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## FORENSIC ANALYSIS OF BLACK MASCARA VIA MICROSCOPY, FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR), SCANNING ELECTRON MICROSCOPY-ENERGY DISPERSIVE SPECTROSCOPY (SEM-EDS), AND VISIBLE MICROSPECTROPHOTOMETRY (MSP)

presented by

JoAnn I. Bilek

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## FORENSIC ANALYSIS OF BLACK MASCARA VIA MICROSCOPY, FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR), SCANNING ELECTRON MICROSCOPY-ENERGY DISPERSIVE SPECTROSCOPY (SEM-EDS), AND VISIBLE MICROSPECTROPHOTOMETRY (MSP)

By

JoAnn I. Bilek

## A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

## **MASTER OF SCIENCE**

School of Criminal Justice

#### ABSTRACT

## FORENSIC ANALYSIS OF BLACK MASCARA VIA MICROSCOPY, FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR), SCANNING ELECTRON MICROSCOPY-ENERGY DISPERSIVE SPECTROSCOPY (SEM-EDS), AND VISIBLE MICROSPECTROPHOTOMETRY (MSP)

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#### JoAnn I. Bilek

Four analysis techniques were utilized in this project in an attempt to differentiate between twenty-four black mascara samples from fourteen different brands. A sample of black lash tint was analyzed along with the mascara samples, but was easily distinguished from the mascara samples by each analysis technique.

Microscopic examination by traditional light microscopy separated the mascara samples into four groups. Analysis by Fourier transform infrared spectroscopy (FTIR) was able to separate the samples into twenty-two groups. The analysis by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) excluded and distinguished between 99.67% of the 600 comparisons based on calculated weight percent ratios. The elements which were quantified and compared were iron, silicon, magnesium, aluminum, and sodium. The analysis performed by visible microspectrophotometry (MSP) did not yield any discriminatory data for the mascara samples in this study.

A proposed analysis scheme for black mascara based on the findings of this study involves a preliminary examination by microscopy, followed by FTIR and SEM-EDS analysis. The overall incorporation of these three techniques to this study left only two samples from being distinguished from one another. The indistinguishable samples were from different mascara brands, but both share the same manufacturer.

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#### **1. INTRODUCTION**

Cosmetics are potentially important forensic evidence because most women apply them on a daily basis. Cosmetics may not be found at every crime scene, but when they are present it is important to have a scheme for the analysis of or knowledge of the significance of such evidence. A woman who is sexually assaulted, or the victim of a violent crime, may transfer her cosmetics to her attacker. If a woman is smothered with a pillow or blanket, the transfer of her cosmetics may help identify the murder weapon or even the murderer.

Trace evidence is seldom used to positively identify a questioned sample as having originated from a known sample and no other source. However, if the examiner concludes that the questioned sample could have originated from the known sample, along with additional circumstantial evidence, than the likelihood of guilt will also accumulate. Although trace evidence may not always lead to a positive or negative conclusion by itself, it should not be disregarded or deemed irrelevant.

## 1.1 Mascara

Mascara is a commonly used eyelash cosmetic. The purpose of mascara is to darken, thicken, and lengthen eyelashes (1). Liquid mascara is the modern formulation used today, while cake and cream mascaras are the older, less encountered formulations. Liquid mascara can be divided into water-based, solvent-based, and water/solvent hybridbased (1). Water-based mascaras are classified as oil-in-water emulsions, and are formulated by dispersing waxes, pigments and resins in water. Solvent-based mascaras are formulated with petroleum distillates or other hydrocarbon solvents to which

pigments and waxes are added, making them waterproof. Hybrid-based mascaras combine both water and solvent-based formulations to form either a water-in-oil or oil-inwater emulsion (1). The common waxes used in the formulation of mascara are beeswax, carnauba wax, candelilla wax, and paraffin. Some examples of pigments used in the formulation of mascara are iron oxides, chromium oxides, ultramarine blue, carmine, and titanium dioxide.

Mascara is best classified as a surface coating. The following terminology from the coatings industry pertains to mascara: surface coating, pigment, and vehicle. A surface coating is a suspension of a pigment in a vehicle designed for the protection of a surface or for decoration, or both (2). A pigment is a finely powdered solid that is insoluble in the coating in which it is dispersed. Pigments impart color to a coating and are usually inorganic (2). A vehicle is a portion of a surface coating other than the pigment, which enables the pigment to be dispersed over the surface (2). Mascara is hence a suspension of pigments in a wax vehicle.

In a case submitted to the Michigan State Police (MSP) forensic science division a few years ago, a wash cloth with what was believed to be mascara was submitted as evidence along with numerous mascara tubes belonging to a murder victim and several suspects. The deceased was found in her bathroom, which is where the homicide was thought to have occurred. The investigators hypothesized that the murderer may have used the wash cloth to clean blood off of her face and in the process transferred her mascara. The forensic scientist who was given the case, Ms. Cheryl Lozen of the Northville MSP Forensic Laboratory, analyzed the questioned and known mascara samples, along with ten known outside samples, using Fourier transform infrared

spectroscopy (FTIR) in order to compare the chemical composition of each sample. Ultimately, Ms. Lozen determined that all of the samples could be differentiated from one another based on their spectra and that the mascara submitted as evidence could have originated from one of the mascara tubes belonging to the victim (3). Had the determination been that the mascara evidence could have originated from one of the mascara tubes belonging to a suspect, the evidence could have assisted the investigators had it supported other circumstantial evidence.

There are hundreds of black mascaras on the market today, and these same types of mascara are also available in numerous colors. An exploratory study to determine the best approach(s) to examining mascara is required prior to a more exhaustive sample study of the cosmetic. Black mascara is the most commonly worn mascara, hence was chosen to be analyzed in this study.

#### 1.2 Analysis techniques

## A. Microscopic Examination

Traditional light microscopy is used as an analysis technique in this research because it is an important tool for the preliminary examination of trace evidence. The microscope is a standard piece of equipment in a forensic laboratory, which will not consume or change the evidence sample being examined.

#### B. FTIR

Fourier transform infrared spectroscopy (FTIR) is a common technique used for analyzing trace evidence in forensic laboratories. FTIR is used to analyze paint, fibers, adhesives, as well as other substances.

In infrared spectroscopy, infrared light is focused on the sample being analyzed. The molecules in the sample absorb the infrared radiation causing the chemical bonds in the sample to vibrate. Functional groups tend to absorb infrared radiation in the same wavenumber range regardless of the remaining structure of the molecule. Thus, the vibrations detected at specific frequencies are characteristic of certain functional groups creating a correlation between the wavenumbers at which a molecule absorbs radiation and its structure. A spectrum is then produced that displays the wavenumbers vs. the percent of light transmitted for the sample analyzed.

Infrared spectroscopy is used in the analysis of coatings, such as paint, to characterize the vehicle, which is primarily organic. The wax vehicle used in mascara is comprised of hydrocarbons. FTIR is a technique that can identify such functional groups and allow for the comparison of samples based on chemical composition.

Unfortunately, there are also disadvantages to this analysis technique. Pigments and other inorganic components are weak absorbers in the infrared region and are not often detected. In addition, the analysis of complex mixtures gives rise to complex spectra leading to difficult spectral interpretation and the masking of minor component peaks by major components.

## C. SEM-EDS

Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) may also be used for forensic trace evidence analysis. This technique is commonly used for the elemental analysis of glass, explosives and gunshot residue.

In SEM, a beam of electrons is used to excite the electrons on the surface of a sample. These excited electrons, or secondary electrons, are emitted from the sample and collected by a detector that ultimately generates a magnified image of the sample. X-rays, which have a specific energy characteristic of the element that produced it, are also emitted from the sample surface. The SEM may be coupled with an x-ray detector allowing for the detection and spectroscopic analysis of the emitted x-rays. The elements in a sample can then be determined by measuring the energy of the x-rays produced, also referred to as energy dispersive spectroscopy (EDS).

The use of elemental analysis by SEM-EDS will allow for the quantitation of inorganic components in mascara, such as elements present in the pigments and additives. Unfortunately, this technique will not produce quantified data for specific compounds; however the overall weight percent of specific elements in a sample will allow for the differentiation of samples if properly compared.

#### D. Visible MSP

Visible microspectrophotometry (MSP) is a technique used to discriminate between samples on the basis of sample interaction with light in the visible region (400-700 nm). This instrument yields absorption spectra displaying the wavelengths at which a sample absorbs visible light. The MSP is used for distinguishing between closely colored materials, which could not be distinguished using the naked eye. This technique may be applied to evidence such as paint, fibers, and ink.

The mascara samples to be analyzed in this project are black. Typically black objects are referred to as achromatic, because they absorb all wavelengths and lack what is perceived as color. In a recent forensic science thesis by Kristen Kopchick, several apparently black, achromatic automotive paint samples displayed positive spectral results when analyzed using visible microspectrophotometry (4). The positive spectral results were the consequence of chromatic secondary pigments added to the paints for an intended color effect or the formulation of a unique coating (4). Although chromatic pigments may not be observed in achromatic paint samples in reflected light, discriminating data may be obtained by utilizing visible MSP. The application of chromatic secondary pigments may also occur in the formulation of black, achromatic, mascara samples.

## 2. MATERIAL AND METHODS

# 2.1 Samples

Twenty-four different mascaras and one lash tint from fourteen brand names were purchased from a local store. All of the samples are labeled as a shade of black. Detailed sample and manufacturer information is displayed in Table 1 and 2.

Brand	Mascara Name	Based	Color	
Almay®	Bright Eyes®	Water	Black	
Almay®	Bright Eyes® Waterproof	Hybrid	Black	
Aziza II	Lengthening	Water	Black	
Bonne Bell®	Eye Style <sup>™</sup>	Water	Basic Black	
Cover Girl®	Professional Smudgeproof	Water	Black	
Cover Girl®	Professional Waterproof	Solvent	Black	
Jane	Hi-Fiber	Water	Basic Black	
Jane	Hi-Fiber Waterproof	Hybrid	Basic Black	
L'Oreal®	Voluminous®	Water	Black	
L'Oreal®	Voluminous® Waterproof	Hybrid	Black	
Max Factor®	2000 Calorie	Water	Rich Black	
Max Factor®	2000 Calorie Aqua Lash®	Solvent	Rich Black	
Maybelline®	Great Lash®	Water	Very Black	
Maybelline®	Great Lash® Waterproof Solv		Very Black	
Maybelline®	Volum' Express® Ultra-Thick Waterproof	Hybrid	Very Black	
Neutrogena®	Full Volume®	Water	Rich Black	
Physicians Formula	To Any Lengths®	Water	Midnight Blk.	
Revlon®	Colorstay® Extra Thick Lashes	Water	Black	
Revlon®	Colorstay® Ex. Thick Lashes Waterproof	Hybrid	Black	
Revlon®	Colorstay Overtime <sup>™</sup> Lash Tint	Solvent	Black	
Rimmel	Extra Super Lash	Water	Black	
Rimmel	Extra Super Lash Waterproof	Solvent	Black	
Sally Hansen®	Maximum Growth®	Water	Blackest Blk.	
Wet'n'Wild®	Protein Mascara	Hybrid	Black	
Wet'n'Wild®	H2O Proof <sup>™</sup> Waterproof	Solvent	Blackest Blk.	

Brand	Manufacturer/Distributor
Almay®	Revlon, Inc.
Aziza II	Inter Parfums, Inc.
Bonne Bell®	Bonne Bell Company®
Cover Girl®	Procter & Gamble
Jane	Jane and Company LLC
L'Oreal®	L'Oreal Group
Max Factor®	Proctor & Gamble
Maybelline®	L'Oreal Group
Neutrogena®	Neutrogena Corp.
Physicians Formula	Physicians Formula Cosmetics, Inc.
Revlon®	Revlon, Inc.
Rimmel	Coty, Inc
Sally Hansen®	Del Laboratories, Inc.
Wet'n'Wild®	Markwins® Beauty Products, Inc.

Table 2: Brands and manufacturer information for mascara samples

The samples were labeled as water, hybrid or solvent-based according to the ingredients listed on the manufacturer packaging of each mascara tube. A sample was designated as water-based if water was listed as the primary ingredient. The solvent-based samples listed petroleum distillates or isododecane as the primary ingredients and did not contain water. The hybrid-based samples listed water and a hydrocarbon solvent, such as isododecane, as the primary ingredients.

Thirteen of the samples are water-based, six of the samples are solvent-based, and the remaining six samples are a water/solvent hybrid-based. In order to simplify the nomenclature involved in sample identification, the mascara samples will be designated by brand and either water, solvent, or hybrid-based as opposed to the actual mascara name.

## 2.2 Microscopic Examination

Microscopy is a technique that may be employed quickly, without sample consumption. Sample preparation for analysis was achieved by spreading a thin layer of each sample onto a clean microscope slide using the applicator wand located on the inside cap of each mascara tube. A cover slip was placed onto the slide with enough pressure to flatten the sample. The samples were allowed to dry and then examined via traditional light microscopy with an Olympus BX41 microscope at 400x magnification. Polarized light microscopy (PLM) was not implemented in this study due to the author's limited experience with the technique.

## 2.3 FTIR

Three studies were performed using FTIR to analyze the mascara samples. As before, sample preparation involved spreading a thin layer of each mascara sample onto a clean microscope slide using the mascara tube applicator wand. Each sample to be analyzed was transferred from the microscope slide with a metal spatula and smeared onto a potassium bromide (KBr) disk. The KBr disk was then placed onto the FTIR microscope stage for analysis. The instrument utilized was a Perkin Elmer Auto Image FTIR Microscope. A background scan was performed prior to the collection of each sample spectra, which was acquired with 16 scans. The baseline of each spectrum was corrected using the Spectrum 1.0 software.

Initially a drying study was performed to determine when each type of mascara sample would be dry; this would be based on the lack of excessive variation between spectra over time. The water, hybrid, and solvent based mascaras of the Maybelline

brand were analyzed at the following time increments: fresh, after four hours, eight hours, one day, two days, three days, and four days.

After the appropriate drying time was determined, a reproducibility study was performed. This study was conducted to assure that the three types of mascara would give consistent spectra between analysis of the same sample smear and different smears of the same sample. The water, hybrid, and solvent based mascaras of the Maybelline brand were analyzed on ten separate occasions within the same day to determine whether the spectra were reproducible.

Finally each of the twenty-five mascara samples was analyzed using the instrument after the time period determined by the drying study.

