AD NUMBER

AD293464

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational use; Dec 1962. Other requests shall be referred to Army Biological Labs, Frederick MD.

AUTHORITY

SMUFD D/A ltr 4 Feb 1972

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

METHODS OF PREPARATION OF SILICA-CONTAINING DUSTS FOR USE IN BIOLOGICAL RESEARCH

TRANSLATION NO.
698

DECEMBER 1962

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

CCBL: RD2-3742
JPRS: R-2876-D

13 November 1962

METHODS OF PREPARATION OF SILICA-CONTAINING DUSTS FOR USE IN BIOLOGICAL RESEARCH

by E. Occella and G. Maddalon

ASTIA AVAILABILITY NOTICE

U. S. JOINT PUBLICATIONS RESEARCH SERVICE
BUILDING T-30
OHIO DRIVE & INDEPENDENCE AVENUE, S. W.
WASHINGTON 25, D. C.

METHODS OF PREPARATION OF SILICA-CONTAINING DUSTS FOR USE IN BIOLOGICAL RESEARCH*

by

E.Occella, Mineralogical Engineer and G.Maddalon, Industrial Consultant

Med. Lavoro, Vol 52,
No 5 (1961); pp 382-390.

1. Introduction

The major part of the fundamental research on the etiology of silicoses begun at the Occupational Clinic of the University of Milan (Clinica del Lavoro dell'Universita di Milano) in 1951 has been completed with the investigation of fine silica-containing or inert dusts prepared at the Laboratory of Mineralogy and Petrography (Laboratoric di mineralogia e petrografia) of this same Clinic; important contributions toward the classification and analysis of these dusts have been furnished by the chemical- and x-ray analytical laboratories of this same university institute **.

In the course of the past few years there were prepared and distributed to the researchers 25 sampl s of classified and analyzed fine quartz dust (weighing between 0.5 and 100 g); three samples of chalcedony, one sample of opal, six samples of diatomaceous earth (amorphous organogenic silica), two samples of silica glass, 22 samples of tridymite, three samples of cristobalite and finally, 20 samples of inert powder used for control (containing carbon and the oxides, silicates and

---

* Work carried out with the financial aid of the High Authority of the European Community of Coal and Steel (Alta Autorita della Comunita Europea del Carbone e dell'Acciaio) and the Study and Research Center on Occupational Diseases (Centro Studi e Ricerche sulle Malattie Professionali) of the INAIL. G. Maddalon is responsible for the writing of Section 2 and the aquisition of the illustrations, E. Occella wrote the other sections and coordinated the entire study.

** In this connection we must mention and express our thanks to Chemists Dr N. Zurlo, A. M. Griffini and L. Metrico and Physicist Dr. S. De Petris, who also helped us in this study.

carbonates of calcium, magnesium and iron).

Altogether there were prepared: 480 g silica-containing dusts with particle sizes predominantly under 3 microns, and 6,500 g silica-containing dusts with particles under 100 microns; 50 g inert dust with particles under 3 microns and 3,500 g inert dust with particles under 100 microns.

These ultrafine dusts have been extensively used in investigations carried out by numerous biological research groups; about 200 g silica-containing and inert powders (mostly under 3 µ) have been sent to the United States at the request of 17 different research institutes located in that country.

In view of the fact that the methods of preparation of these materials have not been described in the reports of the investigations just completed, we feel obliged to describe the sources of prime materials and the methods of preparation and analysis of the powders employed in this very important piece of basic research.

2. Prime Materials

For the preparation of dusts of silicic nature the following prime materials were used: quartz, chalcedony, amorphous hydrated silica (opal, diatomaceous earth), anhydrous silica glass, tridymite, cristobalite; the three latter forms of natural silica were obtained by synthetic methods.

a) Quartz (silica crystallized at the ordinary temperature in the holoaxial rhombohedral system).

