SEM Analysis of Paints, Tapes, and Polymers

1 Scope

This procedure applies to Chemistry Unit caseworking personnel who analyze Paints and Polymers evidence via Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS). This document describes the sample preparation and suggested instrumental parameters for the SEM analysis of paints, tapes, and other polymeric materials. In this document, the term SEM refers to the SEM imaging system as well as the Energy Dispersive X-ray Spectrometer (EDS).

2 Equipment/Materials/Reagents

a. Scanning Electron Microscope with Backscattered Electron (BE) and Secondary Electron (SE) detector: JEOL model JSM 6510LV, TESCAN model Vega 3 XMU (or equivalent)

b. Energy Dispersive X-ray Spectrometer (EDAX Apollo or equivalent)

c. Spectral Library Identification and Classification Explorer (SLICE) (xk, Incorporated)

d. Energy Dispersive X-ray processing software (EDAX Genesis or equivalent)

e. Stereomicroscope (~ 6X to ~ 100X) with appropriate lighting

f. Glass microscope slides

g. Compressed gas duster

h. Acetone (Reagent grade)

i. Distilled water

j. Cotton tipped applicators

k. Double-sided adhesive tape, clear/colorless or carbon

l. Graphite paint (Ted Pella, Inc. or equivalent)

m. Embedding molds (Ted Pella, Inc. or equivalent)

n. Epoxy resin and hardener (Buehler EPO-KWIK® or equivalent). For EPO-KWIK®, mix 5 parts resin to 1 part hardener and blend gently but thoroughly with a stir stick. The pot life

of the mixture is ~5 minutes. The epoxy will cure at room temperature; however, curing in a moderate oven (~65ºC) for two or more hours is preferable.

o. Oven with temperature capability ~ 100°F -150°F (40°C -65°C)

p. Analytical balance (up to 50 grams)

q. Microtome (Leica Ultracut UCT or equivalent)

r. Carbon coater (Cressington 108carbon or equivalent)

s. Carbon rods (SPI Supplies or equivalent)

t. Scalpel handle with blades

u. Pyrolytic carbon planchets (Ernest F. Fullam, Inc. or equivalent)

v. Adhesive (e.g., Durotak 387-2287 (National Starch) or equivalent)

w. Wood applicator sticks

x. Jeweler's saw

3 Standards and Controls

3.1 Standards

Manufacturer-supplied and commercially available paints, tapes, and polymers are maintained in within the FBI Laboratory. These materials are used in casework in accordance with the *Chemistry Unit Procedures for the Use of Reference Materials and Known Materials*.

3.2 Performance Check

Refer to the *Performance Monitoring Protocol (QA/QC) Scanning Electron Microscope (SEM)/Energy Dispersive X-ray Spectrometer (EDS)* for details on the performance checks and necessary supplies to conduct the check and operate the instrument.

4 Sampling

Refer to the current version of the relevant material's *General Approach* Paints and Polymers Standard Operating Procedure (P&P SOP) (i.e., PPSU 100, PPSU 101, PPSU 102) for guidelines on sample(s) selection. Record the sample(s) selected for analysis in the case notes.

5 Procedure

5.1 Sample Preparation

1. The choice of a specific method for sample preparation will depend on the size and condition of the specimen. As needed, use multiple preparation methods in order to determine all sample characteristics. For an accurate comparison of elemental composition and sample layer structure, samples must be prepared in as similar a manner as possible.

2. Samples are first examined with a stereomicroscope. If extraneous (contaminant) materials are present, remove using the tip of a scalpel blade, by taking a series of thin peels, or with a cotton-tipped applicator moistened with water or a suitable solvent. When extraneous materials cannot be removed, note their location during light microscopy or backscatter electron SEM observations and avoid these areas during subsequent SEM analysis.

3. Attach small samples or shavings directly to a pyrolytic carbon planchet using double-sided tape or a thin adhesive layer. Attach a tape backing to the planchet using its own adhesive. If the backing has been separated from the adhesive or if a cross section of the backing has been prepared, mount these using double-sided tape or a thin adhesive layer. Remove adhesives from the tape backing and spread into a thin layer of uniform thickness directly onto a planchet.

4. A paint smear is composed of commingled particles and fragments. Select particles that are approximately 50 µm individually and attach to a carbon planchet for analysis. It is also possible to lift a collection of deposited particles with a sticky material, such as tape adhesive, and attach them to a carbon planchet. Individually analyze such particles.

5. There are a number of sample preparation methods available to expose individual layers in a multilayered sample. Affix a manual cross section, an intact chip oriented on edge, or thin peels of the individual layers to a carbon planchet with double-sided tape or a thin adhesive layer. Alternatively, expose the individual layers in a "stair step" fashion by cutting through and removing the overlying layer(s). Continue carving until a large, flat surface area of each layer is exposed and then affix the sample to a carbon planchet. Alternatively, embed multilayered samples such as paints and tape backings in a medium that hardens and then expose the cross section by microtomy. This technique is described in further detail in steps 6 and 7.