#### 2.4 SEM-EDS

The mascara samples dried on the microscope slides were placed in a desiccator under vacuum for a week in order to maintain them in a dry atmosphere. A small amount of each of the samples was scraped from the microscope slide and mounted on an aluminum stub using double sided carbon tape. The samples were made conductive using an Ernest F Fullam Inc.EFFA MKII Carbon Coater. The sample holder used to place the samples inside the SEM sample chamber held four sample stubs. The samples were analyzed using a JSM-6400 Scanning Microscope coupled with a Thermo Electron Corporation X-ray Detector. The samples were analyzed under high vacuum at an accelerating voltage of 25,000 volts. Samples were analyzed at a working distance of 15 mm at 500x magnification, with a scanning area of 240 microns by 180 microns for 100 live seconds.

The following fifteen elements were detected during trial sample runs: carbon (C), oxygen (O), sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), titanium (Ti), manganese (Mn), iron (Fe), and copper (Cu). However, only the following elements were selected for quantitation: iron (Fe), silicon (Si). magnesium (Mg), aluminum (Al), and sodium (Na). The elements were selected based on the ingredients listed on the packaging of the mascara samples and the abundance of the elements determined during several trial sample runs. Iron is present in iron oxide, a common pigment in mascara, while the remaining elements are present in numerous additives used to manufacture mascara.

The above analysis was performed three times, each time in a different location, for each mascara sample. The calculated weight percent of each element mentioned was provided by the Vontage 1.5.1 software by Noran Instruments, which applies a ZAF correction to compensate for matrix effects and provide accurate quantitation.

## 2.5 Visible MSP

The dried mascara samples on the microscope slides were individually analyzed by Dr. Jay Siegel using a CRAIC QDI 2000, UV-visible-near IR Microspectrophotometer. The instrument was initially calibrated using NIST standards. Dark and reference scans were performed prior to the analysis of each sample. The samples were each analyzed in five different areas using ten scans.

#### **3. RESULTS AND DISCUSSION**

#### 3.1 Microscopic Examination

Microscopic examination of the samples resulted in the differentiation of only some of the samples. All of the samples consisted of black particles, except for the lash tint, Revlon- solvent, sample. This sample contained red, blue, and yellow pigments.

The remaining twenty-four samples could then be separated into the following four groups: contains orange flakes, contains blue specks, contains brown particles, and could not be distinguished. Two of the samples contained orange flakes, the Almayhybrid and Almay- water samples. Two of the samples contained blue specks, the Aziza II- water and L'Oreal- hybrid samples. Two of the samples contained brown particles, the Cover Girl- solvent and Max Factor- solvent samples. The remaining eighteen samples could not be distinguished by microscopic examination. The complete microscopic examination observations are displayed in Appendix A, Table 5.

Four of the samples were observed to contain fibers. Nylon and rayon fibers are sometimes added to mascara to increase the length of eyelashes when applied. These fibers could be identified with the use of polarized light microscopy, which allows for the observation of interference colors in anisotropic substances between crossed polars, and the Michel-Levy chart, which in turn allows the microscopist to identify a substance based on thickness and observed interference colors. The presence of these fibers is significant, however the absence should not be considered important when comparing mascara samples. The fibers may or may not transfer with the mascara, thus the lack of fibers in a questioned sample should not eliminate it from originating from a known sample containing fibers.

## **3.2 FTIR**

The drying study determined that the Maybelline-water, solvent, and hybrid mascara samples displayed the least amount of variation after two days of drying time. The peaks which changed over the time increments in the study were not identified because any questioned mascara submitted as evidence would likely already be dry prior to laboratory analysis. In the event that known mascara samples from manufacturer tubes are analyzed for comparison with a dry questioned sample, these samples must also be dried for two days prior to analysis in order to perform a proper comparison.

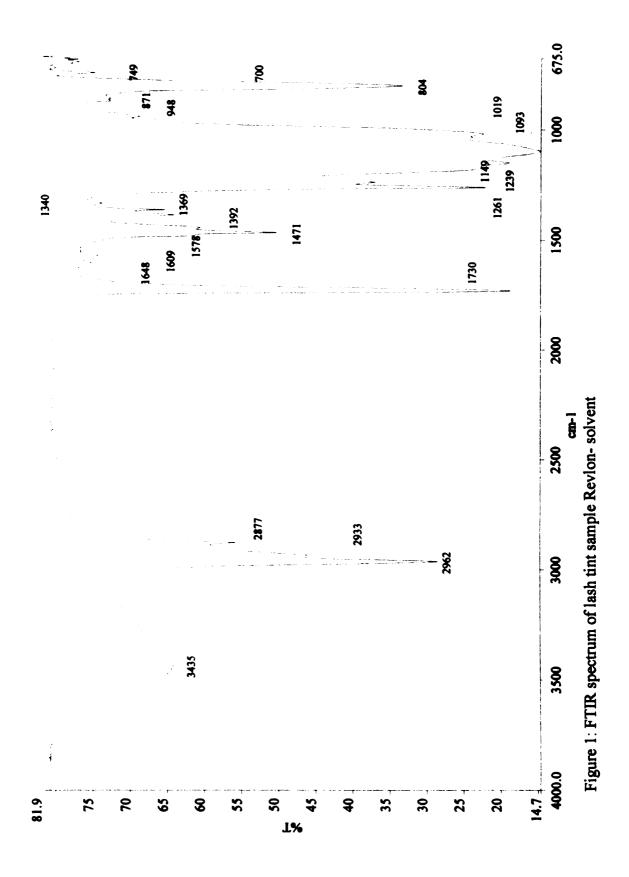
The reproducibility study provided reproducible spectra for the Maybellinewater, solvent and hybrid mascara samples. The ten spectra produced for each of the mascara samples rendered peaks which were consistent within the variation normally observed between spectra in FTIR analysis.

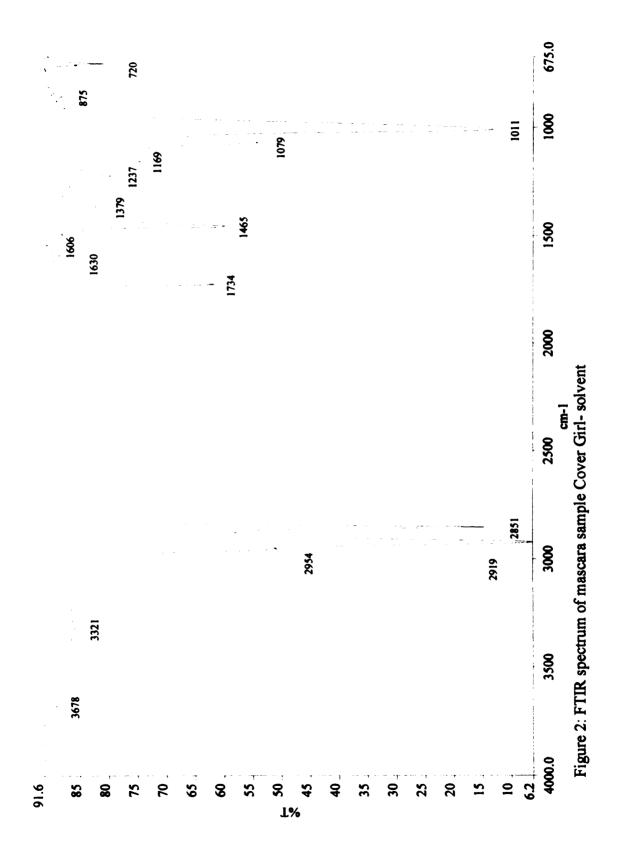
The FTIR spectra of beeswax, carnauba wax, candelilla wax and paraffin wax displayed a strong absorption band in the 2970-2830 cm<sup>-1</sup> range, accompanied by absorption bands in the 1480-1455 cm<sup>-1</sup> and 735-710 cm<sup>-1</sup> ranges (5). These absorption bands are characteristic of long chain hydrocarbons, which are the constituents of wax. Beeswax, carnauba wax, and candelilla wax also display an absorption band in the region between 1750 and 1680 cm<sup>-1</sup>, which is characteristic of a carbonyl group (-COOH). This band may be indicative of cerotic acid, a fatty acid, (CH<sub>3</sub>(CH<sub>2</sub>)<sub>24</sub>COOH), occurring in waxes (6). All twenty-four of the analyzed mascara samples displayed absorption bands in the above mentioned regions, which indicates the presence of 'wax' in each sample. The lash tint, Revlon- solvent, sample (see Figure 1) did not yield a significant absorption

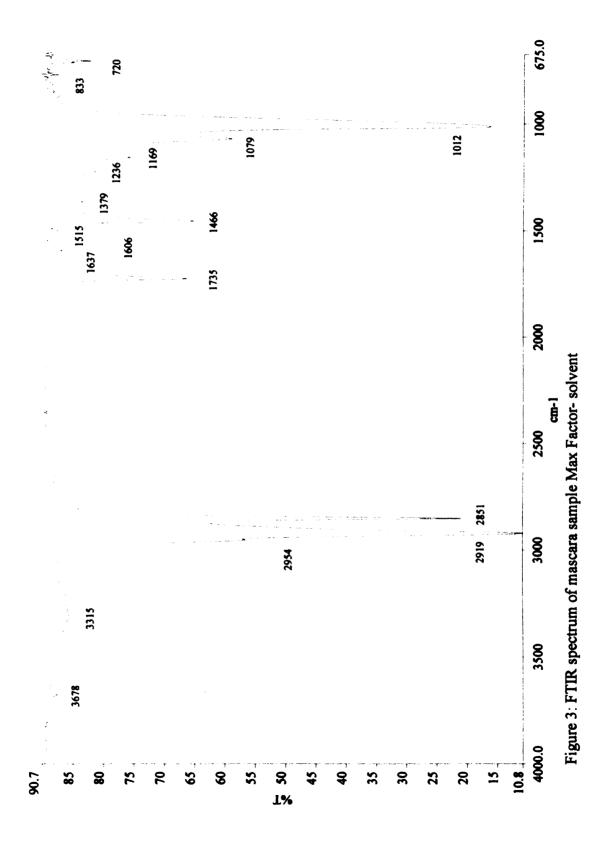
band in the 735-710 cm<sup>-1</sup> range, which was observed in all of the spectra produced from the mascara samples.

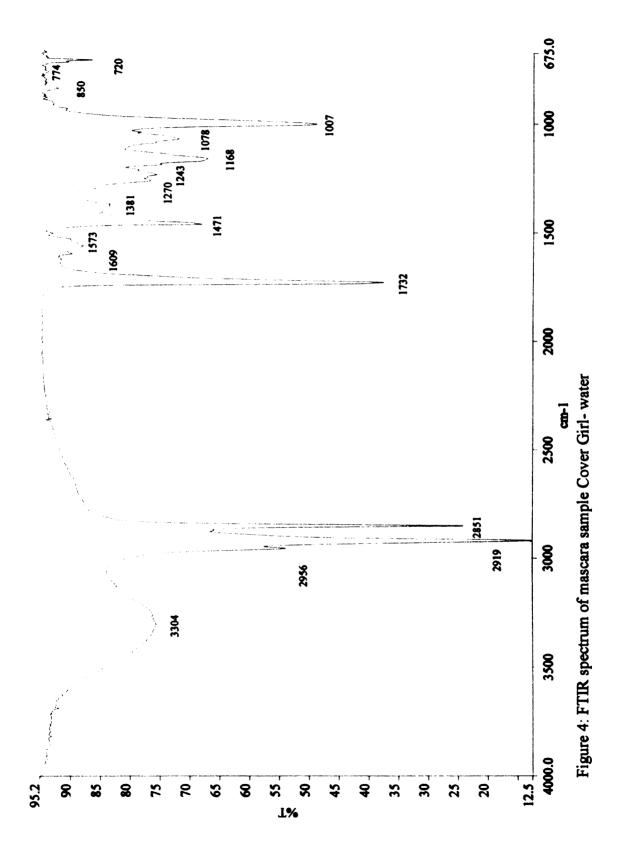
In order to distinguish between the spectra from the twenty-four mascara samples, it was necessary to observe the regions of the spectra where waxes did not have common absorptions. The region between 1650 cm<sup>-1</sup> and 1500 cm<sup>-1</sup>, along with the portion of the fingerprint region in the 1400-750 cm<sup>-1</sup> range displayed absorptions which varied between twenty of the mascara samples. Thus, twenty-one of the samples could be distinguished from one another based on the analysis provided by FTIR.

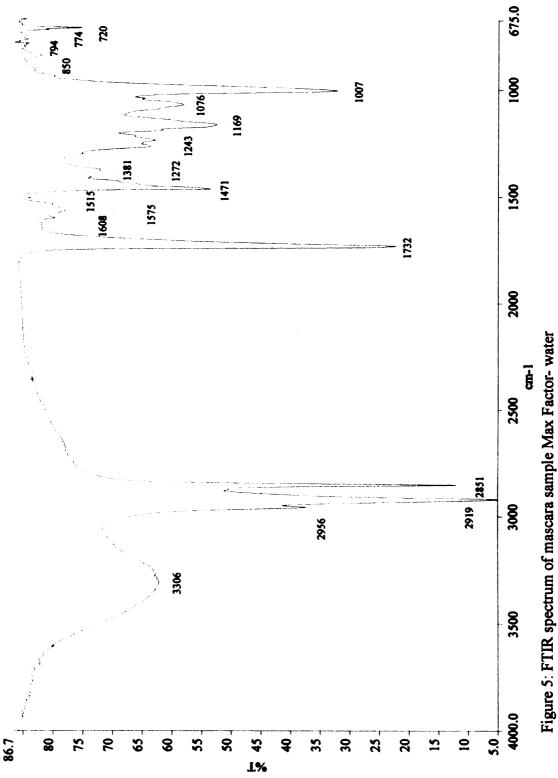
The remaining four samples resulted in two pairs of spectra which were unable to be differentiated from each other. The FTIR spectra of Cover Girl- solvent (see Figure 2) and Max Factor- solvent (see Figure 3) mascara samples could not be distinguished from one another. The spectra of Cover Girl- water (see Figure 4) and Max Factor- water (see Figure 5) mascara samples also could not be distinguished from one another. All of the remaining FTIR spectra of the mascara samples can be found in Appendix B.













