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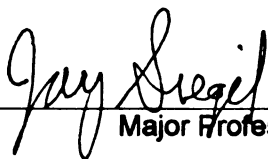
TIFFANY EDWINA CULMER

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**A MICROSPECTROPHOTOMETRIC CHARACTERIZATION OF DYED AND
BLEACHED HAIR**

By

Tiffany Edwina Culmer

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
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2005

ABSTRACT

A MICROSPECTROPHOTOMETRIC CHARACTERIZATION OF DYED AND BLEACHED HAIR

By

Tiffany Edwina Culmer

Microspectrophotometry was used to characterize dyed and bleached human hair fibers. The identification of dyed hair is important when investigating a crime scene. If bleaching or dyeing has artificially altered hairs, their evidential significance will increase. Microscopic techniques allow the analyst to examine and compare hair's structural characteristics but are rather limited for similar color comparison. Color is an important feature in hair dye analysis because it helps in reaching conclusions about similarities and differences.

This research proposed to determine how much dye is required for the visible spectrum of one strand of hair to become consistent with that dye and to compare the microspectra of bleached hair fibers. The results of the hair dyeing analysis determined that the even distribution of the dye varied with each brand name. The results of the hair bleaching analysis demonstrated that there are no significant spectral differences in hair bleaching formulas.

ACKNOWLEDGEMENTS

“I can do all things through Christ who strengthens me...” Philippians 4:13. Nothing is impossible when we put our trust in GOD!

To my parents, **Edwin and Veronica Culmer**, thank you for all the sacrifices that you have made for me so that I can make my dreams a reality. You will always be the wind beneath my winds.

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CHAPTER ONE: INTRODUCTION

Hair evidence is important because it is commonly left at a crime scene without the subject's knowledge. Hair fibers have unique shedding and transfer properties. Hair fibers are constantly shedding and are not easily lost or displaced from fabric and clothing. The types of hair recovered, their condition and number of hairs found all impact their value as evidence in a criminal investigation. The victim's and/or suspect's cosmetically treated hair will influence the significance of the case. Color is the most comparative characteristic available to a forensic examiner.

Hair association made between a suspect and a victim or a suspect and a crime scene can potentially provide very valuable and persuasive evidence in a courtroom. Hair can provide crime investigators with important clues. Apart from burning, hair is virtually indestructible. It remains identifiable even on bodies in an advanced state of decomposition or attached to objects after a crime has been committed.

Hair evidence may be subjected to microscopic examination to determine physical characteristics, biological examination to determine DNA type and toxicological examination to determine the presence of drugs or poisons.

Microscopic analysis is straightforward. Several types of microscopes can be used including stereoscope, compound and comparison.

Stereoscopic microscopy provides a broad overview of the range of characteristics of hairs. They have low magnification capabilities. The compound or polarizing microscopes have a higher magnification. As a result they provide more details about the hair's structural characteristics. If there is a need to make a side by side comparison

between a questioned and known hair sample, a transmitted light comparison microscope is used.

In 1985, Robertson and Aitken conducted a study on the value of microscopic features in the examination of human hairs.¹ The study concluded that microscopic techniques allow the analyst to compare hair's structural characteristics but are rather limited for color comparison.

Visible microspectrophotometry is used to determine the exact color of a material. It is especially useful when comparing two objects that are similar in color. The color of a dyed hair is its most important characteristic. The human eye has the capability to discriminate color better than a microspectrophotometer however, there are a number of problems in the visual comparison of colors under a microscope. It is subjective because of the differences in color sensitivity of the observer and also because of the variations in viewing conditions within the microscope. These variations are due to slight differences in mirrors, optical glass colors and polarizers.²

The identification of dyed hair is important when investigating a crime scene. If bleaching or dyeing has artificially altered hairs, their evidential significance will increase. In this study, human head hairs were analyzed by microspectrophotometry in an attempt to characterize dyed and bleached hair.

The Structure of Human Hair

Each strand of hair grows out of a tiny pocket in the skin called a follicle. Hair is composed of dead cells that are filled with insoluble proteins and held together by strong attachments between the cells. These proteins are hard fibers called keratin. Each hair

has a root and shaft within its follicle. The growth of human hair is cyclic, which involves three stages. Anagen is the stage of active growth. The root is attached to the hair follicle for continued growth. This stage can vary from two to six years. The final resting stage of the hair growth cycle is the telogen stage. This stage normally lasts five to six weeks. In this stage, the hair is pushed out of the follicle and naturally sheds. The transitional period between the growth and the resting stages is known as the catagen stage. This transitional stage can last for one to two weeks. In this stage, hair growth continues but at a very slow rate. The size of the hair root bulb begins to shrink as it is being pushed out of the hair follicle. There is a stop of protein synthesis as the hair follicle retreats towards the surface.

Hair is continually shed and renewed. Growth is not synchronized and each hair passes through the three phases independently. The average human scalp has 100,000 hairs and an average of 30 to 100 hairs are lost per day by shedding.

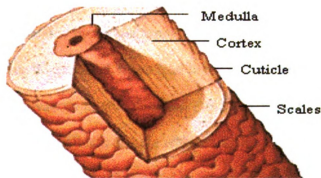
Hair has three layers: cuticle, cortex and medulla. The cuticle is the outermost layer of the hair strand. It serves as a protective sheath around the hair, which is comprised of overlapping scales. It is often compared to roof tiles.

The cortex is made up of spindle-shaped cortical cells, known as keratin, that are aligned in a regular array, parallel to the length of the hair. Single keratin molecules consist of a combination of amino acids, in particular cystine, which form stable disulphide bridges between molecular chains. These disulphide bonds form down the length of the keratin chains, joining them together like rungs of a ladder. Pigment granules containing melanin are embedded within the cortex. These melanin-filled granules are scattered throughout the cortex of the hair. There is no set pattern and no set

amount. This is how nature creates so many variations of hair color. The distribution, shape and color of these granules provide points of comparison.

The medulla is a shaft that runs through the middle of the hair. The medulla can either be continuous, interrupted, fragmented or absent. One important aspect of the medulla is that the presence and appearance vary from individual to individual and even between hairs of a given individual. It may also differ from one area of a hair strand to another.

Figure 1 – Cross-Section of Human Hair³



Hair as Trace Evidence

Edmund Locard first recognized the value of 'trace' forensic evidence in 1910 when he proposed his 'exchange theory'. It simply stated that 'every contact leaves a trace'. It means that anybody entering a crime scene will take something of that crime scene with them, as well as leaving something behind. This can constitute something as

obvious as a fingerprint or a shoe impression. It can even be microscopic evidence such as hairs or a piece of fiber from clothing.

Shed human hairs are one of the most commonly secured biological evidence materials at crime scenes.⁴ Hair often can be found on the floor near the weapon or point of impact between suspect and victim. These hairs may have fallen out naturally or forcibly removed, which may suggest a violent confrontation. Human hair is useful associative evidence because it originates directly from the individual.

The analysis of hair typically involves the inspection by microscopic techniques to determine distinguishing characteristics that may help to associate an individual to the hair sample collected. However, absolute personal identification cannot be provided from microscopic analysis of hair evidence. If hair is pulled out during the anagen phase, the root may be surrounded by a translucent tissue that is known as the follicular tag. If nuclear DNA can be extracted, it can lead to the identification of an individual. In addition, recent advances in mitochondrial DNA (mtDNA) has enabled genetic characterizations of small hair sample with or without root cells.⁵

Forensic analysts are often asked to compare hair found at a crime scene with hair from a particular individual. Some factors that must be taken into account by the examiner include; species, racial origin, body location, the presence of cosmetic treatments and the manner in which the hair was removed.

Hair Dyes

Chemistry is the basis behind all cosmetics. It is through modern chemistry that has made it possible to change hair color for a day or forever. Hair dyes can be compared

to paint because they cover hair strands by chemical coloring or by mixing with the melanin granules without altering them. The main components in most hair dyes are phenylenediamines, aminophenols, dihydroxybenzenes, nitro compounds and their salts. These dyes facilitate simple and rapid hair coloring.⁶ There are three general categories of dyes on the market. They are defined by the duration the color remains on the hair.

Temporary dyes (rinses) comprise of water-soluble acid dyes and water-soluble pigments, which are deposited on the surface of the hair with no penetration into the cortex. These dyes do not lighten or change the structure of the hair. The color is removed within one to three shampoos.

Semi-permanent dyes contain medium sized, basic molecules. These molecules contain simple derivatives of nitroanilines, nitrophenylenediamines and nitroaminophenols. The pH of the dye causes a minor alkaline reaction to take place that swells the hair shaft and causes the cuticle to rise. This allows some of the dye molecules to enter and diffuse throughout the cortex. These dyes cause mild chemical and physical changes in the hair shaft. They usually wash out after five to ten shampoos. Semi-permanent dyes come in the form of liquid, gel or aerosol foam.

Permanent dyes are marketed as two component kits. One component is a combination of dye precursors or intermediates (such as 2,5-diaminotoluene, N,N-bis(2-hydroxymethyl)-p-phenylenediamine and p-aminophenol) and couplers (such as resorcinol, chlororesorcinol, methyl resorcinol and m-aminophenol) in an alkaline base. The other component is a stabilized solution of hydrogen peroxide. The two components are mixed together prior to use. The precursors and peroxide diffuses into the hair shaft by an alkaline reaction that causes the cuticle to swell. The hydrogen peroxide oxidizes

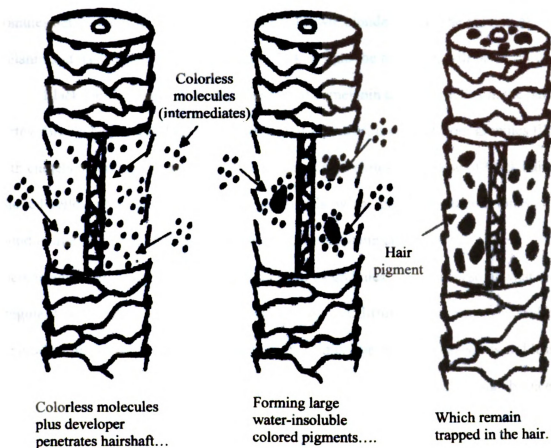
the melanin and lightens the color of the hair. This lightening action depends on three things: the strength of the developer, the level of the ammonia in the product and the processing time.

Peroxide breaks chemical bonds in the hair, releasing sulphur, which accounts for the characteristic odor of hair color. As the melanin is decolorized, a new permanent color is bonded to the cortex.

The cuticle closes and the color molecules are trapped inside with the help of a neutral or slightly basic shampoo which stops the alkaline reaction. These dyes damage the hair shaft by increasing its porosity causing it to become harder to untangle. Permanent dyes cannot be washed out with shampoo, however the color can fade with repeated shampooing.

Hair should be bleached before dyeing if the color of the hair is darker than the desired hair color.

Figure 2 – Stages of Permanent Hair Coloring⁷



Hair Bleaches

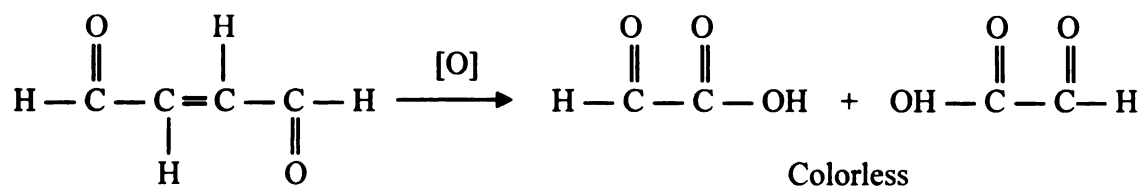
Hair bleaching is a process of lightening the hair by changing part or all of the melanin pigment in the cortex into a colorless substance. This process occurs in two steps: the degradation of the granules and the decoloration of the solubilized pigments.⁸ In most cases, bleaching is used to prepare the hair for dyeing. During bleaching the melanin pigment undergoes irreversible physiochemical changes which result either in the lightening or complete elimination of the original fiber color.⁹

All natural hair colors are created from two types of melanin: eumelanin (black pigment) and pheomelanin (red/yellow pigment). The type of melanin and the size of the granules determine whether hair will be black, brown, blonde or red. The amount of melanin and its distribution determine how dark or light the hair color will be.

Black hair is created from granules full of eumelanin densely packed in the hair's cortex. Brown hair, depending on its darkness or lightness, is created from granules filled with eumelanin and more sparsely distributed along the cortex than those of black hair. The red/yellow pheomelanin is believed to cause the warm, golden, or auburn tones found in most brown hair. Granules filled with pheomelanin create red hair. The pheomelanin in red hair is less densely packed in its granules. Its shape is somewhat more irregular than eumelanin. It is slightly rounder and more diffused. Gray hair is caused by the gradual reduction of melanin production over time. The body produces less and less melanin, and the result is a loss of color strength. Some hairs may reach a total absence of color (white).

Hydrogen peroxide is one of the most common lightening agents. It is a very powerful oxidizing agent because it reacts directly with double bonds in large organic molecules to form organic peroxides. Double bonded molecules have a tendency to absorb light and therefore provide the molecule with its color. When the double bonds are destroyed, the color is removed.

Figure 3 – Breakage of double bonds by oxidizing bleaches¹⁰



All bleaching methods are oxidative-alkaline treatments.¹¹ The solution that is used to bleach hair contains alkali to soften the cuticle and hydrogen peroxide to release oxygen. This oxygen penetrates the hair and reacts with the natural pigments in the cortex, oxidizing them and breaking them down. The melanin is still present, but the oxidized molecule is colorless. Bleached hair tends to have a pale yellow tint however, the degree of color left after bleaching depends on the production of eumelanin and pheomelanin pigments. The yellow color is the natural color of keratin, the structural protein in hair. The bleach mixture also contains modifiers to slow the release of oxygen so that the reaction happens over a period of about an hour.

