor and pulmonary fibrosis. Great attention has been paid to the investigation of morphorology, function and metabolism of AM and its interrelation to other kinds of pulmonary cells. Phagocytosis to 15 kinds of dust in AM in vitro has been investigated by successive photography by Diavert revert phase contrast microscope.[5] During the process of phagocytosis the change of cell membrane and response of cells were obvious. In order to know the mechanism of phagocytosis the effect of quartz dust etc. on organellae has been investigated. The results obtained demonstrate that the toxic effect of SiO_2 on lysosome is present most early. The toxicity of quartz is closely related to its concentration and the duration of action.

The response of cell membrane to different kinds of dust or different length of fiber dust particles of the same dust was different.[6] Brains[7] has reported that when the cells contact with foreign body, absorption occurs first and the change of the absorption depends on the chemical and physical properties of the dust particles. During phagocytosis, the increasing of the cell volume, energy metabolism and membrane receptors were observed. In this investigation the holes of cell membrane at the site of absorption and formation of phagocytosis vacuoles to quartz were observed in the process of AM.

The way of short asbestos fiber phagocytosis is the same to that of SiO_2. But the phagocytosis was slow and only a slight change of cell membrane was observed; while the long

asbestos fiber can be phagocytosed by one or more than one cell which caused aggregation of cells. The ability of phagocytosis of AM to dusts of quartz and asbestos may be related to C_3 receptor and Ig G receptor.[8-10] Sb dust caused the formation of a large amount of filopodia, intensive cell response and obvious morphological changes of cell membrane. Be dust caused the formation of more straw-hat-like cells which will be studied further.

In the aspect of morphological change of the cells the membrane change caused by SiO_2 was the fastest one and damage was obvious. Asbestos had less effect on AM. After phagocytosis of a large amount of fibers only less changes of cell membrane were observed until 24 hr after addition of dusts of asbestos. During the process of examination of AM cells in vitro by successive photography we found that cells can still move in peristalsis way and trasmigrate. There are some reports on interaction between mineral dusts and the membrane. The common viewpoint is that the change of membrane is concerned with the electronic structure at the surface of the mineral particle.

Both VitE and SOD are removing agents of free radicals. The picture of SEM demonstrates that they can protect the membrane from being damage. Schlipköter has demonstrated that P_{204} can form hydrogen bonds with SiO_2 in priority competitively to protect cell membrane piperaquie can make the membrane of lysosome stable. In the investigation of the effect of quartz and coal on phagocytosis, Comolli et al[11] found that labeled leucine tracer technique was the most sensitive one for the determination of protein synthesis. After addition of dusts, protein synthesis was observed 2 hr later. The effect of asbestos dust on protein synthesis was the most obvious one while the effect of quartz dust came the second. The mechanism of suppression of protein synthesis is waiting for further study.

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SUPPRESSION OF QUARTZ CYTOTOXICITY BY PULMONARY SURFACTANT—ELECTRICAL EFFECTS

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ABSTRACT

It is known that respirable native quartz particles are cytotoxic to erythrocytes. Coating the quartz particles with lecithin, a major component of pulmonary surfactant, reduces hemolysis significantly. The zeta potential of lecithin coated quartz, in physiological saline, is significantly less than that of native quartz. A biophysical model for erythrocyte cell—quartz particle interactions, in physiological conditions, has been developed. The model predicts that native quartz particles, approaching an erythrocyte to distances below 10 nm, induce, in the erythrocyte cell membrane, large electrical fields (400 kV/m to 600 kV/m), sufficient to rupture the cell membrane and cause hemolysis. Because of their low zeta potential, lecithin coated quartz particles, on the other hand, do not induce large membrane electrical field strengths. This result adds to the evidence supporting the electrical nature of the mechanism of hemolysis by mineral dusts.

No Paper provided.

PHYSICOCHEMICAL CHARACTERISTICS OF QUARTZ DUST WHICH CONTROLS ITS BIOLOGICAL ACTIVITY

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ABSTRACT

The biological activity of quartz is controlled by specific and defined characteristics of its respirable dust. Virtually the totality of the evidence concerning the fibrogenic activity of quartz dust shows that it is dependent on particle-size distribution, surface properties of the constituent particles and dose delivered to pulmonary tissues. All of these characteristics have been tested *in vivo* and *in vitro*, and have been demonstrated to be the most important variables which control biological activity. The progression of human lung pathology, and fibrosis, are strongly dependent upon the intensity of exposure and these physicochemical characteristics.

Although historically considered a fibrogenic agent, recent reports have suggested that quartz, as well as other forms of crystalline silica, may be an animal carcinogen. Some citations have extended this so that quartz and other silica polymorphs are considered possible human carcinogens. The properties of quartz found to be important in fibrogenesis will be reviewed and extended to include its proposed carcinogenicity.

No Paper provided.

ALTERATION OF RESPIRABLE QUARTZ PARTICLE CYTOTOXICITY BY THERMAL TREATMENT IN AQUEOUS MEDIA

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ABSTRACT

Respirable quartz cytotoxicity, as measured by erythrocyte hemolysis and pulmonary macrophage release of lactate dehydrogenase *in vitro,* is neutralized by boiling in water in glass test tubes for 10 to 40 minutes. The cytotoxicity is reduced to near zero by boiling 1 to 10 mg quartz per milliliter 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 boiling results in full detoxification through 20 mg quartz per milliliter water. Pre-boiling the medium with silica reduces the detoxification effect. Detoxification persists after mild drying at 110°C for 8 hours, and persists after three days of resuspension in water at room temperature.

INTRODUCTION

Reasearch underway to determine interactions of quartz and other mineral dust surfaces with pulmonary fluids and alveolar macrophages in culture led to the observation 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. This finding was in direct contradiction to earlier results from both short term macrophage lysosmal 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 but with steam present.[1,2] However detoxification under boiling conditions has been reported in other research.[3] It was decided to use the hemolysis assay to further investigate these findings, because of its sensitivity, simplicity and cost.

RESULTS AND DISCUSSION

Respirable quartz dust used in this study was taken from a stock of crystalline silica, Min-U-Sil, obtained from Pennsylvania Sand Glass Corporation, fractionated in air with a particle classifier. The small size fraction retained for use was 80% less than 5 micrometer particle diameter, with an area equivalent median diameter of 1.24 micrometers as estimated by automated image analysis. The silica was at least 98.5 mass percent silica as determined by X-ray energy spectrometric analysis; and the crystalline form was alpha-quartz as determined by X-ray diffraction. It's specific surface area was 3.97 square meters per gram as determined by nitrogen adsorption isotherm methods.[1]

To measure the erythrocyte hemolytic potential of treated and untreated dusts we use the method of Harington et al,[4] with minor modification.[1] Briefly, dusts suspended in buffer are mixed with an equal volume of 4% sheep red blood cells, and incubated 60 min. at 37°C with periodic mixing. Next the cells are 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 the dry dust (12 mg), vortexing, and boiling for periods up to 60 min., without stirring. This was done in flint glass tubes for samples with dust concentrations of greater than 1 mg/ml, and in polycarbonate tubes for lower concentrations. After the boiling period was completed, sample tubes were spun down for 60 sec., the supernatant discarded, and the dust resuspended in phosphate buffered saline (PBS) 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. (Figure 1).

