CH SOP-25: General Scanning Electron Microscopy (SEM)

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Page **1** of **7**

A. Purpose

The analysis of samples by scanning electron microscopy (SEM) using energy dispersive X-ray spectroscopy (EDS) is a method employed by the Chemistry Unit of the laboratory. The following procedure shall serve as a guideline for analyzing and interpreting results from sample analyses.

B. Scope

This procedure serves as a general guideline for all procedures that involve the use of SEM analyses. Application of this procedure to evidence requires knowledge of general concepts, methods of a scanning electron microscope (SEM), and understanding of an energy dispersive X-ray spectrometer (EDS). Methods requiring use of SEM/EDS require operational proficiency of each instrument. This knowledge can be obtained from the references provided, training from manufacturers or principal operators within the Unit, and schools in SEM and EDS.

C. Responsibility

Analysts authorized to use the SEM/EDS instrument.

D. Equipment/Materials/Reagents

Listed below are general supplies required for electron microscopy (EM). Additional supplies unique to a particular method are indicated within that topic.

1. Scanning Electron Microscope with Backscattered Electron (BE) detector (Hitachi S-3700N or equivalent)
2. Energy Dispersive X-ray Analyzer (EDAX Genesis or equivalent)
3. Aluminum Stubs (Electron Microscopy Services)
4. Adhesive-backed tabs or double-sided adhesive tape (Electron Microscopy Services)
5. Copper pure element reference material (Sigma-Aldrich or equivalent)

E. Standards/Controls/Reagents

A copper (Cu) pure element reference material is analyzed each day on which casework is performed in order to check the SEM and to measure the performance of the EDS system. The quality control (QC) data from the Cu reference material will be placed into the QC logbook for the instrument. This QC check should be performed prior to evidentiary analyses.

F. Calibration

Not applicable.

G. Sampling

Not applicable.

H. Procedure

1. Ensure instrument is ready for analyses. Never turn on the electronics to the EDS detector without an adequate level of $N_{2(l)}$.
2. Vent the system and open chamber.
3. Insert the sample into the specimen holder. Never touch the surfaces of the discs with bare hands - use gloves appropriately. Use tweezers around step of stubs to manipulate discs.
4. Note position of samples.
5. Close chamber and evacuate.
6. After the chamber is properly evacuated (i.e., green light) turn on the power at the main SEM console. Ensure that the filament, operating voltages, working distance, magnification, and spot size are all at their correct settings and are operating properly.
7. Ensure the correct detector is selected (backscatter electron (BSE) versus secondary electron imaging (SEI)).
8. Adjust brightness and contrast as needed in order to obtain an optimal SEM image.
9. Obtain a good overall view of the sample. The raster area of the SEM is selected in order to yield the best possible sample area. If it is not possible to select one large area, several smaller areas can be analyzed and the data can then be compared and/or summed.
10. If needed, specific particles can be magnified and analyzed.
11. Capture appropriate images, as needed.
12. Collection parameters should be captured, recorded, or noted.
13. Ensure proper collection times are selected (e.g., 2 minute live time) and collect spectral data, as needed, using the EDS detector.
14. Save spectra using appropriate filenames. Using generic filenames will overwrite spectra and data may be lost.
15. Evaluate spectra for elemental information. Final elemental peak determination must be based on user interpretation of valid energy values appropriate to each element's theoretical values.
16. Document appropriate images and spectral data (i.e., hardcopies).
17. Analyze appropriate controls (positive, negative), as necessary.
18. Turn off electronics, properly vent system, and remove sample(s).
19. System should remain under vacuum with electronics off when not in use.