## 3.3 SEM-EDS

The elemental weight percentages determined from the three analyses of each sample were averaged to ascertain which quantified elements were most abundant in each sample. Iron (Fe) was determined to have the largest average weight percentage in twenty-three of the twenty-four mascara samples. The Maybelline-water (see Figure 6) mascara sample was determined to have the highest percentage of iron, 96.7%. The lash tint, Revlon-solvent (see Figure 7) sample, had a very minute percentage of iron (0.4%) and in contrast had the largest percentage of silicon (85.3%) when compared to the mascara samples. The percentage of iron is so minute in the sample that the iron peak is not visible and was not labeled on the SEM-EDS spectrum. Silicon (Si) was determined to be the second most abundant element in the mascara samples based on the average weight percentages calculated for the quantified elements. Calculated averages of the elemental weight percentage data for the mascara samples can be found in Appendix C, Table 6. All of the remaining SEM-EDS spectra of the mascara samples can also be found in Appendix C.

Ratios were then calculated from the elemental weight percents determined from each SEM-EDS sample run. The following elemental ratios were calculated: Fe/Si, Si/Al, Mg/Al, Si/Mg, Fe/Na, Si/Na, Mg/Na, Al/Na, Fe/Mg, and Fe/Al. The three ratios calculated for each sample were averaged and a standard deviation was determined. In order to establish whether the twenty-five mascara samples could be differentiated from one another using SEM-EDS data, an inclusion rule was implemented from a previous forensic study involving crayons (7). Each of the ratios of one sample will be determined to be either inclusive or exclusive of another sample based on whether the ratio average

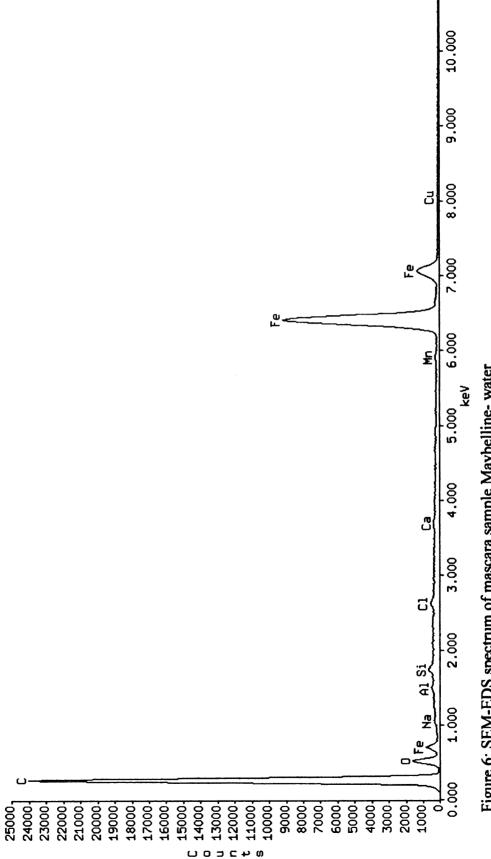
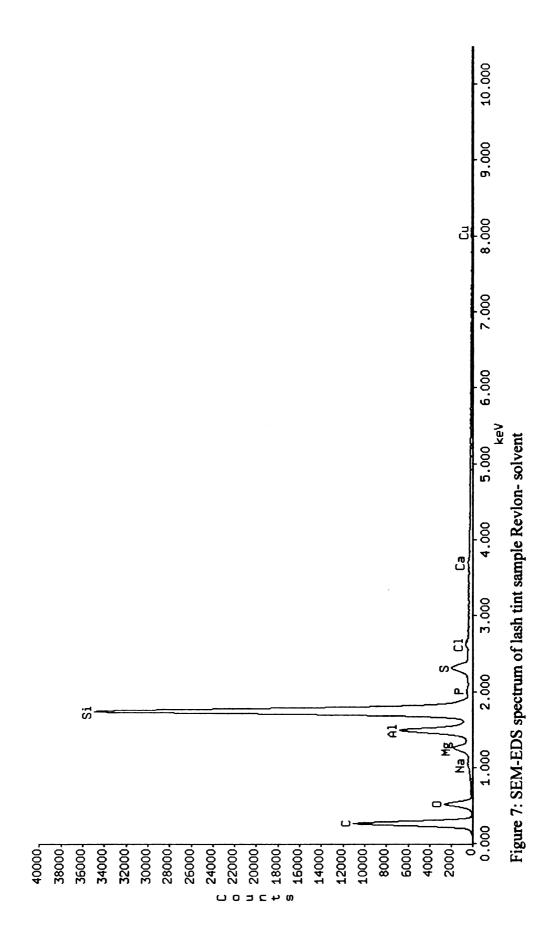


Figure 6: SEM-EDS spectrum of mascara sample Maybelline- water



falls within (inclusive) or out (exclusive) of a low and high range within three standard deviations of the sample to which it is compared. Examples of these elemental ratio calculations and inclusion rule comparisons are displayed in Tables 3 and 4. Samples were labeled as lower case letters to allow for a more simplified nomenclature during sample comparisons. SEM-EDS ratio calculation results for all of the mascara samples can be found in Appendix C, Table 7.

Sample	Calculations	Fe/Si	Si/Al	Mg/Al	Si/Mg	Fe/Na
Maybelline-hybrid	Ratio Average	1.48	32.14	18.23	1.76	20.14
	Standard Dev	0.18	5.14	2.00	0.12	1.47
(m)	Low	0.95	16.71	12.25	1.40	15.73
	High	2.01	47.57	24.22	2.11	24.56
Maybelline-solvent	Ratio Average	2.71	3.00	2.20	1.36	45.86
	Standard Dev	0.11	0.22	0.10	0.05	15.42
(n)	Low	2.39	2.35	1.91	1.22	-0.39
	High	3.04	3.65	2.49	1.51	92.12

Table 3: Example of elemental ratio calculation results for two mascara samples

Table 4: Example of inclusion rule comparison for two mascara samples

Comparison	Fe/Si	Si/Al	Mg/Al	Si/Mg	Fe/Na
(m) in (n)	Exclusive	exclusive	exclusive	exclusive	inclusive
(n) in (m)	Exclusive	exclusive	exclusive	exclusive	exclusive

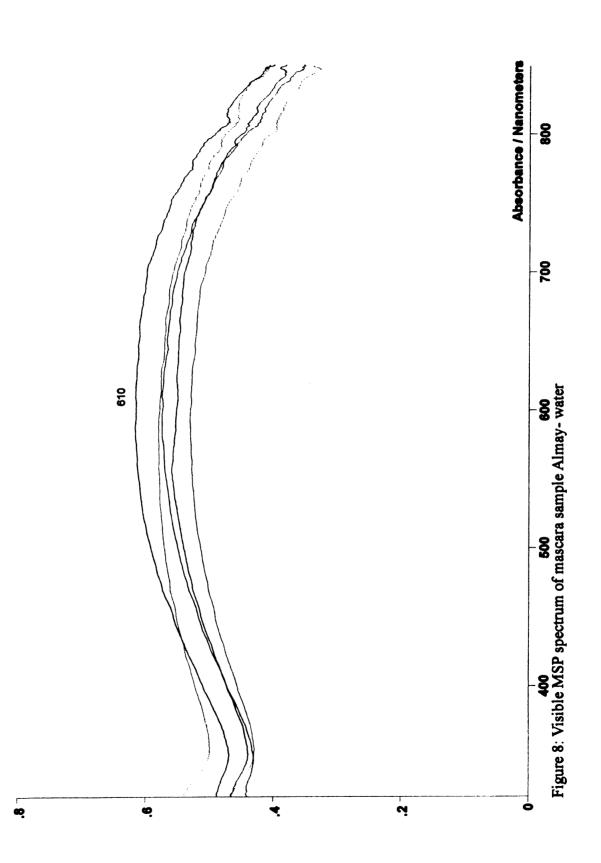
The elemental ratios of the twenty-five samples were compared to each other based on the inclusion rule which resulted in a total of 600 separate sample comparisons. Two comparisons were all inclusive for the ten ratios, (q) in (d) and (e) in (k). Thus, Physicians- water(q) could not be distinguished from Bonne Bell- water (d), and Cover Girl- solvent(e) could not be distinguished from Max Factor- solvent(k) based on the SEM-EDS data compared using the inclusion rule.

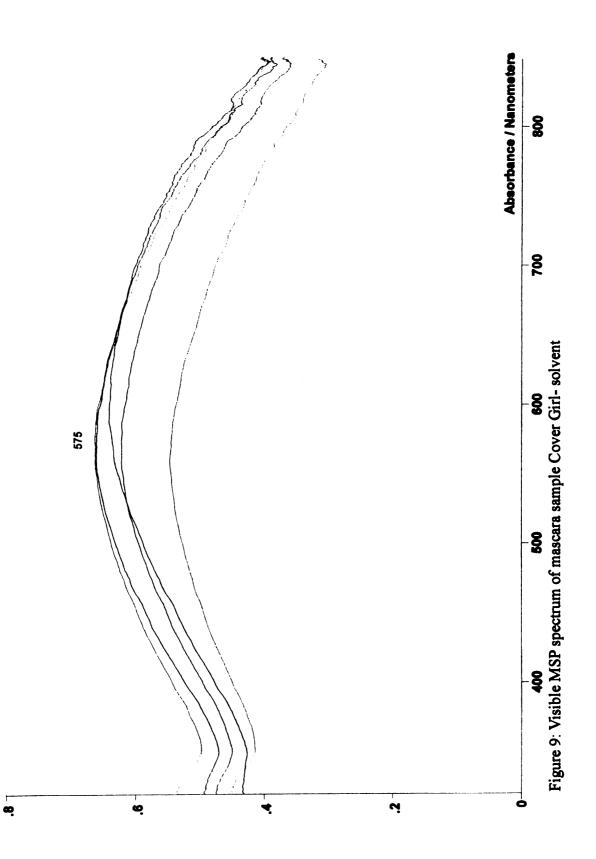
If at least one of the ratios from each sample is outside of the range of another sample, the two samples are excluded as originating from the same source. Two comparisons resulted in a single exclusion for the ten ratios, (d) in (q) and (d) in (o). Thus, Bonne Bell- water(d) is distinguished from Physicians- water(q) by the Al/Na ratio, and Bonne Bell- water(d) is distinguished from Maybelline- water(o) by the Mg/Na ratio. Ten comparisons resulted in two exclusions from the ten ratios. Considering all 600 comparisons: 0.33% was all inclusive, 0.33% had one exclusion, and 1.67% had two exclusions.

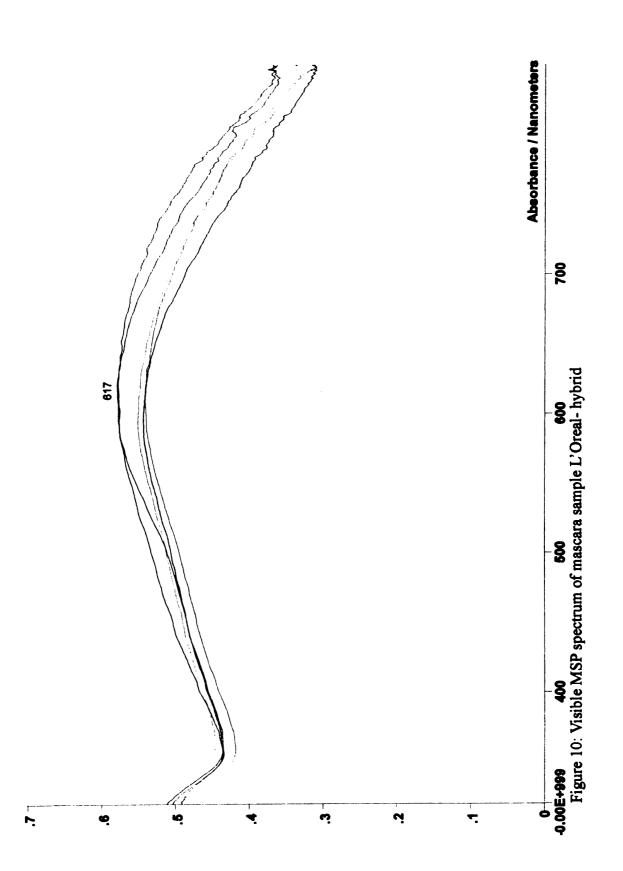
#### 3.4 Visible MSP

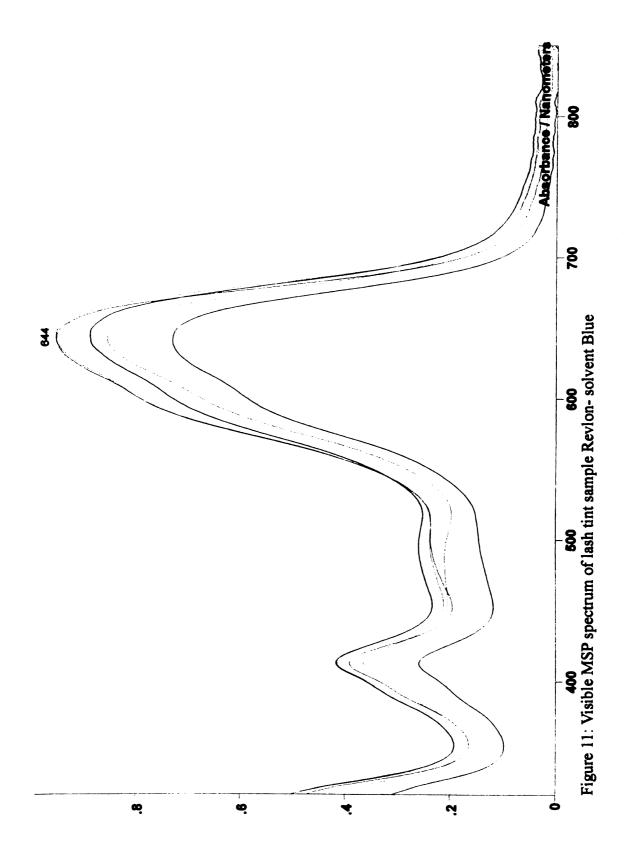
All twenty-four of the mascara samples provided a very broad absorbance curve in the visible region, which is between 400 and 700 nm. This 'flat' absorption is characteristic of achromatic samples. None of the mascara samples could be differentiated based on the acquired spectra. Three of these spectra have been displayed and are labeled as Figures 8, 9, and 10.