When individual magnetic stirrers were used in each sample, results were similar, except the toxicity was reduced to virtually zero at concentrations to 10 mg/ml, and then increased in a linear fashion. Samples were also boiled for half the specified times, centrifuged, the medium changed to fresh water, and boiling continued for the rest of the period. The toxicity was reduced to very low levels through the highest

concentration tested. (Figure 2) As also shown, pre-boiling the water with a separate quartz sample before using the still hot supernatant to boil the test sample, somewhat diminished the detoxification phenonenon. We observed this diminution also in the case of pre-boiling the water with silica gel.

Experiments involving various boiling times showed a weak dependence of detoxification with time, except at 1 mg/ml, where detoxification progressed with boiling time. (Figure 3)

Limited tests of the persistance of the detoxification have been made and are continuing. One question was whether or not the passivation effect was due to some gel or other coating which might not withstand drying and resuspension. Samples were vacuum dried after boiling, and assayed the following day. Fully detoxified samples remained the same, and partially detoxified samples had slightly less toxicity after drying than replicate samples promptly assayed. (Figure 4) Fully detoxified samples boiled at 1 and 10 mg/ml which were decanted and placed in fresh distilled water or PBS did not retoxify over a 3 day period. (Figure 5) Other samples were left standing after boiling in the supernatant from the boiling water at room temperature. Fully detoxified samples boiled at 1 mg/ml remained at zero toxicity after 4 days. Samples at higher concentrations showed some increase with time; the sample boiled at 10 mg/ml was essentially fully retoxified. (Figure 6)

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 flint glass, polycarbonate, and Tefzel tubes, only partial detoxification was seen. When boiled in polycarbonate tubes using water that had been boiled only in polycarbonate, no detoxification was seen at any concentration. (Figure 7)

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 water in polycarbonate tubes with varying amounts of 3 mm soda-lime glass beads. (Figure 8) There is a roughly proportional dependence of detoxification on the number of glass beads, and thus the glass surface area present. The effect was investigated also by using shards of glass cover slips in polycarbonate tubes during boiling. In general detoxification occurs with increasing glass content, but with a lessening of the effect seen at the highest glass content level. (Figure 9) 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. (Figure 10)

An additional anomolous result occured when there was a failure in the reverse osmosis water purification cartridge in our laboratory 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; but detoxification was not complete for any treatment, even using flint glass tubes. (Figure 11) When the water system was restored to proper operation, the results

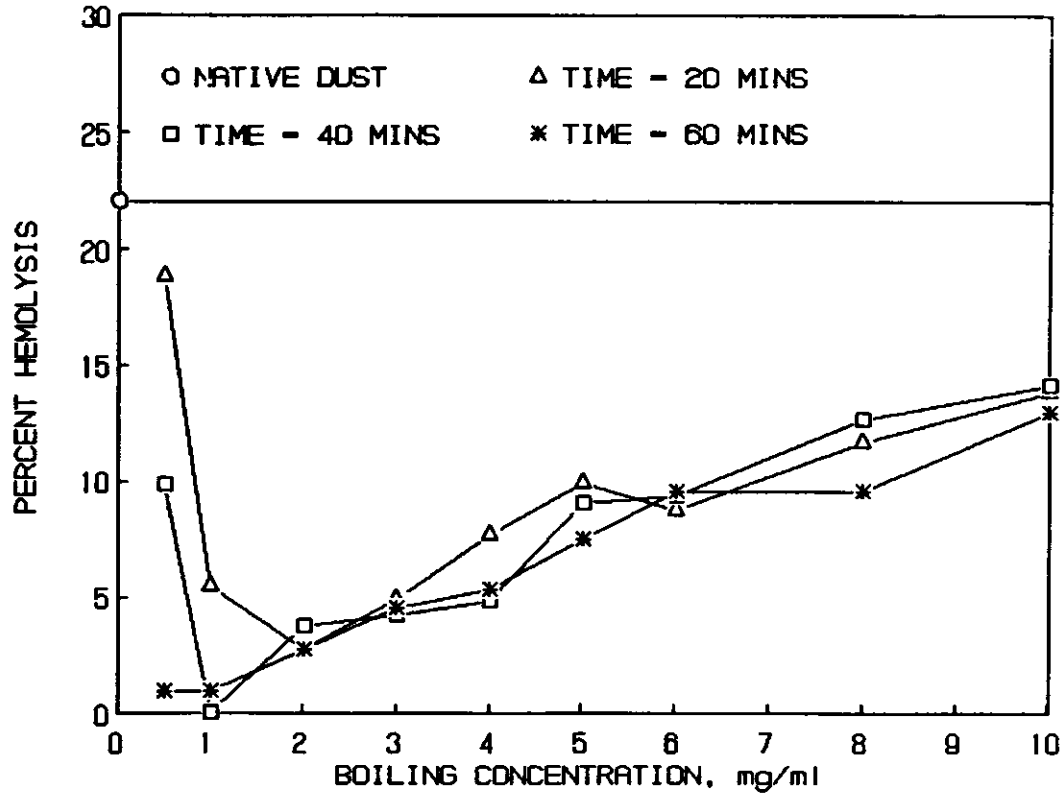

Figure 1. Silica cytotoxicity with boiling.

