# DETECTION OF HYDROXYL RADICALS IN AQUEOUS SUSPENSIONS OF FRESH SILICA DUST AND ITS IMPLICATION TO LIPID PEROXIDATION IN SILICOSIS

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## INTRODUCTION

Despite considerable effort over the years, the mechanism by which the quartz particles exert their toxic action on cells and the processes by which these actions progress to fibrosis are still not fully understood.[1,2] It is generally thought, nevertheless, that the interaction of the quartz particles with the cell membranes is the starting point of the silicotic process.[3] We felt that the mechanism of the membrane damage by quartz might involve oxygenated free radicals because (a) a suspension of quartz particles in contact with alveolar macrophages has been reported[4,5] to initiate an enhancement of lipid peroxidation, defined broadly as the oxidative deterioration of polyunsaturated components of lipids, and (b) hydroxyl (•OH) radicals are known to be capable of peroxidation by abstracting hydrogen atoms from cell-membrame Lipids[6] and initiating lipid peroxidation in lysosomal membranes.[7] Moreover it is known that exposure of cell membranes, fatty acids and unsaturated food oils to ionizing radiation, which generates •OH radicals, causes rapid peroxidation.[6] Earlier studies of the aqueous chemistry of quartz suspensions have reported detection of $H_2O_2$,[8] implicating the formation of •OH radicals as transient species, but, we are not aware of any report of the detection of •OH radicals in quartz suspensions and this provided the motivation for the present undertaking. Since it is known that, because of their high reactivity (hence short life time) in aqueous media, the •OH radicals cannot be detected via electron spin resonance (ESR) directly,[9,10] we have used ESR combined with the spin-trap methodology[9] for studying the •OH formation.

## MATERIALS AND METHODOLOGY

Crystalline silica with particle sizes of 0.2 to 2.5 mm was obtained from the Generic Respirable Dust Technology Center, Pennsylvania State University, University Park, Pennsylvania. Particles in the range of smaller than 25 microns were produced by hand grinding in air, using an agate mortar and pestle because of the structural similarity of agate to that of quartz. Also a rather mixed particle size, rather than a specific range, was employed, with a view to roughly approximate the random particle-size distribution in the mining atmosphere. ESR spectra were obtained at X-band (~9.7 GHz) using a Bruker ER 200D ESR spectrometer. For accurate measurements of the g-values and hyperfine splittings, the magnetic field was calibrated with a self-tracking NMR gaussmeter (Bruker, model ER035M) and the microwave frequency was measured with a frequency counter (Hewlett-Packard, Model 5340A). 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) was purchased from Aldrich and used without further purification, since very weak or no ESR signals were obtained from the purchased sample when used by itself. If necessary the background signals were subtracted from those related to quartz by using an Aspect 2000 microcomputer.

## RESULTS

Some typical results of the ESR spin-trapping studies are shown in Figure 1. We found that a 0.1 M aqueous solution of the spin-trap DMPO alone, with unground particles or with $TiO_2$ powder did not give a detectable ESR spectrum. $TiO_2$ was used as a control because it is known not to be fibrogenic[11] and has a structure resembling quartz ($SiO_2$). However, when quartz was ground in a 0.1 M DMPO (aqueous) solution or when ground quartz particles were mixed with 0.1 M DMPO (aqueous) solution, an ESR spectrum ($g = 2.0059$), consisting of a 1:2:2:1 quartet pattern with a splitting of 14.9 G, was observed (Figure 1a). Based on earlier work,[9,12,13] this spectrum was considered to be due to the DMPO-OH adduct.

Two further tests were made to identify the spectrum. First, the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\bullet}OH + OH^-$),[14] known to produce •OH radicals, was used as a standard. The ESR spin-adduct spectrum obtained by mixing 0.085 M $H_2O_2$, 0.0165 M $FeSO_4$ and 0.1 M DMPO was the same as that of Figure 1a (obtained with ground quartz), thus attesting to the formation of the •OH radical in the quartz suspension.

