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Comparison of Minute Smears of Lipstick by Microspectrophotometry and Scanning Electron Microscopy/Energy-Dispersive Spectroscopy

ABSTRACT: Minute lipstick smears are sometimes found as evidence on clothing, cigarette butts, bedding, and miscellaneous crime scene surfaces. Traditional techniques, such as visible-absorption spectroscopy, and paper and thin-layer chromatography, are not always suitable for comparison of the trace amounts of lipstick which usually occur in actual casework. Microspectrophotometry and scanning electron microscopy (SEM), in combination with energy-dispersive spectroscopy (EDS) were evaluated for comparison of minute lipstick samples. Microspectrophotometry proved very promising in differentiating lipstick samples of nearly the same color but with minor shade differences. Backscattered electron imaging followed by X-ray microanalysis was used as an additional method for discrimination purposes. Both of these techniques can rapidly analyze microscopic samples of lipstick.

KEYWORDS: forensic science, lipstick, microscopy, spectroscopic analysis, scanning electron microscopy, backscattered electron imaging, X-ray microanalysis, microspectrophotometry

When lipstick stains are found on fabric or paper-like materials, the usual question is whether or not the stain or stains could have originated from a control lipstick. Such information can suggest a link connecting the victim and the subject, or the victim and the crime scene, or all three. As such, lipstick comparisons can be of considerable value, depending on the circumstances of a case.

In forensic science, comparative examinations are usually based on the physical or chemical nature of a substance, or both. Lipsticks are composed of waxes, oils, organic dyes, and inorganic pigments [1]. In addition to visual or microscopic examination, and paper or thin-layer chromatography [2], a variety of instrumental techniques have been used for comparison of lipstick samples. These methods include visible-absorption spectrophotometry [1] and high-performance liquid chromatography (HPLC) [3–5] for the analysis of color or dye components in the lipsticks. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) [6,7] have been used for the characterization of waxes, whereas X-ray fluorescence and X-ray microanalysis by scanning

electron microscopy (SEM) [8] have been reported for the study of elemental compositions of inorganic pigments. All these techniques are widely accepted and can produce valid results. However, most are complicated or have limitations due to the sample size requirements or to background interference, or both.

This report describes the application of microspectrophotometry and X-ray microanalysis for characterization of two major features of lipsticks: the color and the elemental composition of pigment particles in lipsticks.

This study was initiated when a questioned document with a lip impression bearing lipstick and a control lipstick were submitted to this laboratory for comparative examination. Both of the methods were successfully used to resolve the case.

Material and Methods

Thirty samples of lip impressions bearing lipstick were obtained on white sheets of bond paper using various brands and types of lipsticks, which were available from volunteers. The colors and respective brands of these lipstick samples are given in Table 1.

Mounting of Specimens

A minute section (approximately 1 mm^2) from the upper surface layer of the paper bearing a lipstick smear was removed with the tip of a surgical blade. This section was then divided into two halves. One half was mounted on a microscope slide for micro-

TABLE 1—*Summary of lip impressions bearing lipstick.*

Sample No.	Lipstick Color	Manufacturer/Brand Name
1	Ripe Nectarine (A46)	Clinique
2	A Different Grape (AB2)	Clinique
3	Golden Raisin (A40K)	Clinique
4	Honey Ginger	Clinique
5	Roseberry Stain	Clinique
6	Grape Iris	Clinique
7	Wine-on-Ice No. 78	Maybelline
8	Wine and Roses	Maybelline
9	Moisture Whip Frost 87	Maybelline
10	Long-Wearing Lipstick	Maybelline
11	Moisture Cream	Revlon
12	Super Lustrous Cream 59	Revlon
13	Super Frost Strawberry Ice	Revlon
14	Iced Sangria 38	Lucy Holliday's Cosmetics
15	Frosted Russet	Merle Norman
16	Sky Red (1B)	l'Erin
17	Nutmeg No. 215	l'Oreal
18	Lip Swirl	Anathashia
19	Lip Swirl No. 22	Anathashia
20	Lip Swirl No. 6	Anathashia
21	Crystal Pink Frost 08461	Quencher Lipstick
22	Romantic Rose CI 8078	Fashion Fair Dist.
23	Pure Plum 8025	Fashion Fair Dist.
24	Color-Fast Daring Rose	Max Factor & Co.
25	Wine	Max Factor & Co.
26	French Raspberry	Aloette
27	Raspberry	Cardeaux Cosmetics
28	Twilight Pink 2975	Avon Products, Inc.
29	Lotus Pink	Avon Products, Inc.
30	Pink	Avon Products, Inc.