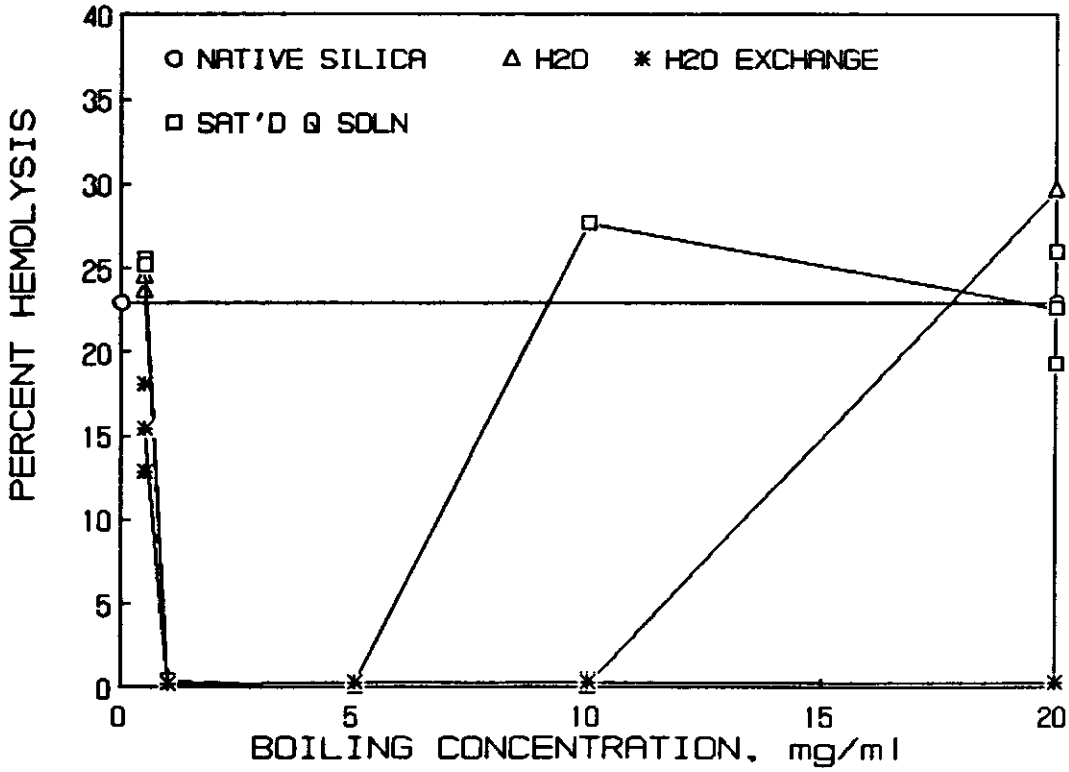

Figure 2. Quartz boiled 40° in various solutions.

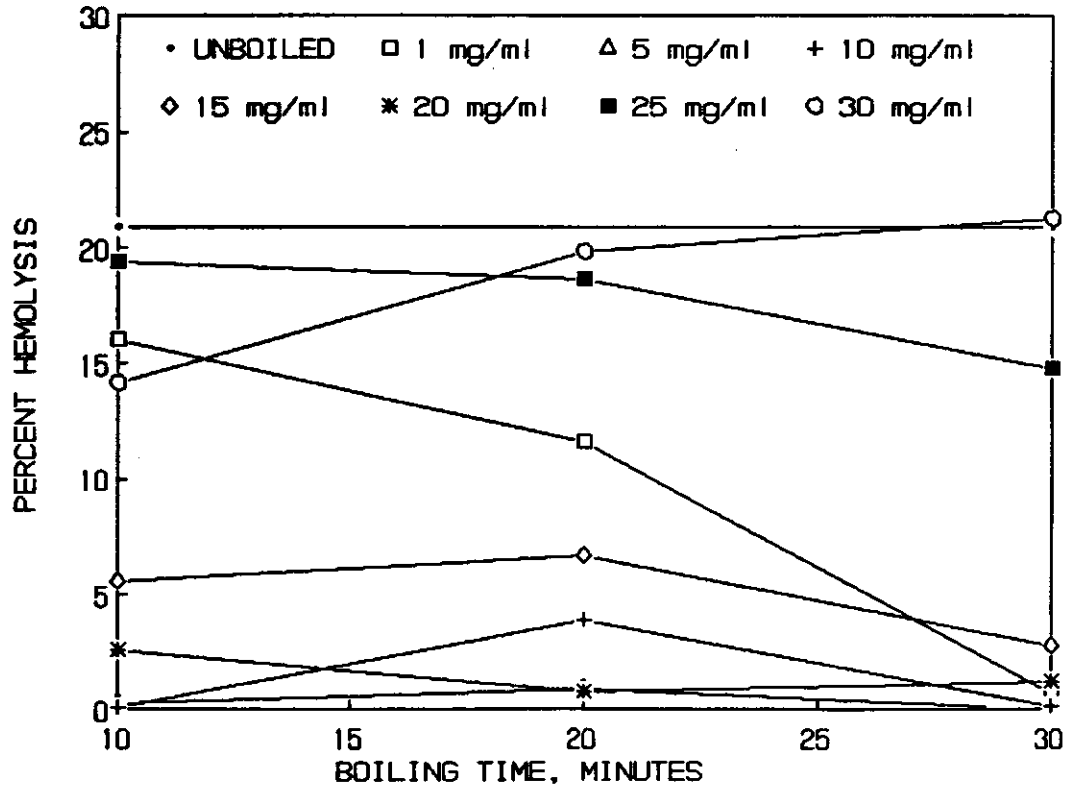

Figure 3. Hemolysis vs. boiling time.

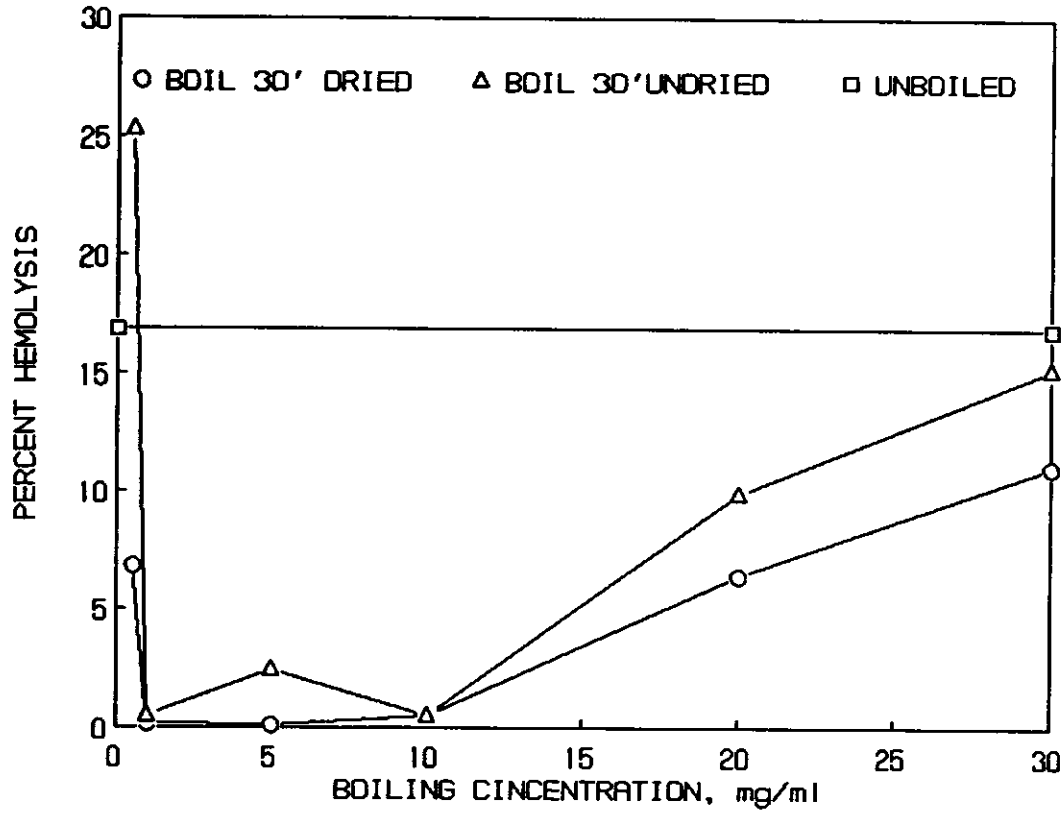

Figure 4. Hemolysis vs. post-boiling drying.

