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Analysis Protocol for Discrimination of Automotive Paints by SEM-EDXA Using Beam Alignment by Current Centering

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REFERENCE: Beam, T. L. and Willis, W. V., **"Analysis Protocol for Discrimination of Automotive Paints by SEM-EDXA Using Beam Alignment by Current Centering,"** *Journal of Forensic Sciences*, JFSCA, Vol. 35, No. 5, Sept. 1990, pp. 1055–1063.

ABSTRACT: A testing protocol for elemental analysis of automotive paint by scanning electron microscopy/energy-dispersive X-ray analysis, based on beam alignment by current centering and using an attached optical microscope was developed to improve the reliability of sample comparisons. Six light-green paint samples (three color pairs) from the 1983 Reference Collection of Automotive Paints were used for developing this protocol. X-ray spectral data were acquired using a JEOL JSM-35CF scanning electron microscope with an attached optical microscope and a Tracor Northern energy-dispersive X-ray analyzer and software. The instrumental conditions for the analyses were as follows: 20-kV accelerating voltage, 0.5-nA beam current, 30-mm detector distance, 35° detector take-off angle, 0° sample tilt, and ×200 magnification. The software acquisition parameters were 1 000 000 integral counts per spectrum and a 0.2 to 10-eV spectral range. Statistical analyses were used to examine the reliability of the X-ray spectral data acquired using this protocol. A one-way analysis of variance (ANOVA) indicated that the elemental means of all paint samples were significantly different than the means of the same sample in replicate analyses. Simple visual comparison of data between samples, along with ANOVA, provided discrimination of all paint samples.

KEYWORDS: forensic science, chemical analysis, paint, paint analysis, energy dispersive X-ray-analysis, automotive paint

The matching or confirmation of suspect paint samples with control paint samples is often necessary in a forensic science laboratory, since approximately 45% of all physical evidence analyzed by spectroscopy in certain laboratories is paint in some form or another [*1*]. A reliable, nondestructive method of paint analysis is desirable in these situations. The complete analysis of paint can involve many analytical steps to determine all of the components. In general, a paint has four components: pigment, binder, solvent, and various additives. Methods used for analysis of the organic constituents include pyrolysis gas chromatography, the Wick-Stick infrared spectrometry technique [*1*], high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) spectrometry, ultraviolet spectroscopy, and thermal analysis. All of these methods destroy the paint sample. Some of the newer Fourier transform infrared spectroscopy instruments have the capability of analyzing the organic portion of a paint sample nondestructively through reflectance measurements. But, in order to analyze the inorganic pigments in paint, elemental analysis is required. Inductively coupled plasma

Received for publication 18 May 1989; revised manuscript received 22 Sept. 1989; accepted for publication 26 Sept. 1989.

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spectrometry, atomic absorption spectroscopy, and emission spectrography will also destroy the sample. Neutron activation analysis is useful, but overly sensitive for the elemental levels ordinarily found in paint [2]. At the present time, the most prevalent nondestructive method of analyzing paint samples employed in forensic science laboratories is X-ray fluorescence (XRF). Research involving XRF on paint samples indicates a need for improved testing methods in order to produce more reliable data for case comparison and confirmation purposes. For example, Howden et al. from the Home Office Central Research Establishment (HOCRE) in Berkshire, England, reports up to 30% variability in replicate measurements in XRF analyses of modern household paints [3]. They attributed the large variability to counting errors, heterogeneity of the sample, and variations in the presentation of the sample. Their XRF data were collected on somewhat large (for microscopy) samples, 2 by 2 mm, so their error may lie more in the technique than in the inhomogeneity of the paint samples. Reeve and Keener [4] report that comparisons of XRF spectral data of suspect and control samples in repeated measurements showed gross elemental differences, and they therefore reported most of their results based on single analyses. They reported that the paint samples were homogeneous as long as they were received from the same source.

For paint samples of distinguishably different colors of paint, XRF can probably provide adequate discrimination. However, in order to discriminate between paints of the same or nearly the same color, especially in quality control or forensic laboratories, a more reproducible and reliable method for analysis is needed. Scanning electron microscopy/energy-dispersive X-ray analysis (SEM-EDXA) with electron beam alignment by current centering (BACC), utilizing an optical microscope for this purpose, provides a method of elemental analysis that can distinguish between different automotive paint samples of the same or nearly the same color.