spectrophotometry. A stereomicroscope was used throughout the process of specimen removal and subsequent mounting on microscopic slides or graphite specimen mounts. Permount (refractive index 1.51; Fisher Scientific Co.) was used as the mounting medium. The mounting medium helps to eliminate the opacity of the fibrous material.

A strip of approximately 6 by 8-mm Double-Sided Coated Tape® (3M Co.) was applied to standard graphite 10-mm SEM specimen mounts [9,10], and the other half of the lipstick sample was mounted. In order to make the mounted samples conductive, a carbon coating of appropriate thickness was applied using a carbon-evaporating accessory (Model No. Hummer VI of Anatech LTD., Alexandria, Virginia).

The mounts with the lipstick samples were placed in the specially designed specimen holder described in the next section.

Design of the SEM Specimen Holder for X-Ray Microanalysis

When samples are analyzed using a brass SEM specimen holder, it introduces its own background of copper and zinc. This background is more pronounced when analysis is conducted at a shorter working distance (15 mm) and with a higher accelerating voltage (25 kV). Therefore, a special specimen holder, capable of holding four standard 10-mm graphite specimen mounts (Fig. 1), was fabricated from an acrylic rod of 31.93-mm diameter. The top surface and all four wells of the specimen mounts were coated with graphite conductive paint. The lower surface and surrounding walls were coated with gold/palladium to make the specimen holder fully conductive and grounded through the SEM stage.

Microspectrophotometric Analysis of Lipstick Samples

The minute differences in the colors of the lipstick samples were studied using a Nanospec 10S microspectrophotometer (Nanometrics, Inc.) in combination with a Reichert Zetopan light microscope. Transmission spectral studies were conducted from 400 to 700 nm at a speed of 240 nm/min using a ×40 objective and a 7 by 35-μm projected slit image. The full-scale and zero suppression values were varied for each spectrum. These
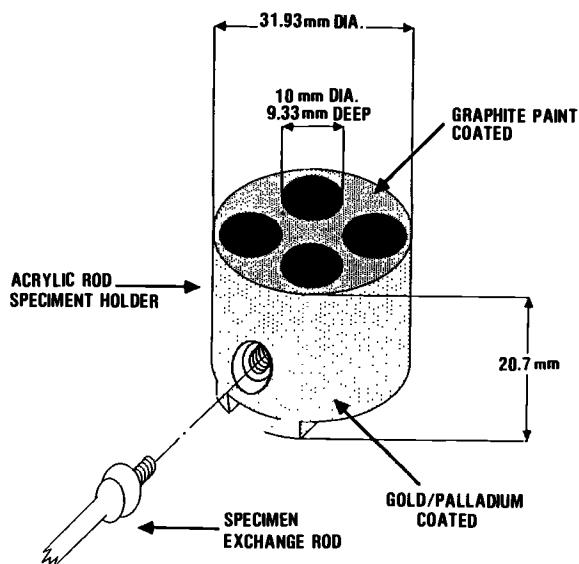

FIG. 1—Nonmetallic SEM specimen holder fabricated from acrylic material.

values were determined by scanning each sample from 400 to 700 nm and then subtracting the observed minimum output intensity from the maximum output intensity ($I_{max} - I_{min} = X$). The full-scale value was then established by adding an arbitrary factor of 10 to the difference in the maximum and minimum intensity values ($X + 10$), whereas the zero suppression value was determined by subtracting 5 from the minimum intensity output. The blank spectrum of the slide, cover slip, and Permount was obtained from an area directly adjacent to the lipstick sample and stored in the memory. This blank spectrum was then automatically subtracted from the subsequent absorbance spectrum of the sample.