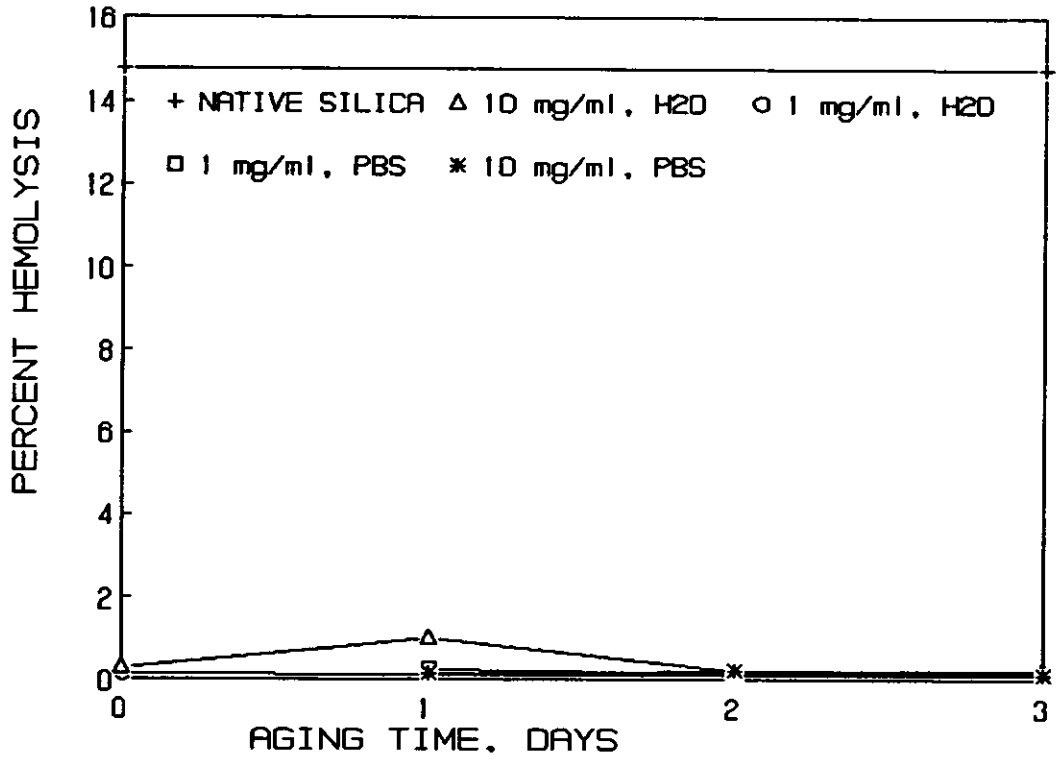

Figure 5. Quartz aged in H_2O and PBS.

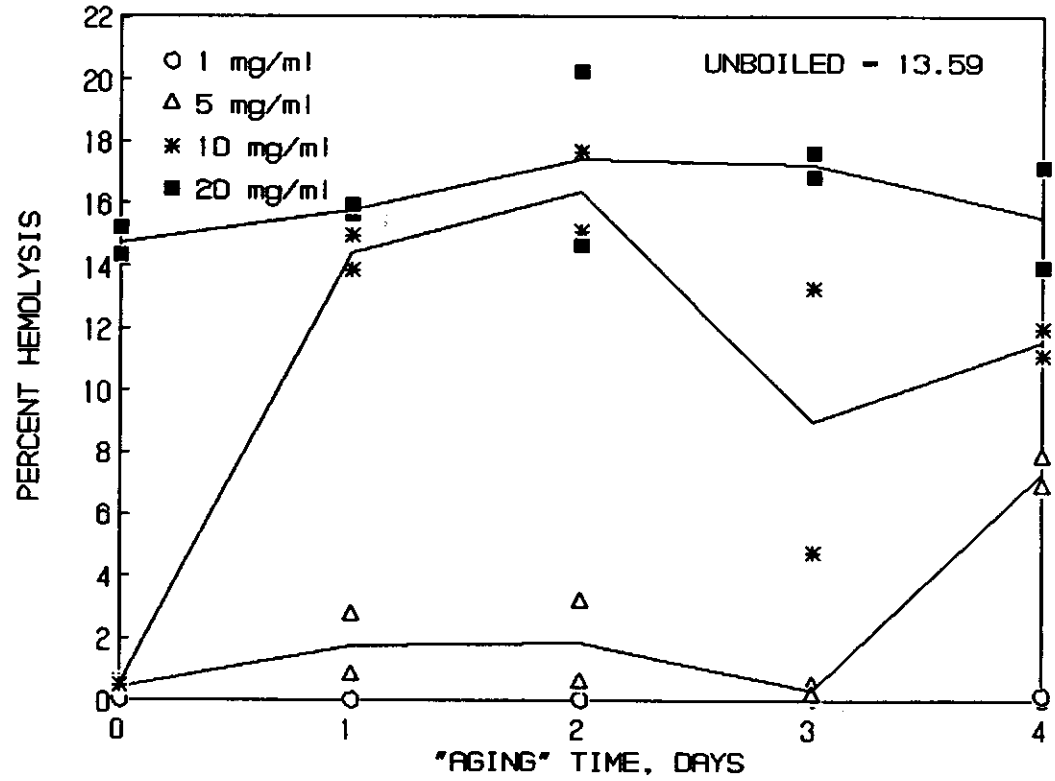

Figure 6. Hemolysis vs. time in supernatant after boiling.

