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Development of a non-destructive micro-analytical method for stable carbon isotope analysis of transmission electron microscope (TEM) samples

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ABSTRACT

The biogenicity of ancient morphological microfossil-like objects can be established by linking morphological (e.g. cell remnants and extracellular polymeric matrix) and chemical (e.g. isotopes, biomarkers and biominerals) evidence indicative of microorganisms or microbial activity. We have developed a non-destructive micro-analytical ion beam system capable of measuring with high spatial resolution the stable carbon isotope ratios of thin samples used for transmission electron microscopy. The technique is based on elastic scattering of alpha particles with an energy of 2.751 MeV. At this energy the ^{13}C cross section is enhanced relative to the pure Rutherford cross section for ^{13}C, whereas the ^{12}C cross section is reduced relative to its pure Rutherford cross section. Here we report the initial results of this experimental approach used to characterize ultramicrotomed sections of sulfur-embedded graphite and microbial cells.

1. Introduction

Analysis of the bulk stable carbon isotopic composition of preserved organic matter in purported cellular remains can reveal whether ancient cells used the citric acid cycle, the reversed tricarboxylic acid cycle, or the 3-hydroxypropionate pathway [1]. When such chemical information is combined with morphological features and paleoenvironmental setting, the biogenicity of ancient microbial-like object may be rigorously assessed [2].

Here we discuss the development of a new micro-analytical method to determine stable carbon isotopes of biological and mineralogical specimens prepared for characterization using transmission electron microscopy. The reader is referred to Kristiansson et al. [3] for information regarding the development and application of nuclear microprobe technology. To test the experimental set-up, the energy interval at which there is a resonant behavior was investigated, data were collected, and data handling routines developed. The method is based on Rutherford backscattering (RBS), which is non-destructive and not limited by matrix effects, and may therefore act as a complement to standard micro-analytical mass spectrometry methods.

The method described in this paper is based upon using a resonance effect to determine the $^{12}C/^{13}C$ ratio of small (i.e. a few micrometers wide) biological and mineralogical electron transparent samples. When alpha particles accelerated to 2.751 MeV encounter a carbon-bearing sample, ^{13}C displays a resonant behavior that significantly increases its effective cross section relative to its Rutherford cross section (Fig. 1), whereas the cross section of ^{12}C simultaneously decreases [4–6]. Thus, if the resonance effect is utilized, and at least 250.000 counts of ^{13}C nuclei–ion interactions (assuming a normal Poisson distribution) are obtained, it is possible to separate distinct ^{12}C and ^{13}C peaks and achieve a ±2 per mil accuracy in the ^{13}C analysis. This requires non-interference from other spectral characteristics and e.g. background contributions have to be minimized.

In addition to the incoming alpha-particle energy, the resonance yield is affected by the scattering angle. To optimize the statistics it is therefore necessary to measure the backscattered particles over the entire solid angle for which there is a resonant behavior. As previously shown [3], this angular interval reaches from 180° to at least 150°, with the highest $^{13}C/^{12}C$ yield present at 180° (relative to the incident beam). Because the $^{13}C/^{12}C$ yield and the energy of the recoiling particles are affected by the scattering angle, there is an energy overlap between recoils from ^{12}C at 180° and ^{13}C at 150°. Therefore, it is not possible to separate the energy spectra of ^{12}C and ^{13}C using a single annular detector. A

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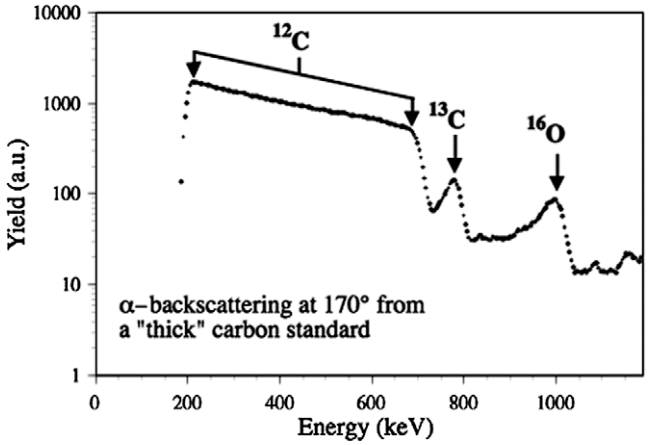

Fig. 1. A RBS spectrum obtained by irradiating a graphite rod showing with 2.751 MeV a-particles. The two marked peaks come from surface oxygen and from the ^{13}C resonance while the continuous distribution comes from bulk scattering on ^{12}C. The good separation between the ^{12}C edge and the ^{13}C peak should be noted.

system of four annular detectors, where each detector covers a dedicated angular interval, was developed to eliminate this problem [3].

The Rutherford backscattering (RBS) method is depth-sensitive because of alpha particle–electron interactions. As a result, there is an increase in the range of energy lost by the alpha particles with depth, hence an increase in the width of the energy spectral peak with increasing thickness of the sample. For most applications this phenomenon is an advantage since it enables monitoring of the element composition in different layers of the sample. In general, however, as the peaks widen with increasing sample thickness, elements with higher cross section will impose a spectral background. In the case of carbon isotope analyses, this background effect prevents an accurate determination of the relative $^{12}C/^{13}C$ ratio unless the sample is pure carbon. To avoid problems associated with sample thickness, it is necessary to analyze samples on the order of 100–200 nm thick. This sample thickness is the same as that obtained during sample preparation for transmission electron microscope (TEM) analysis.