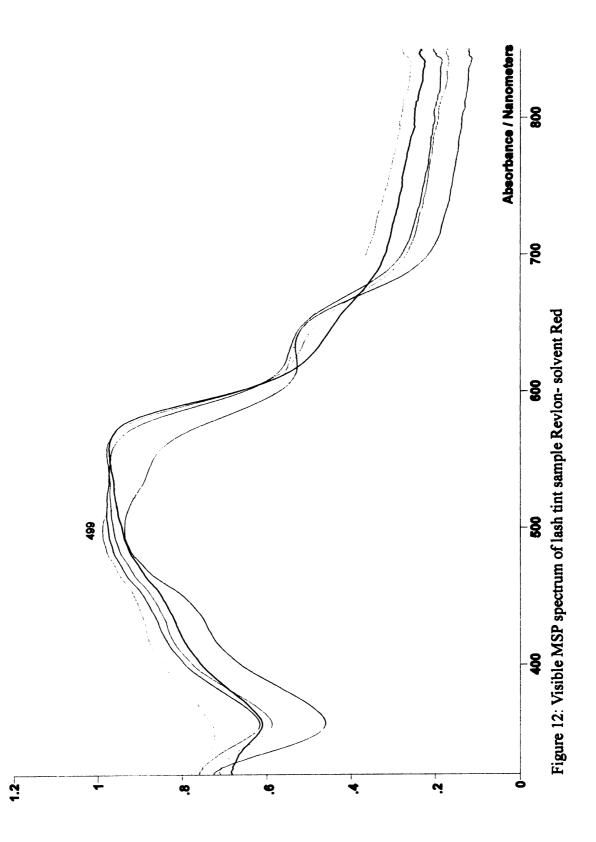
However, the lash tint, Revlon- solvent sample, yielded a different spectral result for each of the three colored pigments observed: blue, red, and yellow. These spectra are labeled as Figures 11, 12, and 13.













#### **4. CONCLUSION**

Each of the four techniques utilized in this project provided a unique level of discrimination for the twenty-four mascara samples. The microscopic examination technique was able to separate the samples into four groups which could not be distinguished from one another; three groups of two samples, and one group of eighteen samples. The FTIR analysis technique was able to separate the samples into twenty-two groups; two groups of two samples and twenty groups of one sample. The SEM-EDS analysis was able to exclude and distinguish between 99.67% of the 600 comparisons based on weight percent ratios. The visible MSP analysis did not yield any discriminatory data for the samples analyzed.

Overall, the incorporation of microscopic examination, FTIR analysis, and SEM-EDS analysis left only two samples, the Cover Girl- solvent and Max Factor- solvent samples, from being distinguished from one another. Proctor & Gamble Inc. is the manufacturer of both the Cover Girl and Max Factor brands, which explains the inability to differentiate the two samples. There are several other mascara brands on the market that also share the same manufacturers (see Table 2). Thus, caution must be taken when comparing questioned mascara samples with such brands.

The lash tint, Revlon- solvent sample, was easily distinguished from all of the mascara samples by each of the analysis techniques.

In regards to the forensic analysis of black mascara evidence, the best technique to employ would be SEM-EDS, followed by FTIR, and finally microscopy. However, in regards to an analysis scheme, this order would not be recommended. Microscopy is a preliminary examination tool and should remain as such. FTIR would be employed next,

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due to the lack of sample consumption, sample preparation and time required for analysis. Although questioned mascara submitted as evidence would likely be dry prior to laboratory analysis, samples should be dry for two days prior to analysis in order to perform a proper comparison. Finally, SEM-EDS should be utilized last in the analysis scheme because it requires sample alteration. The sample preparation of mascara for SEM-EDS analysis involves carbon sputter coating, which will alter the chemical composition of the sample.

#### **5. FUTURE WORK**

-As a continuation of this research, more black samples of different brands and mascaras of the same brand could be analyzed and compared with the results of this study.

-Analysis should also be performed on different colors of mascara, such as brown, navy, auburn, etc. This research will likely yield more useful results with visible microspectrophotometry.

-The analysis of mascara via pyrolysis gas chromatography-mass spectroscopy should be attempted.

-Blind studies using samples from within and outside of this study should be attempted to determine the accuracy in comparing and identifying mascara samples.

-A study involving the transfer of mascara from one individual to another, in order to compare the transferred samples to pristine samples, such as the samples analyzed in this study.

**APPENDICES** 

### **APPENDIX A.**

## **Microscopic Examination Data**

Brand	Based	Observation
Almay	Hybrid	Black particles, orange flakes
Almay	Water	Black particles, orange flakes
Aziza II	Water	Black particles, blue specks
Bonne Bell	Water	Black particles
Cover Girl	Solvent	Black particles, few brown particles
Cover Girl	Water	Black particles
Jane	Hybrid	Black particles, fibers
Jane	Water	Black particles, fibers
L'Oreal	Hybrid	Black particles, blue specks
L'Oreal	Water	Black particles
Max Factor	Solvent	Black particles, few brown particles
Max Factor	Water	Black particles
Maybelline	Hybrid	Black particles
Maybelline	Solvent	Black particles
Maybelline	Water	Black particles
Neutrogena	Water	Black particles
Physicians Formula	Water	Black particles
Revion	Hybrid	Black particles, fibers
Revlon	Solvent	Red, blue, and yellow spots
Revlon	Water	Black particles, fibers
Rimmel	Solvent	Black particles
Rimmel	Water	Black particles
Sally Hansen	Water	Black particles
Wet'n'Wild	Hybrid	Black particles
Wet'n'Wild	Solvent	Black particles

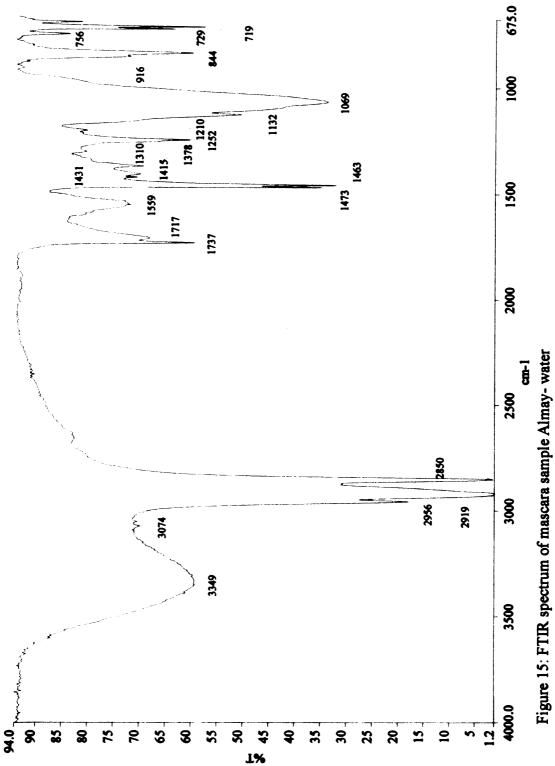
Table 5: Observations from the microscopic examination of mascara samples

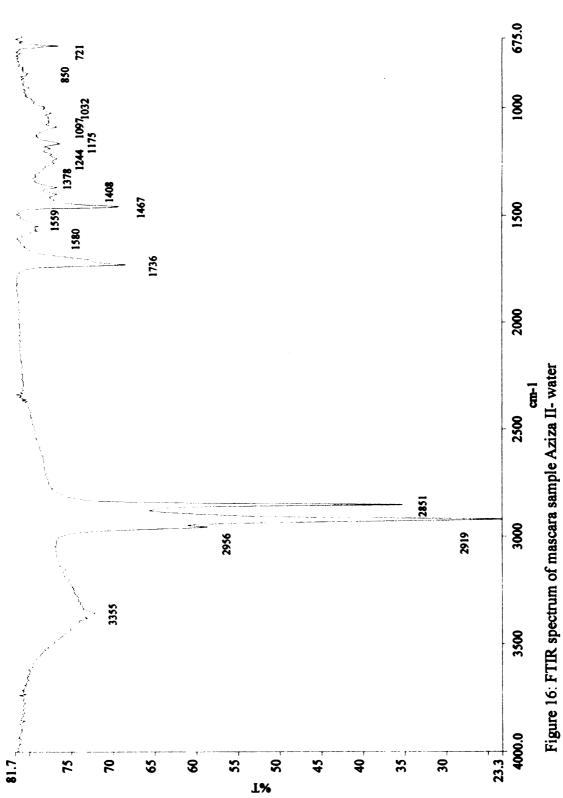
### APPENDIX B.