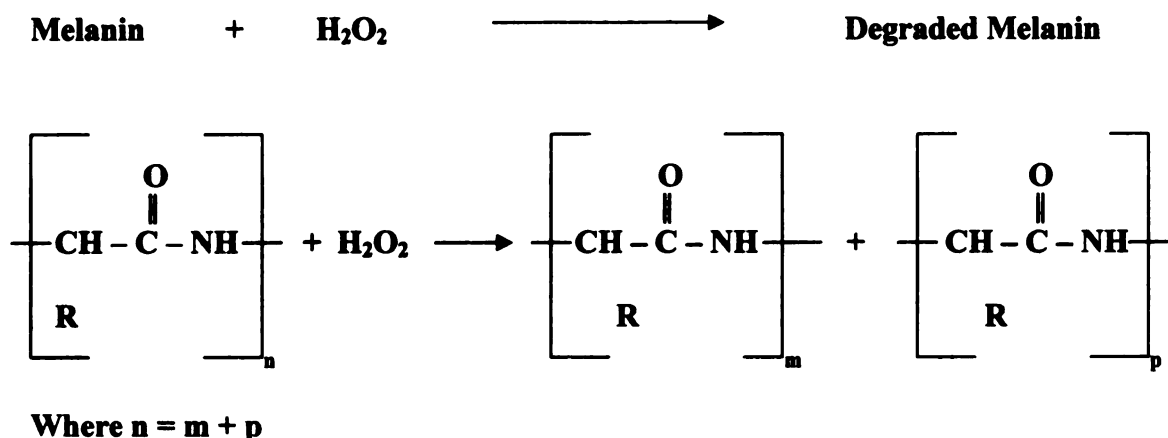
What is Melanin?

Melanin is the natural hair color pigment in the cortex layer. Specialized cells, called melanocytes that are contained in the papilla of the follicle produce melanin granules. Melanin is a family of polymers that are formed as an end product during the metabolism of the amino acid tyrosine. As a result of its high molecular weight, this color cannot be changed except by intense oxidation or concentrated alkaline solutions.

Melanin is an ill defined and an insoluble macromolecule with many alternating single and double bonds. This organic molecule has the ability to absorb light at many frequencies. Melanin absorbs light energy very efficiently and disperses it as heat.

Color in organic materials is the result of light absorption by certain chemical configurations called chromophores in molecules. $C = C$ and $C = O$ bonds are examples of chromophores. A chromophore is a part of a molecule that is able to absorb UV or visible light thus producing color in organic compounds.

Figure 4 – Redox Reaction of Melanin¹²



Review of Literature

There are many published journal articles on cosmetically treated hairs. Past research have utilized methods such as stereoscopic microscopy, polarized light microscopy (PLM), ultraviolet light microscopy, scanning electron microscopy (SEM/EDX), Fourier transform infrared spectroscopy (FT-IR), gas chromatography-mass spectrometry (GC-MS), pyrolysis gas chromatography (PGC), thin layer chromatography (TLC), microspectrophotometry and secondary ion mass spectrometry (SIMS) to determine physical and chemical properties. This research was necessary to provide a qualitative and quantitative comparison of permanent hair dyes and hair bleaches.

N. Tanada et al. have done extensive research of oxidation dyes on human hair. In one study, N. Tanada et al. purposed to improve the reliability of identification of dyed hair in addition to conventional procedures, using gas chromatographic-mass spectrometric analysis (GC-MS) and secondary ion mass spectrometry (SIMS) to analyze nine common components of permanent hair dyes. It was concluded that hair dye

component analysis of a dyed hair specimen could serve as an index for use in forensic hair comparison.

Another study conducted by N. Tanada et al. involving selective ion monitoring (SIM) analysis demonstrated that stained hair confirmed to be very beneficial for the identity of human hair in practical cases. The five identifiable components in this study were, p-phenylenediamine, toluene-2,5-diamine, o-aminophenol, m-aminophenol and p-aminophenol. The peak area percentage of the aminophenols versus diamines is thought to be effective for the identification of the brand of oxidation hair dyes.¹³

A comprehensive study on the mechanism of hair bleaching was conducted by Wolfram et al. This investigation aimed at obtaining a better understanding of the complex processes that are associated with both the melanin pigments and hair proteins. This study employed both microspectrophotometry and thin-layer chromatography techniques. Microspectrophotometry was utilized to obtain the visible and UV spectra of melanin and thin-layer chromatography was employed to separate the melanin oxidation products. It was discovered in this study that the solubilization of melanin by hydrogen peroxide is the first step in the reaction sequence. It also showed that prolonged treatment resulted in the decolorization of hair fibers. The reaction between keratin and hydrogen peroxide is confined mainly to the cystine residues.

Purpose of Study

This study is an attempt to determine the utility of microspectrophotometry as an analytical tool in the characterizing of permanently dyed and bleached hair. There are two purposes of this study. The first is to determine if the amount of dye that is required for

one strand of hair to become consistent varies between dyes that are all produced by the same manufacturer, L'Oreal.

The second purpose is to determine whether bleaching is spectrally unique. In this study, the SEE 1100 was used to obtain the absorbance spectra in the visible regions of hairs that were treated with permanent dyes and bleaches.

Limitations

This study focuses exclusively on the analysis of Caucasian hair. This is clearly an artificial concept in casework situations as the population of most countries is of mixed ethnic origin.

CHAPTER TWO: INSTRUMENTATION

History of Microspectrophotometry

Caspersson reported the first literature reference, which described the combination of a microscope to a spectrophotometer between 1925-1940. The spectrophotometer consisted of a sector photometer, monochromator, and a photocell. A microscope with quartz optics permitted both transmission of the source (visible, ultraviolet, or fluorescence) and visual examination of the sample.

With the emergence of lens technology, the Cassegrainian objective provided a high quality visual image, reasonable magnification of up to 18x, and transparency in the UV, visible, near and mid-IR regions. The instrumental advances in the development of ultraviolet microscopy during the 1940s laid the foundation for commercial UV microscopy. A quality spectrum could be obtained within several minutes to several hours depending on the size and sensitivity of the specimen.

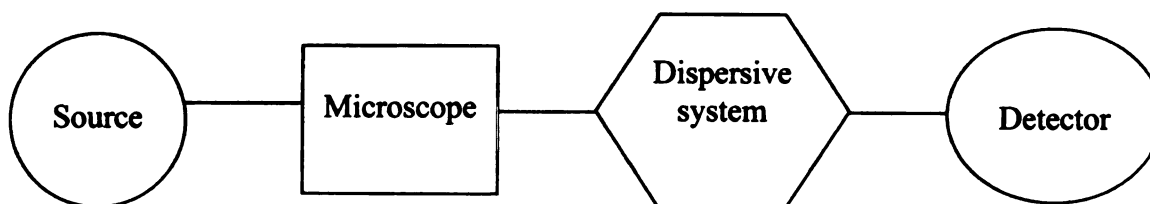
Uses of Microspectrophotometry

This instrument was primarily introduced for the identification of tissue section and single cells in biological studies. Today this analytical technique is being utilized extensively in many areas. Quantitative absorbance data are attainable for many applications in the field of forensic science, specifically in the area of trace evidence. A forensic examiner can employ this technique to analyze fibers, paint chips and questioned documents. This technique is also being applied to the cosmetic industry to help provide an understanding of how cosmetic treatments affect human hair properties. This knowledge is critical for the development of successful products.

Microspectrophotometry

A microspectrophotometer is an instrument that is a combination of a microscope and a computerized spectrophotometer. A spectrophotometer measures the intensity of a light beam before and after it passes through a sample and compares these two intensities. Spectrophotometry is based on the wave theory of light and the principle that substances selectively absorb certain wavelengths and reflect or transmit others. It provides an absolute measurement because it compares the light that has been transmitted or reflected at each wavelength with the intensity of light incident on the object, making the result independent of the type of illumination used.¹⁴

Figure 5 - Basic Components of a Microscopical Spectrophotometer System.¹⁵



A pictorial representation of the manner in which a certain colored object reacts to various wavelengths of light is referred to as a spectrum. Microspectrophotometry has been widely utilized in forensic examination of trace evidence for objective evaluation of colors of minute materials.¹⁶ Different substances react to light in different ways. The spectra of different objects can be compared to each other to determine similarities and differences.

An advantage of using microspectrophotometry as opposed to comparing colors by the eye via microscopy is that phenomenon of metameric colors is eliminated. Metamerism occurs when two colors match in one light but fail to match in another. The spectral comparisons that are produced using microspectrophotometry provide more improved and objective conclusions than by the comparison made by visual examination.

In this study, the SEE 1100 microspectrophotometer was utilized in conjunction with the Grams/32 program software. Grams/32 is an integrated suite of instrument controls, spectral data analyses programs and a fully relational database. To compare the precise color of the hair dyes in this project, the transmittance method was employed utilizing a halogen lamp.

Combined with its high discriminating power, its non-destructive and rapid method of analysis that requires little sample preparation, microspectrophotometry can provide excellent results. Data from both instruments are stored as spectra. The instruments can load up to thirty spectra in the memory stack. These stored spectra can then be retrieved for future comparison if it is necessary.

The determination and accuracy of the instruments were determined by routine calibrations with the aid of filters. The wavelength accuracy is determined through holmium oxide and didymium filters. The spectra of these filters display sharp peaks at well-documented wavelengths. The holmium oxide filter is used to calibrate wavelength from 280 nm to 640 nm in the visible and UV regions. The didymium filter has peaks in the visible and near IR wavelength regions, which is used to calibrate wavelengths from 440 nm to 880 nm. The photometric accuracy is determined through a sequence of neutral density filters (OD = 0.1, 0.5, 1.0). These filters are characterized by a flat optical

response in the wavelength region from 250 nm to 1000 nm. Each filter comes with NIST traceable wavelength values. The NIST traceable set that is provided by SEE Incorporated adheres to the ASTM standard practices for describing and measuring the performance of UV, visible and near IR spectrophotometers.

CHAPTER THREE: METHODS AND MATERIALS

Sample Preparation

All of the hairs used in this study were head hairs cut from one Caucasian female individual with clean scissors and stored, until use, at room temperature. The hair fibers had only been subjected to normal wear, which included shampoos, conditioners, water settings, hair dryers, combing, brushing and atmospheric exposure. The hair fibers were not chemically treated prior to this experiment. The samples were washed twice with a non-conditioning commercial shampoo to eliminate external contaminants and allowed to dry at room temperature for 3 days. The hair samples were divided into two equal batches and were kept separate from each other.

Preparation of Smears

Eight bottles of permanent red hair dyes were purchased from a local beauty supply store. Table 1 lists the brands and colors of the dye. 0.5 ml of each dye was added to its corresponding developer in a ratio of 1:1. The solutions were thoroughly mixed and allowed to process. Small amounts of each mixture were smeared on glass microscope slides and were allowed to dry at room temperature. Once dried, five sample scans were run on each sample in different areas of the hair dye smear. Images in this thesis are presented in color.

The Hair Dyeing Process

In order to establish practical and reliable dyeing and bleaching methods, the manufacturers' instructions were carefully followed. Initially, the hairs were bleached prior to dyeing because the present hair color was much darker than the desired hair

color. Hair fibers were stained with eight permanent hair dyes. Once the hair fibers were bleached, they were evenly separated into batches. Hairs were randomly selected out of each batch and nine sample scans were obtained from different locations on the hair. The spectra from the scans were then overlaid and printed for further examination.

The hair bundle was composed of dark brown virgin hair 5 cm in length. The hair fibers were bleached in order to lighten the natural hair shade and to prepare it for dyeing. 14 g of a bleaching powder, L'Oreal Quick Blue, was added to 29 ml of its corresponding developer. Table 2 lists the names of the bleaches and their corresponding developers. The powder and developer were thoroughly mixed and applied to the hair using the foil wrap technique. The hair was placed on a small piece of aluminum foil and covered with the bleach mixture. The foil was then folded into a small package and processed until the hair was white in color. The hair was checked every 10 minutes to determine the final color. When the desired shade was obtained, the hair fibers were carefully rinsed with water, shampooed and air-dried. These hair samples were divided up into 8 different batches that each contained 50 strands of hair and were assigned numbers 1 to 8.

Batches 1 to 8 were now ready to be dyed. The hair fibers were stained with 1ml of dye according to the manufacturer's directions and were stored at room temperature until the time of analysis. Five hair fibers were randomly selected from each batch and were individually mounted on using microscope slide/cover slip preparations in 1.539 Cargille refractive index fluid.

Nine sample scans were obtained from different locations on each hair fiber. The scans were overlaid and examined for consistency. If the color was not consistent the remaining hair fibers in all batches were stained with the dye corresponding to its batch

number in increments of 0.5 ml. This process was repeated until the dye had been equally distributed throughout the hair fibers. The spectra were overlaid and printed for further examination.

Table 1 – List of Brands and Colors of L’Oreal Dyes

Brands of L’Oreal Dyes	Name of Color
Excellence	Red Penny
Féria	All-Out Red Copper Red
Féria	Double Intensity Auburn Red
Féria	Glowing Red-Hot Red
Preference	Red Penny
Preference	Smoldering Red
Preference Mega Reds	Dark Intensity Copper Red
Preference Mega Reds	Medium Intense Red Copper

Table 2 – Dye Processing Data

Batch Number	L'Oreal Brand	Color	Developer	Processing Time (min)
1	Excellence	Red Penny	L'Oreal 20 Vol. Oreor Crème Developer Oxydant	25
2	Féria	All-Out Red Copper Red	L'Oreal Féria Multi- Tone Color Developer Plus	25
3	Féria	Double Intensity Auburn Red	L'Oreal Féria Multi- Tone Color Developer Plus	25
4	Féria	Glowing Red- Hot Red	L'Oreal Féria Multi- Tone Color Developer Plus	25
5	Preference	Red Penny	L'Oreal 20 Vol. Oreor Crème Developer Oxydant	25
6	Preference	Smoldering Red	L'Oreal 20 Vol. Oreor Crème Developer Oxydant	25
7	Preference Mega Reds	Dark Intensity Copper Red	L'Oreal 20 Vol. Oreor Crème Developer Oxydant	25
8	Preference Mega Reds	Medium Intense Red Copper	L'Oreal 20 Vol. Oreor Crème Developer Oxydant	25

The Hair Bleaching Processes

Six commercially available bleaching formulas for home use were individually applied to the batches of hairs, labeled numbers 9 to 14, according to the manufacturer's instructions via the foil wrap technique. Refer to Table 3 for bleach processing data.

Five hair fibers were randomly selected from each batch and analyzed by wet-mount comparison with 1.539 Cargille refractive index fluid mounting media. Five sample scans were obtained from different locations on each hair. The scans were overlaid and

examined for similarities and differences. The spectra were overlaid and printed for further examination.

Table 3 – Bleach Processing Data

Batch Number	Powder Bleach	Developer	Amount of Powder Bleach (g)	Amount of Developer (ml)	Processing Time
9	Clairol Kaleidocolors	Salon Care 20 Volume Crème	8	17	20
10	Clairol BW2	Clairol 30 Volume Clairoxide	8	13	50
11	L'Oreal Quick Blue	L'Oreal 30 Volume Oreor Crème Developer Oxydant	8	17	50
12	L'Oreal Super Oréal Blanc	L'Oreal 30 Volume Oreor Crème Developer Oxydant	8	19	50
13	Salon Care Quick White	Clairol 30 Volume Clairoxide	8	13	50
14	Wella Wellite	L'Oreal 30 Volume Oreor Crème Developer Oxydant	8	16	50

Microspectrophotometric Analysis

Microspectrophotometric analysis was performed on the SEE 1100 (version 2.0) in the forensic science laboratory at Michigan State University. The initial calibration of the instrument was performed prior to the initiation of this microspectrophotometric

analysis. Each filter was measured 50 times over the course of 25 days in order to establish a baseline average for each specific microspectrophotometer.