The quartz labeled "analytic grade" by large pharmaceutical companies has been found to contain between two and four percent impurities consisting of various minerals (feldspar, mica, oxides); the quartzites and quartz-containing sands of Italian origin contained, on the other hand, accessory minerals in proportions even higher than 10 percent. In view of the fact that a product of the highest purity was needed due to reasons of differential fragility (Occella and Henking), we decided on the use of the hydrothermal crystalline quartz of the veins of Nomaglio (Quincinetto - Ivrea) and of Viu (Lanzo - Turin). In these mineral deposits the quartz content attains 99.8 percent, with only traces of muscovite mica, metal sulfides (galena, chalcopyrite, zinc blend) and of ferric oxides (hematite, ochre) being present.

b) Chalcedony (mineral silica of fibrous-radiating structure, crystallized in an as yet unclarified system, possibly monoclinic).

From fragments remaining after crushing by means of an agate mortar and pestle it is possible to isolate almost pure samples of chalcedony, hardly containing more than two to four percent hydrated amorphous silica. An optical control was necessary to exclude the pseudochalcedony-containing materials (microcrystalline quartz cemented by hydrated amorphous silica); control by means of x-ray diffractography does not make such a discrimination possible since the diffraction spectrum of chalcedony is nearly identical with that of quartz (Fig.1).

The chalcedony may contain small amounts of microcrystalline carbonates; these can easily be eliminated directly from the ground products by means of a weak attack with dilute hydrochloric acid, followed by filtration and drying of the insoluble residue.

c) Opal (hydrated amorpous mineral silica); Diatomaceous Earth; Silica Gel.

For amorphous silica we utilized opal mined at Casellette (Turin); an accurate manual choice has enabled us to isolate samples of 97-98 percent hydrous silica content and to exclude fragments contaminated by magnesite. Any accessory materials (ferric oxides, carbonates) were eliminated by means of dilute hydrochloric acid.

The diatomaceous earth ("rotten-stone"), made up of organogenic residues of silica sponges, algae and diatoms and containing hydrous silica, could not be employed as conveniently in the experiments as the fine opal powder, partly because of the normal presence of extraneous substances (quartz, silicates), partly because of its resistance to grinding caused by its softness, and partly because of the difficulty of estimating the specific surface area of the very minute and complex dendritic structures (Fig.2).

Silica gel (amorphous hydrous silica, obtained by chemical precipitation) presents, analogously to diatomaceous earth, noteworthy difficulties in regard to the determination of the specific surface area.

d) Anhydrous Silica Glass (synthetic amorphous form of free silica deprived of its water of crystallization; rarely found in nature in the form of lechatelierite).

From fragments of industrial "quartz glass" (obtained by fusion of quartz) used as refractory there have been obtained samples of the highest purity, containing about 98 percent of free silica. It was necessary, with this type of material, to carry out the following controls:

1. Chemical analysis of the residue remaining after the action of hydrofluoric acid, in order to determine the total silica content; and

2. X-Ray spectrographic analysis in order to exclude the presence of crystalline silica which could result from a recrystallization following the thermal stresses to which the material is subjected during

production and eventual use cycle.

e) Tridymite (a form of free crystalline silica, rarely found in nature, pseudohexagonal orthorhombic at ordinary temperatures).