Elemental Analysis of Pigment Particles

The scanning electron microscope (JEOL Model JSM35CF), which was equipped with an annular type backscattered electron detector, was used to visualize the lipstick pigment particles. Subsequently, these particles were analyzed at higher magnification ($> \times 4000$) for their elemental compositions, using a Tracor Northern energy-dispersive system (Model TN 5500). All of the specimens were analyzed at a 15-mm working distance, a takeoff angle of 14°, and an accelerating voltage of 25 kV.

Results and Discussion

Comparison of the 30 samples of lipstick by their visible absorbance spectra disclosed that they were distinguishable from each other. For example, Fig. 2, *a* and *b*, shows the spectral analysis of two different areas of the same red color lipstick sample (No. 1). The identical patterns indicate the reproducibility of the method, and that the substrate material did not interfere with the analysis. The Spectrum 2c from another lipstick of a similar red color (Sample No. 4) from the same manufacturer was clearly distinguishable from Spectra 2a and 2b. This indicates that the technique is capable of discriminating visually indistinguishable samples from the same manufacturer.
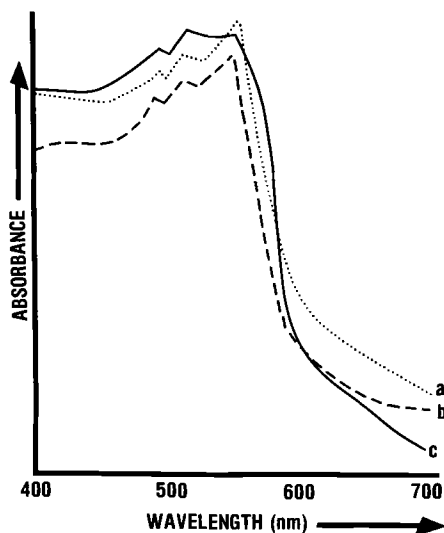

FIG. 2—*Visible absorbance spectra (a,b) of different areas of a sample (No. 1) showing reproducibility; Spectrum 2c illustrates the variability in a sample of similar color from the same manufacturer.*

Spectra a, b, and c in Fig. 3 were obtained from Samples No. 7, 25, and 29, respectively. All were a similar violet-pink shade but were from different manufacturers. These spectra are clearly dissimilar in their spectral pattern even though they were indistinguishable microscopically.

The method was successfully applied to distinguish the control and questioned lipsticks from the aforementioned case. Figure 4 shows spectra of the (a) questioned and (b) control samples. The grossly dissimilar pattern of these spectra suggests that the questioned stain was not produced by the control lipstick.

Unlike microspectrophotometry, substrate paper analysis presented a background problem when samples were analyzed by energy-dispersive X-ray microanalysis. The elemental compositions of minute pigment particles in the samples were not detectable because of the high background of the paper material when analyzed in the energy-dispersive spectroscopy (EDS) or "area scanning" mode of the SEM. As shown in Fig. 5, no major differences were noted between the elemental profiles of the paper material and the control lipstick smear present on the same bond paper. Since the relative amounts of elements in paper varies from one area to another, simple subtraction of background spectra, as suggested by Andrasko [8], could not be applied to resolve the background problem. Furthermore, there is no known way to determine the exact composition of the paper under a questioned lipstick smear.