The instrument was calibrated before the dye smears and hair mounts were analyzed. Prior to calibration, there was a stabilizing period for the instruments of 30 minutes. The first component to be calibrated was the microscope. It was adjusted for Köhler illumination. This term refers to a type of optically centered and focused illumination that is essential to the transmission of the maximum amount of analytical beam energy through the system.¹⁷

It was necessary that all the wavelength measurements stayed within the designated range of ± 3.0 nm of the National Institute of Science and Technology (NIST) values on the calibration certificates when the same measurement conditions are utilized. All photometric measurements were also required to remain within the range that is specified on the certificates when the same measurement conditions are utilized. The instruments were calibrated with the NIST Traceable Filter Set on a daily basis prior to use of the instruments. The calibrations were archived for future instrument evaluation.

The instruments had to go through another series of steps before a sample spectrum can be taken. The first step was to apply the autogain function in order to optimize the instruments. This feature automatically adjusts the instrument. The light source shutter was closed and a dark scan was taken. This was done to ensure that the microspectrophotometers were free of light leaks and were not affected by stray light. The light path was opened and the reference scan was taken. This scan is performed within the field of area that does not contain the sample. The sample was then positioned so that it could be analyzed. The sample scan button was pressed and the transmittance

(%T) spectra option was chosen. A reference scan is always obtained before every sample scan that is taken outside of the previous reference area.

CHAPTER FOUR: RESULTS AND DISCUSSION

Results

Hair Dyed Samples

Upon comparison of the spectrum of the dye smears, it was observed that the spectrum varied slightly in shape and relative peak heights. The wavenumbers of the most predominant peak in the microspectrophotometry spectrum for the 0.5 ml dye smears were compared to each other. The spectra were all consistent with each other (see Figures 6 – 13), with some variation. The range of the maximum absorbance peaks ranged from 468.9 – 481.2. This is a difference of 12.3 absorbance units. Some of this variation could have resulted from the manual markings of the peaks.

The amount of dye that was required to obtain equal distribution throughout one strand of hair was determined by microspectrophotometry. There were little variations in intensity across the spectral region as expected due to dye diffusion across the hair fiber. This reinforces the importance of taking multiple scans of one object in order to determine the overall variation among spectra.

The evaluation of the recorded spectra obtained by microspectrophotometry determined that the amount of dye needed to obtain a consistent color on a hair fiber varies with the product. The amount of dyes that was required for consistency are listed in Table 3.

The microspectrophotometry absorbance spectra of the dyed hair fibers were compared and classed based upon the overall shape of the spectral curve. Feria All-Out Red Copper Red, Feria Glowing Red-Hot Red, Preference Mega Reds Dark Intensity

Copper Red and Preference Mega Reds Medium Intensity Copper Red spectrum could not be distinguished from each other.

Excellence Red Penny and Preference Red Penny spectrum also could not be distinguished from each. Feria Double Intensity Auburn Red and Preference Smoldering Red spectrum were the only two dyes that were easily identifiable from all the other dyes analyzed in this study.

It was predicted that the products that had the same sub-brand should have required the exact amount of dye to produce consistency, however this study did not support that hypothesis in all cases. There were four sub-brands of dyes that were manufactured by L'Oreal that were analyzed in this study. They were Excellence, Feria, Preference and Preference Mega Reds.

Table 4 - Results of Dye Amounts for Consistency

Batch Number	L'Oreal Brand	Name of Color	Developer	Amt of Dye (ml) for Consistency
1	Excellence	Red Penny	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	1.5
2	Féria	All-Out Red Copper Red	L'Oreal Féria Multi- Tone Color Developer Plus	1
3	Féria	Double Intensity Auburn Red	L'Oreal Féria Multi- Tone Color Developer Plus	1.5
4	Féria	Glowing Red- Hot Red	L'Oreal Féria Multi- Tone Color Developer Plus	1
5	Preference	Red Penny	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	1.5
6	Preference	Smoldering Red	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	1
7	Preference Mega Reds	Dark Intensity Copper Red	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	1.5
8	Preference Mega Reds	Medium Intense Red Copper	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	1.5

Although all of the dyes examined in this experiment were marketed as red in color, there were color differences before and after the addition of the developer. These color differences are listed in Table 4. This proves that that there are many different formulas that are used to alter hair color.

Table 5 – Dye Color Differences Before and After the Addition of Developer

L'Oreal Brand	Name of Color	Developer	Color of Dye	Color of Mixture
Excellence	Red Penny	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	Light Yellow	Dark Purple
Féria	All-Out Red Copper Red	L'Oreal Féria Multi-Tone Color Developer Plus	Light Brown	Dark Purple
Féria	Double Intensity Auburn Red	L'Oreal Féria Multi-Tone Color Developer Plus	Light Brown	Dark Purple
Féria	Glowing Red- Hot Red	L'Oreal Féria Multi-Tone Color Developer Plus	Light Brown	Dark Purple
Preference	Red Penny	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	Light Brown	Dark Purple
Preference	Smoldering Red	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	Light Brown	Dark Purple
Preference Mega Reds	Dark Intensity Copper Red	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	Light Brown	Dark Purple
Preference Mega Reds	Medium Intense Red Copper	L'Oreal 30 Vol. Oreor Crème Developer Oxydant	Light Brown	Dark Purple

Hair Bleached Samples

This study was based more on quality rather than quantity. The hair fibers were separated into batches 9-14 however, the number of hair fibers in each batch were not always equal as those in the hair dyeing process. Each batch was bleached with a different bleaching formula using the foil wrap technique. Hairs were randomly selected out of each batch and five sample scans were taken from different locations on each hair.

After representative hair samples were scanned from each batch, the most compatible spectra was chosen and overlaid.

Figure 24 shows an overlay of hair samples from batches 9-14. All six of the bleaching formulas demonstrated a similar spectral pattern. The evaluation of the transmission spectra obtained from analyzing the bleached hair fiber samples was based upon the peak maxima and overall shape of the spectral curve. All the bleaching formulas used in this spectral comparison showed that there is no spectral uniqueness about any particular bleaching formula.

Discussion

Forensic analysis of hair centers on color and structure that is determined through microscopic magnification. Head hairs are usually the longest hairs on the human body. These hairs are subjected to more alterations than hairs from any other body part. The most popular hair treatments are bleaching and permanent dyeing. Nature develops its own precursors within melanocytes, as a result the effects of hair treatment depend on the characteristics of the individual hair fibers.

The primary purpose of this study was to determine if the amount of dye that is required for one strand of hair to be consistent varies between permanent dyes that are produced by the same manufacturer. The quantitatively determined values were achieved by adding increments of 0.5 ml for the amount dye as needed.

When it comes to normal hair care coloring, permanent color is the most popular choice because it lasts the longest and gives the most dramatic change of tone. Hair care products are available in permanent hair colors that will last until the hair is cut and/or the roots grow out.

Permanent hair dyes start out as colorless molecules that are small enough to squeeze between the cuticle cells and get inside the hairs. These tiny molecules react with hydrogen peroxide to become colored molecules. They also form clusters that are too large to wash out of the hair, leaving your hair permanently colored. A hair color can't be judge by looking at the dye mixture. The color of the pigments will change as the colorless molecules react with hydrogen peroxide. At the end of a successful coloring operation, the giant pigment molecules are securely in place behind the translucent cuticle, which is once again protecting the cortex.

Permanent hair dyes are also referred to as oxidative hair dyes. Hydrogen peroxide in the oxidative hair dye formulae serves as a bleaching agent for the natural pigment of the hair. The color formation is dependent on precursors present in the dyeing solution, its pH and the time of contact with the hair.^{18,19} Oxidative hair dyes are resistant to fading by shampooing, but re-coloring of hair is required approximately every six weeks due to hair growth. The time of contact of the dyeing solution on the hair can vary from 15 - 50 minutes. Permanent hair dyes were chosen because these products provide a lasting color effect, 100% grey coverage and represent a large percentage of the hair coloring market.

A comparison of all the active ingredients of each dye used in this study was done. The result corresponded with the findings from the analysis that although these dyes were marketed as the same color, they were different in their chemical composition. The components in all of the dyes (see Appendix V) were listed on the dyes' labels, however there were a few active ingredients that were either present and/or absent in the some of the dyes. These ingredients will determine the final color, its lightness or

darkness. All of the labels read one of the following phrases; “individual shades do not contain every ingredient” or “may contain.”

There was one active ingredient that was listed as may or may not being present in seven of the dyes however, it was not listed at all on the label of Preference Smoldering Red. This ingredient was propylene glycol.

Propylene glycol is a petroleum derivative that is formulated into hair care products and serves as a humectant or a wetting agent. The role of a humectant is that it retains the moisture content of cosmetic products by preventing the escape of moisture or water. This substance causes the hair to swell due to the excess amount of water as a result the amount of dye intermediates and precursors can not enter to develop. Hence, after all things considered, hair fibers dyed with Preference Smoldering Red required less dye for consistency to be achieved. In addition to Feria All-Out Red Copper-Red and Feria Glowing Red-Hot Red, which may not have contained this ingredient. All three of these dyes that were previously listed only required 1 ml of dye for consistency to be achieved.

The secondary purpose of this study was to determine if there were any significant spectral differences in hair bleaching formulas. A qualitative analysis of the bleaching formulas illustrated that there were not any significant spectral differences, therefore for the most part their chemical composition is similar.

When the hair fibers were bleached in this study, the pigments in the hairs were eliminated. This is visible when looking at the overlay of spectra in Figure 24. There is no definite shape to any of the spectra.

CHAPTER FIVE: CONCLUSION AND FUTURE RESEARCH

Conclusion

This study demonstrated that visible transmission microspectrophotometry was shown to be an effective method for the examination of pigment distribution throughout cosmetically treated hair fibers. The presence of artificial treatments such as dyes and bleaches can be identified through microscopical examination. The microscopic association of bleached hairs is rather difficult because these hair types have less distinctive pigment patterns. Head hairs grow at a rate of one centimeter per month. The approximate time of this treatment can be determined by measuring the length of the untreated area of the hair.

Microspectrophotometry is a non-destructive method that provides useful structural information and has the ability to accurately discriminate between similar dyes using only a single hair fiber. This method does not provide conclusive forensic evidence, but can serve as a screening test for hair identification with other analytical and chemical techniques such as gas chromatography and thin layer chromatography.

This study carried out by microspectrophotometry has revealed that it can serve as a useful tool in to provide an objective rather than subjective information about color in both the areas of forensic science and the cosmetic industry.

Future Research

Hair growth occurs both longitudinally and in diameter. It is well known from the literature that the rate of hair growth varies from person to person. Hair growth is a function of individual factors. Other factors such as age, gender, hair color, the presence of drugs, hormones and nutrition can either accelerate or prolong hair growth. While

some of the factors may be or may not be known in an individual case, hair analysis from a particular individual can be rather challenging.

Upon conclusion of this study, there are several possible research projects that could derive from the present work. There is a need for research furthering the inclusion of all racial hair types, since only Caucasian hair samples were used in this study. There are striking and well documented differences between hair types of varying ethnic origins, including Negroids, Caucasoids and Mongoloids. Hair from these groups differs in numerous properties including maximum attainable length, fiber shape, thickness, density, amino acid content, and tensile properties such as elasticity and percent elongation at break. The effects of hair treatment depend on the characteristics of the individual fibers. The criteria required to perform racial hair analysis are summarized in Table 6.

Table 6 – Racial Characteristics of Hair

Race	Cross Section	Diameter	Pigmentation	Cuticle	Appearance
Negroid	Flat	60-90 μm	Dense	Thin	Wavy
Caucasoid	Oval	70–100 μm	Even	Medium	Straight/wavy
Mongoloid	Round	90-120 μm	Auburn	Thick	Straight

Before any permanent color can penetrate the hair shaft, the cuticle must be opened so that the chemicals can get into the natural pigment molecules. When comparing the racial characteristics of hair from Table 6, it is likely that the Caucasoid or

the Negroid hair types would always require less dye to achieve consistency than the Mongoloid. The Mongoloid hair type possesses a thick cuticle and a larger cross section as a result more dye molecules will be needed to penetrate the hair shaft and for the equal distribution of the dye molecules throughout the entire hair strand. Additional research is needed to draw a general conclusion about ethnic dependency.

As a continuation of the present research, different brands as well as colors of permanent hair dyes could be analyzed to compare the amount of the dye that is need for consistency to be achieved. These spectra of dye smears can be compiled to establish a library for comparison of permanent hair dyes.

Additional research needs to be performed on developing microspectrophotometry as a better discriminatory technique. Microspectrophotometry was not able to conclusively distinguish between permanent hair dyes that had different sub-brands.

Environmental factors have the ability to change the color of hair both physically and chemically. When dyed or bleached hair is exposed to environmental factors such as sun, water and air, it begins to oxidize and the dye pigments are released. The cosmetic value of hair can be diminished through excessive exposure to the sun. Prolonged sun exposure to hair is an example of photochemical change to the hair shaft. The UV light in direct sunlight affects the cuticle in a similar way to bleach, by ultimately breaking down the keratin protein in the hair.