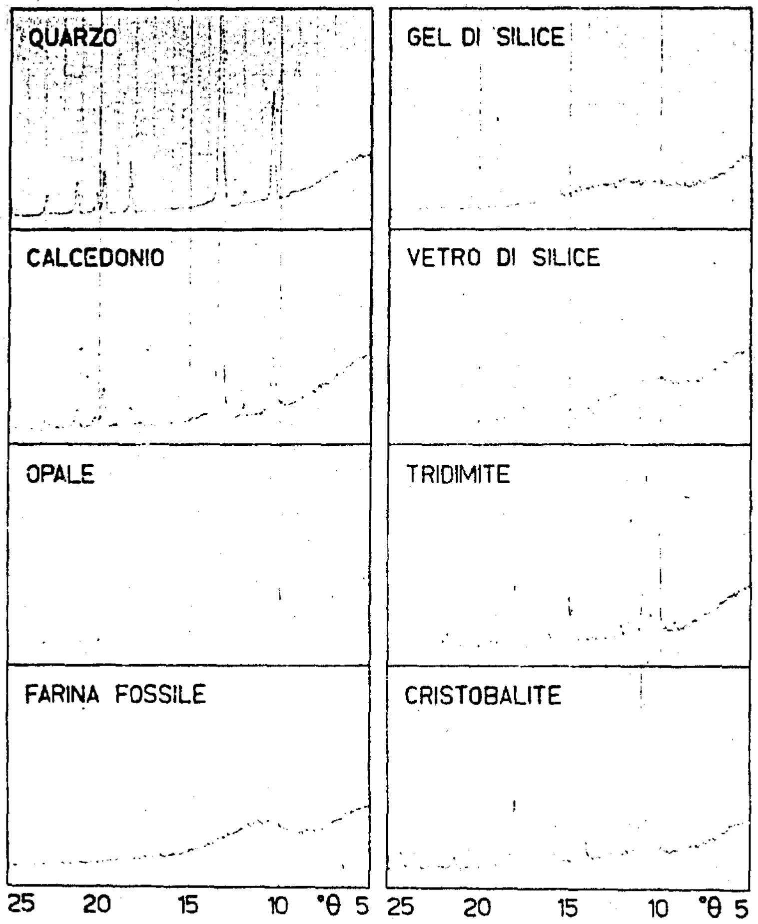

Fig.1. Diffractograms of the Principal Forms of Free Silica (Apparatus: Siemens Kristalloflex III; Voltage Applied: 35 KV; Anodic Current: 13 mA; Copper Anticathode; Measurement of Diffraction Intensity by Means of Geiger Counter and Recorded on Paper Ribbon; Velocity of Excursion of Geiger 1 degree/minute; Bottom Scale of Registration 1000 Impulses/Sec).

1) Quartz 2) Chalcedony 3) Opal 4) Diatomaceous Earth 5) Silica Gel 6) Silica Glass 7) Tridymite 8) Cristobalite.

In view of the limited practical possibility of utilizing natural tridymite crystals exceptionally present in druses and geodes within effusive acid rocks (liparites, trachites), we employed tridymite obtained synthetically. The literature (Peretti) cites the possibility of obtaining tridymite by heating quartz to approximately 1000°C in presence of suitable fluxes. Zwetsch and Bucking have prepared tridymite by prolonged heating of silica glass at 1000°C in presence of sodium tungstate.

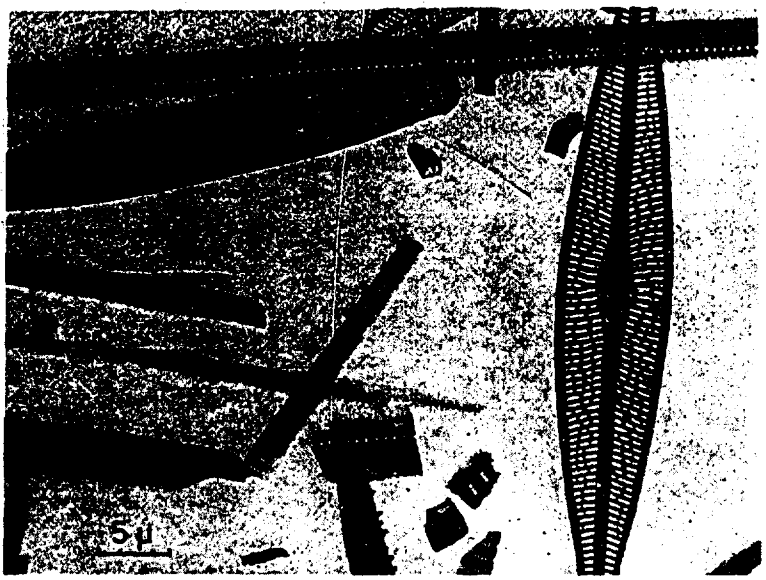

Fig.2. Diatomaceous Earth (Photograph by Frigerio)

After a series of exploratory experiments we worked out the following preparation*:

A sample of quartz powder composed of particles predominantly under 100 µ is mixed with 10 percent by weight of anhydrous sodium sulfate (flux) and placed in a cavity excavated within a silica-alumina refractory brick, insulated from the surrounding atmosphere by means of a refractory cover. The sample is introduced in a glassmaker's oven and maintained at a temperature of 1450°C for a period of about seven weeks. At the end of this period it is withdrawn and allowed to cool to room temperature by simple exposure to the atmosphere; the nucleus of the fused and porous briquette obtained in this manner (of the purest white color) is composed of pure tridymite practically free from impurities, as is demonstrated by subsequent debyegraphic (Fig.1) and petrographic tests.

f) Cristobalite (a form of free crystalline silica rarely encountered in nature, crystallized in the tetragonal system, pseudocubic at ordinary temperatures).