References that discuss the principles, theory, and applications of SEM-EDXA are available [5–7], but it is beyond the scope of this paper to discuss SEM-EDXA in such detail. Information regarding paint analysis by XRF and SEM-EDXA is also available in the literature [3,4,8]. More studies have been done with XRF then with SEM-EDXA, but little emphasis has been placed on validation of the analytical protocol or the quantitation of elemental composition in paints. The difference in the two methods lies in the theory, sensitivity, reproducibility of analysis, and cost of instrumentation. XRF will generate spectra with a greater sensitivity for the elements of higher atomic number. Russ [9] reports that XRF data from paint chips can vary as little as 1% for the elements producing strong emissions (higher atomic number), but that this kind of precision is not possible for elements producing weaker emissions (lower atomic number elements). This is inadequate for paint analysis since many important low atomic number elements such as aluminum, silicon, sulfur, and chlorine may not be detected by this technique. Conversely, SEM-EDXA has a greater sensitivity to the lower atomic number elements and can distinguish between different concentrations of these particular elements. Ryland and Kopec [10] in 1978 conducted SEM-EDXA studies on automotive paints and were able to distinguish between all different colors of paint, using a comparison of spectral peak heights. They pointed out that there are few studies on automotive paint samples and suggested that more statistical studies were needed to assess the value of SEM-EDXA properly as an analytical tool for paint analysis.

Our study was designed to improve upon the capabilities of SEM-EDXA as an analytical technique in the identification of automotive paints, and to develop a valid and reliable testing protocol for distinguishing between automotive paints of the same or nearly the same color. SEM-EDXA/BACC was expected to be an improvement upon already existing XRF and EDXA protocols for paint analysis, and statistical studies indicate data generated through this protocol (see below) are reliable with an assigned certainty. SEM-EDXA/BACC is a rapid, nondestructive, and reliable method for ana-

lyzing the elemental composition of automotive paints. Computerized data interpretation gives results in elemental weight percentages, which facilitates distinguishing between paint samples of the same color but from different manufacturers or between the same color paints with different bases.

Experimental Procedure

Sample Preparation

Six green paint samples were taken from the 1983 Reference Collection of Automotive Paints, set up by the Collaborative Testing Services, Inc., McLean, Virginia. The samples were labeled G1 through G6; G1 and G2 were considered to be a color pair by the Reference Collection, as were G3 and G4 and also G5 and G6. The two paint chips in each color pair were made by different manufacturers (the G1/G2 and G3/G4 color pairs) or with different paint bases (the G5/G6 color pair). All three paint pairs were visibly very similar in color—light green. Paint samples from each color pair and from two different color pairs were difficult to distinguish by visual examination alone.

In preparation for analysis, each paint sample was scraped off one corner of each reference paint plate. The thickness of the sample was not measured, since X-ray penetration depth was controlled by the accelerating voltage. The resulting paint chip, approximately 2 by 2 mm, was mounted onto a graphite stub and was coated with a 500- to 600-Å layer of carbon. Carbon coatings, as opposed to other commonly used coating materials (gold and platinum), do not interfere with the detection of the X-rays of interest in paints [*11*].

Instrumentation

X-ray analyses were performed with a JEOL (U.S.A.) Inc., (Peabody, Massachusetts) JSM-35CF scanning electron microscope, equipped with a Tracor Northern (Madison, Wisconsin) Model 2000 energy-dispersive X-ray analyzer. An optical microscope attached to the SEM permitted exact alignment of the electron beam onto the specimen (BACC). A Digital Equipment Corp. PDP-11 computer and Tracor Northern software were used for data manipulation and storage.

Testing Protocol

The SEM was warmed up for at least 1 h before any X-ray analyses were performed. During warm-up, the following procedures were performed: filament load current saturation, BACC, and aperture alignment at $\times 10\,000$ magnification. BACC entailed the insertion of an optical lens in the direct line of the electron beam in the SEM column. (See Fig. 1 for the placement of the optical microscope with respect to the SEM column). By optically viewing the fluorescent pattern of the beam on a zirconium oxide crystal, the examiner can optimize its shape and alignment with the gun axis. By adjusting several solenoids (four in this instrument) and "wobbling" the condenser current, the beam can be exactly aligned within the column so that an object in correct focus would move no more than 1 μm when the beam current was changed. This permitted extremely stable beam currents throughout replicate measurements and provided the basis for reliable data. After BACC, the optical lens was removed from the SEM column. The conditions for all analyses were the following: 20-kV accelerating voltage, 0.5 nA beam current, 30-mm detector distance, 35° take-off angle (fixed on this instrument), 0° tilt, and $\times 200$ magnification (0.54 by 0.54 mm). The software parameters used were 1 000 000 integral counts per spectrum, and 0 to 10.2-eV spectral energy range. After SEM warm-up, an