I. Evaluation of Data

1. <u>Elemental Determination</u>:

a. While preliminary elemental peak information can be initially obtained using automated features, final elemental peak determination must be based on user interpretation of valid energy values appropriate to each element's theoretical values.
b. Use a systematic approach to elemental identification so that misidentifications or omissions of minor peaks does not occur. This approach includes consideration of X-ray line families, spectral artifacts, escape peaks, sum peaks, and overlaps.
c. Reference lines, or energies, may be obtained from several sources including energy slide rules, computer generated 'KLM markers' that may be superimposed on the spectrum, and published tables. Additionally, manufacturers often provide an automatic element identification application. Often these aids are used to complement one another.
d. Identification can begin with high-energy peaks and major peaks. High-energy peaks will typically have less overlap than lower energy peaks within a spectrum. If a major peak is present, then a complete family of peaks should be able to be observed. Each line within the family should be labeled with elemental symbols. Spectral artifacts, including sum peaks and escape peaks associated with major peaks, should be identified and labeled.
e. As spectral interpretation alternates between the identification of major and minor peaks, the vertical (counts) scale should be adjusted to reveal the required detail. In addition to the higher energy peaks, note the presence of any lower energy families and their expected relative intensities. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap. Elemental identification is aided by superimposing and scaling KLM reference lines on the spectrum or referencing the actual spectrum of an elemental standard. The analyst should become familiar with the characteristic pattern and relative intensities of peaks of various atomic numbers.
f. Following the identification of major components, lower intensity peaks and overlapped peaks are identified. Identification of minor elemental components is often limited by the number of characteristic peaks present in a spectrum.
g. The presence of an element can be considered unequivocal only when a distinctive unique set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or spectral artifact. Unequivocal identification may not be possible if an element is present in low concentration or if lines required for confirmation are overlapped with the lines of other elements.
h. If an automatic identification application is used, peak identification must be confirmed by the analyst.
i. If identification is unequivocal, then each individual peak should be labeled with the corresponding elemental symbol. If the identification is probable but not unequivocal, then the peak label should indicate so (e.g., by parenthesizing the elemental symbols or by putting a 'poss' for possible).
j. For documentation, spectra should be displayed on a scale that clearly demonstrates the peaks identified. Multiple scales may be necessary.

2. Spectral Comparison:
   a. Comparisons of elemental composition can be made by the direct spectral comparison of materials. These spectra can be collected at the same time as that of the questioned material.
   b. Spectra are acquired from materials to be compared, using similar analytical conditions.
   c. Overlay spectra of materials to be compared. Vertical offset and horizontal and vertical scales should be adjusted to permit thorough spectral comparison.
   d. Depending upon their correspondence, the spectra can be characterized as similar, generally similar, and different.
      i. Spectra should be considered "similar" if they only exhibit channel-to-channel intensity variations.
      ii. Spectra should be considered ‘generally similar’ if they exhibit only a few slight differences. These differences may be qualitative or quantitative (intensity). Permissible qualitative differences in materials considered to be generally similar may consist of the absence of trace amounts of common elements. Permissible quantitative differences may consist of slight ratio differences in the peaks heights of major or minor elements.
   e. Spectra should be considered ‘different’ when they exhibit distinct and significant differences. These differences may be qualitative or quantitative. Significant qualitative differences may consist of the absence of trace amounts of rarely occurring elements or the absence of a significant peak from a more commonly occurring element. Quantitative differences may consist of significant ratio differences in the peak heights of major and minor elements.
   f. There is not an absolute distinction between the definitions, ‘generally similar’ and ‘different.’

J. Instrumental Conditions

1. General operating conditions can be:

Beam Voltage	Variable depending on sample (e.g., 25 kEV)
Beam Current	Adjusted to yield 30% deadtime
Live counting time	~200 seconds (bulk analysis) ~100 seconds (specific particle)
Pulse Processor time constant	Mid-range value
Spot-size	Variable
Working Distance	Variable (e.g., 10mm, 11.2mm)

2. Changes to General Operating Conditions:

   Changes to suggested instrumental conditions may be needed under the following circumstances:

   a. Beam voltage is increased when higher energy line excitation is required
   b. Beam voltage is decreased when greater spatial resolution is required
   c. The pulse processor time constant is lengthened when greater spectral resolution is required
   d. The pulse processor time constant is shortened when a greater count rate is required (e.g., for trace element analysis or construction of elemental distribution maps)
   e. Detector-to-sample distance can be reduced to increase X-ray collection efficiency
   f. Beam current is increased when the X-ray count is too low. Beam current may be increased by decreasing the condenser lens current and/or increasing the final aperture size.
   g. Beam current is decreased when the X-ray count is too low. Beam current may be decreased by increasing the condenser lens current and/or decreasing the final aperture size.

