X-ray Microanalysis of Biological Samples by High-resolution Energy Dispersive Microcalorimeter Spectrometer Using a Low-voltage Scanning Electron Microscope

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A transition edge sensor (TES) microcalorimeter detector with an energy resolution of 17.3 eV@1.74 keV mounted on an SEM has been newly developed and applied for the first time to reveal the cellular distribution of Cd in a cadmium hyper-accumulating plant. The elemental maps of the light element P as well as the heavy elements Cd and Zn were also successfully obtained. Similarly, the simultaneous analysis of As Lα and Mg Kα in an arsenic-accumulating fern was possible with this system. It is expected that the microcalorimeter EDS system has the potential to open a new area of the X-ray microanalysis of biological samples.

A half century has passed since the development of the solid-state detector (SSD), which is widely used as a detector of energy dispersive X-ray spectroscopy (EDS) mounted on a scanning electron microscope (SEM). The energy resolution of the SSD is in the range of 130 eV, which is not suitable to carry out a low acceleration voltage analysis using an SEM. A detector resolution of better than 30 eV is required in order to discriminate among the K-, L-, and M-lines of all elements in the low energy portion of the spectrum. This problem can be solved by using a superconductor transition edge sensor (TES) microcalorimeter (μ-cal) with an energy resolution of one order of magnitude better than the SSD.

The microcalorimeter consists of an X-ray absorber contact with a thermometer, which is a superconductor with a transition temperature of ca. 100 mK and the thermometer is maintained at a temperature within the transition from the superconducting to the normal state, thus it is called a TES. Hitting the absorber by a single X-ray photon causes a temperature increase in the thermometer, which results in an electrical resistance increase. Since the circuit voltage remains constant, the current decreases. This current change is a function of the X-ray energy and is readout by a SQUID (superconducting quantum interference device) amplifier.

The pioneering works of the NIST group[1–3] developed the first practical μ-cal EDS system utilizing TES, which is still the only system available for material studies. The users reported typical samples important in material science[1–6] showing the advantage of the system. Recognizing the importance of this detector, we have started the development of the μ-cal system including TES and SQUID based on domestic technology.[7,8] We now report the performance of the newly developed prototype μ-cal SEM–EDS system for heavy element analysis as the first successful application of the μ-cal system to biological samples. The maximum count rate is over 300 cps estimated from the pulse decay time (50 μs).

Figure 1 shows an EDS spectrum of WSi_2 measured by the TES microcalorimeter. The EDS spectrum measured by a conventional Si(Li) detector with a typical measurement time of 300 s and a probe current of 300 pA is also shown for comparison. The data clearly demonstrate the ability of the detector to resolve important peak interference problems and the energy resolution

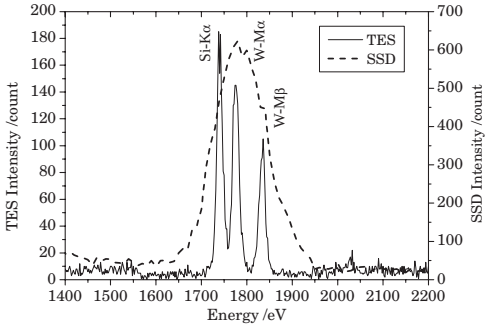

Figure 1. EDS spectrum of WSi_2 on Si substrate acquired at 5 kV, total count of 20 kcounts. (Measurement time = 500 s, count rate = 40 cps for TES).

was 17.3 eV measured at 1.74 keV. The EDS spectrum of euxenite, (Y,Ca,U,Th)Nb_2O_6, was measured and compared with the SSD data in Figure 2. TES gives well-resolved peaks of the heavy elements Y-Lα_1,Y-Lβ_1, Nb-Lα_1, Nb-Lβ_1, Nb-L$\beta_{2,3}$, Nb-Lγ, Th-Mα, U-Mα, and U-Mβ, as well as the light element of Ca Kα showing the high potential of the heavy element analysis by μ-cal system.

Since biological samples have a complex structure from a histological level to an organelle level, and the chemical compositional analysis of the sample under SEM observation has a strong demand in biology. Especially, observation at a low acceleration voltage yields a high-resolution SEM image and therefore is suitable for biological samples. Thus, we considered that biological and clinical samples are some of the important targets of the present analytical system despite the absence of any preceding research. As a first sample, we chose unique plants containing both heavy and light elements.

Some types of plants can grow in heavy element contaminated soils and absorb a large amount of heavy elements such as Cd, As, and Pb in their bodies.[9,10] This characteristic is used for the

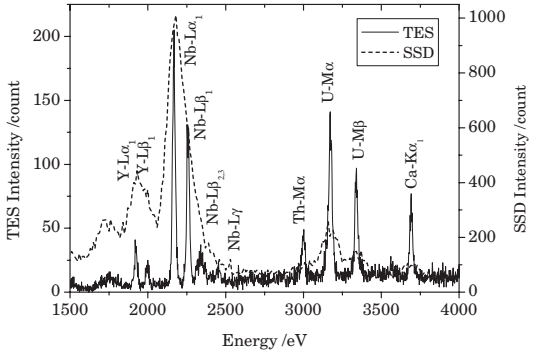

Figure 2. μ-cal EDS spectrum of euxenite, (Y,Ca,U,Th)Nb_2O_6, measured for 1000 s at 15 kV, total counts = 40 kcounts.