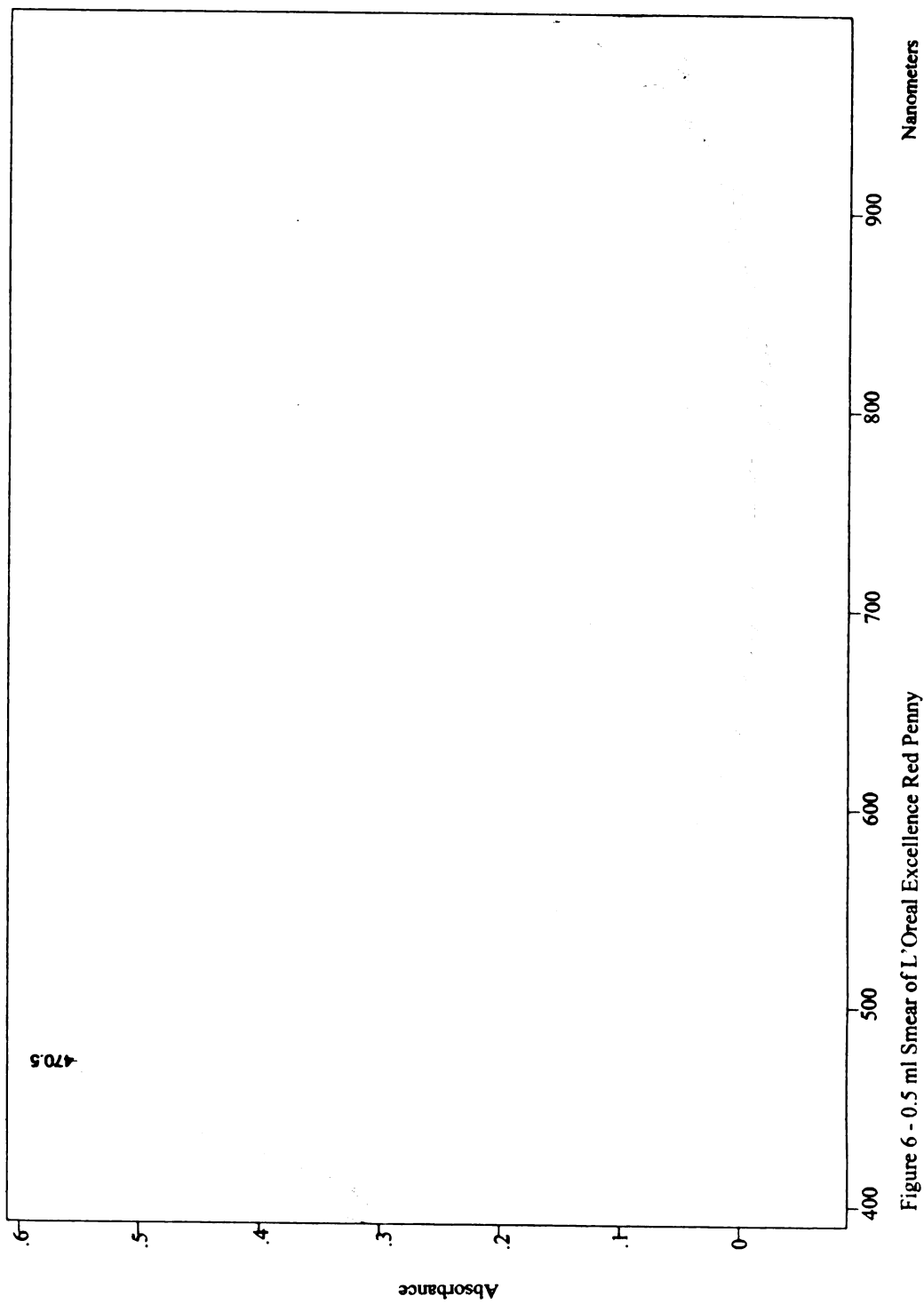
Hair discoloration also occurs from both chlorinated and salt water. In dyed blonde or bleached hairs, the chemicals in pool water tend to bind to the hair and cause the hair fibers to become green. Mineral deposits from salt water severely dehydrate hair.

The best way to minimize weathering damage is to use color safe shampoo and deep conditioning treatments that contain keratin, jojoba oil and wheat germ. These natural ingredients increase moisture and shine. After all things considered, two areas of potential research are suggested: to determine what length of time is required for cosmetically treated hair to become physically and chemically changed while being exposed to these external factors and how well do these products work to minimize or prevent damage from environmental exposure.