6. Embedment provides mechanical support for subsequent sample preparation. Begin by attaching the sample to the bottom of a mold with a thin adhesive layer. Position the sample in the mold to reveal the structures of interest when subsequently cross sectioned. A sample identification label can also be placed in the mold. Add the embedding

medium, such as two-part liquid epoxy, to the mold slowly so as to prevent air bubble entrapment and allow it to cure.

7. Once embedded, cross-section the samples by hand or by microtomy, to produce a flat block and/or thin cross-sections of the sample. Trim the block face to an area of approximately 2 x 3 mm with a pyramidal shape using a jeweler's saw and/or a razor blade. For microtomy, clamp the block into a holder that is attached to the microtome arm. Adjust parameters such as cutting speed, cutting thickness, and knife angle to optimize the resulting sample. Use the knife to trim the block face first with rough cuts followed by fine cuts. If sections are desired, remove these from the knife face; if a faced block is desired, remove the block from the holder and process for analysis.

8. Apply a conductive layer (e.g., carbon) to the sample surface of polymeric materials in order to minimize sample charging. Place carbon rods in the electrodes of the vacuum evaporator, place the sample on the base plate, and cycle the evaporator to high vacuum. Current is induced through the carbon rods in order to evaporate the carbon onto the sample. Then, the chamber is pressurized and the sample removed.

9. When analyzing multiple samples, construct a map identifying sample location on or within the sample holder.

5.2 Analytical Procedure

1. Structural imaging:

    a. Light microscopy demonstrates layer structure as well as some structural detail within each layer of a multilayered sample when examining either a thin cross-section or the prepared block.

    b. Collect a backscatter electron image when elucidating layers and structures, and/or for defining distribution of particulate components.

    c. When collecting SEM micrographs, include a measuring scale or magnification scale or both. Also include a display to document which signal (e.g., back scattered electron or secondary electron) was used to produce the image.

2. Collection of a "bulk" EDS spectrum permits determination and comparison of average elemental compositions of a material. Collect a bulk spectrum after the raster area of the SEM is selected to yield the largest sample area possible. If it is not possible to select one large area, several small areas are analyzed, and the data from each are summed.

3. Particle analysis is performed when bulk analysis alone is insufficient to discern adequate structural and compositional discrimination of select components (e.g., aluminum flakes, decorative flakes, $CaCO_3$, $BaSO_4$). Perform a particle analysis by directing the electron

beam of the SEM directly onto the structure of interest, either by increasing the magnification.

4. Once an X-ray spectrum is collected, perform a spectral peak identification in order to determine the elements present.

    a. Spectral peak identification is best achieved through SLICE. The algorithms used for peak identification consider factors such as escape peaks, sum peaks, peak overlap, and X-ray line families.

    b. Regardless of which automatic element identification application is utilized, peak identification must be confirmed by the examiner by superimposing and scaling KLM reference lines on the spectrum and/or referring to published tables.

    c. The presence of an element is considered unequivocal only when a distinctive set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or artifact. The peak(s) are labeled with the corresponding elemental symbol.

    d. 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 (an)other element(s). When identification is probable, but not unequivocal, the elemental symbol is parenthesized.

5. Direct spectral comparisons can be achieved using SLICE. The compositional similarity of questioned materials to reference materials can also be performed using SLICE.

6 Instrumental Conditions

The following operating conditions are meant as general guidelines for starting conditions. Actual requirements can vary as determined by specific analytical needs.

Beam voltage:	20-25 kV
Beam current:	adjusted to yield at least 5000 CPS
Live counting time:	100 - 200 seconds
Amp Time:	6.4µS
Working distance:	13-18 mm
Take off angle:	~35-40^0

Generally, changes in the suggested instrumental conditions, listed above, are required under the following circumstances:

a. The beam voltage is increased when higher energy X-ray line excitation is required.

b. The beam voltage is decreased when greater spatial resolution is required.

c. The EDS detector-to-sample distance is reduced to increase X-ray collection efficiency.

d. The spectral energy display scale is expanded when sufficient detail is not evident in order to determine if instrumental conditions need adjustment.

7 Decision Criteria

a. Spectral comparisons should be conducted with spectra collected using similar sample preparations, similar sample characteristics (e.g., thickness, topography), and similar instrumental parameters, as appropriate.

b. Spectra are compared and interpreted based on the observation of spectral differences, or lack thereof, between the sets of elemental data.

    1. Spectral overlay is a recognized approach for comparing data where the presence or absence of peaks, peak shapes, and relative intensities are all considered in the evaluation as to whether exclusionary differences exist between compared samples.