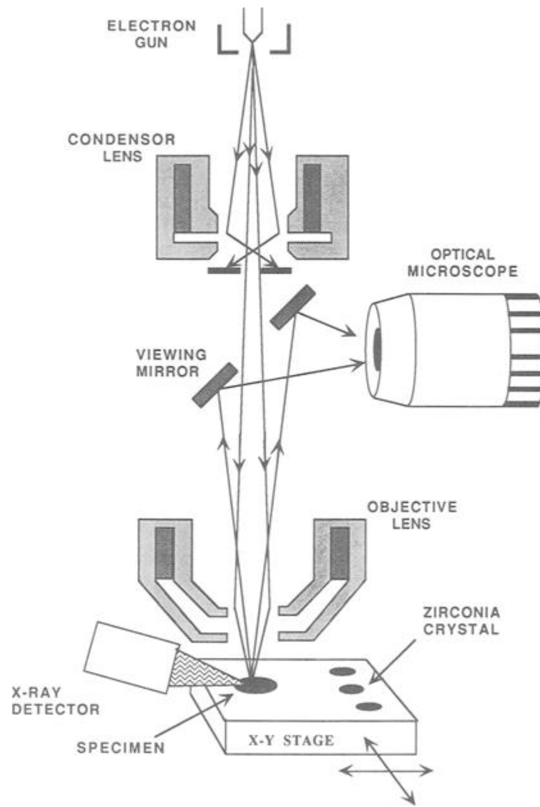

FIG. 1—*Placement of the optical microscope with respect to the SEM column.*

area of the paint sample was brought to image on the SEM screen. Fine adjustment of the beam current was made and acquisition of the X-ray data was started. Then, the paint sample was moved slightly so that another area (with the possibility of some minor overlap) was imaged, and another X-ray data acquisition was started. Five spectra from each paint sample were acquired, analyzed, and stored using this testing protocol.

Software Manipulation

The IDENT software was used to identify and display spectral peak energies and peak areas. Another program, standardless semi-quantitative analysis (SSQ), provided spectral background fit and subtraction, assignment of correction (ZAF) factors, and conversion of spectral data to elemental weight percentages.

Results and Discussion

Optimization of Experimental Parameters

A primary goal of this work was to develop a valid testing protocol for analyzing paint samples using SEM-EDXA. The various instrumental control parameters which could affect the resultant X-ray spectra were systematically studied. The total acquired X-ray

counts for the entire spectrum was chosen as a convenient parameter to hold constant (at one million) in the testing protocol. This facilitated direct comparison of spectral data and required a compromise between the parameters of magnification, accelerating voltage, beam current, and detector distance. Optimization of the magnification parameter was determined through studying X-ray spectral data acquired under several different raster areas, from ×20 to ×1000. In order to minimize the variations in elemental spectral data, the lower magnification of ×200 was chosen as the optimum magnification.

The peak-to-background ratio is a concern in validating a testing protocol. Generally, an X-ray peak height should be greater than three times the standard deviation of the bremsstrahlung height. This can be achieved through optimization of acquisition parameters, namely, by accelerating the voltage, beam current, and detector distance. Optimization of these three parameters can best be performed by close monitoring of the detector count rates and detector dead times during X-ray acquisition. Count rates can be calculated on a single channel of the energy spectrum or over the entire region of the spectrum. Basically, the greater the count rate, the greater the peak-to-background ratio. Excessive dead time (>50%) necessitates longer acquisition times, as does very low dead time (<20%). A compromise between acquisition time and dead time (33%), which gives sufficient count rates when the detector position is optimized, is generally made by the operator. Initial studies optimizing the accelerating voltage parameters were conducted at 25 kV. Most of the literature reports accelerating voltages of 25 kV with SEM-EDXA [*3,4,12*], probably to ensure that the incident electron energy is sufficient to overcome the critical excitation potential of some of the higher atomic number elements. No more elements were seen in the X-ray data using 25 kV that in the data acquired at 20 kV, so 20 kV was chosen to reduce thermal and radiation damage to the sample. The beam current optimization studies used 0.5 to 2.0 nA initially. A beam current of 0.5 nA was chosen because it produced an acceptable dead time (30%). In the JEOL JSM-35CF, the detector is fixed at 35° take-off angle but is permitted to move through a range of 17 to 90 mm from the specimen. A detector position of 17 mm created extremely high detector dead times, and so was not used. Refocusing and aligning of the electron beam within the SEM column (necessary before X-ray acquisitions on each paint sample) required that the optical microscope be moved into the direct line of the beam over the specimen. With the optical microscope in the SEM column, the X-ray detector may be only as close as 30 mm to the specimen. In a compromise between sufficiently high count rates, manageable dead times, and operator convenience, 30 mm was chosen as the best detector position for X-ray acquisition.