As a second, confirmatory, test of the •OH radical. formation, spin-trap ESR experiments were performed in which ethanol was added as a secondary trap. It has been shown[10,15] that in the presence of ethanol, the intensity of the DMPO-OH signal decreases, because ethanol scavenges some of the •OH radicals to form the ethanolyl radicals[12] which react with DMPO to give the spin-adduct DMPO-

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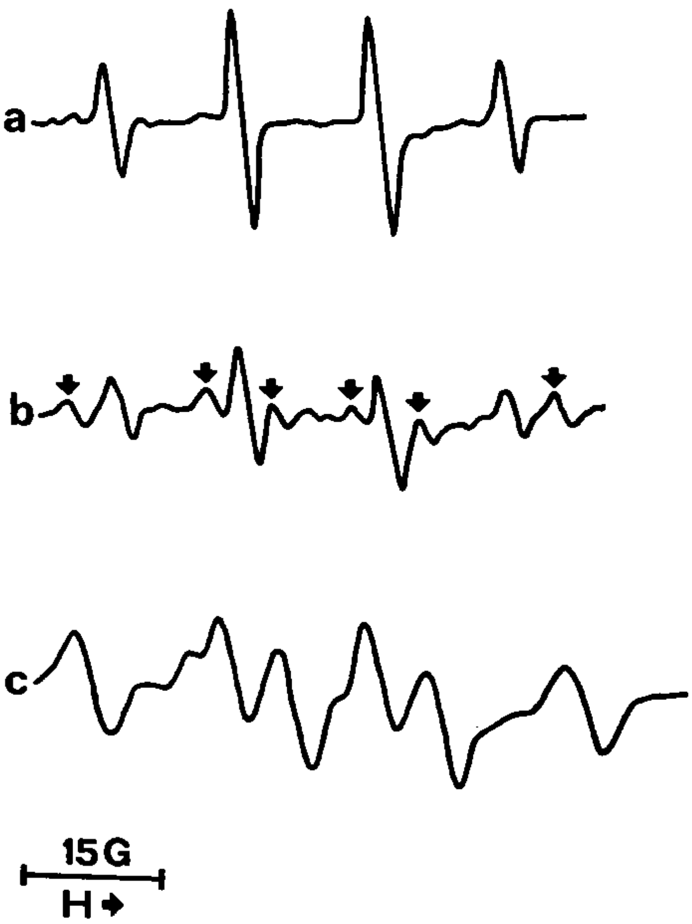

Figure 1. ESR spectra recorded 2 minutes after mixing 100 mM DMPO aqueous solution with (a) freshly ground quartz particles; (b) same as (a) but with 30% ethanol added; (c) same as (a) but with excess ethanol. Receiver gain, 5 × $10^5$; modulation amplitude, 2 G; scan time, 100 seconds; field, 3460 ± 75 G.

$CHOHCH_3$. The ESR spectrum of the spin-adduct DMPO-$CHOHCH_3$ was indeed observed as indicated by arrows in Figure 1b (for 30% ethanol) and more clearly in Figure 1c, obtained in the presence of excess ethanol, thus confirming the •OH radical formation in the quartz suspension.

The intensity of the •OH radical adduct signal increases with the amount of grinding (Table I), thus showing that the •OH radical generation is related to some surface property of the freshly made dust, most likely the silicon-oxygen radical sites known to form on grinding.[16-21] Additional spin-trap measurements as a function of the time of "aging" of the dust after grinding showed that freshly generated quartz dust produces more •OH radicals than that which had been stored in air after grinding (Table II). In order to characterize the kinetics of the dust's aging on its ability to generate •OH radicals, attempts were made to determine whether the reaction was of the first order (a straight line plot for log (con.) vs. time) or second order (straight line plot for $(\text{con.})^{-1}$ vs. time). The analysis indicated the kinetics to be neither first nor second order but of a more complex nature. Thus while it was not possible to define a unique half-life for the decrease in the •OH radical producing potential of the quartz dust on storage after grinding, we note that, on the average, freshly ground quartz dust loses its •OH-generating capacity to about 50% in approximately 1 day.

## DISCUSSION

It is clear that the breakage of quartz crystals implies the homolysis of Si-O-Si bonds and the generation of silicon-based radicals (≡Si • , ≡SiO • , ≡SiOO •)[8,16-21] We have indeed verified that Si • and SiO •-type of radicals are produced by grinding in air, and that the radicals decay as a function of time when the dust is stored in air after grinding,[17] with a half-life of about one and a half day. Earlier workers[16] have reported that the crushing of quartz under vacuum produces SiO •-type radicals whose concentration decreases drastically on exposure to atmosphere with a half-life of about 30 hours.