APPENDICES

APPENDIX I

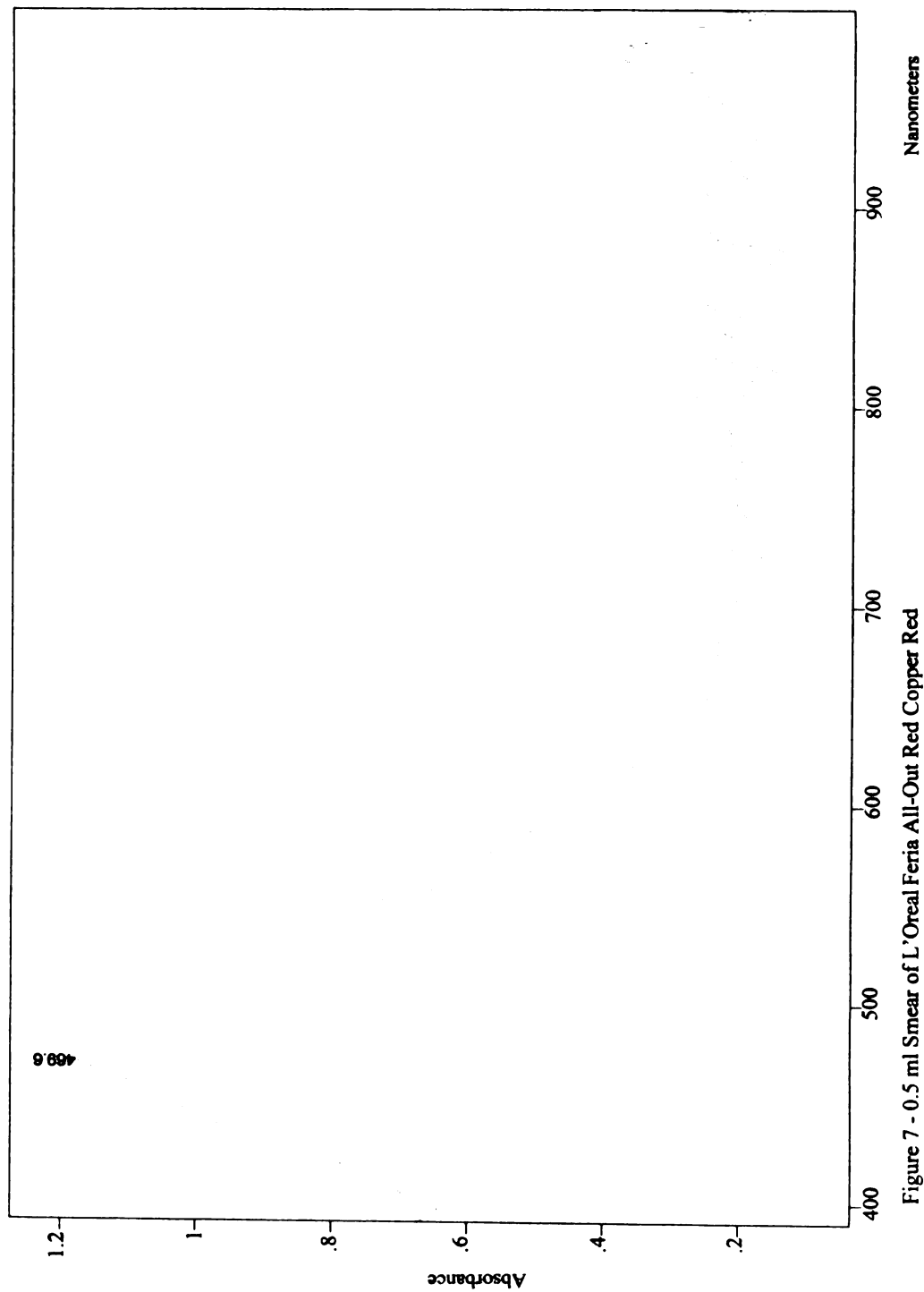
SPECTRA OF DYE SMEARS



Nanometers

Figure 6 - 0.5 ml Smear of L'Oreal Excellence Red Penny





Nanometers

Figure 7 - 0.5 ml Smear of L'Oreal Féria All-Out Red Copper Red



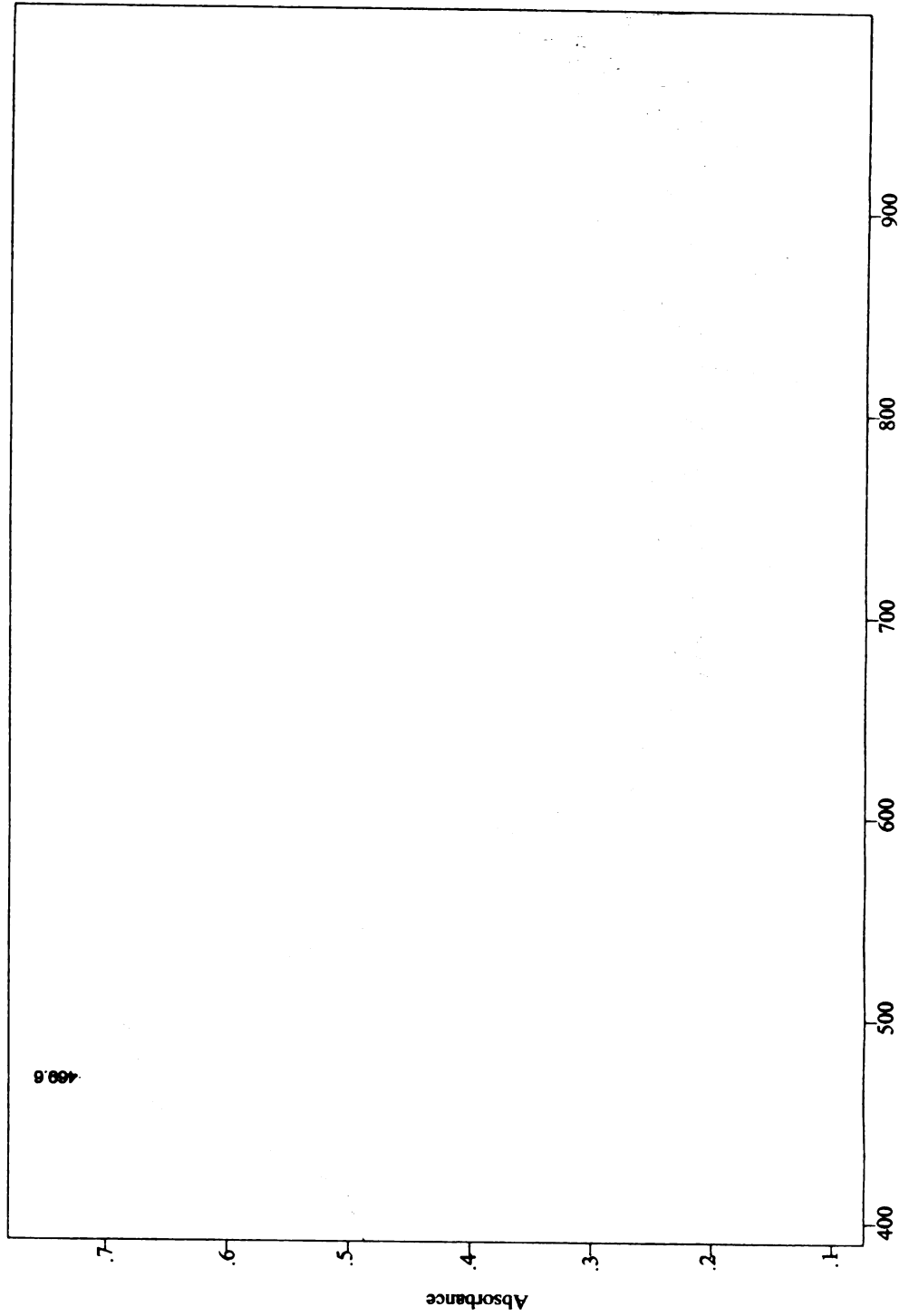


Figure 8 - 0.5 ml Smear of L'Oreal Fera Double Intensity Auburn Red



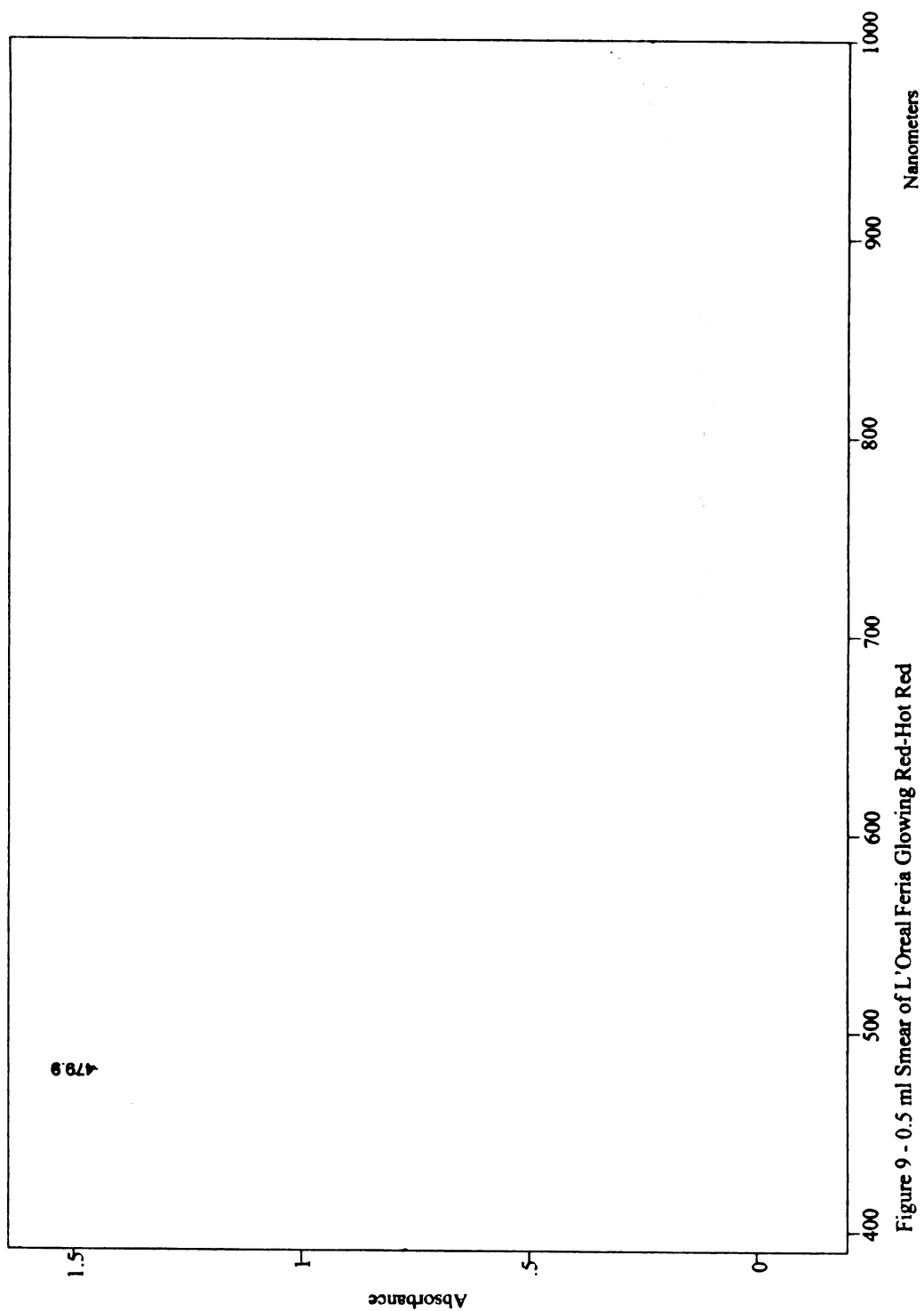


Figure 9 - 0.5 ml Smear of L'Oreal Féria Glowing Red-Hot Red



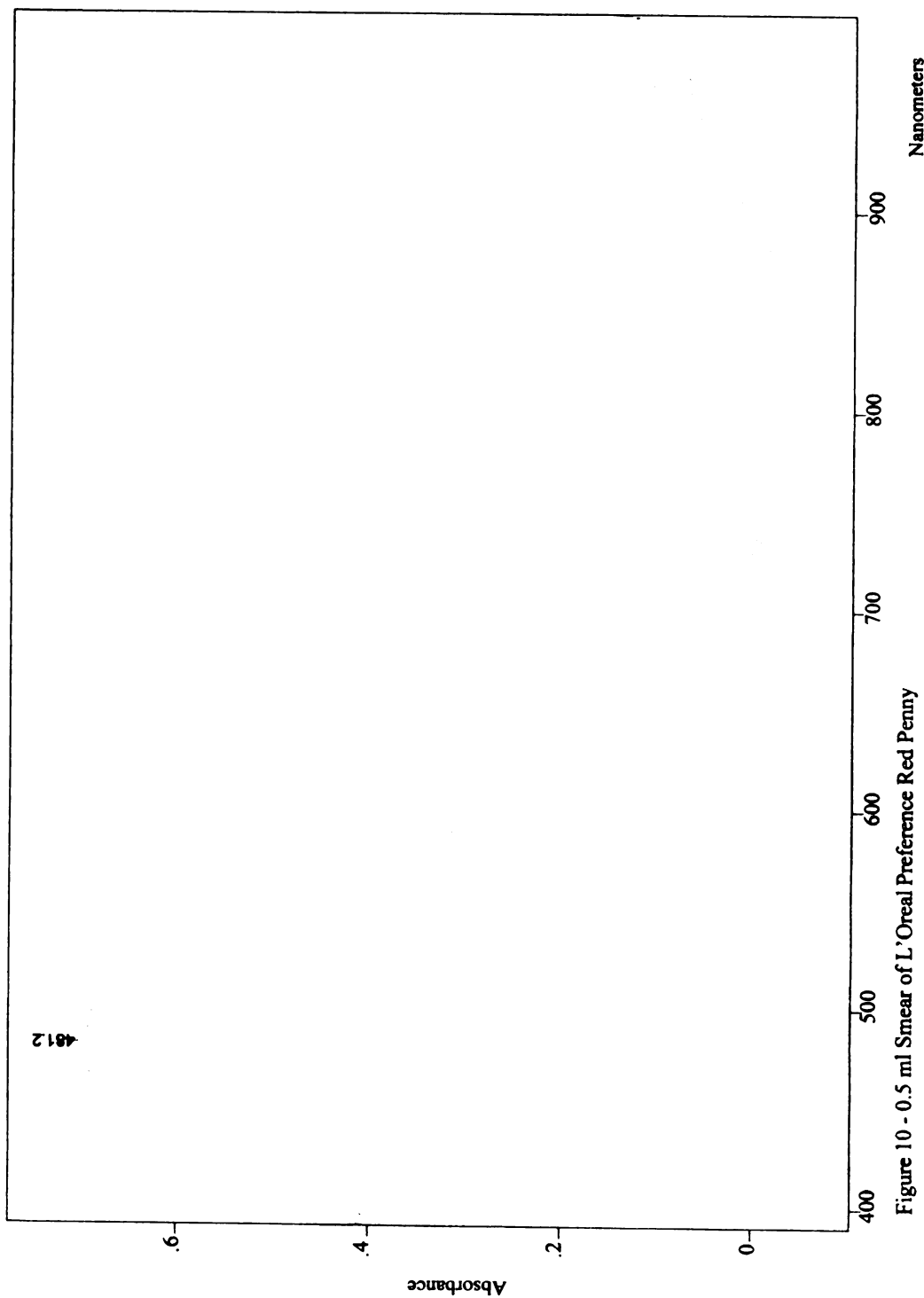


Figure 10 - 0.5 ml Smear of L'Oreal Preference Red Penny

Nanometers



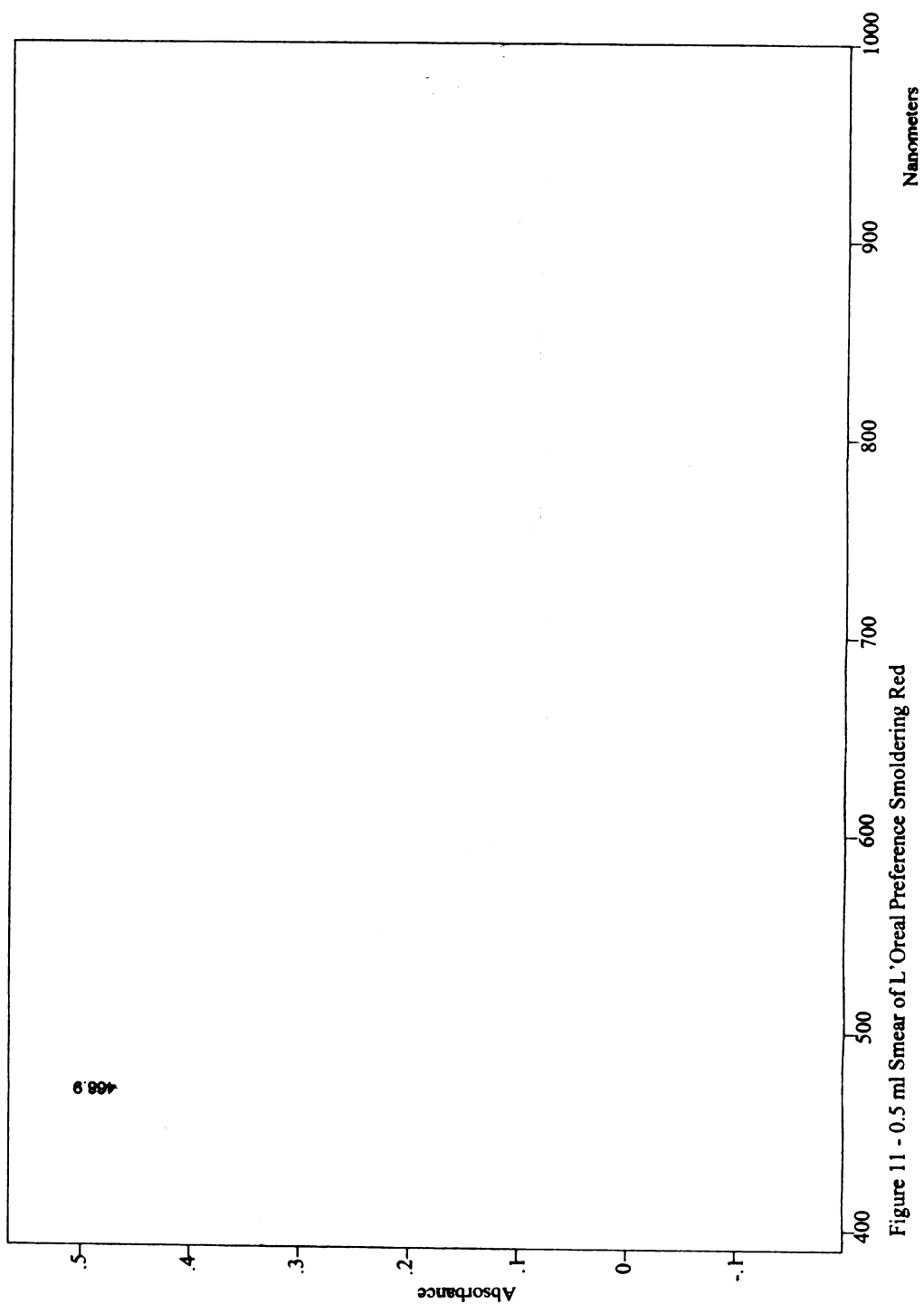
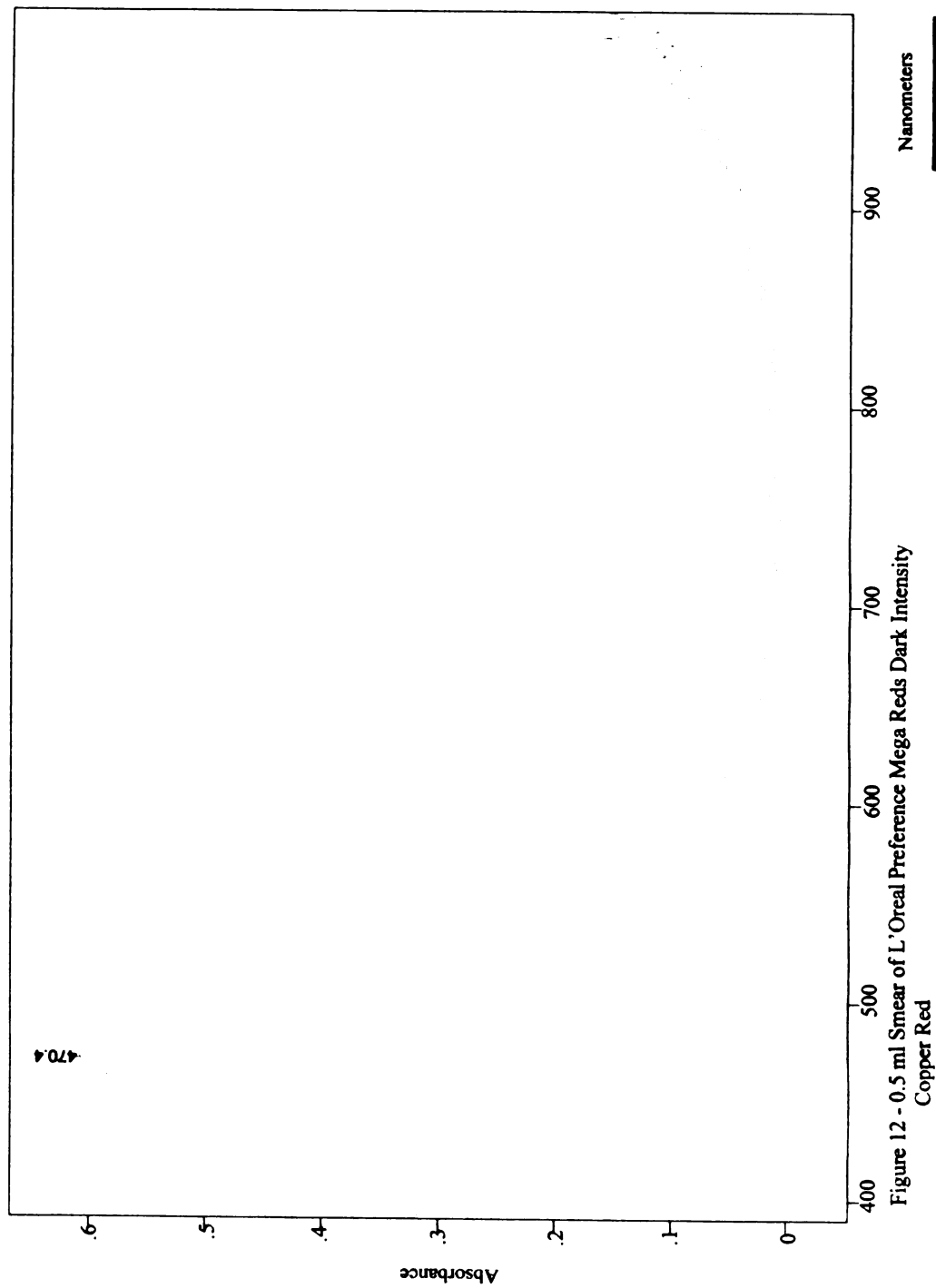


Figure 11 - 0.5 ml Smear of L'Oreal Preference Smoldering Red





Nanometers

Figure 12 - 0.5 ml Smear of L'Oreal Preference Mega Reds Dark Intensity
Copper Red



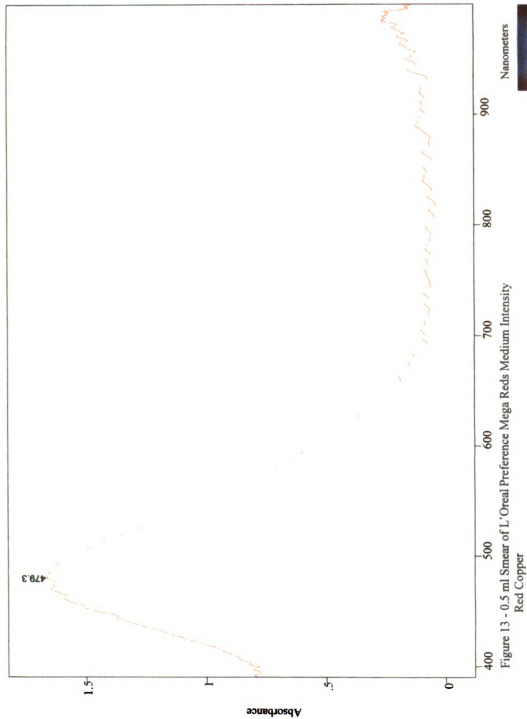


Figure 13 - 0.5 ml Smear of L'Oreal Preference Mega Reds Medium Intensity Red Copper

