Chapter 13

A Micro-hemolysis Assay for Monitoring Mineral Dusts

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Abstract

The hemolytic activity of milligram-sized mineral dust samples from coal mines collected in personal dust samplers were measured by a modified Harington assay method which increases the sensitivity of the technique. Measurements were conducted on a large number of samples from different mines characterized for gravimetric quartz content and the relative bulk concentration of common contaminants (Al,Ca,Fe). The results of these measurements were compared to the hemolytic activity of pure quartz and of mineral samples obtained from roof-bolter dust boxes of two coal mines. No correlation could be established between hemolytic activity and the compositional variables tested. The results suggest that the distribution of minerals among individual particles and the surface characteristics of particles, rather than the bulk concentration, determine the hemolytic activity of respirable mine dusts.

Introduction

The crystalline silica (quartz) content of respirable dusts from mines and other occupational environments is measured frequently as an index for potential respiratory health hazard. This is attributable to the well-recognized effect that quartz particles have in causing silicosis, pneumoconiosis, and other respiratory ailments. Ideally, a direct measure of the disease-inducing potential of a dust should be assayed. The in vivo cytotoxicity of dusts may be evaluated by long term animal inhalation exposure experiments. These, however, are costly, time-consuming, and require large amounts of test material. Epidemiological studies, on the other hand, are unreliable because of the difficulties in obtaining representative populations and the inherently large statistical deviations in the results.

In vitro cytoxicity testing using pulmonary macrophage, and erythrocyte hemolysis assays, are frequently used in a research setting to provide a measure of the direct prompt cell-damaging potential of dusts. The hemolytic potential only measures the membranolytic activity of a dust sample and, therefore, is not completely predictive of dust cytotoxicity. In fact, hemolysis measurements fail to distinguish the pathogenicity of several minerals of widely different pulmonary disease-inducing potential (Vallyathan, et al., 1988). For example, layered aluminosilicate kaolin clay, generally recognized as a non-toxic material, has been shown to possess an in vitro cytotoxic potential comparable to quartz on a specific surface area basis (Wallace, et al., 1985). Thus neither hemolysis nor other cytotoxicity measures can be used as an absolute index of pulmonary disease-inducing potential for any material. Nevertheless, within a set of comparable materials, e.g., respirable particulate silicates and aluminosilicates, or asbestos minerals of a given type, hemolytic activity will generally correlate with their level of cytotoxicity (Heppleston, 1973, and Langer, 1980). It was thus deemed of interest to compare on this basis the respirable mineral dusts collected in mine respirators which are used to evaluate the levels of exposure of miners.

The conventional method of Harington et al (Harington, et al., 1971) for the measurement of hemolytic activity of dusts requires samples of several milligrams for a single test, an amount in excess of that routinely gathered by personal or area sampling collectors for determination of respirable dust exposure levels and dust composition.

In order to provide the capability for membranolytic toxicity analysis of conventional samples of respirable dusts taken for gravimetric analyses, we have modified Harington's hemolysis assay method to increase the sensitivity for quartz to the microgram range. This test permits the analysis of the hemolytic potential of dusts routinely collected for occupational exposure monitoring.

Materials and Methods

Low temperature ashed coal mine dust samples from personal dust samplers were provided by MSHA laboratories in Pittsburgh, PA, together with quartz content data obtained by IR measurements. These samples, identified by MSHA code numbers, proceed from various coal mines across the United States. The low temperature ashing process removes the organic components of the dust while retaining the mineral fraction with minimum alteration by thermal treatment. These samples are routinely examined for their quartz content, which when combined with the weight loss by ashing gives the quartz content of the initial dust sample. In the present experiments the hemolytic activities of the ashed materials were compared both to samples of pure quartz of respirable particle size, and to dust samples from mine roof-bolter dust boxes which are almost entirely mineral in character and representative of the typical hard rock structure of mines.

For comparisons with pure quartz Min-U-Sil (quartz) dust obtained from Pennsylvania Sand and Glass Corporation, Pittsburgh, PA was used. The particle size of the Min-U-Sil used was in the respirable range, and consisted of 98% less than 5 micrometers diameter particles. The specific BET surface area was measured as 5.0 cm^2/g with a median area-equivalent diameter of 0.23 micrometers.

The roof-bolter dust box samples were obtained from two different mines (Mathies and Rushton) representing two different geographic and geological areas (western and eastern Appalachia, respectively) in which different mineralizations may be expected. The dust samples were characterized by specific surface area measurements. The particles tested were smaller than 38 um with BET specific surface areas of 7.4 cm^2/g and 6.9 cm^2/g and estimated median area-equivalent diameters of 0.34 um and 0.36 um, for the Mathies and Rushton mine samples respectively.

