*Acknowledgement.* Support for this work by the NSF grant No. CHE-8207175 is gratefully acknowledged.

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## V22

## Physico-Chemical Aspects in Silicosis

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Silicosis is a pulmonary disease which afflicts persons who inhale over long periods of time freshly-ground silica-containing dust. Its mechanism of action at the molecular level is not yet understood.

The majority of crystalline $SiO_2$ forms are pathogenic whereas the amorphous silicas are largely inactive. The primary cause of silicosis has thus to be sought in the structural and surface characteristics of the $SiO_2$ particles.

Recent biological work envisages in the engulfment of the $SiO_2$ particles into a macrophage the first step ending up with the formation of the silicotic nodule in the lung [1–3]. Intermediate stages of the process are illustrated in Fig. 1. Any silica particle exhibits a membranolytic action on the phagolysosome, probably related to the surface hydroxyls configuration, with consequent release of lytic enzymes into the cytoplasm, death of the macrophage and release of the free $SiO_2$ particle in the lung tissue where it can be phagocytosed by another macrophage. This does not imply any fibrotic action *per se*. If the process takes place with *freshly ground, crystalline* $SiO_2$, namely quartz, tridymite and cristobalite, within the phagolysosome a fibrogenic factor is also formed, which, when released in the lung tissue, stimulates fibroblasts to an abnormal production of collagen, and hence the formation of the silicotic nodule. Membranolysis can be reduced or blocked by chemical modification of the surface, however the intimate cause of silicosis relies on the catalytic role of crystalline $SiO_2$ in the production of the 'factor'.

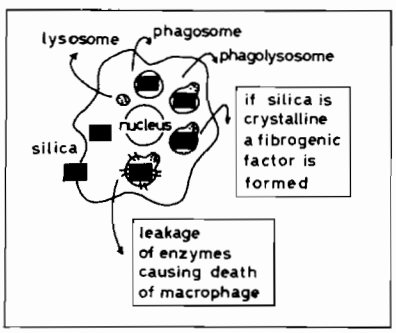


Fig. 1. Membranolytic effect of silica on macrophages.

Virtually any difference in surface properties between amorphous and crystalline silicas may account for their different biological activities.

We have investigated, so far, the formation of free radicals at the crushed surface (E.S.R.), the heat of interaction with water molecules (adsorption microcalorimetry) and the kinetics and energy of interaction with some aminoacids (immersion calorimetry) on amorphous and crystalline silicas of comparable size [4].

Free radicals are produced by mechanical grinding of quartz, and readily react with various atmospheric components yielding paramagnetic species such as $SiO_2^*$ and $SiCO_2^*$, possible intermediates in the formation of the fibrogenic factor.

Water vapour reacts with silicas in various ways depending on the dehydration degree of the surface. The heat of adsorption on micronized quartz (~5 $m^2$ $g^{-1}$), low and high surface area amorphous silicas (porasil ~ 16 $m^2$ $g^{-1}$), (aerosil ~ 380 $m^2$ $g^{-1}$) all outgassed *in vacuo* below 423 K (in order to prevent elimination of silanols), are reported in Fig. 2 as a function of water uptake. Micronized quartz exhibits at low coverages an interaction energy (~210 kJ $mol^{-1}$) which is much higher than the corresponding one on the two amorphous silicas (~125 kJ $mol^{-1}$).

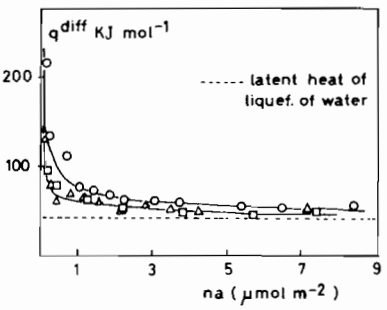


Fig. 2. Heat of adsorption *vs* water vapour uptake: ○ Quartz, □ Porasil, △ Aerosil.

Crystalline structure rather than particle size thus dictates the binding energy of surface sites.