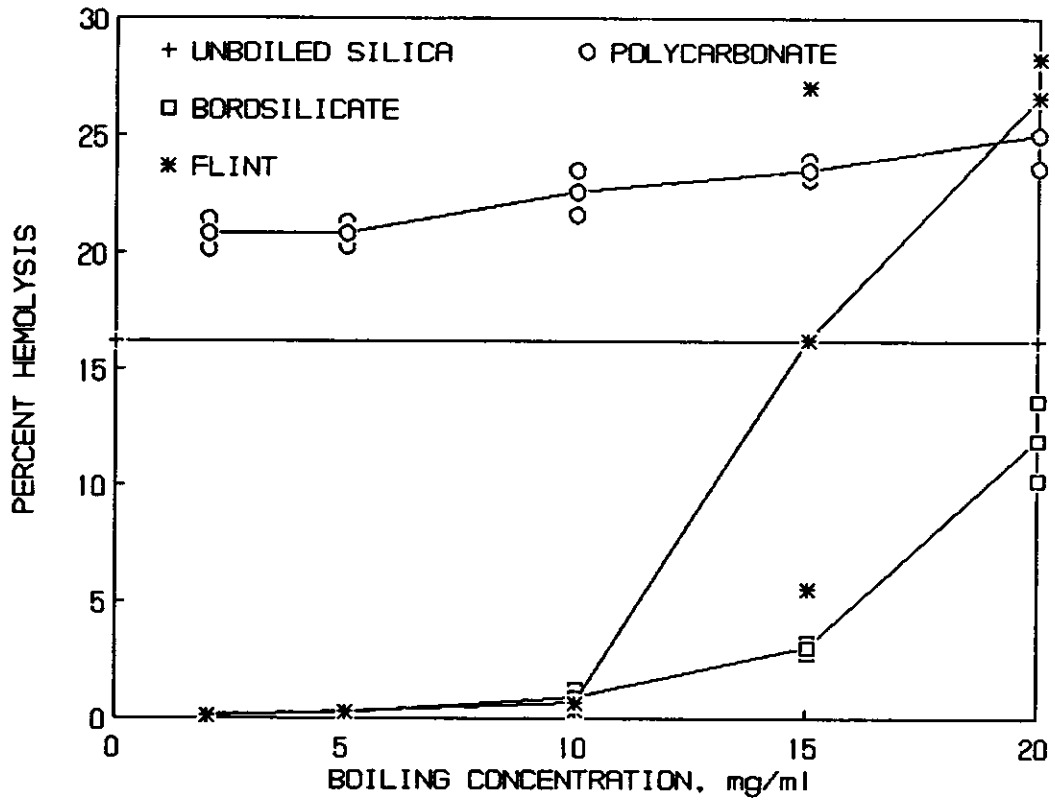

Figure 7. Hemolysis: quartz boiled in various tubes.

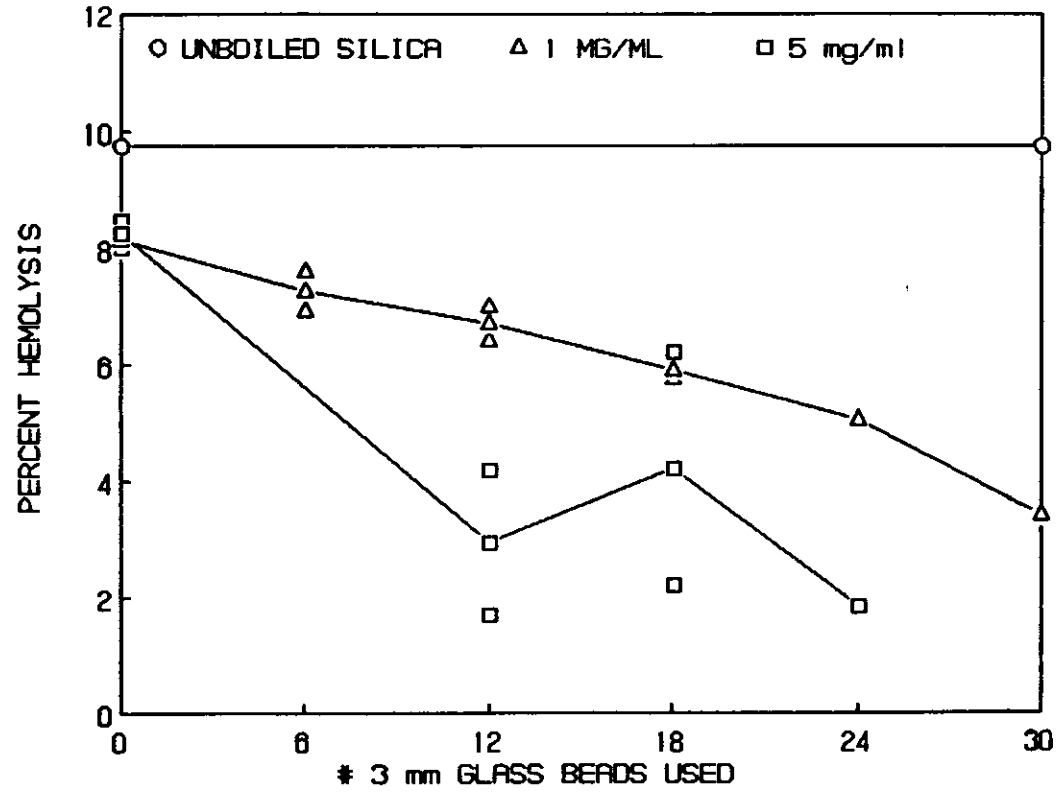

Figure 8. Quartz boiled 30° in polycarb W/glass beads.