Renouncing, also in this case, the use of mineral crystals which are isolated with difficulty from certain rocks of volcanic origin, we found it preferable to employ cristobalite obtained by synthetic methods. Peretti mentions the possibility of obtaining cristobalite by calcification of amorphous hydrous silica (diatomaceous earth in par-

---

*The principle of this method has been used in the production of tridymite by G. Nagelschmidt, Safety in Mines Research Establishment, Sheffield (England); it is incorporated in the scheme presented below.

ticular) at about 1000°C for six to eight hours, in presence of small amounts of fluxes (iron or aluminum oxides, sodium sulfate). The final product is handicapped by the uncertainty regarding the chemical composition of the diatomaceous earth and the relative difficulty of grinding and of estimating the specific surface area.

Cristobalite is present in a high proportion in an internal portion of refractory silica bricks used in the Martin-Siemens furnace, after a normal castic operation (Occella and Maddalon); this portion, of a greyish-blue color and located immediately below the crust glassified on contact with the fusing bath, is composed of 85 to 90 percent cristobalite, plus oxides and silicates of iron. The material ground to coarse particles may be subsequently purified by means of separation in heavy liquids; the enriched sample may attain cristobalite contents above 95 percent.

For the enrichment by means of dense liquids the following method has been found convenient: The powder, composed mainly of particles under 100 µ is immersed in a mixture of xylene and bromoform (having a density of about 2.5); then bromoform is added drop by drop while at the same time the density is verified by means of a Westphal balance. The fraction of the powder floating between densities 2.58 and 2.64 represents a high concentration of cristbalite, since the impurities have densities lying outside the interval between these two values.

3. Grinding, Classification and Purification of the Various Materials

The various coarse samples -previously triturated in cast-iron mortars or laboratory crushers- were ground in a Linatex* cylindrical drum mill with rubberized walls (length and diameter: 300 mm) and with a grinding charge consisting of coarse fragments of the same sample and metallic spheres lined with rubber. The time of operation for the various samples varied between four and eight hours at a velocity of 50 rpm. The resulting products were much enriched in fine particles and were used directly in the appropriate dust chambers for the inhalation experiments with laboratory animals. If it is desired that the samples be classified according to grain size, the ground material may be sieved, using DIN 300 or 400 mesh sieves with openings of 55 and 38 µ, respectively. The classification of sizes within the limits of inhalability of the particles (three to five microns) was carried out by means of Zurlo's pneumatic classifier (with linear or cylindrical fissures) capable of separating classes of two to five microns and under two microns. It must be pointed out that if the original product is

---

* Provided for this study by the Institute of Mining Arts (Istituto de Arte Mineraria) of the Turin Polytechnic Institute (Politecnico di Torino), to whose director Prof. Lelio Stragiotti we wish to express our sincere thanks for having authorized the grinding department associated with the laboratory to prepare the minerals used in the present investigations.