APPENDIX II

SPECTRA OF DYED HAIR FIBERS

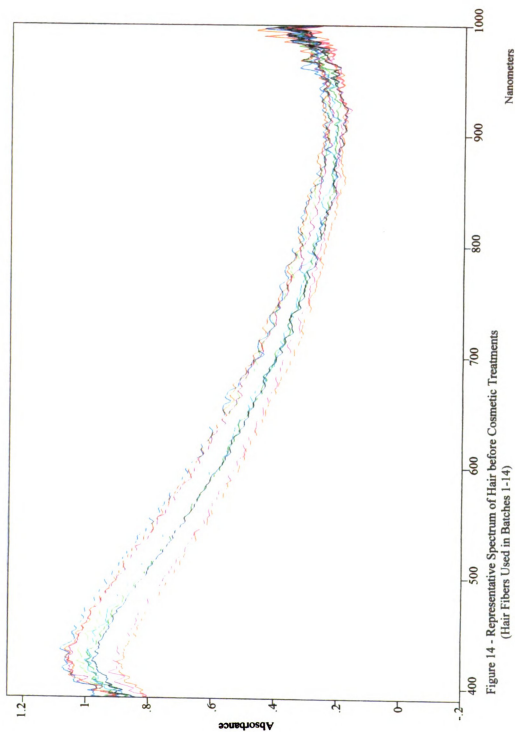
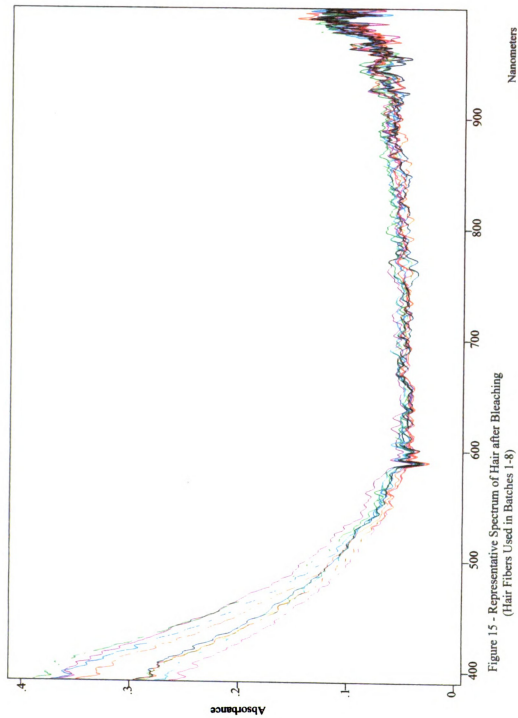


Figure 14 - Representative Spectrum of Hair before Cosmetic Treatments
(Hair Fibers Used in Batches 1-14)



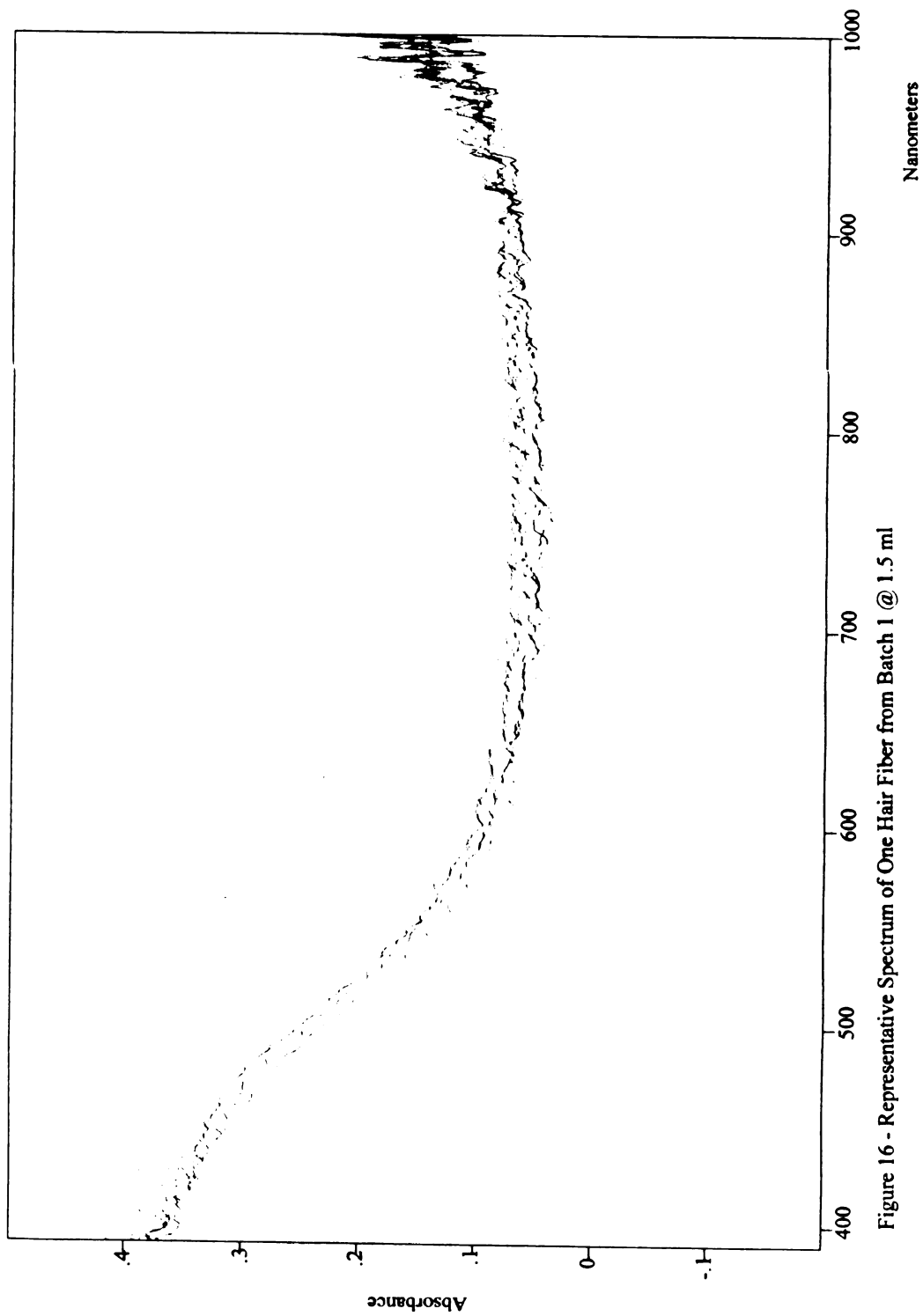


Figure 16 - Representative Spectrum of One Hair Fiber from Batch 1 @ 1.5 ml



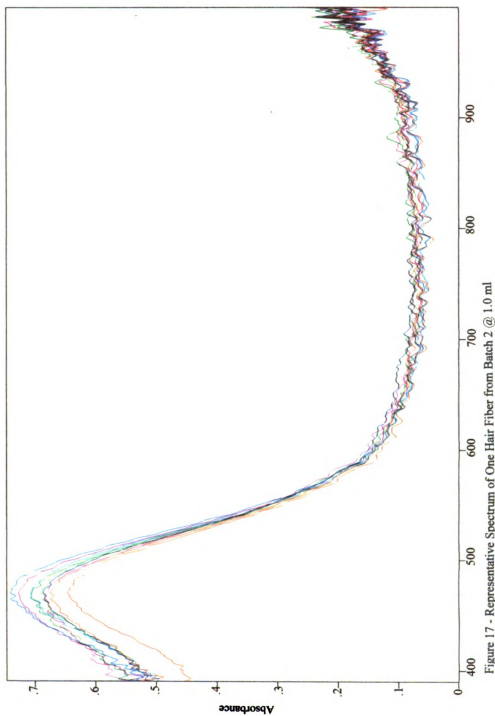


Figure 17 - Representative Spectrum of One Hair Fiber from Batch 2 @ 1.0 ml



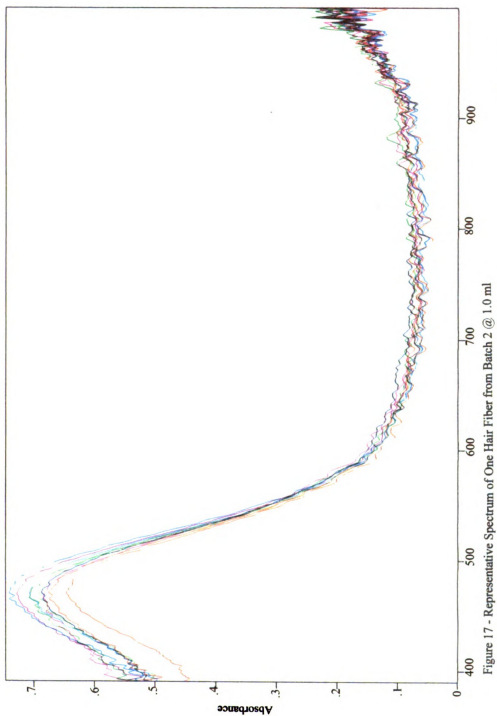


Figure 17 - Representative Spectrum of One Hair Fiber from Batch 2 @ 1.0 ml

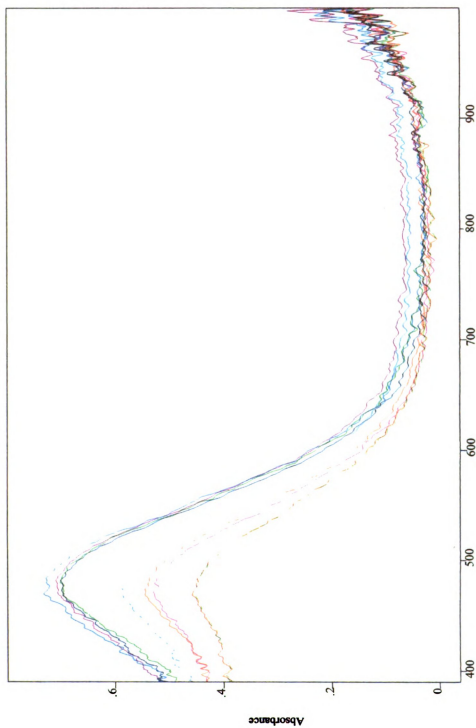
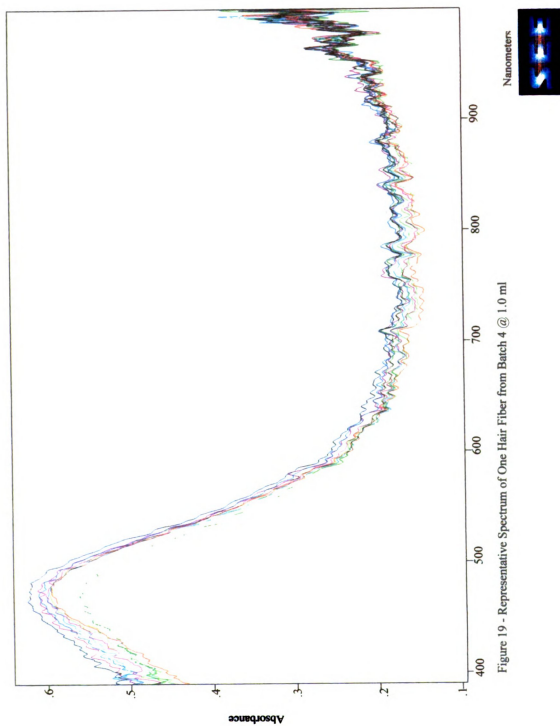