    2. When assessing differences between spectra, consider sample limitations (e.g., small samples, thin layers, dirty samples, sample smears that eliminate layer structure) and instrumentation limitations (e.g., sampling size, limits of detection).

c. Possible reasons for spectral differences include dissimilar sample conditions (e.g., size, thickness, surface topography), lack of representativeness of the specimen or source material, contribution from extraneous materials, or origination from different source materials. Additional samples can provide supplemental data to assist in assessing such differences.

d. If suitable spectra are produced, comparisons can provide information regarding the potential relationship of the sources of the samples.

e. Distinguishable sources: When exclusionary differences are observed between compared spectral features, the sources of the samples are considered distinguishable by SEM energy dispersive spectroscopy. Exclusionary differences in spectral comparisons: 1) are outside the variability of spectra originating from the same source; and 2) cannot be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories.

f. Indistinguishable sources: When no exclusionary differences are observed between compared spectral features, the sources of the samples are considered indistinguishable

by SEM energy dispersive spectroscopy. Differences that are not considered exclusionary: 1) are within the variability of spectra originating from the same source; or 2) can be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories. If no exclusionary differences are observed in a SEM comparison, samples can be analyzed by other analytical techniques to provide additional information about the potential relationship between the sources of the samples.

g. SEM spectral comparison is one part of a multi-analytical comparative approach. SEM data alone can be used to distinguish the sources of compared samples, but is otherwise not used independent of data obtained from other analytical techniques to reach an overall opinion regarding the potential relationship between the sources of the samples. An overall opinion that sources are indistinguishable is only reported when no exclusionary differences are observed in the analytical techniques that were applied.

8 Calculations

Not applicable.

9 Measurement Uncertainty

Not applicable.

10 Limitations

The methods described in this guide can have some limitations, including the inability to detect elements in trace concentrations, the need for a conductive coating on the sample, and the discoloration of materials by irradiation.

a. The information available from a specimen can diminish as its size is reduced and its condition degrades. As specimen size is reduced or the material becomes degraded, it may no longer be representative of the original material.

b. A disadvantage of embedment is the inability to remove a sample from most embedding materials after analysis.

c. Although the natural X-ray line width is approximately 2 eV, EDS resolution is generally no better than approximately 140 eV. As a result, overlap of peaks in the EDS spectrum of materials containing several elements can occur. The following are some commonly occurring overlaps encountered in EDS: TiKα/VKα, VKβ/CrKα, CrKβ/MnKα,

MnKβ/FeKα, FeKβ/CoKα, PbMα/SKα/MoLα, BaLα/TiKα, KKβ/CaKα, ZnLα/NaKα, PKα/ZrLα, and AlKα/BrLα.

d. Any individual particle or fragment from a heterogeneous material may not be compositionally representative of the bulk and therefore would not be expected to produce spectra similar to the bulk material.

11 Precautionary Statements

a. As with any procedure involving trace evidence, ensure actions minimize the potential for loss or contamination of the sample.

b. Orientation of the sample area of interest perpendicular to the electron beam is critical for accurate and reproducible EDS results. As such, extreme care should be taken when embedding a multilayered sample or when placing an unembedded chip or manual cross section on an SEM mount.

c. When analyzing a cross-section of a thin layer, such as a factory-applied automotive basecoat (~10 µm), care must be taken to ensure that the excitation volume does not extend into adjacent layers.

d. With a "stair step" preparation, the excitation volume can penetrate through a thin layer to an adjacent underlying layer.

e. 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. These include dissimilar sample geometry, heterogeneity of the sample, and X-ray contribution from extraneous material.

12 Safety

Use standard precautions for the handling of potentially biohazardous materials, chemicals, or sharps. Refer to the *FBI Laboratory Safety Manual* and appropriate Safety Data Sheet(s) for further details. Personal radiation monitors (dosimeters) are administered by the Health and Safety group to monitor exposure to ionizing radiation. Operators should familiarize themselves with the specific User's Guide safety section of the instrument prior to use.

13 References

A Guide to Scanning Microscope Observation, JEOL

ASTM E1610 Standard Guide for Forensic Paint Analysis and Comparison. ASTM International, West Conshohocken, PA

ASTM E2809 Standard Guide for Using Scanning Electron Microscopy/ X-Ray Spectrometry in Forensic Polymer Examinations. ASTM International, West Conshohocken, PA

ASTM E3260, Standard Guide for Forensic Examination and Comparison of Pressure Sensitive Tapes. ASTM International, West Conshohocken, PA.

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3	09/18/18	Deleted Introduction and Principle sections and renumbered. Revised Scope to describe who document applies to. Updated Equipment Section, deleted Calibration Section and renumbered, Updated remaining sections in the document for clarity including expanding decision criteria. Updated references.
4	04/06/21	Clarified Scope and added items to equipment list to align with procedural guidance. Clarified procedural guidance in Section 5. Removed the term "reference collection" from Section 3. Clarified display change guidance for instrumental condition settings in Section 6. Revised Section 7 to align with OSAC revisions to E2809 Standard Guide for Using SEM/EDS in Forensic Polymer Examinations, updated references. Removed QA approval line.

Approval

Redacted - Signatures on File

Paints and Polymers
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