K. Decision Criteria

If spectral differences are detected, it is likely that the materials that produced them are not similar in composition; however, several alternative explanations are possible:

1. Dissimilar sample geometry.
2. Spectral collection from non-representative areas of an inhomogeneous sample.
3. X-ray fluorescence contributions from elements in adjacent structures.
4. X-ray contribution from extraneous material.
5. Contribution of carbon from a carbon SEM mount.

L. Uncertainty of Measurement

Not applicable.

M. Limitations

1. Adhesive may transfer to the sample, adversely affecting subsequent organic examinations.
2. While considered a non-destructive technique, samples usually are not removed and reanalyzed using other methods after adhered to an SEM stub.
3. If the dimensions are limited, a sample may be difficult to orient precisely.
4. This technique is limited to solid samples.
5. X-ray fluorescence may excite characteristic X-rays from structures remote from the area visibly selected for analysis.
6. Elements present in trace concentrations, under average conditions, are generally not detected below 0.5%.

7. Although the natural X-ray line width is approximately 2eV, EDS resolution is generally no better than approximately 140eV. As a result, overlap of peaks in the EDS spectrum of materials containing several elements may occur. Some commonly occurring overlaps encountered in EDS are TiK_{α}/VK_{β}, VK_{β}/CrK_{α}, CrK_{β}/MnK_{α}, MnK_{β}/FeK_{α}, FeK_{β}/CoK_{α}, $PbM_{\alpha}/SK_{\alpha}/MoL_{\alpha}$, $BaL_{\alpha}/TiK_{\alpha}$, KK_{β}/CaK_{α}, $ZnL_{\alpha}/NaK_{\alpha}$, PK_{α}/ZrL_{α}, and $AlK_{\alpha}/BrL_{\alpha}$.
8. Similar spectra do not necessarily indicate identical composition. They are similar only down to the detection limits of this analytical method.
9. The particular methods employed for each sample examined will depend upon sample size, sample suitability, analytical request, and available accessories. Variations to sample preparation may occur.

N. Safety

1. General precautions common to EM laboratories include minimization of contact with cryogens, venting or filtering of rough pump discharge, and avoidance of direct exposure to beryllium metal.

2. Use universal precautions when handling potentially biohazardous materials. Refer to the GL *Safety Procedure* and appropriate MSDS/SDS documents for additional information. Take standard precautions for the handling of chemicals and sharp cutting instruments.

O. References

1. Hitachi S-3700N operator’s manual.
2. EDAX Genesis Manual(s)
3. Bearden, J.A. "X-ray Wavelengths," *Reviews of Modern Physics*, 39 (1), No. 1, 1967.
4. Goldstein, et al. *Scanning Electron Microscopy and X-ray Microanalysis*, second edition, Plenum Press, New York, 1992.
5. Ward, D.C. "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-ray Analysis," *Forensic Science Communications*, 1(2), 1999
6. Ward, D.C. and Sibert, R.W., "The Use of Vacuum Evaporation of Metals for Surface Feature Enhancement," *AFTE Journal*, 18(4), 1986.

Rev. #	History
1	Old procedure (CH-SOP-25) was accidentally retired and this will serve as a new document with new title. Added 'Scope' section. Significant format and verbiage changes throughout document. Added more specification to the 'Procedure' section. Added the following sections to the document: Scope, Standards/Controls/Reagents, Evaluation of Data, Instrumental Conditions, Decision Criteria, Limitations, and Safety. Added more references. Added Revision History section.