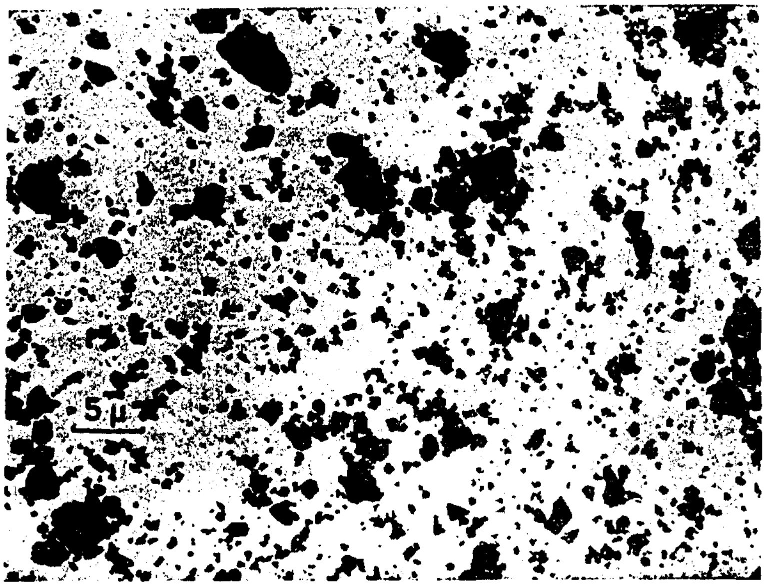

Fig.3. Tridymite Powder Obtained by Grinding in Colloid Mill.
(Photograph by De Petris)

not absoluely free of associated impurities, the mechanically more fragile components will separate, on grinding, in the form of predominantly minute particles which then accumulate in the finer classes of the separated products, introducing thereby an appreciable factor of contamination. The production of ultrafine powders is at the rate of 0.1 to 0.2 gram per hour of operation of the pneumatic classifier.

If it is desired to grind samples of the order of a few tens of grams, one may use a laboratory ball mill in which the centrifugal action increases the force of mass. We adopted a Hormuth colloid mill with cups of agate, corundum and special steel, of a volume of 250 cm^3, rotating at 400 rpm: this mill is particularly suitable for the grinding of siliceous materials when cups and spheres made of agate are used, since the wear and tear on the latter is negligible and so is the subsequent contamination of the products.

The grinding operation lasted for about 50 hours, obtaining from original crudes 0.5 to 1 mm in diameter grain classes with 80 to 90 percent (in number) of the particles under 3 µ, depending on the characteristics of the crude: the highest degree of fineness was obtained in the case of cristobalite dust and the lowest in the case of chalcedony. The samples were then separated by means of Zurlo's pneumoseparator.

It must be pointed out that the protracted operation of the mill over several hours makes it possible to obtain final products of ultrafine granulometry which may immediately be used for the various biological experiments. The tridymite dust (ground for 200-250 hours in the colloid mill) was examined under the electron microscope (Fig.3) and found to consist of particles practically always under 5 µ, with 95 percent in number under 2 µ. In this way one has the advantage of avoiding the necessity of all subsequent classifications, having obtained a final product of the same composition as that of the crude, while preventing the eventual segregation of the various constituents into various granulometric classes according to their differential friability. The production of powders of the highest degree of fineness is at the rate of 0.3 g per hour of operation of the colloid mill.

When it is found necessary to eliminate the amorphous layer present under the surface of the crystalline silica particles obtained by grinding -especially when dealing with very fine powders, under 10 µ- one may resort to a mild attack with hydrofluoric acid according to the following procedure:

5 g powder is dispersed in 200 cm^3 distilled water; 50 cm^3 48 percent hydrofluoric acid is added and the mixture left for 90 seconds. Thereupon the action of the acid is arrested by the addition of 1250 cm^3 3.5 percent boric acid. The mixture is filtered, the product on the filter washed with excess distilled water and calcined for 8-10 hours at 650°C. The final weight of the residue amounts under these conditions to about 75 percent of the original sample, if the latter has the above-mentioned granulometry. The product subjected to microanalysis by the colorimetric method (distillation and coloration by Eriochrome Cyanine R) shows the presence of fluoride to the extent of approximately 0.1 percent.

4. Granulometric and Analytical Examination

All the samples of fine powders were regularly subjected to granulometric examination under the optical microscope and in certain cases under the electron microscope.

The following types of microscopes were used: Leitz polarization microscope MOP, Leitz polarization microscope Panphot, Zeiss-Winkel bright field microscope. The following observational conditions were used: Objectives: dry, with a numerical aperture of 0.65 and a magnification of 40 diameters; immersed, with a numerical aperture of 1.30 and a magnification of 100 diameters; Eyepieces: periplanatic with a magnification of 10 and 20 diameters. In this way effective magnifications of 400, 800, 1000 and 2000 were obtained.

