Identification of Inclusions in Lung Tissue with a Raman Microprobe

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Inhaled particles smaller than 4 μm can cause damage to lung tissue, a disease called silicosis. We present an investigation on the use of a Raman microspectrometer for the identification of inclusions in lung tissue. We measured Raman spectra of such inclusions in lung tissue of a patient whose probable cause of death was silicosis. Most of the inclusions we could identify were calcite particles.

Index Headings: Raman; Microprobe; Silicosis; Lung tissue; Inclusions; Pathology.

INTRODUCTION

In recent years several techniques have been developed for the identification of small amounts of material. Some of these techniques make use of microbeams (ion, electron, or proton); others use secondary electrons or x-ray scattering. Usually with these microprobes only the nature of the atoms in the sample is determined. Recently a Raman microprobe was developed which has the advantage over other microtechniques that chemical identification is possible without causing damage to the sample or the necessity of staining the sample. Out of an increasing number of applications, we mention the identification of airborne particles by Etz and the analysis of silicone inclusions in lymph tissue by Abraham.

The results presented here were obtained from a study of lung tissue using a Raman microprobe. The lung tissue belonged to a patient who probably died from silicosis or pneumoconiosis. This is caused by small airborne particles embedded in the lung tissue. Some of these particles are removed by macrophages, but others may stay in the lung and cause damage to the tissue. Materials that may cause the disease are silica, silicates, asbestos, or carbonates and related minerals. Identification of the inclusions in the lung tissue is of importance because this might provide a possibility of determining the source of the pollution which caused the disease. In this way one might prevent other exposures.

EXPERIMENTAL

Thin (5–15 μm) paraffinized unstained sections of lung tissue were deposited on normal microscope slides. Before measurement the sections were deparaffinized in xylol, which evaporated afterwards. The sections were analyzed by using polarized light microscopy. This was
done to locate the inclusions in the sample. Once a particle was found, its Raman spectrum was recorded.

The measurements were performed using a Raman microspectrometer similar in principle to the one described by Delhaye. We used a coherent Ar laser (CR 3) operating at 514.5 nm as a light source. The laser light passed through the epi-illuminator of a Nikon Optiphot microscope. A 100× objective (NA = 0.90) was used to focus the beam on the sample (spot diameter 1.6 μm). The same objective was used to collect the scattered light and direct it onto an entrance slit of a Jobin-Yvon monochromator (HG 28). An EMI 9863QB photomultiplier was used for detection. The apparatus was tested with polystyrene spheres of 1- and 10-μm diameter (Dow Chemical). A normal polystyrene spectrum was recorded for both types of particles. With other measurements using polystyrene spheres it was possible to estimate that the effective depth in the particle from which the light was collected lies between 1 and 2 μm.

The recording of the Raman spectrum of each separate particle in the lung tissue was repeated several times to be sure that no sample deformation took place in the laser spot. No effects of heating or deformation could be detected at the power densities used (≤0.5 × 10⁹ W·m⁻²). The tissue itself was more sensitive to heating caused by the laser beam. Intensities exceeding 0.5 × 10⁹ W·m⁻² caused visible damage to the tissue. A Raman spectrum of the tissue was not recorded because of the high fluorescent background.

RESULTS AND DISCUSSION

Because with stained sections the Raman spectrum has a high background caused by fluorescence of the staining dyes, we used unstained specimens. However, with unstained sections the localization of inclusions by birefringence, using polarized light microscopy, can be very difficult.

In Fig. 1A the Raman spectrum of one of the types of inclusions in the lung tissue sample under investigation is shown. Raman lines are present at frequency shifts of 155, 281, 711, and 1085 cm⁻¹. This spectrum is in very good agreement with a reference spectrum of calcite.
Raman spectroscopy is shown to provide a rapid and definitive analysis of the nature of urea inclusion compounds. It is demonstrated that the photodecomposition of included alkanones does not destroy the urea lattice, but the inclusion compounds of n-decane are destroyed by ultrasonic agitation.

Index Headings: Raman spectra; Urea inclusion compounds.

Urea forms inclusion compounds with paraffin-like molecules (n-alkanes, fatty acids, etc.). This property has been used successfully to separate linear and branched compounds. While pure urea forms tetragonal crystals, in the inclusion compounds it crystallizes in an hexagonal lattice forming channels of ~0.5 nm diameter. These channels accommodate linear chain molecules permitting free chain rotation to occur.

Recently, urea inclusion compounds have been shown to provide a unique environment for photochemical reactions. The photodecomposition of included n-alkanones was found to occur with greatly differing consequences than is the case for n-alkanones in solution.

During the course of these photochemical studies and other investigations on the motions of included molecules, it became necessary to determine unequivocally if inclusion of the molecules of interest had indeed occurred and if the crystalline lattice had been destroyed during the photochemical reaction.

The crystal structures of free urea (tetragonal) and of the inclusion compounds (hexagonal) present hydrogen bonds of different lengths. As a result, the vibrations (external and internal) of the urea molecules are different.

CONCLUSIONS

It was shown that by using Raman microspectrometry the character of inhaled particles (diameter 1–4 µm) embedded in lung tissue can be revealed. Some problems arise if the sections are stained, because of the fluorescent background. In unstained sections the localization of inclusions may give rise to difficulties. Further investigations are in progress.

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