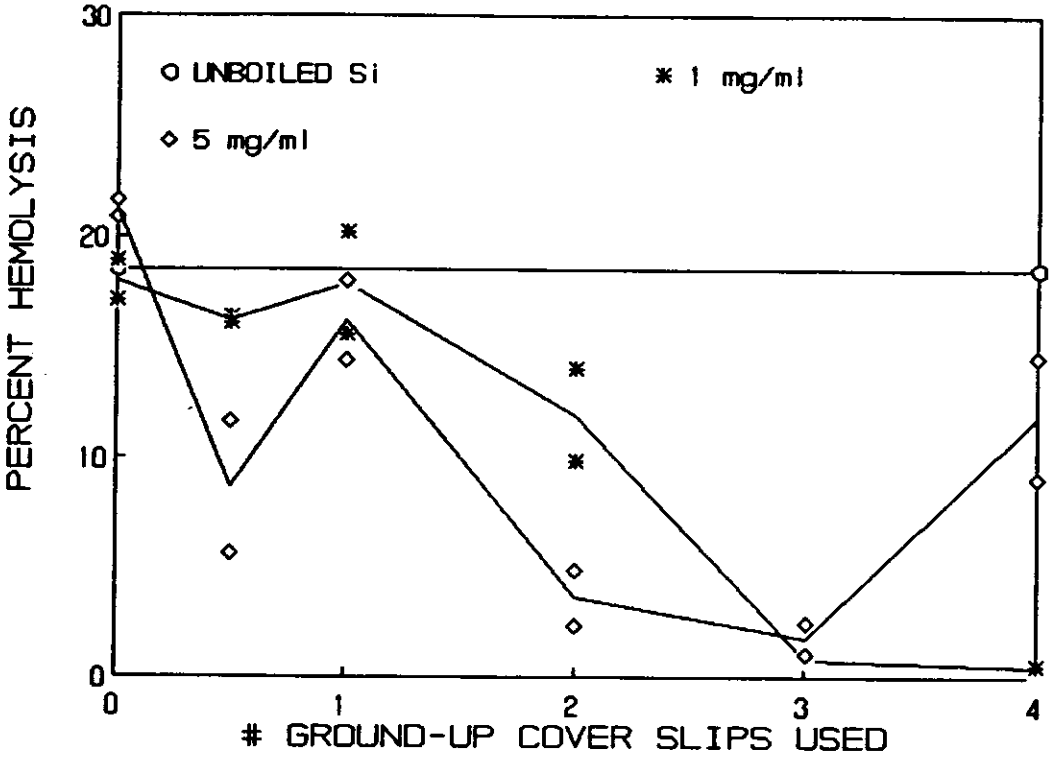

Figure 9. Quartz boiled 30° in polycarb W/glass pieces.

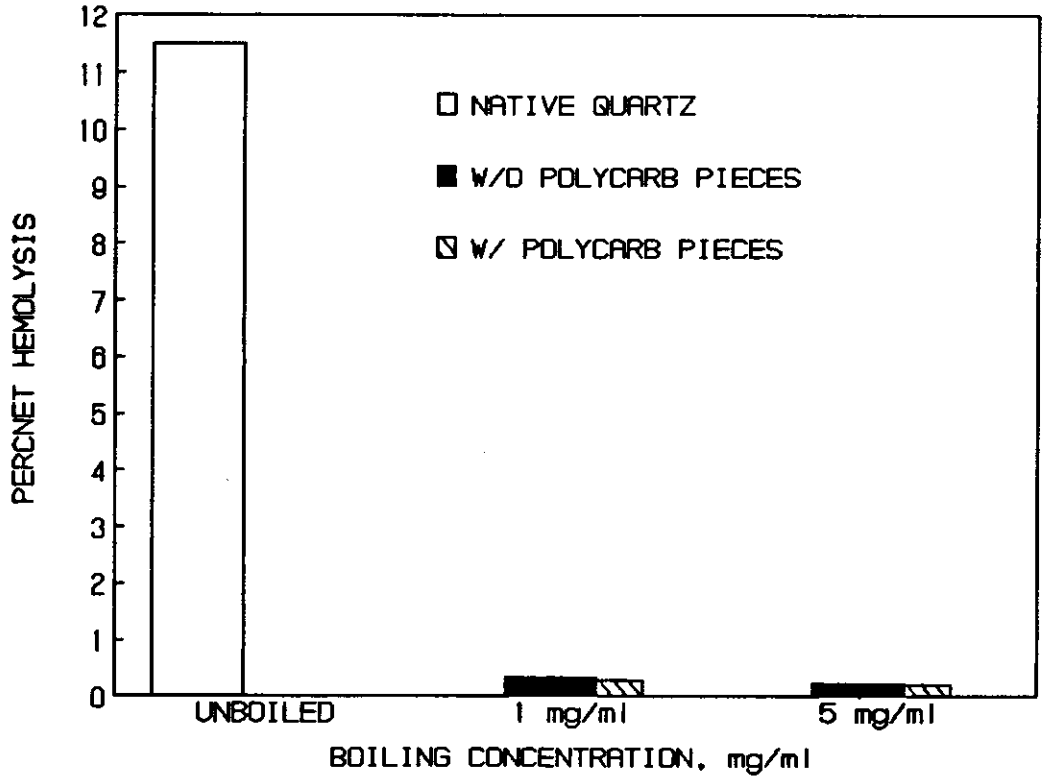

Figure 10. Hemolysis of quartz boiled in flint glass with and without polycarbonate pieces.

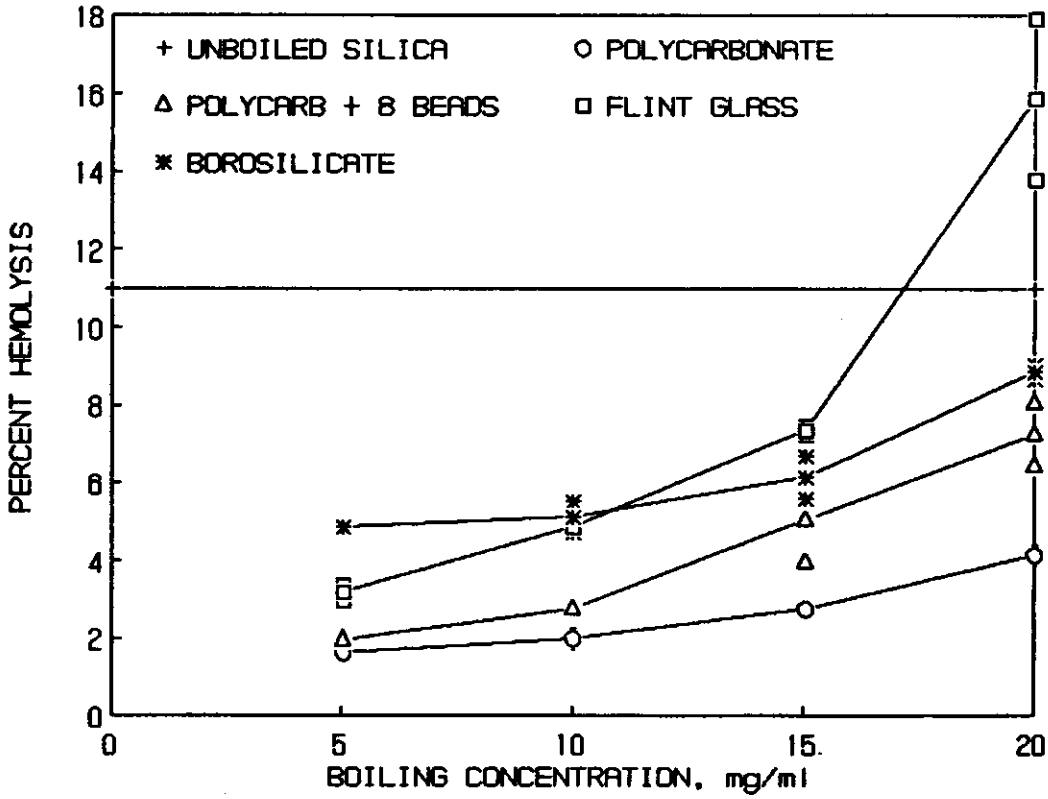

Figure 11. Hemolysis: quartz boiled in various tubes in contaminated water.

agreed with previous findings.In a limited investigation of this, quartz samples were boiled with sodium and calcium chloride solutions of several different concentrations; the effects were weak, slightly lessening the detoxification. (Figure 12)