# FTIR Spectra

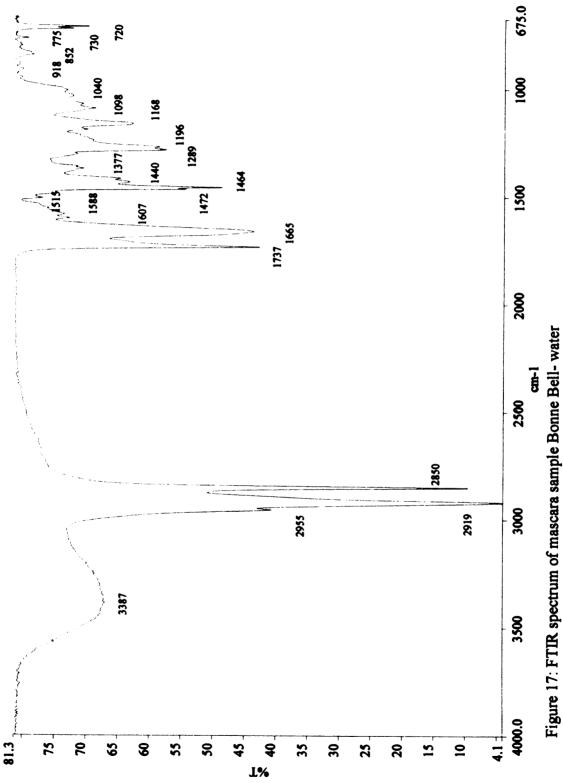


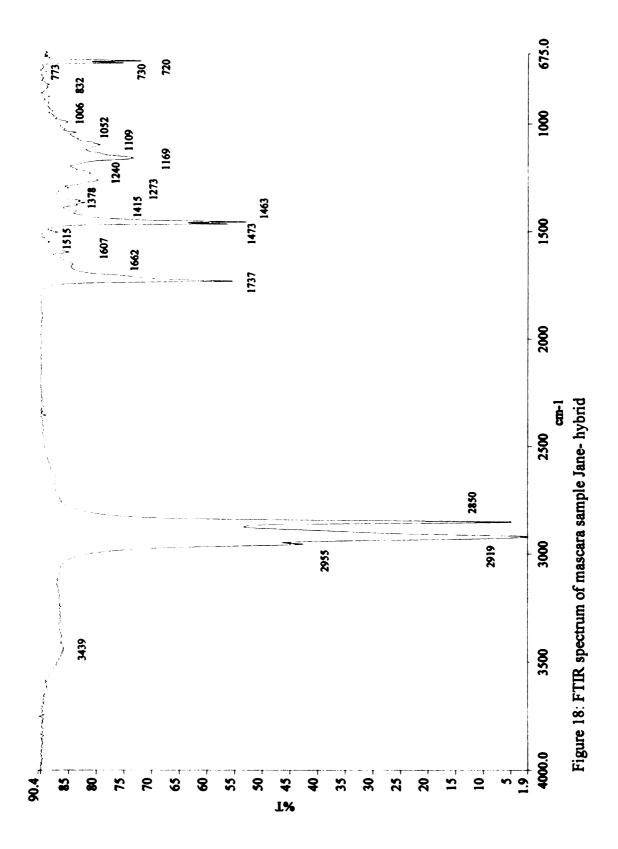


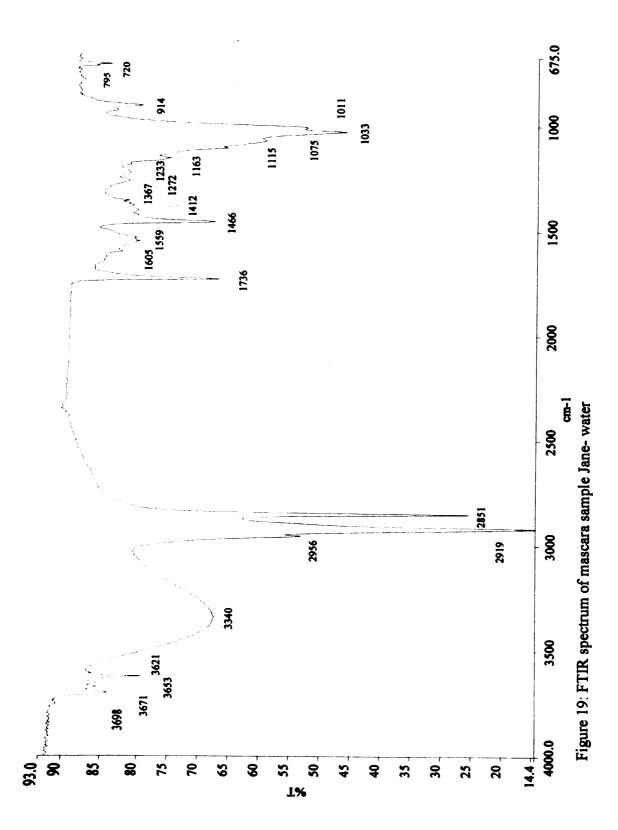






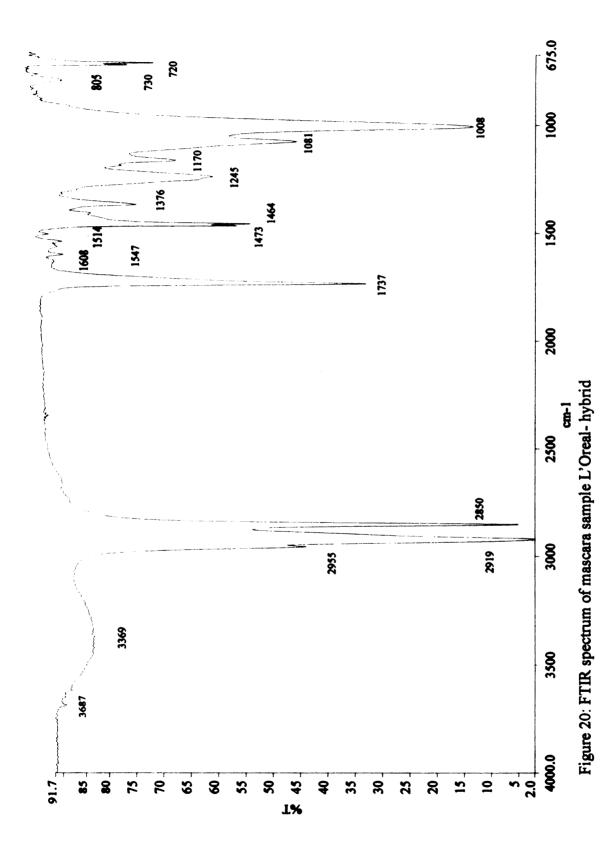






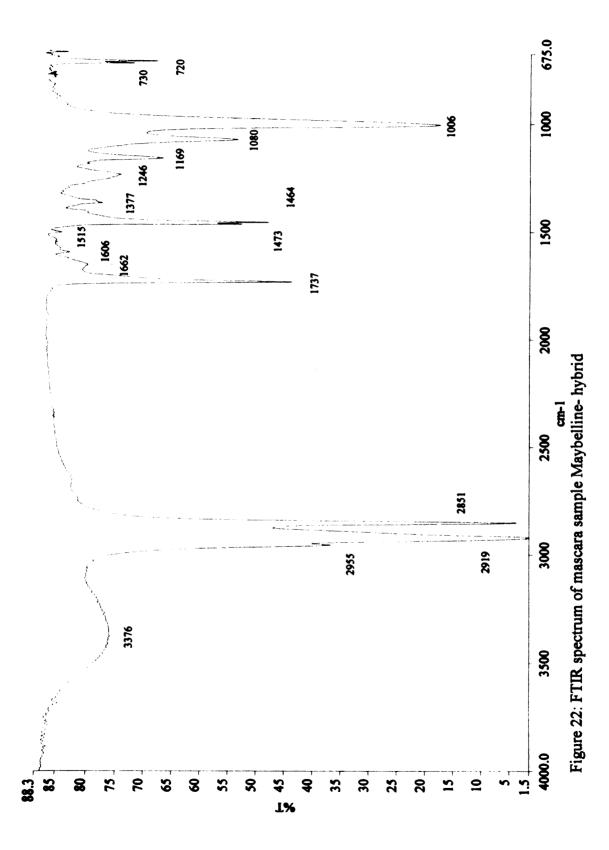
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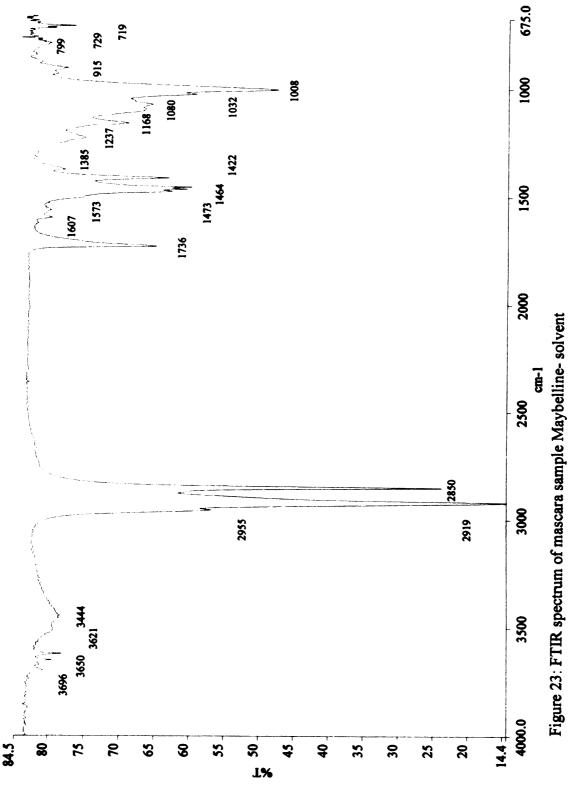


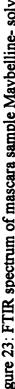






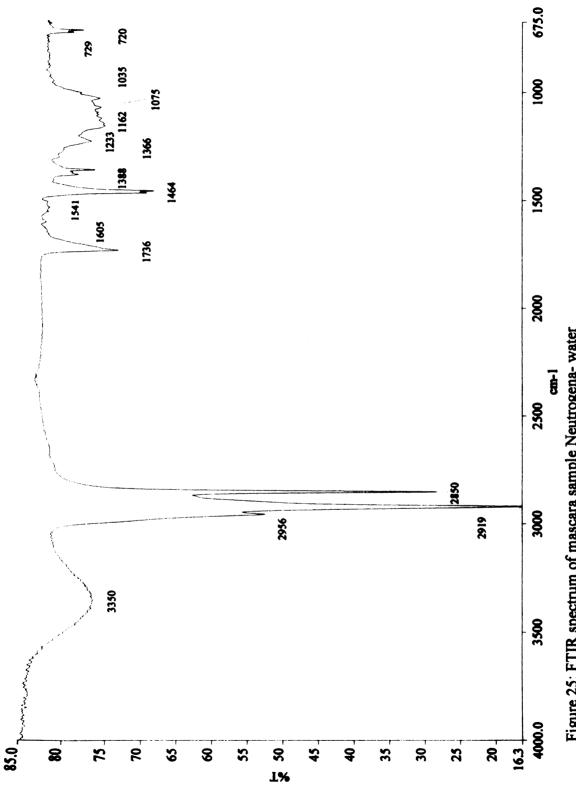












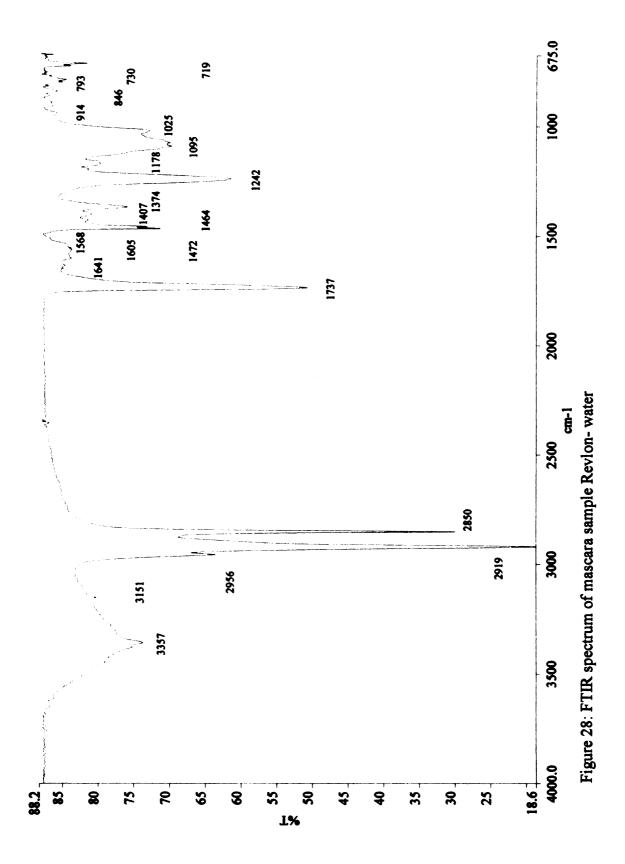


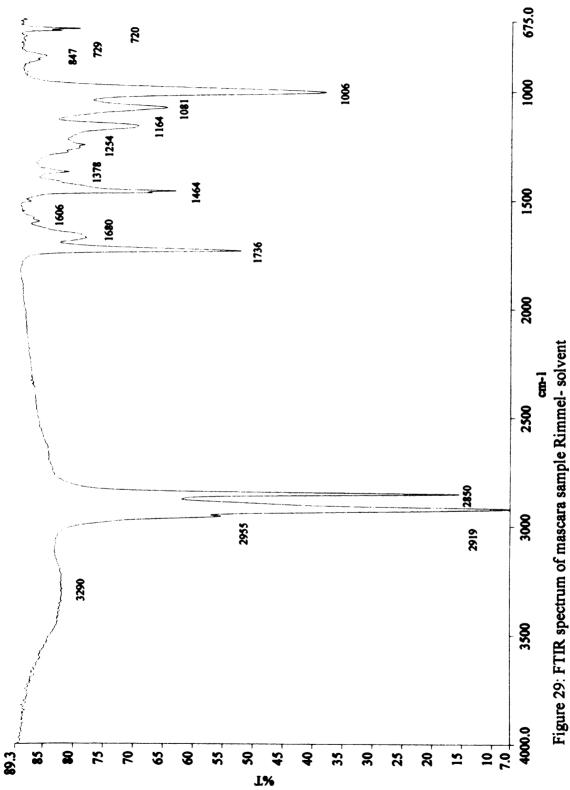






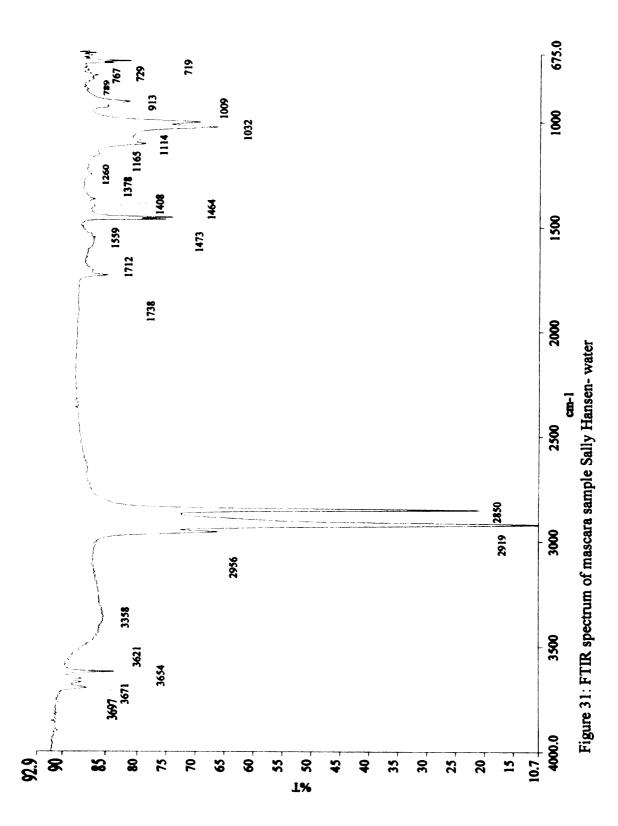












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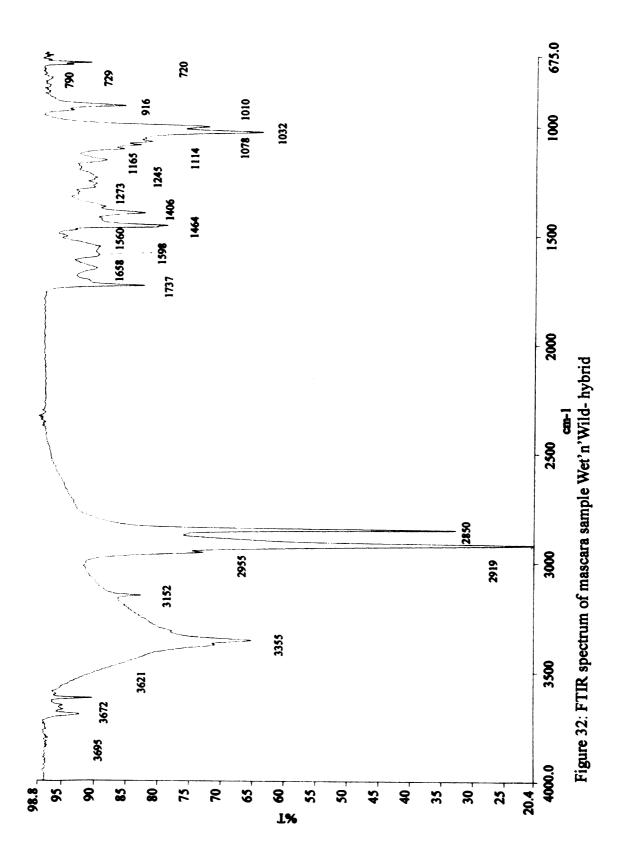




Figure 33: FTIR spectrum of mascara sample Wet'n'Wild- solvent

## APPENDIX C.

# SEM-EDS Data and Spectra

Sample	Avg % Na	Avg % Mg	Avg % Al	Avg % Si	Avg % Fe
Almay- hybrid	0.5	1.5	1.4	50.0	46.5
Almay- water	3.6	0.5	8.3	25.9	61.8
Aziza II- water	2.6	0.1	2.1	2.5	92.7
Bonne Bell- water	2.3	0.8	1.1	3.6	92.2
Cover Girl- solvent	0.7	17.1	1.0	29.4	51.8
Cover Girl- water	2.9	12.8	1.1	18.2	64.9
Jane- hybrid	2.1	0.4	0.4	0.6	96.5
Jane- water	2.3	2.6	10.0	19.0	66.1
L'Oreal- hybrid	3.7	14.1	4.8	36.6	40.9
L'Oreal- water	3.5	4.0	0.6	16.4	75.4
Max Factor- solvent	0.5	15.3	0.9	26.8	56.4
Max Factor- water	2.4	15.7	0.9	19.7	61.4
Maybelline- hybrid	2.3	18.1	1.0	31.8	46.8
Maybelline- solvent	1.3	15.2	6.9	20.6	56.0
Maybelline- water	1.1	0.2	0.5	1.5	96.7
Neutrogena- water	8.6	0.2	1.0	3.6	85.3
Physicians Formula-water	2.3	1.2	1.1	3.8	91.5
Revlon- hybrid	2.1	1.6	3.5	7.4	85.3
Revlon-solvent	0.4	2.7	11.2	85.3	0.4
Revlon- water	3.0	1.2	0.4	10.4	84.9
Rimmel- solvent	1.0	12.2	0.9	25.2	60.8
Rimmel- water	3.4	0.1	0.5	0.3	95.7
Sally Hansen- water	1.0	0.3	7.7	13.0	78.0
Wet'n'Wild- hybrid	1.1	0.3	6.6	7.8	84.1
Wet'n'Wild- solvent	0.4	7.2	0.5	10.9	81.0