Table I

Dependence of the ESR Intensity of the DMPO-OH Adduct (i.e., •OH production) on Size (grinding times) of Quartz Particles

| Grinding times (minutes) | Relative ESR intensity |
|---|---|
| 0.0 | 0.0 |
| 0.5 | 0.3 ± 0.3 |
| 1.0 | 1.1 ± 0.6 |
| 2.0 | 2.3 ± 0.7 |
| 4.0 | 3.4 ± 0.8 |
| 10.0 | 5.1 ± 1.2 |

Table II

Dependence of the ESR Intensity of the DMPO-OH Adduct
(i.e., •OH production) on the "Aging" of Quartz Dust

| Time after grinding | Relative ESR intensity |
|---|---|
| 5 minutes | 5.2 ± 0.8 |
| 1 day | 3.2 ± 0.8 |
| 2 days | 1.9 ± 0.7 |
| 3 days | 1.7 ± 0.8 |
| 4 days | 1.3 ± 0.7 |

Following Kalbanev et al.,[8] we suggest that the •OH radical production might involve the following steps:[8]

$$\equiv SiO\bullet + H_2O \rightarrow \equiv SiOH + \bullet OH \qquad (a)$$
$$\equiv SiO\bullet + \bullet OH \rightarrow \equiv SiOOH \qquad (b)$$

Kalbanev et al. have also suggested[8] that the hydrolysis of SiOOH could produce $H_2O_2$, according to reaction (c):

$$\equiv SiOOH + H_2O \rightarrow \equiv SiO\bullet + H_2O_2 \qquad (c)$$

The yield of $H_2O_2$, depending on the pH and the temperature of hydrolysis, was reported to be as high as $10^{18}$ molecules/g quartz particles,[8] enough to be measured by the standard method of wet analytical chemistry, the $MnO_4$ − reduction:

$$\underset{(pink)}{2MnO_4-} + 5H_2O_2 + 4H^+ \rightarrow 5O_2 + \underset{(colorless)}{2Mn^{2+}} + 8H_2O \qquad (d)$$

We verified the reducing activity of our quartz particle suspension with respect to $KM_nO_4$, although the $H_2O_2$ yield was measured to be about an order of magnitude smaller for our samples than those of Kalbanev et al.[8] Thus experiments were carried out to examine whether the •OH radical formation was through the Fenton reaction,[14] the $Fe^{2+}$ possibly being a trace impurity. The experiments consisted of spin-trap measurements in which diethylenetriaminepenta-acetic acid (DETAPAC) (0.03 − 3.0 mM) was used as a strong metal-ion chelate. it is known that the iron-DETAPAC complex formation stops the •OH generation from $H_2O_2$.[9] On adding DETAPAC the •OH radical-related ESR signals showed no variation in either the g value or the observed splitting pattern but only a small (20 %) decrease in intensity even at the high DETAPAC (3 mM) levels. This result, together with the dependence of the •OH radical concentration on time and surface freshness, suggests that the Fenton-type mechanism is not a major contributor to the •OH radical generation in our quartz suspensions.

After this work was essentially complete,[17-20] two significant reports have appeared. In the first, Fubini et al.,[21] have report the formation of Si •, SiO •, and $SiO_2$ • radicals on quartz particles ground in air, without contact with water. They suggest a possible role of these radicals (or some other surface property) in the mechanism of quartz-induced fibrosis. Our ESR results on the silicon-based radicals,[18,19] agree with Fubini's.[21] We further show that the concentration of the Silicon-based radicals is time dependent[17] and that their reaction with aqueous media generates (perhaps) an even more potent species,[18-20] the •OH radicals. The second paper, by Gulumian and Van Wyk,[22] reported the detection of •OH formation in aqueous suspension of glass and quartz fibres *in the presence* of $H_2O_2$, and the scavenging of the generated •OH radicals by the prophylactic agent (polymer) polyvinylpyridine N-oxide (PVPNO). They suggest that the therapeutic efficacy of PVPNO in silicosis might be related to its scavenging effects on •OH radicals. Our work shows that the grinding process itself causes the quartz surface to be a source of •OH radicals in aqueous media and that this activity decreases with the aging of the dusts.[19,20] This higher toxicity of fresh dust must be taken into consideration in the future *in vitro* or *in vivo* laboratory (e.g., animal exposure) studies of quartz and related mineral dusts.

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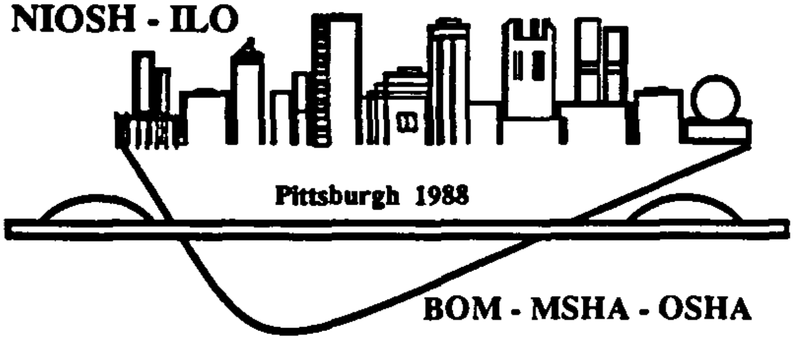

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