As shown in Fig. 6a, the pigment particles present in the lipstick smear on paper were not visible in the secondary electron image but were clearly visible in the backscattered electron image (Fig. 6b). Backscattered electron imaging, by eliminating the undesired background, helped to visualize the pigment particles and thus facilitated the elemental analysis of the microparticles. The elemental composition of Samples No. 1 through 30 is given in Table 2. These samples were distinguished by their major elemental compositions and differences in their approximate relative ratios.

Figure 7, showing the particle analysis of four lipstick samples (Nos. 1 through 4) from the same manufacturer, indicates that the pigment formulation varies in different lipsticks even from a single manufacturer.

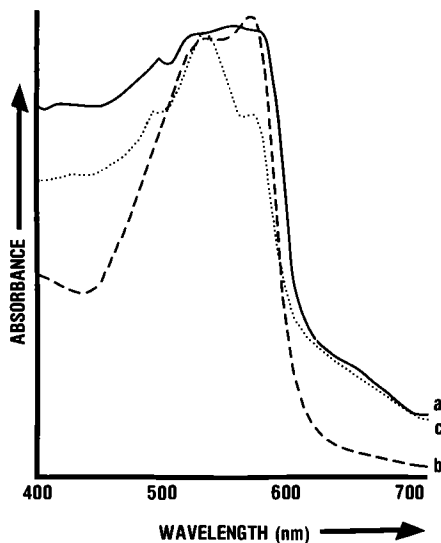Figure 8, a and b, shows the particle analysis of the control and the questioned lipsticks,

FIG. 3—Visible absorption spectra of lipstick samples of similar shade from different manufacturers.
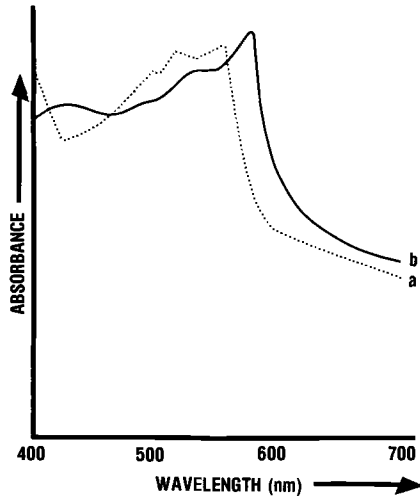
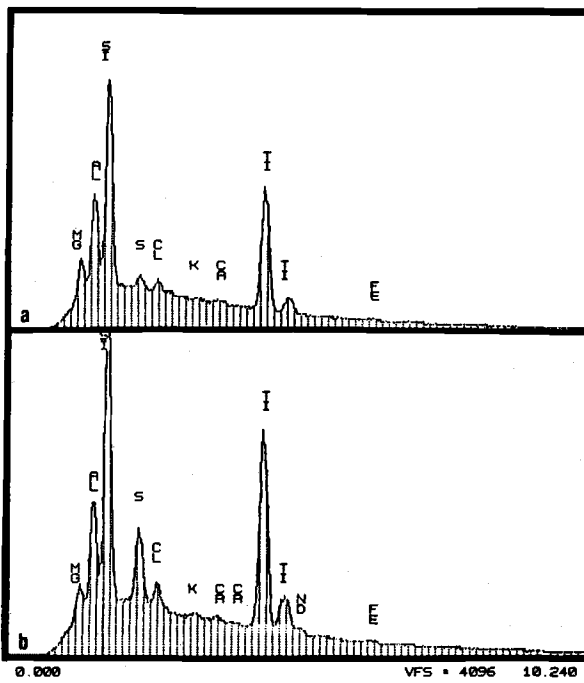FIG. 4—*Comparison between the visible absorbance spectra of questioned and control lipstick samples.*