Hemolytic activities of the samples were compared to their quartz content, as measured by IR spectroscopy by MSHA, and to the impurity content, as subsequently measured by scanning electron microscopy - energy dispersive X-ray elemental analysis (SEM-EDAX).

Table 1 shows the quartz content and relative atomic ratios of major cationic impurities in representative samples of the mine respirator dusts and of the roof-bolter dust box minerals.

Microgram-size quartz samples from low temperature ashed coal dust collected in mine respirators were scraped off the collector's filter into 12x75 mm polypropylene test tubes. Five hundred microliters of Dulbecco's phosphate buffered saline (PBS) were added to each sample, and the mixtures sonicated for 5 minutes. Subsequently, five hundred microliters of a 0.1% suspension of sheep erythrocytes in PBS were added, and the dust-erythrocyte mixtures were then incubated for 15 minutes at 25C. The hemolysate was analyzed with either a Hewlett-Packard 8451A diode array spectrophotometer, or a BIO-TEK EL307C microplate reader at 540 nm.

The percentage of hemoglobin released was calculated as the ratio of absorbance for supernatant using the samples and that obtained by complete lysis with 0.5% Triton-X 100. Standard Min-U-Sil samples were prepared on Nucleopore filters with the use of an eight stage Sierra-Anderson personal impactor. Duplicate silica (quartz) samples were used as calibration standards.

TABLE 1

Quartz (SiO_2) content and relative amount of main cationic impurities in typical mine respirator samples.

District *	SiO2 Content **		Al:Si	Fe:Si	Ca:Si
	wt. ug	wt.%			
624	14	2.8	0.44	0.13	0.13
012	1	0.1	0.49	0.23	0.61
313	53	5.3	0.54	0.10	0.36
524	38	4.7	0.58	0.12	0.02

* MSHA Code Number
** Total weight of SiO_2 from X-ray analysis: wt.% is reported in terms of total sample weight before ashing.

TABLE 2

EDAX analysis of principal cations in respirator samples and roof bolter dust box samples from two mines

CATION	RESPIRATOR SAMPLE MEAN* %	MATHIES RBDB SAMPLE %	RUSHTON RBDB SAMPLE %
Si	46.5	50.9	52.4
Al	20.9	22.0	20.0
Fe	10.4	17.6	16.7
Ca	12.2	0.6	0.8
K	7.2	6.4	7.8
Ti	1.0	1.5	0.9
Mg	0.6	1.1	1.4

* Combined material from 160 individual collectors.

For a comparison of respirator samples with Min-U-Sil (quartz) and mineral dusts from roof-bolter boxes, material from 160 personal dust samplers was combined into a single, mixed sample of sufficient size to allow a determination of hemolytic activity as a function of concentration. Table 2 compares the cationic composition of this aggregate with those of the roof-bolter dust box samples of the two mines.

Results

The hemolytic activity of dust samples from five different mining districts as measured by the micro-hemolysis technique, and the quartz content as measured by IR spectroscopy are compared in Table 3. Comparison of the hemolytic activity per mass of quartz in the sample, expressed as % hemolysis/ ug quartz, shows a three to five-fold variation within each group. That is, hemolytic activity does not correlate with the amount of quartz contained in the dust, even for samples originating in the same district.

TABLE 3

Hemolytic activity of low-temperature ashed residue of mine respirator samples from various mine districts

District *	SiO2 cont. (ug)	Hemolysis %	%Hemolysis per 10 ug SiO2
221	39	17.9	4.6
	43	51.7	12.0
	108	32.3	3.0
321	39	51.3	13.2
	39	34.5	8.9
	108	47.4	4.4
	111	76.8	6.9
624	14	4.1	2.9
	56	48.3	8.6
	93	80.6	8.7
	110	56.1	5.1
012	4	28.8	72.0
	18	23.6	13.1
	30	36.5	13.1
	159	97.8	12.2
723	17	21.7	12.8
	29	60.7	20.9
	48	17.8	7.7

MSHA Code Number

The data of Table 2 are plotted in Figure 1a, together with data obtained on samples from other districts. The wide variability of the hemolysis data is apparent. A linear fit to the 42 points shown results in a correlation coefficient of only 0.67. A second order polynomial fit results in a correlation coefficient of 0.71. In the same figure, the hemolytic activity resulting from increasing amounts of Min-U-Sil quartz is shown. For comparison, first and second order polynomial fits to the Min-U-Sil data result in correlation coefficients of 0.958 and 0.985. Figure 1b shows the hemolytic activity of respirator samples from mines within a single district for which the degree of correlation is not significantly different from that of the combined data of Figure 1a.