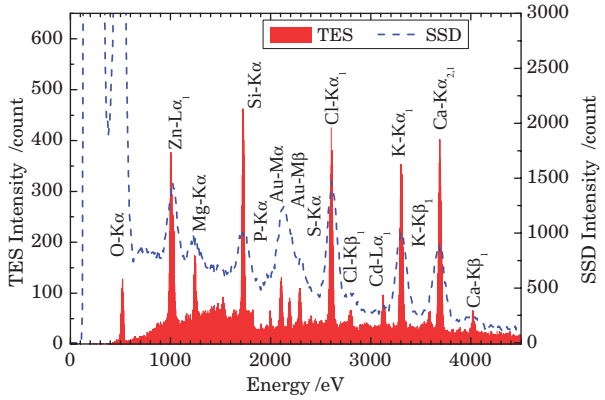

Figure 3. μ-cal EDS spectrum (total 100 kcounts, 131 cps, and meas. time = 1330 s at 10 kV, 0.3 nA) of trichome compared to SSD spectrum (300 s. at 10 kV, 0.5 nA).

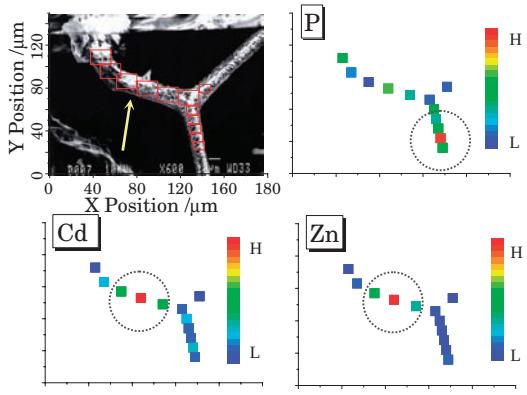

Figure 4. SEM image of trichome showing analytical points and the elemental mapping results of Cd, P, and Zn.

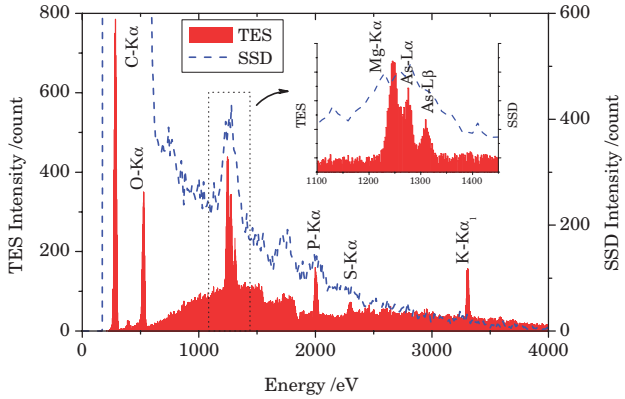

Figure 5. μ-cal EDS spectra (total 100 kcounts) of Chinese brake fern acquired at 5 kV, 3 nA (TES) and SSD spectra at 5 kV.

cleanup of heavy metal-polluted soils and is called phytoremediation technology. To reveal the mechanism of the hyper-accumulations, two-dimensional analysis of the heavy elements in the plant tissues is a key analytical method. However, conventional SEM–EDS is not suitable for the analysis of the K-lines of heavy elements such as Cd and As because of the low sensitivity of the electron beam excitation for the heavy elements. Furthermore, the detection of the L-lines of Cd and As is also difficult because the former and the latter overlap with the K-lines of K and Mg, respectively; both elements are essential elements for plants. This problem was so far only solved by our high energy synchrotron radiation (SR) μ-XRF analysis.[9,10] However, the SR facility is very limited in number and requires many days to obtain beam time. Moreover, it is difficult to carry out SEM observations during the SR–μ-XRF analysis.

The plant samples of Cd-hyperaccumulator, *Arabidopsis halleri* ssp. *gemmifera*, and As-hyperaccumulator fern, *Pteris vittata* L., were subjected to the present analysis.[11]

Figure 3 shows the EDS spectrum of *A. halleri* obtained by the TES compared to that of the SSD. The SEM image of the sample (trichome) and the analyzed point is shown by the arrow in Figure 4. The Cd Lα_1 peak (3134 eV) is well resolved from the K Kα_1 peak (3314 eV), while the SSD is not (Figure 3). Moreover, the Kα peaks of P and S, biologically important elements, are clearly observable and resolved from the Au Mα and Mβ peaks in the coating of the sample.

Elemental mapping of the trichome sample was carried out to locate the high-accumulation point. The analyzed points are shown in Figure 4 (upper left) and the distributions of Cd, P, and Zn are shown by the color scales, which were calculated from the X-ray intensity of each element normalized from the blue (minimum) to red (maximum) colors. It was found that both Cd and Zn highly accumulated in the base of the bifurcation area of the trichome. This result is consistent with that obtained by the SR–μ-XRF analysis.[9]

Figure 5 shows EDS spectra of a fern measured at the rim by TES μ-cal and SSD. It is found that Mg Kα (1254 eV) and As Lα (1282 eV) are clearly resolved in the spectrum while the SSD data gave a broad peak.

The present results demonstrated the superior energy (i.e., elemental) resolution of the TES μ-cal EDS. This system is superior to the wavelength dispersive X-ray spectrometer in biological applications because the latter requires a high beam current which causes serious damage to the samples. Moreover, the low voltage produces low penetration of the electron beam and hence a surface sensitive analysis, which allows a high spatial resolution analysis. These characteristics are advantageous for the analysis of cell or organelle, and this new analytical system will therefore open a new stage for the SEM–EDS analysis of the biological samples.

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