Figure 18 - Representative Spectrum of One Hair Fiber from Batch 3 @ 1.5 ml





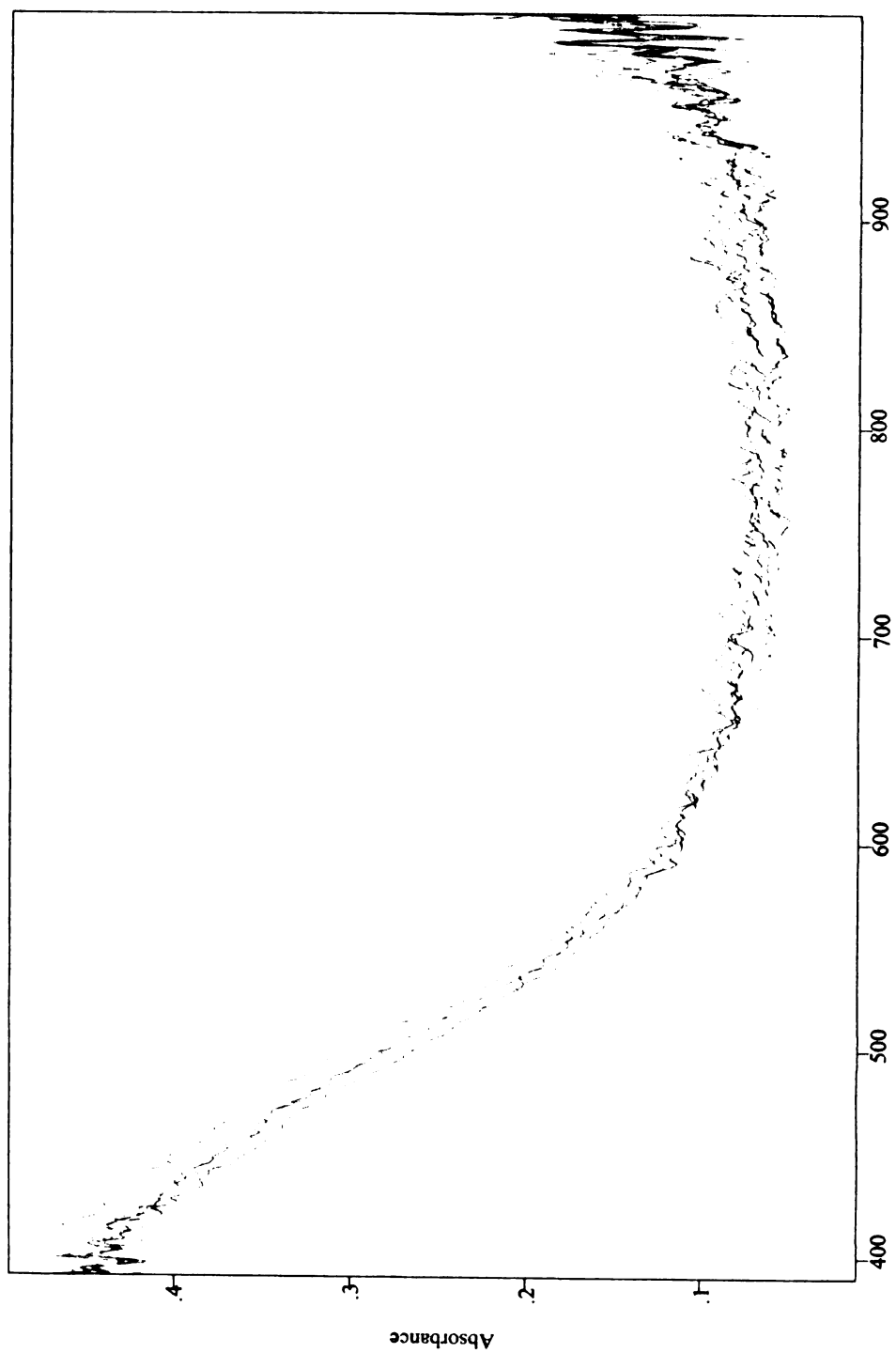


Figure 20 - Representative Spectrum of One Hair Fiber from Batch 5 @ 1.5 ml

Nanometers



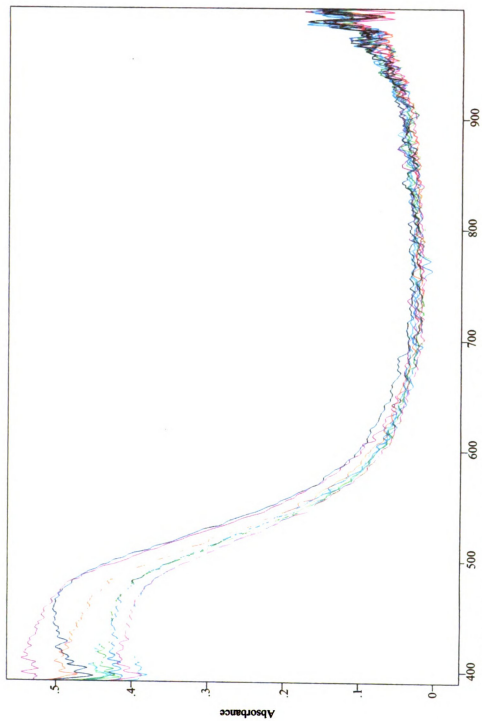


Figure 21 - Representative Spectrum of One Hair Fiber from Batch 6 @ 1.0 ml



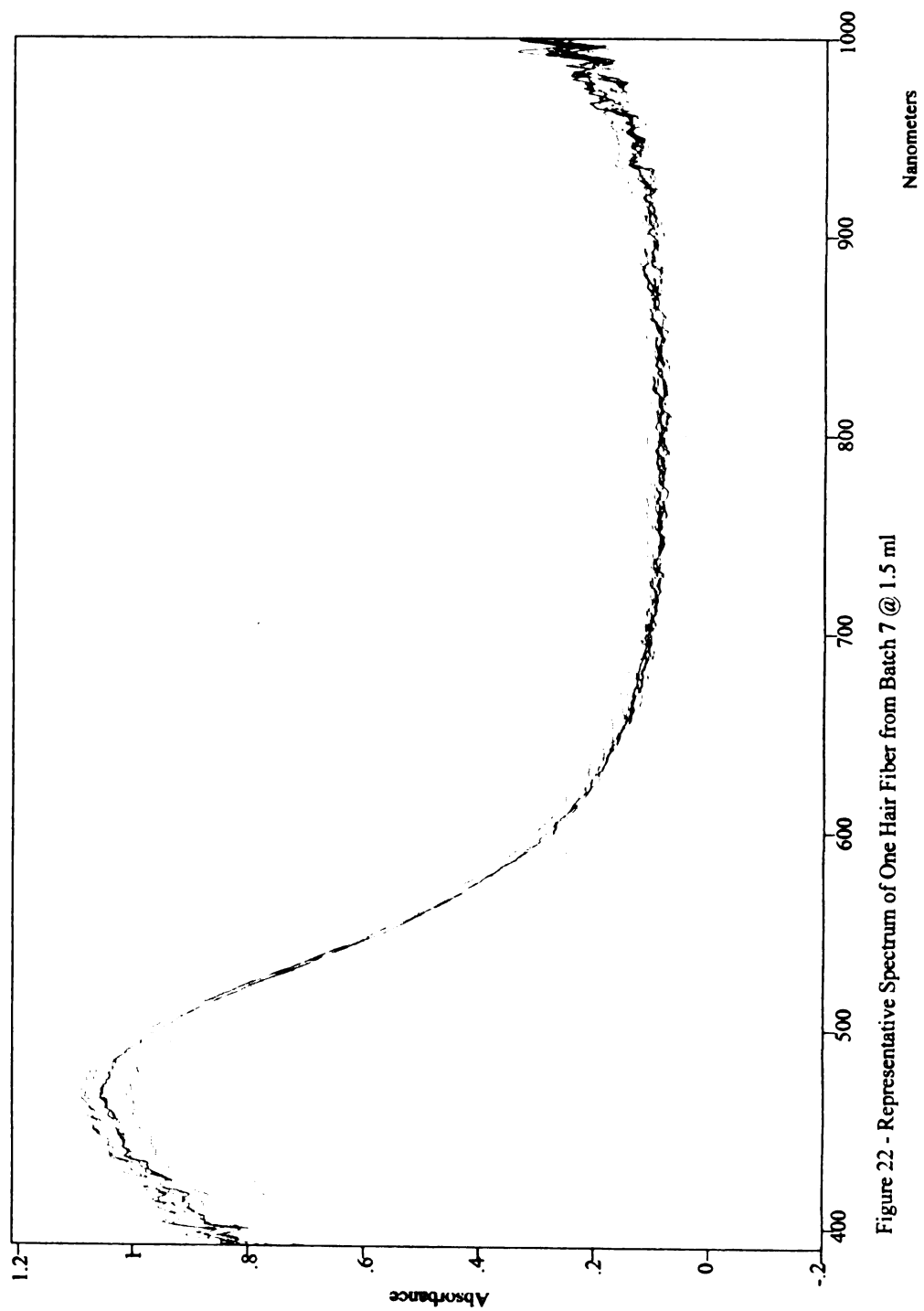


Figure 22 - Representative Spectrum of One Hair Fiber from Batch 7 @ 1.5 ml



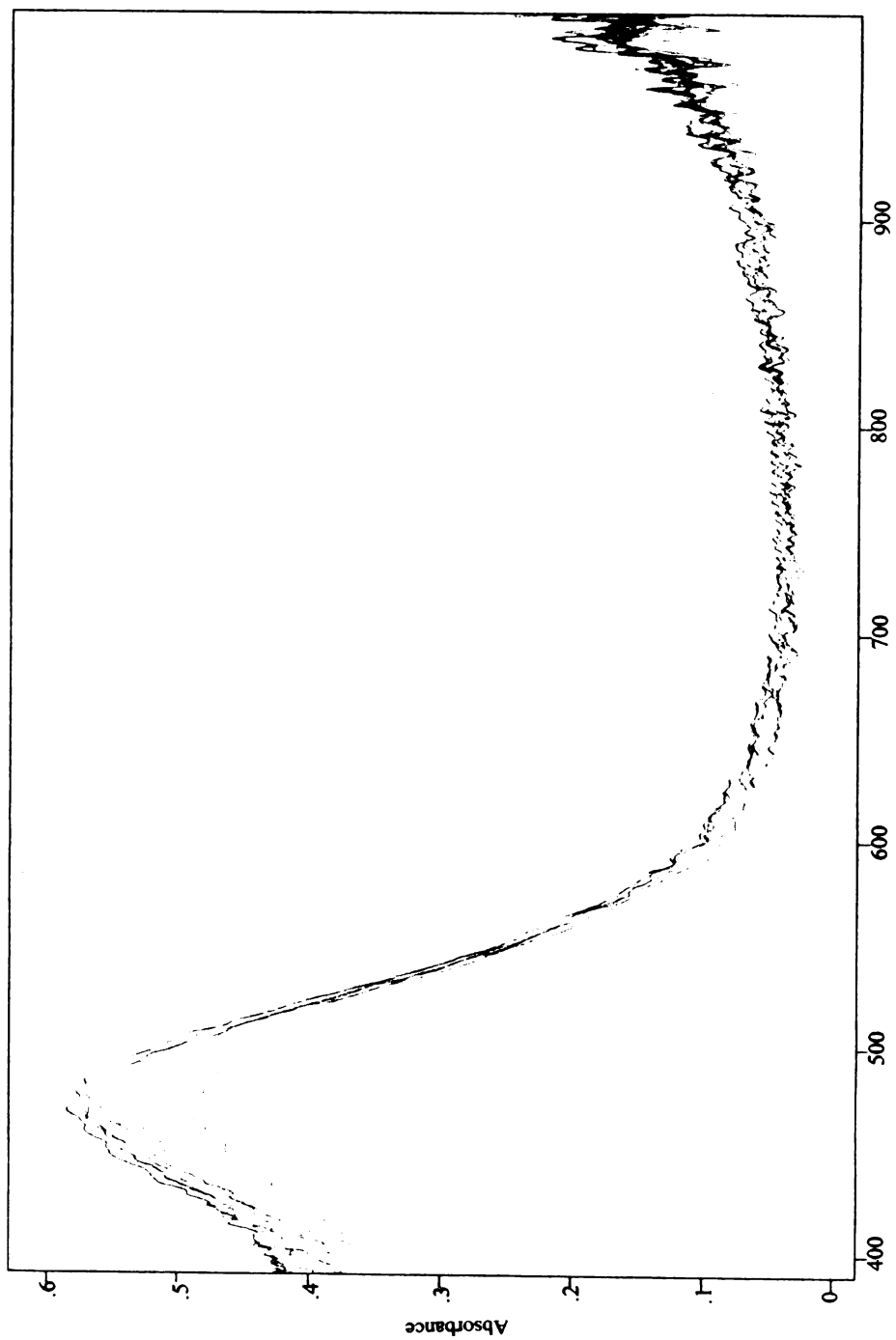


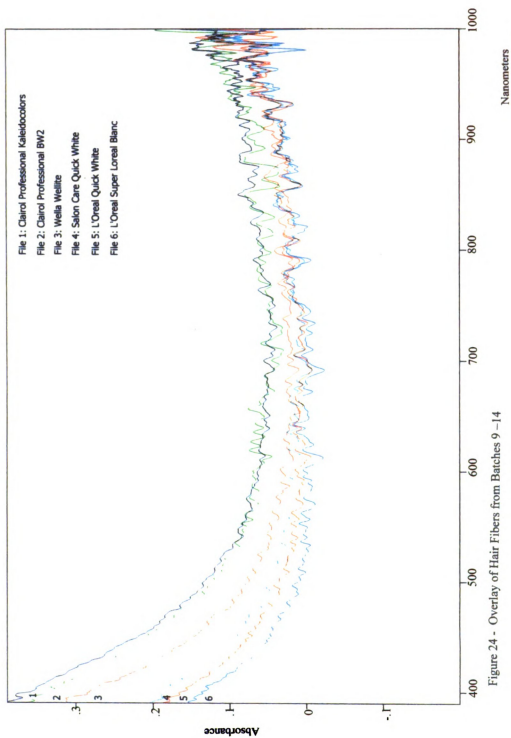
Figure 23 - Representative Spectrum of One Hair Fiber from Batch 8 @ 1.5 ml