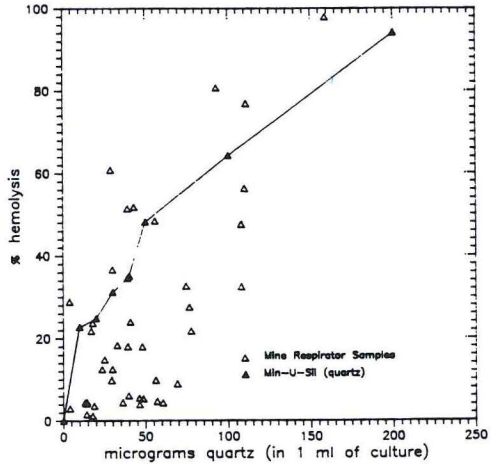

Figure 1a: Hemolytic activity of the mineral fraction of dusts from mine respirators compared to that of pure quartz (Min-U-Sil) dust.

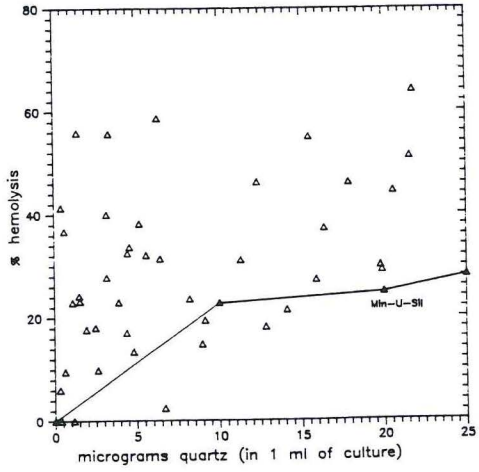

Figure 1b: Hemolytic activity of mine respirator dust samples from a single mining district (MSHA No.432) compared to the activity of pure quartz (Min-U-Sil).

The hemolytic activity in function of the three major impurity elements (Al, Fe, Ca) for the samples of Figure 1a are shown in Figure 2. Again, no definitive correlation between the two parameters is observed. Finally, Figure 3 compares the hemolytic activity of the combined respirator sample with the roof-bolter dust samples and pure quartz (Min-U-Sil). These results show that the difference between the hemolytic activities

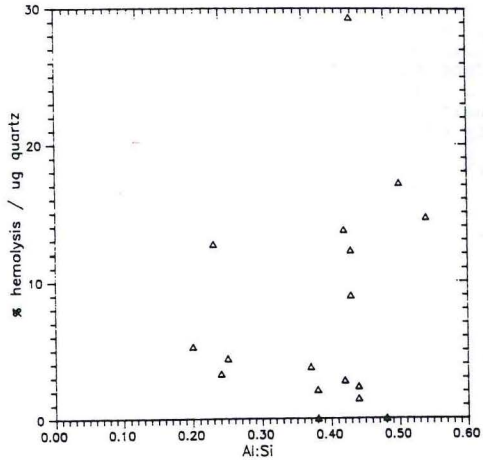

Figure 2a: Hemolytic activity of the mineral fraction of dusts from mine respirators for samples of different Al:Si ratios.

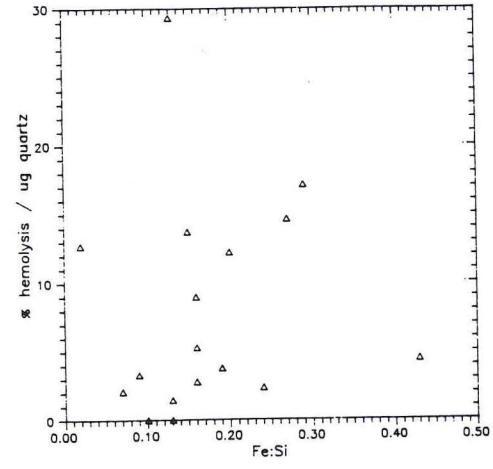

Figure 2b: Hemolytic activity of the mineral fraction of dusts from mine respirators for samples of different Fe:Si ratios.

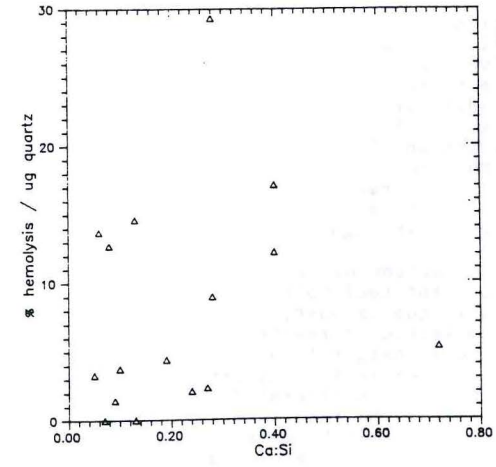

Figure 2c: Hemolytic activity of the mineral fraction of dusts from mine respirators for samples of different Ca:Si ratios.

detected for the two roof-bolter dust box samples is nearly as large as the scatter between the hemolytic activities of individual respirator samples shown in Figure 1.