Initial zeta potential measurements have been made on samples of unboiled quartz and on quartz boiled at 5 mg/ml in flint glass and in polycarbonate tubes. The zeta potentials for unboiled quartz and for quartz boiled in polycarbonate are essentially identical, while the samples boiled in flint glass show a less negative zeta potential. (Figure 13)

The boiling treatment was also applied to kaolin and alumina dusts. The kaolin dust, previously described,[1] was unaffected. A commercially obtained respirable sized alumina expressed hemolytic potential in its untreated state, and was detoxified upon boiling. (Figure 14) The untreated and treated alumina samples were subsequently analyzed by photoelectron spectroscopy, courtesy of the U.S. Department of Energy, Morgantown Energy Technology Center. The intention was to determine if the elemental composition of the alumina surface showed substantial levels of silicon in addition to aluminum after treatment. Results of the test showed, however, that the surface of the untreated alumina itself had a silicon-to-aluminum elemental ratio of about 4-to-1. This was reduced to about 1-to-1 after boiling. Studies using other dusts including asbestiform materials are ongoing.

CONCLUSIONS

At this point, several conclusions may be stated, and a partial 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 glass tubes, flint or borosilicate having been tested thus far; plastic tubes do not show the effect.
- The effect is only moderately time-dependent, tests having been limited to boiling times of 10 minutes or more thus far; at most concentrations the effect seems nearly complete at 10–15 minutes.
- The effect seems to persist on mild drying (overnight vacuum drying).
- Fully detoxified samples appear to show little or no cytotoxicity after soaking at room temperature in the supernatant from boiling for periods up to 4 days; partially detoxified samples show an increase with time.

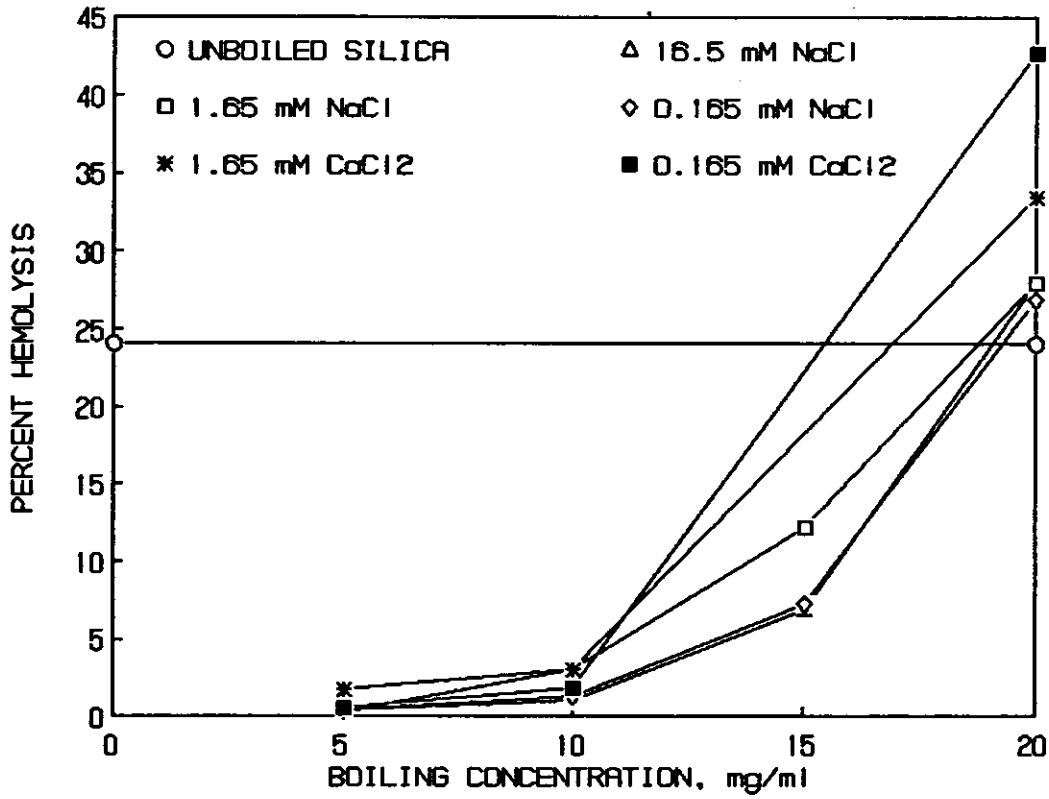

Figure 12. Hemolysis: silica boiled in NaCl and $CaCl_2$.

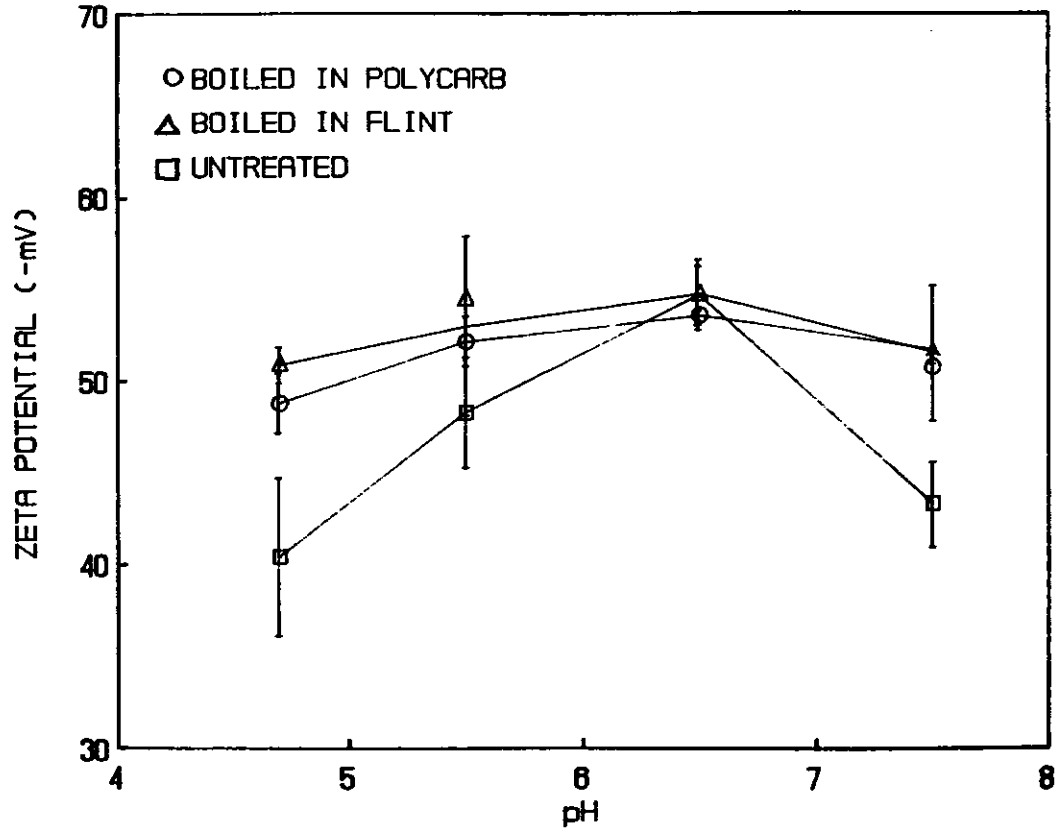

Figure 13. Zeta potential of quartz boiled in flint or polycarbonate.