BACC was found to be a necessary part of the SEM start-up procedures, because it substantially increased the reliability of the data. X-ray analyses performed without BACC can show wide variations in elemental concentration in replicate measurements [*4*]. All paint chips were coated with 600 Å carbon, which reduced the charging effects to acceptable levels.

Reproducibility of SEM-EDXA

XRF studies have shown errors in elemental composition as great as 30% in a paint sample [*3*]. We observed in our data the highest standard deviations when an element is detected in only one or a few of the replicate X-ray acquisitions for a paint sample (see Table 1). The lowest errors are indicated where there are very high counts for a particular element. It is possible to decrease the percentage error for all elements by simply increasing the acquisition times, thereby increasing the elemental peak areas.

The K X-ray lines were the only emissions used in elemental comparisons. The X-ray peak area results from each paint sample normalized to a value of 10 000 for the largest peak element, titanium, are shown in Table 1. Differences in elemental composition

TABLE 1—*Normalized elemental peak area means for six paint samples.*[a]

Sample	Al	Si	S	Cl	Ca	Ti	Fe	Cu	Zn
G1	146	526	0	138	144	10 000	310	84	46
G2	47	282	0	152	80	10 000	600	90	57
G3	294	40	102	84	0	10 000	194	82	42
G4	168	376	0	116	90	10 000	156	68	36
G5	40	474	70	0	0	10 000	210	202	104
G6	138	438	54	0	0	10 000	282	56	32

[a]Normalized to a value of 10 000 for the peak area of titanium.

between all paint samples are readily apparent in the normalized data. Comparison of the data for paired paint samples, for example, G1 and G2, shows even the subtle differences in elemental compositions for chlorine, calcium, copper, and zinc. Although results expressed as relative differences in composition are satisfactory for many purposes, a conversion to weight percentage for each element by software also was done and these results are shown in Table 2. Weight percentage values can be useful when compared with manufacturer's composition values.

Another consideration in the comparison of elemental compositions between samples is the spatial heterogeneity of the paint particles over the surface of a specimen. The use of large-beam raster areas during X-ray acquisition may tend to average out this variation, but some variability is always inherent in the specimen. Automotive paint is fairly homogeneous because of its small particle size and method of application. In rare instances, we observed erratic elemental spectral data. For example, the raw data (not shown) for calcium in G3 or the calcium and sulfur in G5 suggest that the electron beam was in an appropriate position on the sample to detect these elements in only one out of the five X-ray acquisitions. This variation may reflect the inhomogeneity of the paint, rather than instrumental fluctuations in elemental detection. The use of replicate spectra minimizes the possibility of being misled by erratic data.

Discrimination of Paint Samples

Discrimination of all paint samples was possible with SEM-EDXA. Sometimes, discrimination based on the data from one element only was enough to distinguish between the two paint samples in a color pair. For example, G3 contained sulfur which G4 did not, a very obvious difference between the two samples. In other cases, widely different levels of one or many elements served to distinguish between a color pair. For example, the silicon, calcium, and titanium concentrations were much different between G1 and G2. These differences went far beyond the expected 5 to 10% instrumental error limits

TABLE 2—*Elemental weight percentage means for six paint samples.*

Sample	Al	Si	S	Cl	Ca	Ti	Fe	Cu	Zn
G1	1.28	3.02	0.00	0.41	0.94	84.66	5.33	3.00	1.28
G2	1.01	1.39	0.00	0.38	0.67	82.13	9.32	3.48	1.48
G3	2.56	0.48	0.28	0.09	0.00	89.24	3.44	2.75	1.14
G4	1.83	2.46	0.00	0.49	0.75	88.24	2.87	2.27	1.03
G5	0.70	2.40	0.12	0.00	0.00	83.30	3.51	6.29	3.00
G6	1.33	2.73	0.21	0.00	0.00	87.69	5.06	1.94	0.81

earlier reported and observed [*4,11,12*]. We also observed large compositional differences for aluminum, titanium, iron, copper, and zinc in the data for G5 and G6. In most instances, discrimination was based on the statistical one-way analysis of variance (ANOVA) of the data for all elements considered simultaneously (see Table 3). Replicate spectra were required for this purpose. Additional ANOVAs were performed on paint color pairs to distinguish between paints of the same color but from different sources or different paint bases (see Table 4). These ANOVAs also used all of the elements detected in each sample spectrum. This depth of analysis is not necessarily needed in a forensic science laboratory because, as soon as a discrepancy in elemental composition between a control sample and an evidentiary sample is found, the samples are generally not considered a match. But, on the other hand, if a match is made, then this kind of analysis should stand up very well in the courtroom.