The preparations of silica-containing samples to be examined optically were obtained by dispersing 1-2 mg powder in monobromonaphthalene on the glass slide; the perception of the particles under these conditions is very clear, since monobromonaphthalene has an index of refraction considerably above that of the various forms of free crystalline silica. In order to avoid the volatilization of the dispersing agent it is advisable to block off the edges of the slide by a mist of auto-polymerizable resin.

The counting and estimation of the dimensions of the particles were accomplished in all preparations by examining certain zones delimited by given divisions of the linear ocular micrometer; the graphic representation of the results (by means of a granulometric curve) enables one to establish the number of particles comprised in the various fundamental classes, that is, 0.5 to 1 µ; 1 to 2 µ; 2 to 3 µ; 3 to 5 µ; above 5 µ. From the numerical determination the weight distribution of the particles may be established in good approximation in each of the classes, on the basis of the average dimensions.

Whenever one uses separatory equipment according to dimensions (sieves, pneumatic classifier, etc), the determination of weight distribution -obtained by means of direct weighing of the individual classes- is much more precise.

For the numerical counting of the particles good results are also obtained with a Reickert optical microscope with a large projecting screen provided with a linear graduated scale which may be moved in the field.

Statistical accuracy requires that for a correct representation of the analytical results at least a few hundred grains be counted. In the case of optical microscopes, even at the highest magnification it is illusory to attempt to define particle dimensions under 0.5 µ and to estimate the presence of particles under 0.2 µ; hence it is obvious that it is impossible to determine the granulometric distribution with classes of 0.1 in 0.1 or of 0.2 in 0.2 µ.

Whenever the knowledge of a granulometric distribution must be carried to fractions of a micron, an electron-microscopic examination becomes indispensable. In our particular case we used a Siemens Elmiscop I: the samples containing the powder of free silica were subjected to ultrasonic treatment in a mixture of alcohol and distilled water, then deposited in a collodion support on the grid and examined and photographed after the complete evaporation of the dispersing liquid. The granulometric analysis was then carried out by measuring and counting 200 to 400 particles on the microphotographs at magnifications between 2000 and 8000 diameters.

In the case of samples obtained by sieving and pneumatic separation it was necessary to carry out an analytic control in order to find out whether there had been a preferential accumulation, in the fine classes, of some constituents of low mechanical resistance. In first approximation one may resort to a polarization microscope, then -according to the fineness of the dust- follow this up with an analytic determination of the crystalline silica via semiquantitative or quantitative x-ray spectrography with internal standard (Frigerio and Zurlo).

In such cases one may also perform chemical tests according to the techniques already described (Occella, Zurlo and Frigerio), being careful to keep the intensity of the attack below that normally used in order to avoid -in view of the fineness of the dusts- making soluble also the particles of free silica. A good knowledge of the composition of the original materials facilitates in such cases the investigation of the presence of accessory components in the different classes of ultrafine products.

5. Concluding Observations

An exact definition of the origin, composition and granulometric distribution of the individual dusts used in biological research becomes indispensable if one wishes to establish valid elements of comparison between the results of the various studies and experiments.

Inasmuch as the international medical and technical literature has seldom adhered up to now to any uniform criteria regarding the presentation of analytical and granulometric data related to dusts, the authors wish to conclude this illustrative note by stressing the importance of a rigorous precision in the citation of sources, of the compositions and characteristics of all dust samples used, as basic materials, in clinical experiments and research.

Bibliography

[Key to non-English titles: 1) Rapid Routine Analytical Process for the Determination of Quartz by Debyegraphy and Chemical Petrography. - 2) The Silicotigenic Function of Acid and Silica-Alumina Refractory Materials in Ironworking. - 3) Methods of Analysis of Free Crystalline Silica.- 4) A New Pneumo-Classifier for Fine Dusts. IV. Colloquium on Dusts. National Institute of Security, Paris, 1957].

[Contribution is from the Occupational Clinic "L. Devoto" (Clinica del Lavoro "L. Devoto") of the University of Milan (Universita di Milano); Director: Prof. Enrico C. Vigliani].