Aminoacids are adsorbed at the quartz surface [5] but when the process occurs from aqueous solution their interaction energy is competitive with water itself. In the case of proline, however, a specific interaction with quartz was observed: in that case the heat released upon contact with quartz was 30 times higher than on amorphous silica and the interaction lasted many hours indicating an activated process, possibly the oxidation to hydroxyproline specifically catalyzed by the quartz surface.

All these findings may be regarded as a first step in the investigation of the particular reactivity of crystalline $SiO_2$ within the cells.

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## V23

## Divalent Metal Ions in the Pharmacodynamics of Morphine-like Opiates

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Paramagnetic Mn(II) ions were used to sample the interaction of morphine-like opiates with divalent metal ions, which are known, especially $Ca^{++}$, to be involved in the mechanisms of drug dependence and tolerance.

The motional correlation time of the opiate molecule in aqueous solution was determined by selective proton irradiation methods ($\tau_c = 7 \times 10^{-10}$ sec at 22 °C) and a preferred *cis* conformation of $H_5$ and $H_6$ protons was inferred.

The water proton relaxation rates at different metal to ligand ratios were used to evaluate the equilibrium constant ($K_{ass} = 10^{-2}\ M^{-1}$) by assuming a dipolar only interaction with $T_{1p}^{-1}$ modulated by rotational tumbling of the complex (see figure).

The temperature dependence of $T_{1p}^{-1}$ was measured (Table I) in the range 25–60 °C: it turned out that $H_1$, $H_2$, $H_5$, $H_7$ and $H_8$ proton relaxation rates were in the fast exchange region, while the $-NCH_3$ protons were undergoing slow exchange from the metal coordination sphere.

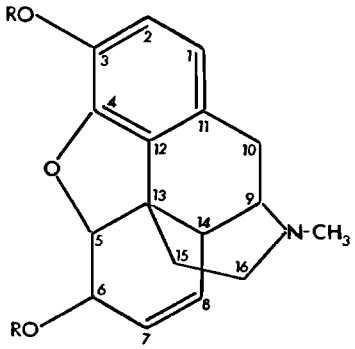


TABLE I. Temperature Dependence of $T_{1p}^{-1}$ of Morphine Protons. [Morphine] = 0.05 *M*, $[Mn^{++}]$ = 0.1 m*M*, pD = 7.

| $1000/T$ ($K^{-1}$) | $T_{1p}^{-1}$ ($sec^{-1}$) | |
|---|---|---|
| | $H_5$ | $-NCH_3$ |
| 3.00 | 0.10 | 0.24 |
| 3.10 | 0.22 | 0.13 |
| 3.20 | 0.37 | 0.05 |
| 3.25 | 0.42 | 0.04 |
| 3.30 | 0.50 | 0.07 |
| 3.35 | 0.56 | 0.12 |

As a consequence, metal–proton distances could be calculated for almost all the protons considered and it was shown that simultaneous binding to the two -OH groups was consistent with the similar measured magnitudes of the Mn–$H_1$, Mn–$H_2$, Mn–$H_7$ and Mn–$H_8$ vectors (r is in the range 3.01–3.16 Å). $H_5$ was found to be the nearest proton to the metal ion ($r$ = 2.84 Å).

The temperature dependence of $T_{1p}^{-1}$ of the $-NCH_3$ methyl protons was taken to evaluate the thermodynamic functions for the exchange process from the metal coordination sphere ($k_{off} = 2.25 \times 10^4$ $sec^{-1}$, $\Delta H^{\ddagger}$ = 14.1 kcal/mol, $\Delta S^{\ddagger}$ = 7.2 e.u.).

The structural and kinetic information was used to suggest that interaction with divalent metal ions is characterized, for morphine-like opiates, by an almost exclusively enthalpic barrier due to binding to charged OH groups. Such interaction can be relevant either in modulating the interaction of divalent metal ions with external groups in membranes or in affecting the receptor–opiate interaction modes.