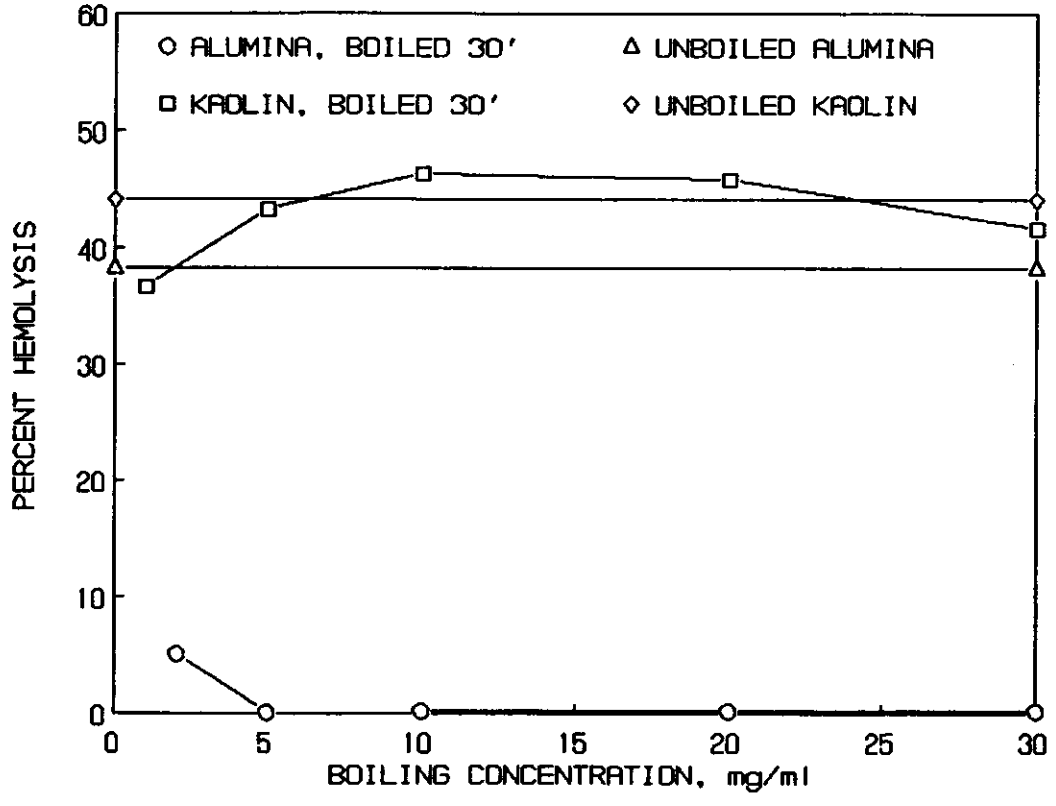

Figure 14. Hemolysis of boiled kaolin and alumina dusts.

- The detoxification shows some proportionality to available surface area of glass present during boiling.
- There is some indication that pre-boiling water with silica partly diminishes the detoxification effect for subsequently boiled quartz.

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

Boiling water releases a soluble or partially soluble factor, possibly silicic acid or sodium and/or calcium silicates or hydroxides, which, in monomeric or polymeric form, react or are physically adsorbed on the quartz surface, which fully or partially detoxify the mineral surfaces, as shown in *in vitro* cellular toxicity assays.

There is a significant amount of discussion in the literature concerning the dissolution of silica in water. Holt and King found that all sizes of quartz particles behave as if a soluble fraction of silica is leached from their surface, and that surface leached at pH 9 will rapidly adsorb the dissolved silica species.[5] Baumann measured the uptake of silicic acid by quartz from aqueous solution prepared by mixing silica gel in water.[6] In general, various silicates, including vitreous glass and quartz, are reported to have slight solubilities in aqueous media. The values found for quartz are on the order of one magnitude lower than the values obtained for glass under the same test conditions.[7] Iler states that the ability of quartz surface to hold water of hydration even after outgassing at 100C, in contrast to the behavior of amorphous silica, suggests a powerful hydrogen bonding capacity of the quartz surface silanol groups. He suggests this may be related to the peculiar power of quartz to adsorb multilayers of silicic acid as noted by Baumann.[8] This seems to favor a hypothesis that some soluble form of silica dissolves from both quartz particles and the glass container; that the "silicic acid" or a polymerized derivative re-adsorbs to the quartz; and this masks or otherwise passivates the quartz surface. Tests using pre-saturated medium raise the possibility that the quartz surface must undergo a desorption step or some conditioning before or in conjunction with adsorption of passivating species.

Suggested strategies for clarifying this would include radiolabel experiments to distinguish the source of surface silica groups after boiling treatment, and to determine if native quartz surface groups are exchanged with the medium in the passivation process; further investigation of the effect of treatment on the zeta potential of quartz; and the attempted use of surface spectroscopy methods, such as diffuse reflectance Fourier transform infrared spectrophotometry to identify surface structural changes following treatment. If acid-base reactions are involved, the pH dependence of detoxification should be looked at in detail.

The prime question raised here is under what moderate treatment conditions will quartz be surface modified so that it becomes biologically inert for cytotoxicity in cellular assays or for fibrogenicity *in vivo*. That is, what physical and chemical conditions are necessary and sufficient to passivate the quartz surface? This study has identified some parameters involved: the process proceeds in aqueous solution; glass surface must be present; there is a concentration dependence; details of the boiling procedure can significantly affect the results.

Another question is whether the passivation effect persists. The effect should be monitored in long term experiments involving physical and/or chemical methods, as well as *in vitro* assays, and possibly *in vivo* bioassays to determine the long term persistance in air and in physiological fluids.

The last question is whether this phenomenon is a feasible basis for prevention strategies. One major unknown here is the long term persistance of the effect under *in vivo* conditions.

In any event, the possibility exists for de-toxification of quartz by relatively mild treatment conditions. Seemingly innocuous preparation procedures used in biological assays of quartz could produce respirable dust surface property changes which are not readily detected by chemical or physical analysis, but which can confound interpretation of bioassay results. The possibility for such should be recognized in research protocols.

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