Reliability of Software

As the SSQ program processes the spectral data, ZAF corrections are made, and a bremsstrahlung spectrum is fitted to the sample spectrum by the software. Sometimes, this fit is not very accurate. The bremsstrahlung spectrum is overlaid on the sample

TABLE 3—*One-way ANOVA results by element for six paint samples.*[a]

Element	Sum of Squares	Mean of Squares	*F* Values
Al	223 525 962	44 705 192	224.37
Si	1 006 000 000	201 102 944	387.87
S	67 759 656	13 551 932	88.68
Cl	130 916 946	26 183 388	289.15
Ca	119 019 120	23 803 824	48.08
Ti	112 200 000 000	22 450 000 000	100.21
Fe	383 352 246	76 670 458	13.15
Cu	23 261 028	4 652 206	10.59
Zn	4 939 835	987 967	7.49

[a]Degrees of freedom: factor = 5, error = 24, total = 29; $F(0.01, 5, 24) = 3.90$ (28).

TABLE 4—P *values from ANOVA by element between color pair samples.*[a]

Element	G1/ G2	G3/ G4	G5/ G6
Al	0.0776	0.0001	0.0001
Si	0.0001	0.0001	0.0001
S	0.0000	0.0001	0.0487
Cl	0.1505	0.0001	0.0000
Ca	0.0001	0.0001	0.0000
Ti	0.0001	0.2363	0.0001
Fe	0.0916	0.0007	0.0001
Cu	0.5255	0.008	0.0001
Zn	0.9697	0.0586	0.0001

[a]Undetected elements return a value of 0.0000.

spectrum using two energy regions chosen by the analyst, on each side of the sample spectrum. The predicted background on the low-energy side of the bremsstrahlung spectrum may not match that of the sample spectrum. However, readjustment of the positions of the chosen regions can improve the background fit. Every spectral fit was checked and adjusted so that the fitted bremsstrahlung closely resembled the baseline of the sample spectrum. The resultant bremsstrahlung spectrum is then subtracted from the sample spectrum, and peak areas are converted to elemental weight percentages.

Statistics

ANOVA is a statistical method [13] used to test the hypothesis that variance (standard deviation squared) of the elemental means between paint chips (with all elements considered simultaneously), s_b^2, is significantly different from the variance of the elemental means obtained within one paint chip, s_w^2. It is based on the ratio F, defined as

$$F = \frac{s_b^2}{s_w^2}$$

ANOVA will return an F value greater than the tabulated F ($F_{critical}$) for a chosen significance level if the hypothesis is true. In other words, the variation in data between paint chips is greater than the variation observed between replicate runs on the same paint chips with a certain statistical probability. The use of BACC reduces this latter type of variability. An $F_{critical}$ value of 3.90 is indicated (Table 3), for a 0.01 significance level (99% probability that the difference is real) with the appropriate degrees of freedom for these analyses. The F values shown in Table 3 are much greater than 3.90. For each color pair in Table 4 there were significant differences in elemental composition; many differences occurred at the 0.0001 significance level (99.99% probability). This confirms that the elemental compositions of the six paint chips are indeed different as analyzed by this SEM-EDXA protocol. This protocol, with BACC, thus produces data from individual paint samples which are statistically reliable and which permit identification/discrimination with an assigned confidence level. Forensic science laboratories often do not report results of examinations with accompanying statistical information, but the results of a BACC study such as this with one's own laboratory equipment can prove to be an invaluable aid in courtroom testimony.

Conclusions

SEM-EDXA as an analytical tool involves the careful control of a number of important instrumental parameters in order to produce reliable spectral data. Development of a testing protocol requires careful study and optimization of each analysis parameter and statistical analyses to validate the resultant data. Integral X-ray counts or acquisition times may be held constant so that valid comparisons can be made between spectral data. Replicate X-ray acquisitions from all samples should be performed under identical protocols. Possible spatial inhomogeneities in sample elemental composition may be reflected in spectral data and can be minimized by the use of low magnifications, longer acquisition times, and a greater number of replications. Most important, beam alignment by current centering provides extremely stable beam currents which produce data of substantially greater reliability. Discrimination of all samples may be accomplished through software manipulation, giving spectral data in elemental weight percentages, through statistical analyses, or through simple relative comparison of normalized data.

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