Table 6: SEM-EDS average elemental weight percent data for mascara samples

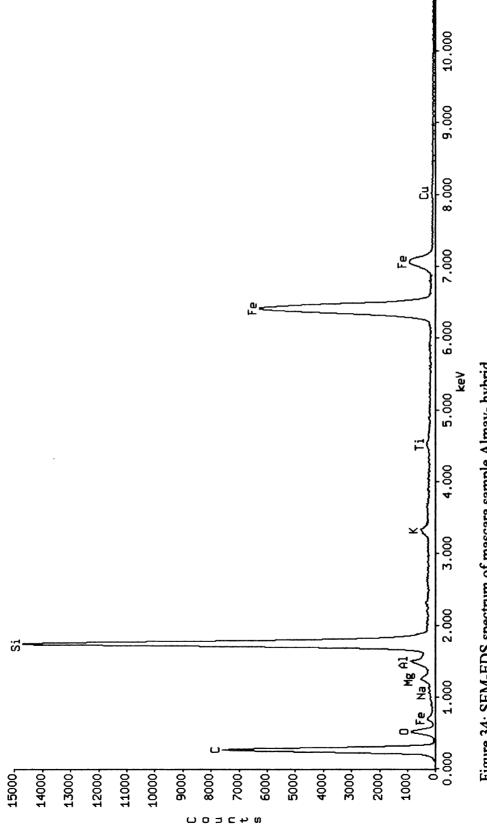
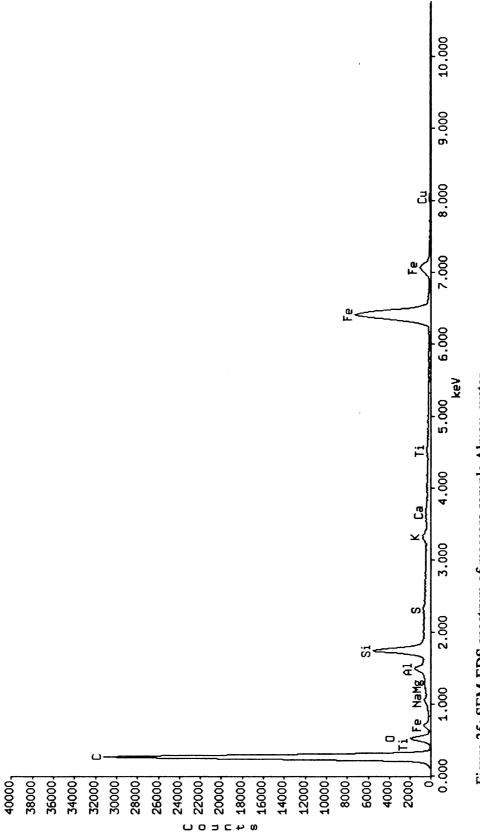
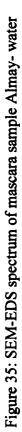
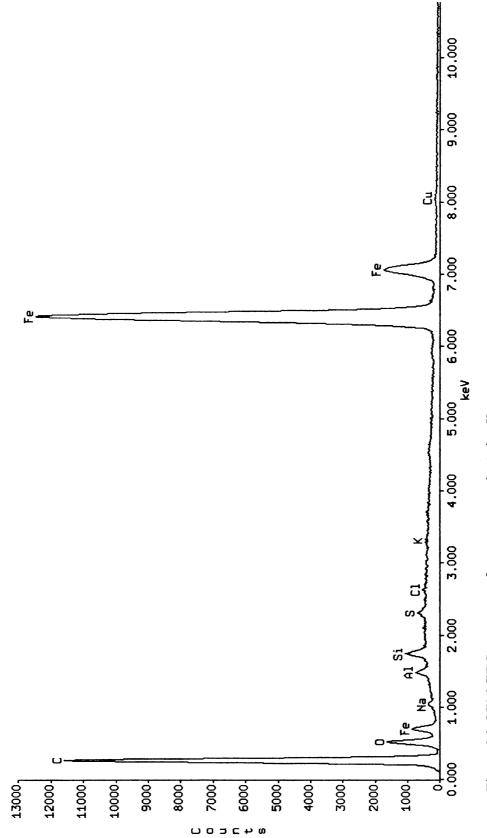


Figure 34: SEM-EDS spectrum of mascara sample Almay- hybrid

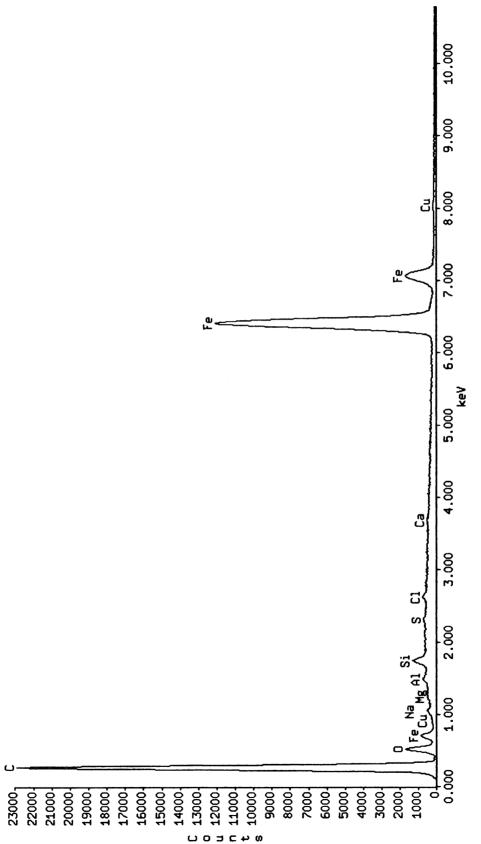
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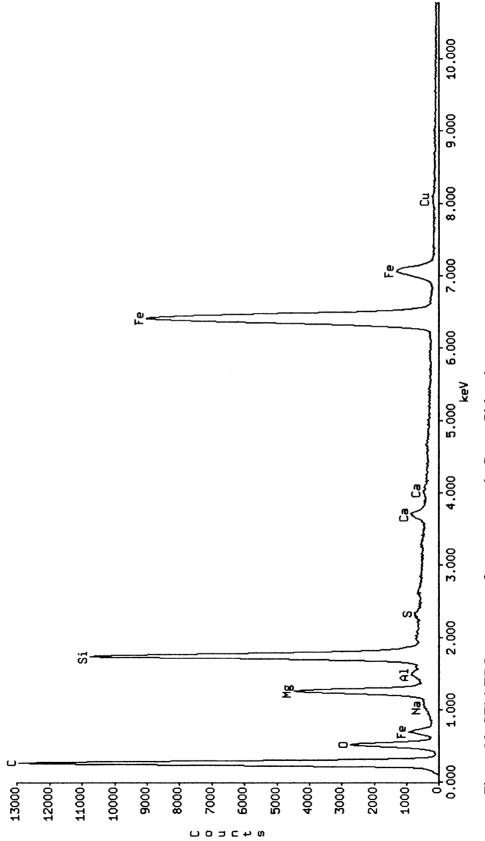