Discussion

The data shown in Table 2 and Figure 1 shows poor correlation of measured hemolytic activity with quartz mass content. It is known that the toxicity of quartz particles is determined by a number of factors such as purity, degree of crystallinity, particle size, and surface condition. The hemolytic activity of quartz particles with clean, hydroxylated surfaces is greatest (Langer and Nolan, 1985, and Stalder and Stober, 1965), but other characteristics such as particle size and chemical modifications of the surface can also affect measured dust toxicity (Harley and Margolis, 1961, and Nolan, et al., 1985). Recent studies have shown very pronounced effects of heat treatment (Pandurangi, et al., 1990), freshness of surface (Vallyathan, et al., 1988), and impurity distribution (Wallace, et al., 1990) on the biological activity of various silica particles. Conventional infrared spectroscopic or X-ray diffraction assays of the quartz content of a respirable dust sample do not address the possible effects of particle size distribution and/or surface composition that may affect the

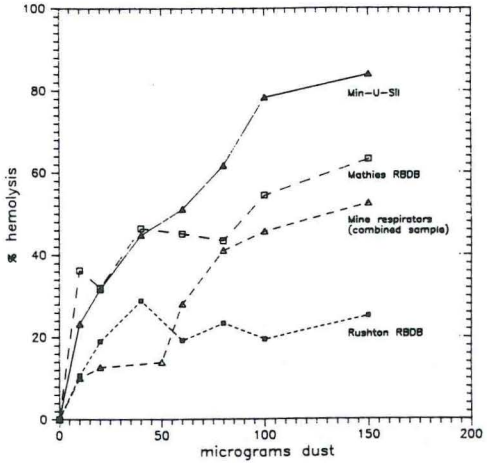

Figure 3: Comparison of the hemolytic activity of four dusts.

biological activity of a particular dust. Although overall chemical analyses such as shown in Table 1 may give a rough indication of the degree of surface contamination of quartz particles, they do not readily provide a quantitative assessment of potential biological activity, which may be linked to a fraction of the particles in the system. This is further confirmed by the lack of any correlation between the hemolytic activity of the samples tested and composition ratios, as is illustrated in Figure 2.

The comparison of hemolytic activities of the two different roof-bolter dust samples with respirable quartz dust, and a mixed sample of dusts collected in respirators shown in Figure 3 offers some insight into the variables that may affect the hemolytic activity and potential toxicity of these mineral dusts. Although a comparison of the results obtained with these materials can only be made semiquantitatively because of their different particle size distributions, it is interesting to note that the roof-bolter dust box samples from the two mines exhibit widely different hemolytic activities in spite of quite similar bulk compositions (see Table 1). The hemolytic activity, on a per unit surface area basis, exhibited by the Mathies mine dust is equal or higher than that of pure quartz, while that of the Rushton mine is significantly lower. The hemolytic activity of the mixed respirator dust sample is intermediate between the two roof-bolter dust samples. This result is in qualitative agreement with the broad scatter of values observed in Figure 1 which combines samples of many different mines and locations within mines. It also confirms the observation of Robock and Bauer in their study of mine dusts that measurements of quartz content are not always predictors of the prevalence of dust-induced disease (Robock and Bauer, 1988).

More critical for the hemolytic activity and potential cytotoxicity of respirable dusts appears to be the amount of biologically available quartz surface in any given sample. Wallace and coworkers (Wallace, et al., 1990) have shown how the particular morphology of multiphase dusts can greatly influence the surface to volume ratios of particular components. Assays of hemolytic activity, pulmonary macrophage tests, and similar in vitro methods may provide a way for assessing the relative amounts of biologically active areas present in dust samples.

The micro-sized hemolysis assay described herein appears to offer a simple solution to one of the problems confronting the use of bioassays for characterization of dust samples. Specifically, it permits hemolysis potential measurements, essentially an assessment of the maximum in vitro membranolytic potential of the dust, on small amounts routinely acquired for gravimetric analysis monitoring of respirable dust exposures.

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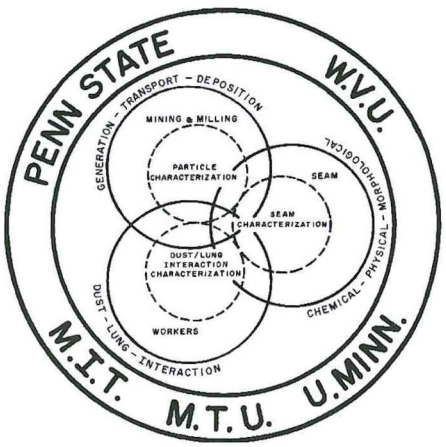

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