FIG. 5—*Elemental profiles of* (a) *blank bond paper and* (b) *a lipstick smear on the bond paper by area scanning mode of the SEM.*
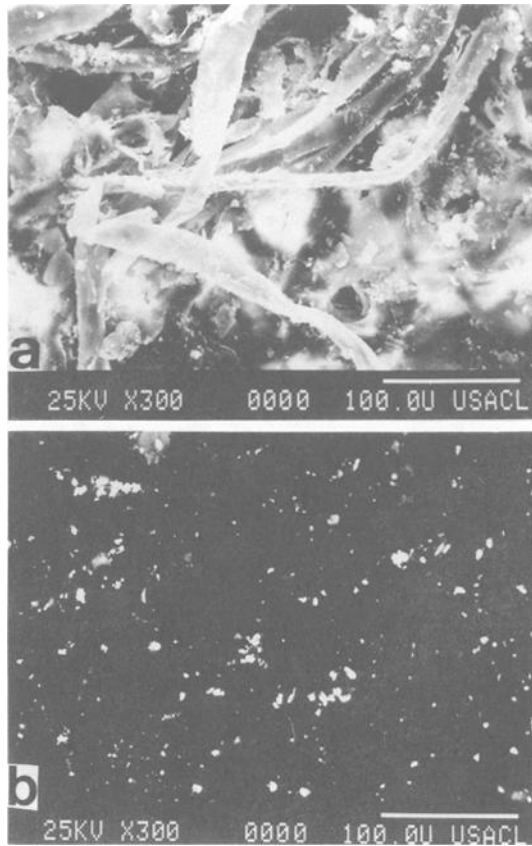
FIG. 6—*Electron micrographs of a lipstick smear:* (a) *secondary electron imaging and* (b) *back-scattered electron imaging of the same area.*

respectively, from the case. The obvious difference between the two spectra was the barium in the control lipstick (Fig. 8*a*), whereas the questioned smear showed bismuth as a major component (Fig. 8*b*). Thus, the lipstick smear present on the questioned document was clearly distinguished from the control lipstick.

Conclusions

Microspectrophotometry offers a rapid and objective means of comparing minute amounts of lipsticks of apparently similar color or with minor differences in shade, even from a single manufacturer. The thin layer of paper material bearing the lipstick smear did not significantly affect the reproducibility of spectra from one area to another.

Backscattered electron imaging proved to be more appropriate for X-ray microanalysis of the pigment particles, especially when a minute amount of lipstick stain is present on paper. Particle analysis by SEM/EDS of lipstick stains can distinguish between samples on the basis of their elemental compositions and differences in their relative ratios. The technique may be useful for identifying samples where different pigment formulations are used for similar shades of lipstick made by different manufacturers. The technique

TABLE 2—*Elemental analysis data.*

SAMPLE NUMBER	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Cr	Fe	Cu	Zn	Mo	Ba	Bi
1			S	S	T	S	S	S	T	M		L	T				
2			L		T	S		T	M			T	T	T	M		
3			T							S			T				L
4			S	S		M										M	
5			T	T		M										M	
6			T						T			T	T				M
7			M	M		M	T	S					T			L	
8		T	S	S		M	S	T	T			S	S	S		M	
9			S	S		M		T								M	
10						M										M	
11			S	S		L	T	T	T							M	
12										S							M
13			S	S		M	T	S				S				M	
14			M	M		S	S	M	L			S	T				
15			L	L		T	T	L	M			S		T			
16	T		S	S		L	T	S	S				T	•		L	
17				S		L										L	
18			S	S		M	S		T			S	T	T		L	
19			S	S		L	S		S				T			L	
20				T	T	T	T				T	M	M	T			
21						M							T			M	
22			T	T		S		T								M	
23			S	S		M										L	
24			S	S		M		S								M	
25		S	S	L		S	S	S	S	S		S					
26		T	T	T								T	T	T			M
27			S	S		L	S		S				T	T		L	
28						M										M	
29		S	S	S		M										M	
30	T		S	S		M										M	