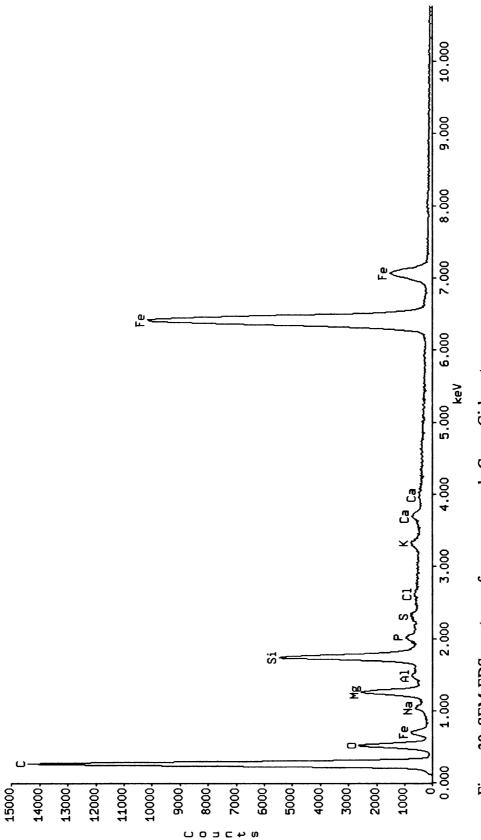




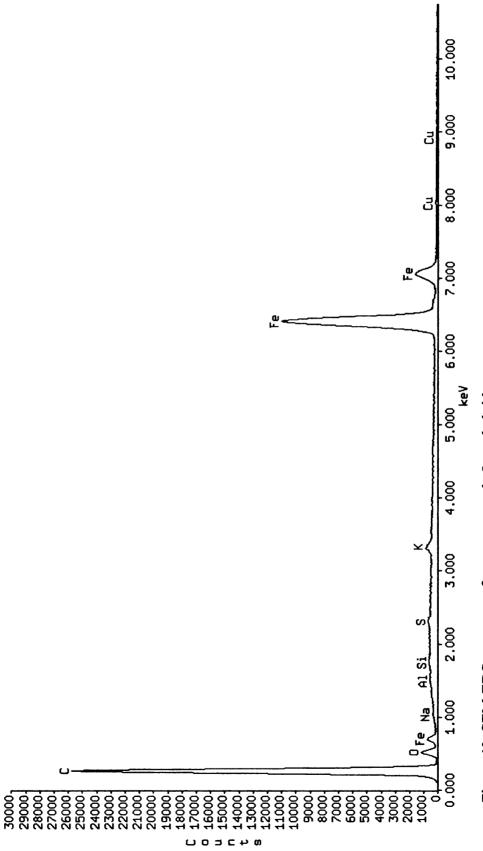




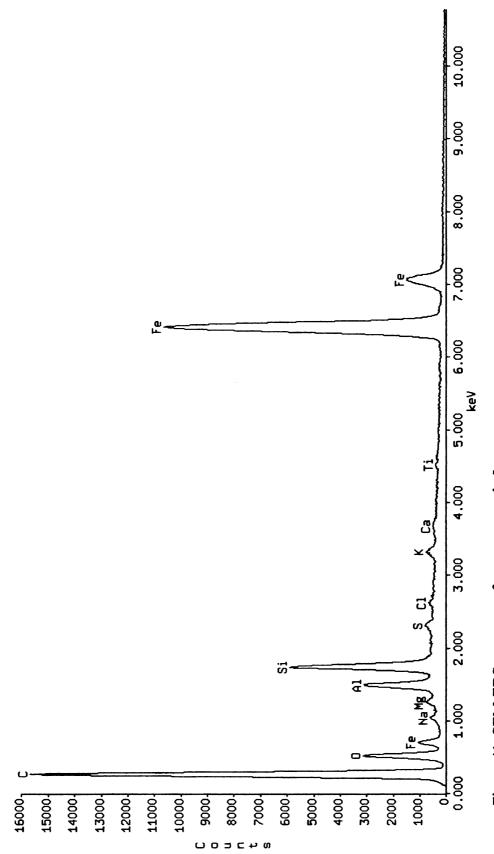




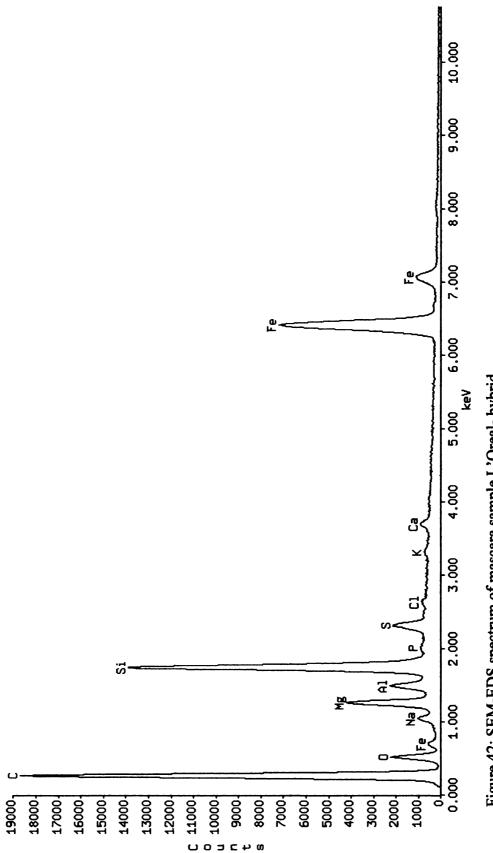




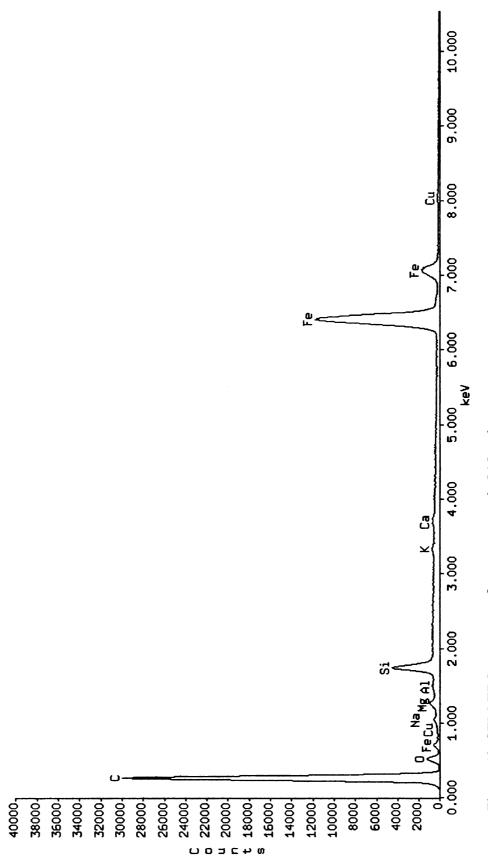




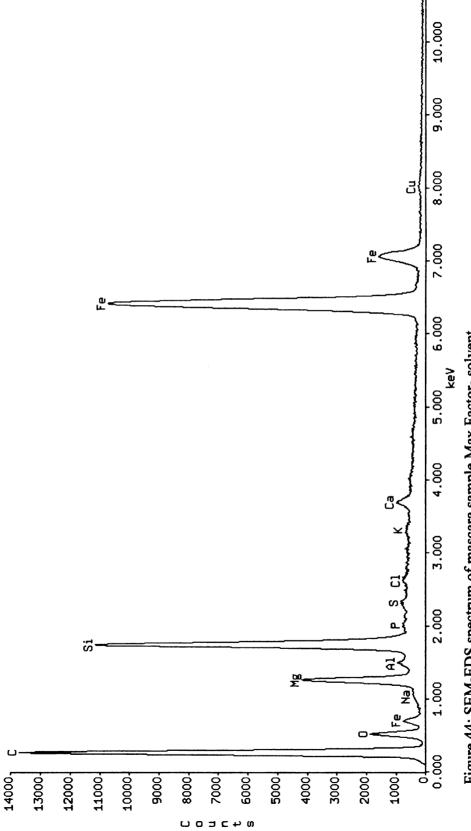






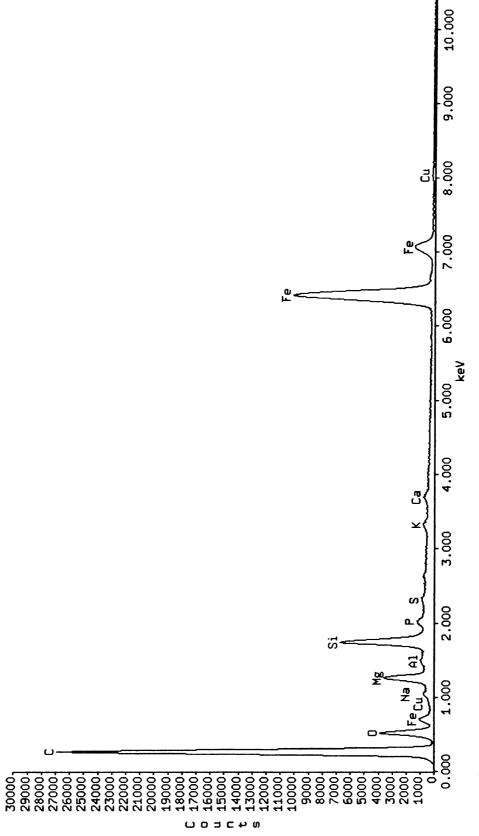




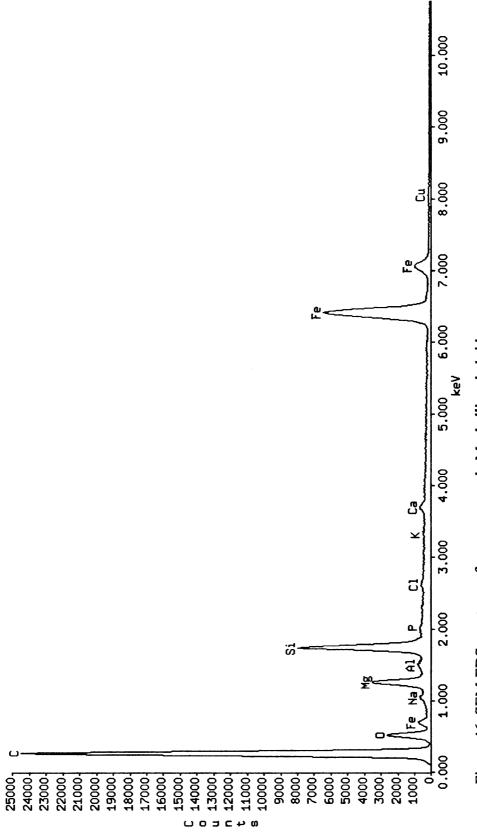




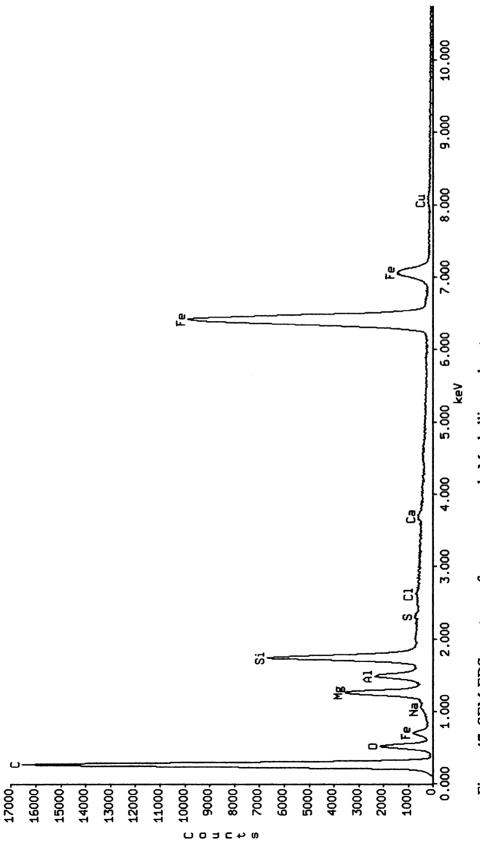




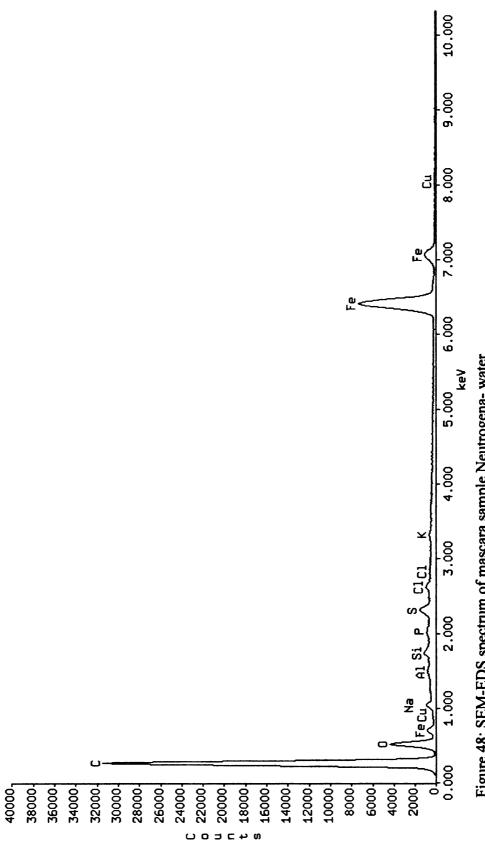




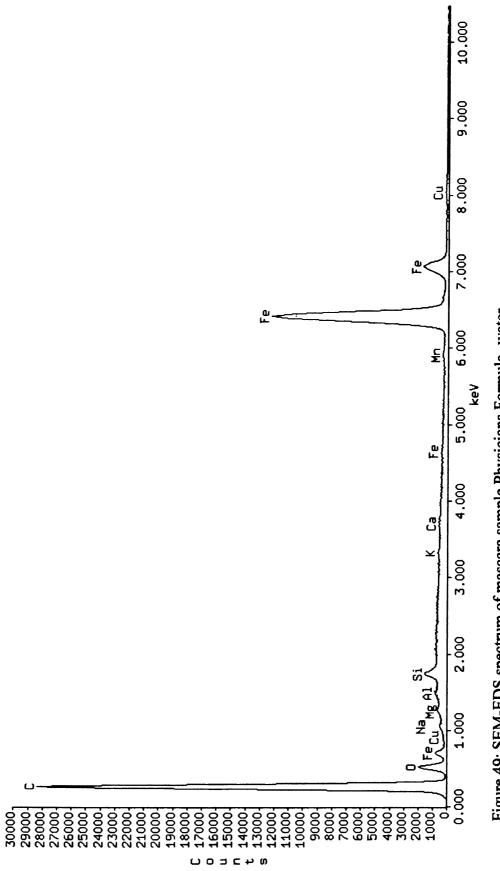


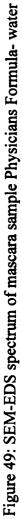


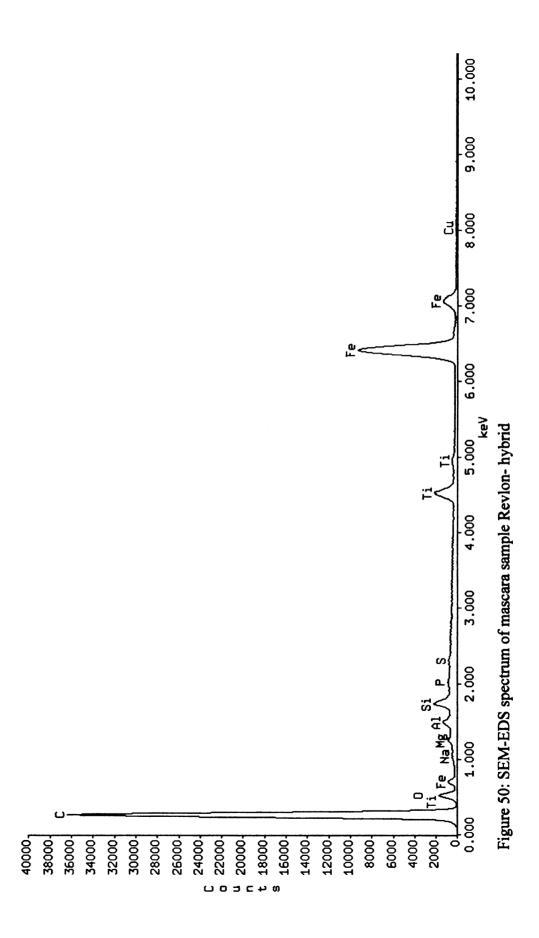




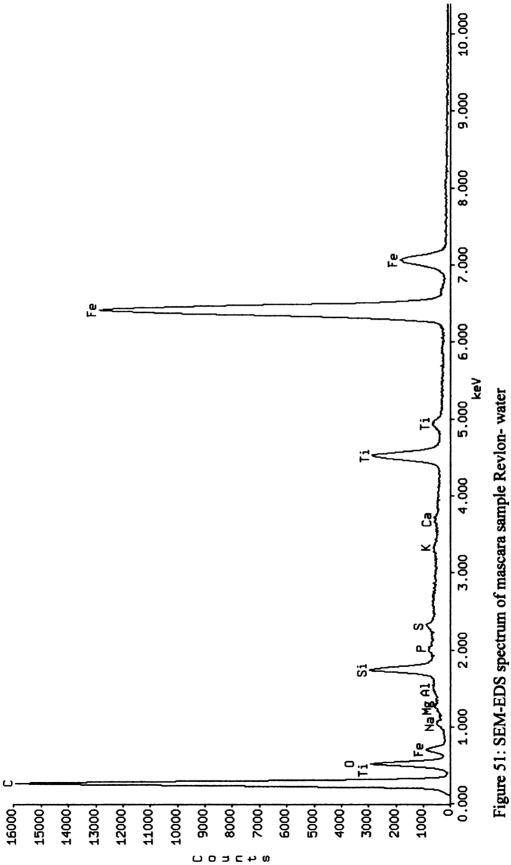




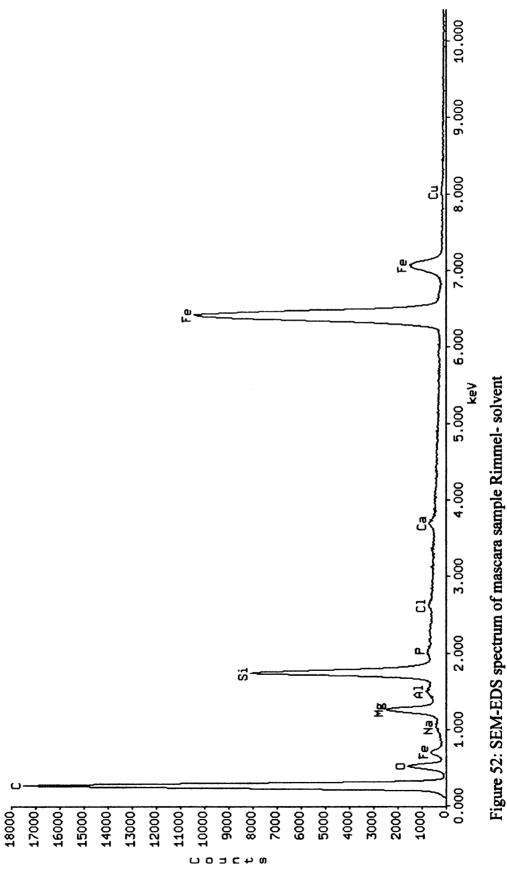




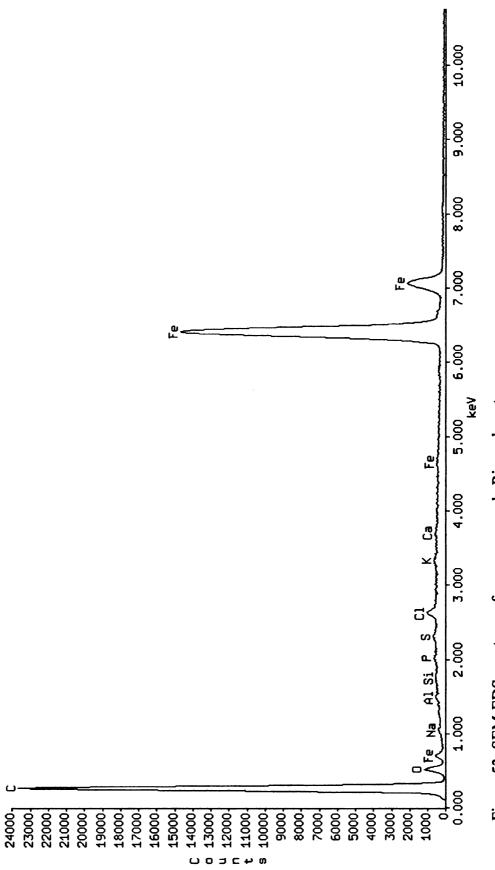


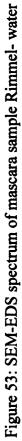


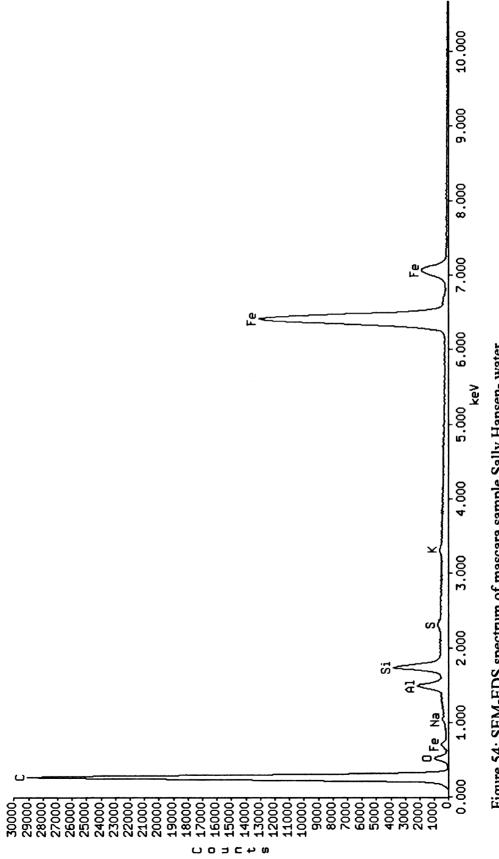




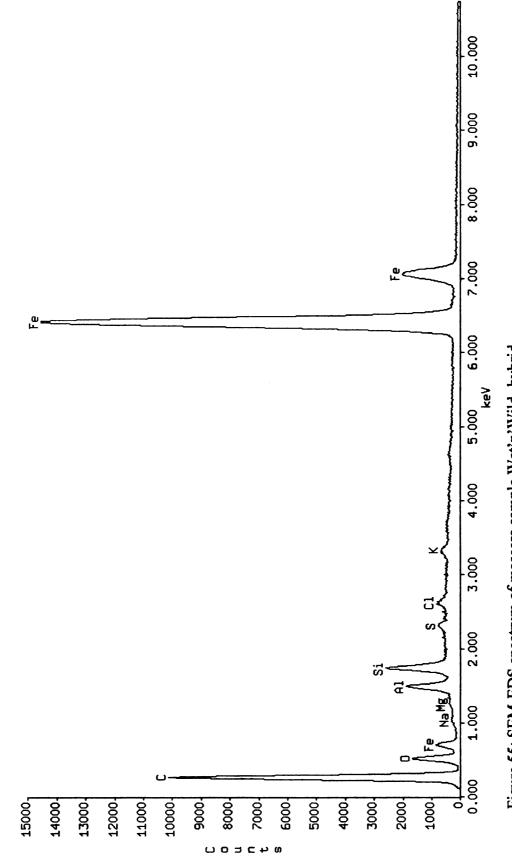




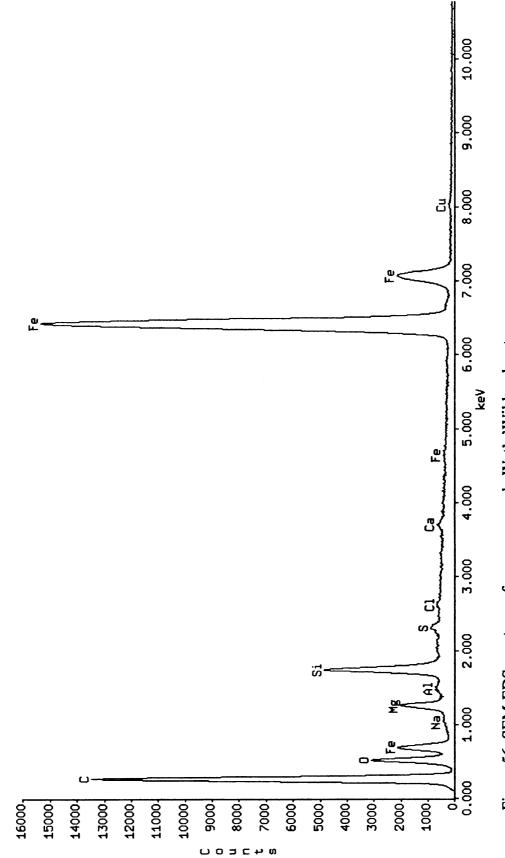














Sample	Calculations	Fe/Si	Si/AI	Mg/AI	Si/Mg	Fe/Na	Si/Na	Mg/Na	AI/Na	Fe/Mg	Fe/AI
Almay- hybrid	Ratio Average		36.26	1.09	33.38	92.73	98.02	2.93	2.94	31.17	33.19
	Standard Dev		9.07	0.28	1.14	30.16	22.05	0.61	1.42	3.87	5.04
(a)	Low	0.59	9.04	0.24	29.95		31.86	1.10	-1.33	19.56	18.07
	High	1.27	63.49	1.94	36.81		164.18	4.76	7.21	42.77	48.31
Almay-water	Ratio Average		3.40	0.06	57.33		7.32	0.13	2.30	138.64	8.40
	Standard Dev		1.14	0.01	12.14		1.01	0.02	0.71	37.84	3.88
(q)	Low	1.43	-0.004	0.02	20.91		4.30	0.07	0.17	25.10	-3.24
	High	3.37	6.81	0.10	93.76		10.34	0.18	4.44	252.17	20.05
Aziza II- water	Ratio Average		1.18	0.05	24.67		96.0	0.04	0.82	927.00	43.86
	Standard Dev		0.25	0.01	2.52		0.18	0.00	0.10	3.00	5.16
(c)	Low	26.08	0.42	0.03	17.12		0.41	0.03	0.53	918.00	28.37
	High	49.61	1.93	0.06	32.22		1.52	0.05	1.12	936.00	59.35
Bonne Bell- water	Ratio Average	26.05	3.37	0.77	4.33	47.55	1.83	0.42	0.52	113.23	87.94
	Standard Dev		0.86	0.14	0.46		0.82	0.16	0.12	21.16	25.58
(q)	Low	17.93	0.80	0.36	2.94		-0.64	-0.06	0.15	49.75	11.21
	High	34.17	5.94	1.19	5.72		4.31	0.90	0.89	176.71	164.67
<b>Cover Girl- solvent</b>	Ratio Average		29.57	17.26	1.71		44.29	25.83	1.50	3.03	52.17
	Standard Dev		3.06	1.93	0.04		4.09	2.40	0.07	0.11	5.08
(e)	Low	1.68	20.39	11.47	1.61		32.02	18.64	1.29	2.68	36.91
	High	1.85	38.75	23.06	1.82		56.55	33.02	1.71	3.37	67.42
Cover Girl- water	Ratio Average		16.53	11.70	1.42		6.30	4.44	0.39	5.06	59.27
	Standard Dev		3.19	2.60	0.04		0.52	0.31	0.11	0.03	13.47
(J)	Low	3.19	6.95	3.92	1.29		4.74	3.52	0.07	4.97	18.85
	High	3.95	26.11	19.49	1.54		7.86	5.36	0.72	5.15	99.68
Jane- hybrid	Ratio Average	174.64	1.51	1.12	1.49		0.29	0.20	0.20	261.08	252.24
	Standard Dev		0.43	0.58	0.45		0.04	0.07	0.05	107.83	66.46
(g)	Low	-14.26	0.23	-0.63	0.14		0.15	0.00	0.05	-62.42	52.85
	High	363.54	2.80	2.87	2.84		0.42	0.41	0.34	584.57	451.64

Table 7: SEM-EDS ratio calculation results for mascara samples

Sample	Calculations	Fe/Si	Si/Al	Mg/Al	Si/Mg	Fe/Na	Si/Na	Mg/Na	AUNa	Fe/Mg	Fe/Al
Jane- water	Ratio Average	3.47	1.91	0.26	8.42	29.05	8.36	1.19	4.35	29.32	6.64
	Standard Dev	0.04	0.10	0.12	3.78	5.54	1.60	0.68	0.66	13.37	0.29
(h)	Low	3.34	1.62	-0.09	-2.90	12.44	3.57	-0.86	2.39	-10.78	5.76
	High	3.61	2.21	0.61	19.75	45.66	13.16	3.23	6.32	69.42	7.53
L'Oreal-hybrid	Ratio Average	1.12	7.67	2.97	2.59	10.95	9.79	3.79	1.28		8.58
	Standard Dev	0.04	0.09	0.17	0.12	0.32	0.07	0.18	0.01		0.32
(j)	Low	1.01	7.41	2.47	2.24	66.6	9.59	3.24	1.24	2.53	7.63
	High	1.23	7.94	3.47	2.94	11.91	10.00	4.34	1.31	3.26	9.52
L'Oreal- water	Ratio Average	4.63	30.83	7.56	4.06	21.82	4.69	1.16	0.17	18.79	140.29
	Standard Dev	0.52	10.98	2.55	0.07	4.17	0.48	0.14	0.06		39.93
(j)	Low	3.08	-2.12	-0.10	3.86	9.32	3.26	0.75	-0.02		20.50
	High	6.18	63.77	15.21	4.27	34.33	6.12	1.56	0.35		260.07
Max Factor- solvent	Ratio Average	2.13	28.74	16.42	1.75	106.58	50.57	28.98	1.77		60.67
	Standard Dev	0.38	2.34	0.58	0.08	14.54	6.28	3.97	0.25		7.26