The use of electron transparent samples does not decrease the ^{13}C yield since the resonance energy interval is narrow (see below). When samples thicker than a few hundred nanometers are used, the alpha particles experience enough energy loss to slip out of the resonance interval somewhere below the surface. In other words, the resonant behavior only occurs at the very surface of a sample during analysis. When the sample thickness is less than the depth interval for which there is a resonant behavior, the count rate of ^{13}C will be reduced, and an increased analytical time will be required. To evaluate whether TEM sections were sufficiently thick for the purpose of this study, we determined the energy interval for which the resonant behavior occurred.

2. Experimental procedure

2.1. Sample preparation

Brittle geological specimens are typically thinned to electron transparency by ion-milling petrographic thin (30 μm) sections, and biological specimens are commonly prepared by embedding pelleted cells or coherent microbial mats in resin and ultramicrotoming them to electron transparency. Given the emphasis in this study on stable carbon isotope analysis of TEM specimens, non-traditional protocols for preparing electron transparent sections for nuclear microprobe analyses were used.

A technique first reported by Bradley et al. [7] and described in detail by Hugo et al. [8] was used to embed samples in liquid sulfur and ultramicrotome them to produce sections ~100 nm thick. In this procedure, a sublimated sulfur crystal was melted at 120 °C on an acid-washed glass slide to form a ~500 μm droplet of liquid sulfur. Meanwhile, a 150 μm particle of the sample to be analyzed was placed on a tapered glass rod with a 1 mm^2 flat work surface that was also held at 120 °C. The molten sulfur droplet was then inverted and lowered onto the work surface to immerse the sample particle in liquid sulfur. The working temperature was lowered to 90–95 °C, and a rod-shaped sublimated sulfur crystal was gently touched to the molten droplet to nucleate solidification of a sulfur block suitable for ultramicrotoming. One of the sulfur blocks produced in this study is shown in Fig. 2.

Once the tapered rod was fixed into an ultramicrotome chuck, sections were cut and collected on 150-mesh Be grids coated with a lacey SiO support film [9]. The Be grids were then placed under rough vacuum (10^{-3} mbar) to sublimate the sulfur. This sample preparation procedure yields a TEM sample that consists of an

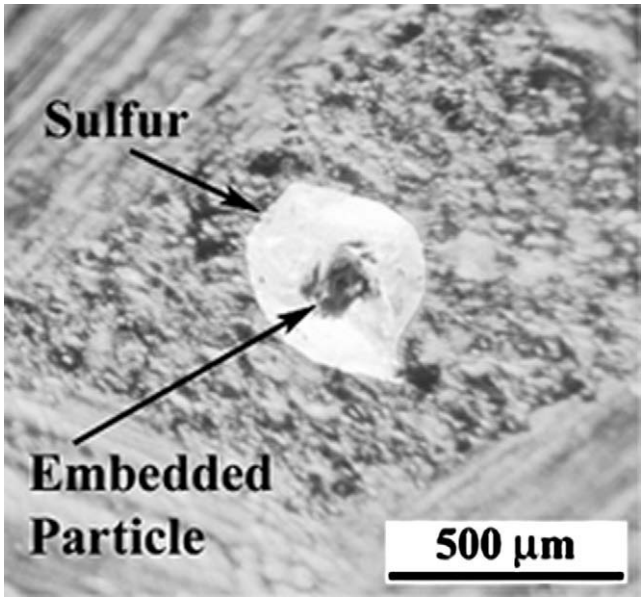

Fig. 2. A ~150 μm particle of a sample embedded in a sulfur block for ultramicrotoming.

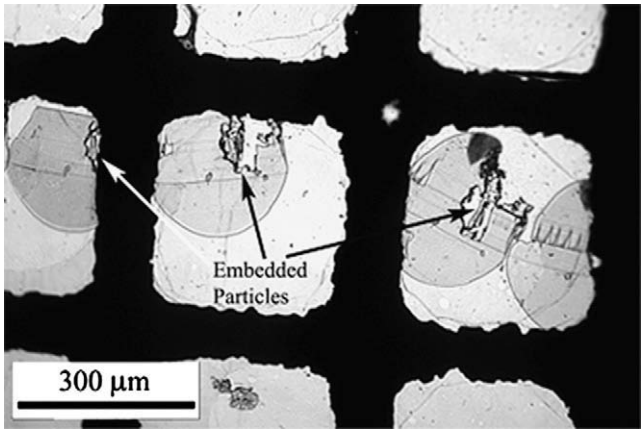

Fig. 3. Optical light microscope image of ~100 nm thick ultramicrotomed sulfur sections collected on a lacey SiO support film. Embedded particles are denoted with arrows. Subsequent to this step, the sulfur sections were sublimated in vacuum, leaving only the 100 nm thick particles on SiO film.