RELATIVE AMOUNTS

L — LARGE
M — MEDIUM
S — SMALL
T — TRACE

of backscattered electron imaging followed by X-ray microanalysis is very useful in eliminating the inherent background problem of the paper material and in determining the actual elemental composition of the pigment particles.
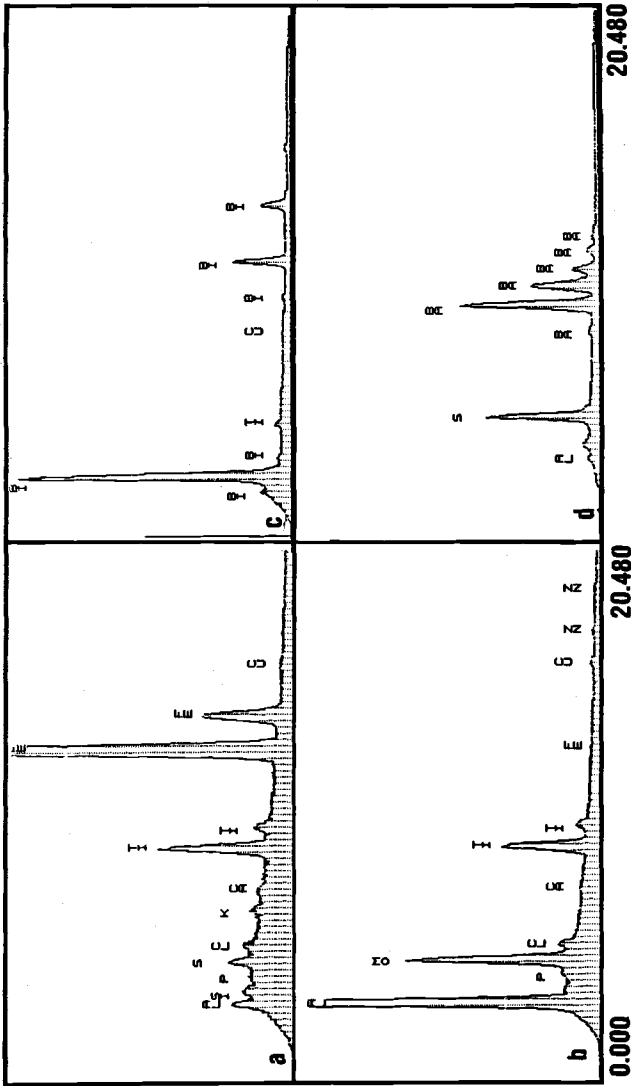
FIG. 7—Particle analysis of four lipstick samples (Nos. 1 through 4) from the same manufacturer (Clinique).
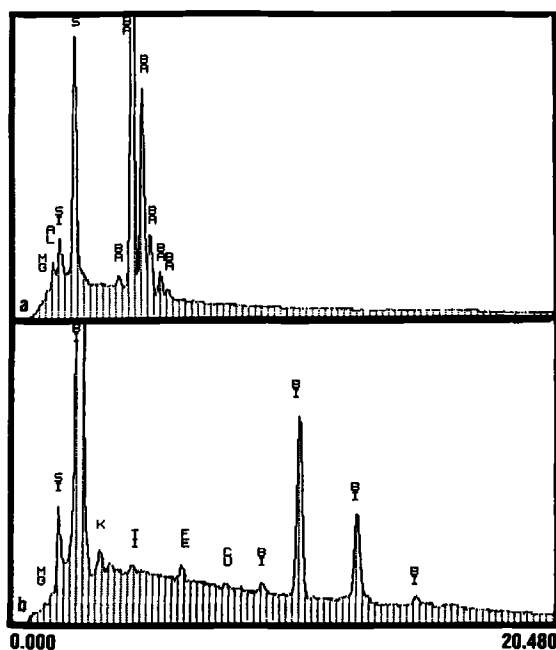
FIG. 8—*Particle analysis of* (a) *control and* (b) *questioned lipstick samples.*

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