(k)	Low	0.98	21.73	14.69	1.50	62.95	31.73	17.08	1.01	2.07	38.88
	High	3.28	35.76	18.15	2.00	150.20	69.41	40.87	2.52		82.46
Max Factor- water	Ratio Average	3.12	21.89	17.41	1.26	26.04	8.34	6.63	0.38		68.22
	Standard Dev	0.14	0.62	0.61	0.03	2.19	0.31	0.31	0.03		1.25
(])	Low	2.69	20.03	15.57	1.16	19.47	7.41	5.70	0.31		64.47
	High	3.55	23.74	19.24	1.36	32.60	9.27	7.56	0.46		71.98
Maybelline-hybrid	Ratio Average	1.48	32.14	18.23	1.76	20.14	13.80	7.82	0.43	2.58	46.96
	Standard Dev	0.18	5.14	2.00	0.12	1.47	2.34	0.83	0.04	0.13	3.16
(m)	Low	0.95	16.71	12.25	1.40	15.73	6.78	5.32	0.30	2.20	37.50
	High	2.01	47.57	24.22	2.11	24.56	20.81	10.32	0.56	2.97	56.43
Maybelline- solvent	Ratio Average	2.71	3.00	2.20	1.36	45.86	16.82	12.34	5.64	3.70	8.16
	Standard Dev	0.11	0.22	0.10	0.05	15.42	5.16	3.63	1.79	0.27	0.83
(u)	Low	2.39	2.35	1.91	1.22	-0.39	1.33	1.45	0.25	2.88	5.66
	High	3.04	3.65	2.49	1.51	92.12	32.31	23.22	11.02	4.51	10.65

Table 7 (cont'd)

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Table

Sample	Calculations	Fe/Si	Si/Al	Mg/Al	Si/Mg	Fe/Na	Si/Na	Mg/Na	AUNa	Fe/Mg	Fe/AI
Maybelline- water	Ratio Average	68.54	3.10	0.39	8.28		1.46	0.18	0.49	593.44	207.62
	Standard Dev	17.71	1.03	0.13	2.84		0.42	0.01	0.16	342.94	
(0)	Low	15.41	0.02	0.01	-0.24		0.19	0.15	0.02	-435.38	23.72
	High	121.66	6.18	0.78	16.80		2.73	0.21	0.96	1622.27	
Neutrogena- water	Ratio Average	26.86	4.22	0.28	17.83	8.87	0.36	0.02	0.10	426.67	
	Standard Dev	12.01	1.73	0.20	6.75		0.14	0.00	0.05	15.05	
(d)	Low	-9.18	-0.97	-0.32	-2.42		-0.05	0.01	-0.04	381.51	
	High	62.90	9.40	0.87	38.09		0.78	0.03	0.23	471.82	
Physicians Formula- water Ratio Av	r Ratio Average	24.27	3.46	1.06	3.31		1.64	0.50	0.47	80.93	
	Standard Dev	2.77	0.33	0.14	0.43		0.18	0.07	0.01	18.92	
(b)	Low	15.95	2.47	0.63	2.01		1.09	0.29	0.44	24.15	
	High	32.60	4.45	1.48	4.61		2.18	0.70	0.50	137.70	
Revlon-hybrid	Ratio Average	11.72	2.29	0.46	4.98		3.70	0.84	1.89	59.12	
	Standard Dev	2.34	0.96	0.13	1.57		0.97	0.46	0.98	22.60	
(r)	Low	4.69	-0.61	0.08	0.26		0.80	-0.54	-1.04	-8.68	
	High	18.75	5.18	0.85	9.70		6.59	2.22	4.82	126.93	
Revion-solvent	Ratio Average	0.005	7.63	0.24	32.57		270.93	8.02	35.10	0.18	
	Standard Dev	0.003	0.30	0.03	5.68		139.83	2.68	16.61	0.12	
(s)	Low	-0.003	6.72	0.15	15.53		-148.55	-0.03	-14.74	-0.20	
	High	0.013	8.53	0.33	49.62		690.42	16.07	84.94	0.55	
Revion-water	Ratio Average	8.22	26.77	3.18	8.73		3.67	0.45	0.14	71.82	
	Standard Dev	1.08	4.15	0.91	1.73		1.01	0.22	0.03	17.26	62.41
(1)	Low	4.97	14.31	0.45	3.53		0.64	-0.21	0.04	20.05	35.38
	High	11.46	39.23	5.91	13.93		6.70	1.11	0.23	123.58	409.87
Rimmel- solvent		2.42	29.06	14.05	2.07		26.09	12.61	0.00	5.02	70.43
	Standard Dev	0.23	1.12	0.53	0.02		2.06	1.08	0.10	0.51	7.24
(n)	Low	1.74	25.72	12.46	2.02		19.92	9.39	09.0	3.48	48.70
	High	3.11	32.41	15.64	2.12		32.26	15.84	1.20	6.56	92.16

(cont'd)
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Tab

Sample	Calculations	Fe/Si	Si/Al	Mg/Al	Si/Mg	Fe/Na	Si/Na	Mg/Na	AUNa	Fe/Mg	Fe/AI
Rimmel- water	Ratio Average		0.57		3.00	31.25	0.10	0.03		957.00	
	Standard Dev		0.06		0.00		0.04	0.01		11.36	
(v)	Low	307.64	0.39		3.00		-0.02	-0.01		922.93	
	High	330.36	0.74		3.00		0.22	0.07		991.07	• •
Sally Hansen-water	Ratio Average		1.69		52.33		13.59	0.28		327.75	
	Standard Dev		0.07		11.98		4.46	0.13		122.56	
(m)	Low	2.82	1.47	-	16.39		0.19	-0.11		-39.93	
	High	9.37	1.90		88.28		26.98	0.66		695.43	
Wet'n'Wild- hybrid	Ratio Average		1.19		24.19		7.40	0.33		256.56	
	Standard Dev		0.02		6.58		1.28	0.15		36.54	
(x)	Low	5.50	1.13		4.46		3.55	-0.12		146.93	
	High	16.33	1.25		43.93		11.24	0.79		366.18	
Wet'n'Wild- solvent	Ratio Average		22.13		1.52		33.60	22.30		11.45	
	Standard Dev		3.44		0.04		13.97	9.92		1.67	
(y)	Low	3.90	11.80	8.75	1.39	. •	-8.31	-7.47		6.43	54.77
	High	11.19	32.46	20.33	1.65	668.45	75.51	52.07	4.16	16.47	•••

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