Nanometers



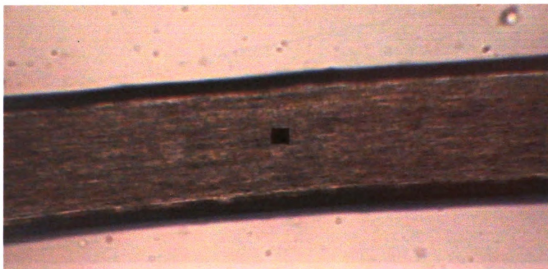
APPENDIX III

SPECTRA OF BLEACHED HAIR FIBERS

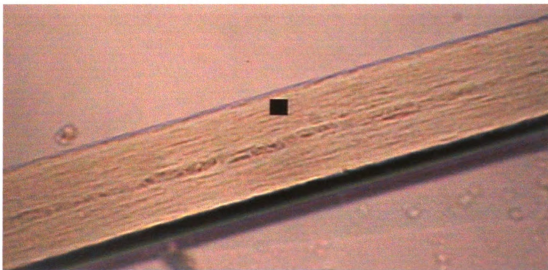


APPENDIX IV

PHOTOGRAPH LIBRARY



**Figure 25– Photo of Hair Fiber before Cosmetic Treatments
(Hair Fibers Used in Batches 1-14)**



**Figure 26 – Photo of Hair Fiber after Bleaching
(Hair Fibers Used in Batches 1-14)**

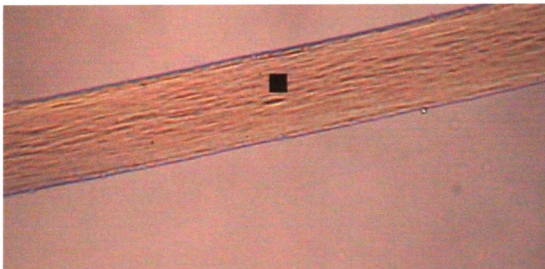


Figure 27 – Photo of Hair Fiber from Batch 1 @ 1.0 ml of dye

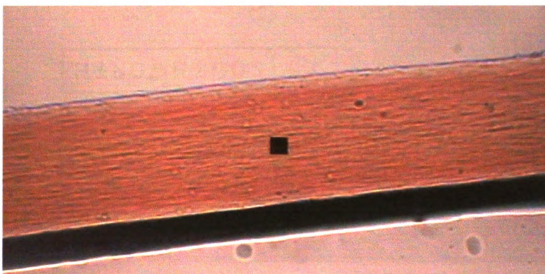


Figure 28 – Photo of Hair Fiber from Batch 1 @ 1.5 ml of dye

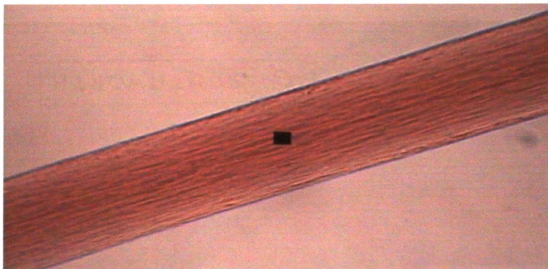


Figure 29 – Photo of Hair Fiber from Batch 2 @ 1.0 ml of dye

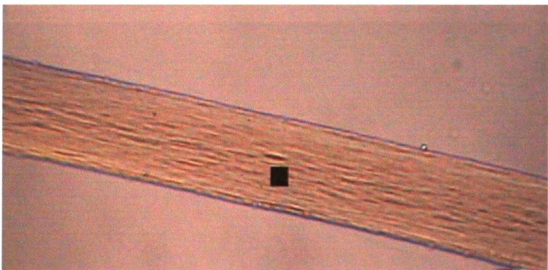


Figure 30 – Photo of Hair Fiber Batch 3 @ 1.0 ml of dye

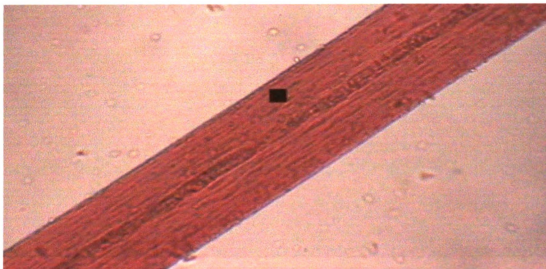


Figure 31 – Photo of Hair Fiber from Batch 3 @ 1.5 ml of dye

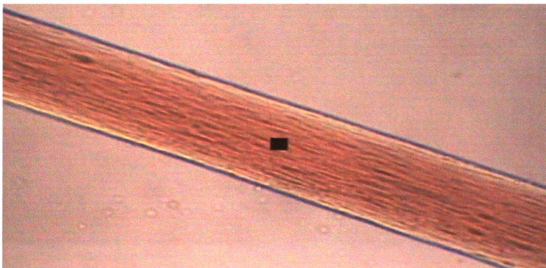


Figure 32 – Photo of Hair Fiber from Batch 4 @ 1.0 ml of dye

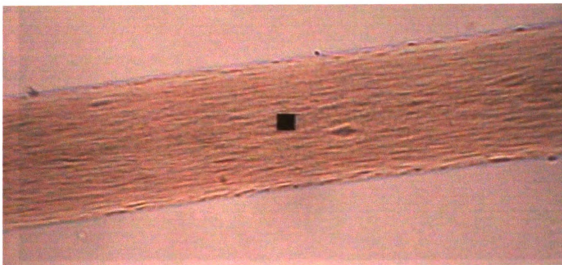


Figure 33 – Photo of Hair Fiber from Batch 5 @ 1.0 ml of dye

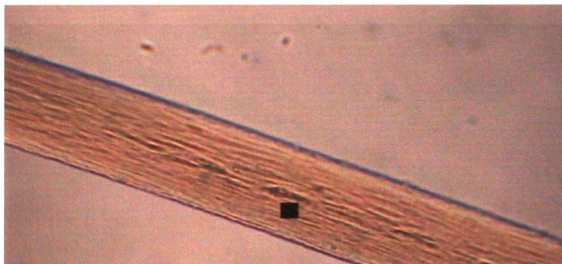


Figure 34 – Photo of Hair Fiber from Batch 5 @ 1.5 ml of dye

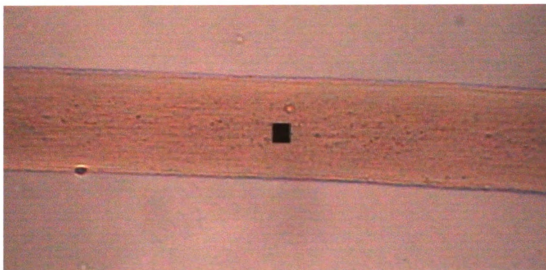


Figure 35 – Photo of Hair Fiber from Batch 6 @ 1.0 ml of dye

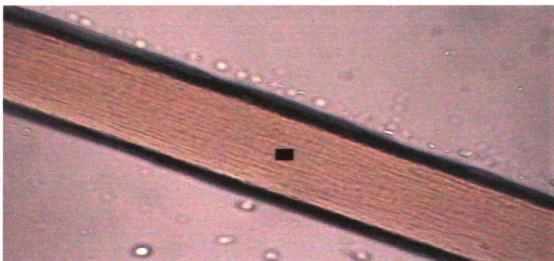


Figure 36 – Photo of Hair Fiber from Batch 7 @ 1.0 ml

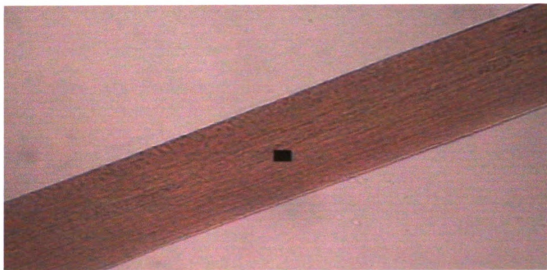


Figure 37 – Photo of Hair Fiber from Batch 7 @ 1.5 ml of dye

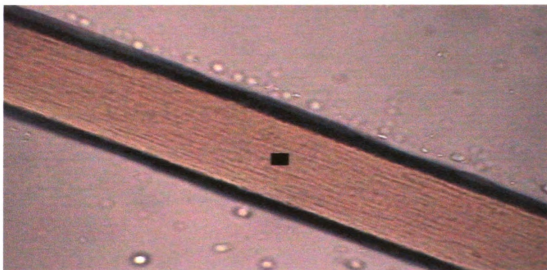


Figure 38 – Photo of Hair Fiber from Batch 8 @ 1.0 ml of dye

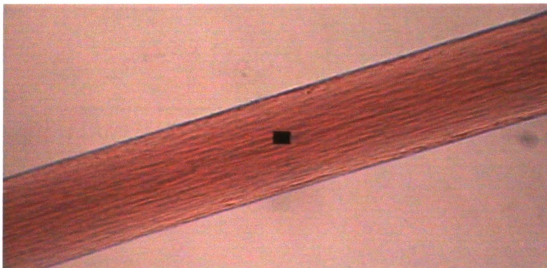


Figure 39 – Photo of Hair Fiber from Batch 8 @ 1.5 ml

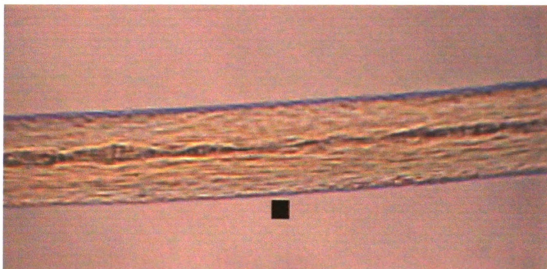


Figure 40 – Photo of Hair Fiber from Batch 9

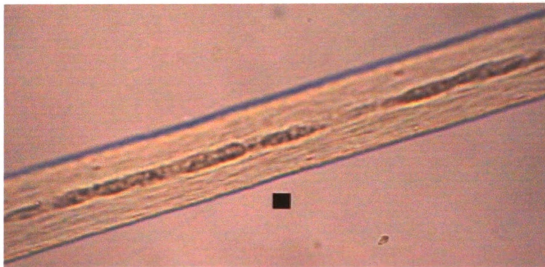


Figure 41 – Photo of Hair Fiber from Batch 10

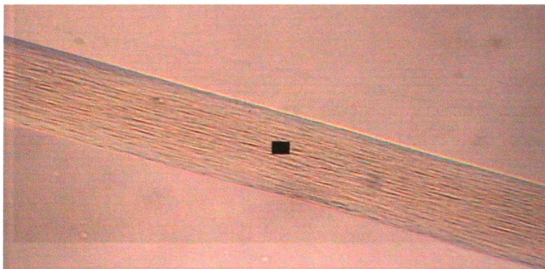


Figure 42 – Photo of Hair Fiber from Batch 11

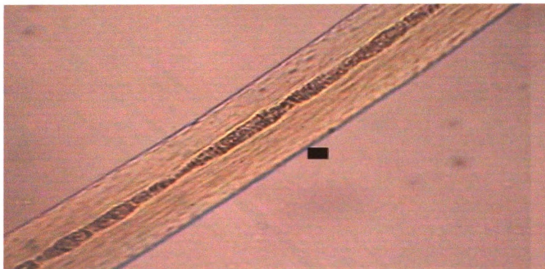


Figure 43 – Photo of Hair Fiber from Batch 12

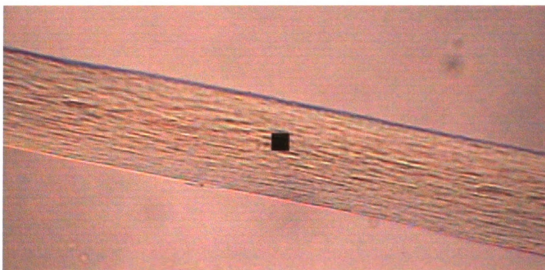


Figure 44 – Photo of Hair Fiber from Batch 13

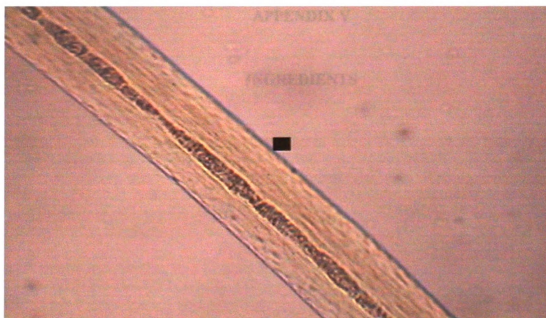


Figure 45 – Photo of Hair Fiber from Batch 14

APPENDIX V

INGREDIENTS

INGREDIENTS

Dye Ingredients

L'Oreal Excellence Red Penny

m-aminophenol, p-aminophenol, o-aminophenol, ammonium hydroxide, butoxydiglycol, cocamide mipa, deceth-3, deceth-5, 2,4-diaminophenoxyethanol HCl, dicocodimonium chloride, EDTA, erythorbic acid, fragrance, hydroxyethyl oleyl dimonium chloride, isopropyl alcohol, p-methylaminophenol sulfate, 2-methyl-5-hydroxyethylaminophenol, 2-methylresorcinol, p-phenylenediamine, propylene glycol, resorcinol, sodium metabisulfite, thiolactic acid, water.

L'Oreal Feria All-Out Red Copper Red

L'Oreal Feria Double Intensity Auburn Red

L'Oreal Feria Glowing Red-Hot Red

alcohol denatured, 4-amino-2-hydroxytoluene, m-aminophenol, p-aminophenol, o-aminophenol, ammonium acetate, ammonium hydroxide, ammonium thiolactate, 2,4-diaminophenoxyethanol HCl, erythorbic acid, fragrance, hexylene glycol, hydroxybenzomorpholine, 6-hydroxyindole, hydroxypropyl bis(n-hydroxyethyl-p-phenylenediamine) HCl, 2-methyl-5-hydroxyethylaminophenol, p-methylaminophenol sulfate, 2-methylresorcinol, n,n-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, oleic acid, oleyl alcohol, peg-2 oleamine, pentasodium pentetate, phenyl methyl pyrazolone, p-phenylenediamine, polyglyceryl-2 oleyl ether, polyglyceryl-4 oleyl ether, propylene glycol, resorcinol, sodium diethylaminopropyl, cocoaspartamide, sodium metabisulfite, toluene-2,5-diamine, trideceth-2 carboxamide mea, water. Covered under U.S. Patents: #5,114,429, #5,279,620, #5,900,028, #5,690,696 and #6,093,220. Individual shades do not contain every ingredient.

L'Oreal Preference Red Penny

water, trideceth-2 carboxamide mea, propylene glycol, hexylene glycol, peg-2 oleamine, polyglyceryl-2 oleyl ether, oleyl alcohol, alcohol denatured, ammonium hydroxide, polyglyceryl-4 oleyl ether, oleic acid, sodium diethylaminopropyl, cocoaspartamide, pentasodium pentetate, ammonium acetate, sodium metabisulfite, fragrance, erythorbic acid, ammonium thiolactate, thiolactic acid. May contain: p-phenylenediamine, 2-methyl-5-hydroxyethylaminophenol, p-aminophenol, resorcinol, phenyl methyl pyrazolone, p-methylaminophenol sulfate, 4-amino-2-hydroxytoluene, m-aminophenol, 2,4-diaminophenoxyethanol HCl, 6-hydroxyindole, 2-methylresorcinol, hydroxypropyl bis(n-hydroxyethyl-p-phenylenediamine) HCl, n,n-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, 2-amino-3-hydroxypyridine, o-aminophenol, hydroxybenzomorpholine. Covered under U.S. Patents: #5,279,620, #5,114,429, #5,690,969 and #6,093,220.

L'Oreal Preference Smoldering Red

alcohol denatured, 2-amino-3-hydroxypyridine, 4-amino-2-hydroxytoluene, m-aminophenol, p-aminophenol, p-phenylenediamine, ammonium acetate, ammonium

hydroxide, butoxydiglycol, 2,4-diaminophenoxyethanol hcl, fragrance, erythorbic acid, hydroxybenzomorpholine, 6-hydroxyindole, hydroxypropyl bis(n-hydroxyethyl-p-phenylenediamine) HCl, p-methylaminophenol sulfate, 2-methyl-5-hydroxyethylaminophenol, 2-methylresorcinol, n,n-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, oleic acid, oleyl alcohol, peg-2 tallow amine, pentasodium pentetate, phenyl methyl pyrazolone, polyglyceryl-2 oleyl ether, polyglyceryl-4 oleyl ether, propylene glycol, resorcinol, sodium diethylaminopropyl cocoaspartamide, sodium metabisulfite, thiolactic acid, trideceth-2 carboxamide mea, water. Covered under U.S. Patents: #5,114,429, #4,361,421 and #5,279,620. Every shade does not contain all of these ingredients.

L'Oreal Preference Mega Reds Dark Intense Copper Red

L'Oreal Preference Mega Reds Medium Intense Red Copper

trideceth-2 carboxamide mea, water, propylene glycol, hexylene glycol, peg-2 oleamine, polyglyceryl-4 oleyl ether, oleyl alcohol, alcohol denatured, ammonium hydroxide, polyglyceryl-2 oleyl ether, oleic acid, sodium diethylaminopropyl cocoaspartamide, pentasodium pentetate, ammonium acetate, sodium metabisulfite, fragrance, erythorbic acid, ammonium thiolactate, thiolactic acid. May contain: p-phenylenediamine, 2-methyl-5-hydroxyethylaminophenol, p-aminophenol, resorcinol, phenyl methyl pyrazolone, p-methylaminophenol sulfate, 4-amino-2-hydroxytoluene, m-aminophenol, 2,4-diaminophenoxyethanol HCl, 6-hydroxyindole, 2-methylresorcinol, hydroxypropyl bis(n-hydroxyethyl-p-phenylenediamine) HCl, n,n-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, 2-amino-3-hydroxypyridine, o-aminophenol, hydroxybenzomorpholine. Covered under U.S. Patents: #5,279,620, #5,114,429, #5,690,969 and #6,093,220.

Bleach Ingredients

Wella Wellite Powder Lightener

potassium persulfate, sodium silicate, magnesium carbonate hydroxide, mineral oil, ammonium persulfate, sodium stearate, xanthan gum, rice starch, algin, disodium EDTA, silica.

Clairol Professional Kaleidocolors

potassium persulfate, sodium metasilicate, sodium stearate, silica, hydrated silica, sodium lauryl sulfate, hydroxypropyl methylcellulose, sodium persulfate, ammonium persulfate, disodium EDTA, aloe, barbadensis gel, fragrance, carbomer, dextrin, ultramarines, ext. D&C violet no. 2.

Salon Care Quick White

potassium persulfate, sodium metasilicate, ammonium persulfate, sodium stearate, silica, hydroxypropyl methylcellulose, aluminium stearate, EDTA, sodium lauryl sulfate.

L'Oreal Super Oreal Blanc

potassium persulfate, sodium silicate, sodium persulfate, acrylates/c10-30 alkyl acrylate, crosspolymer, urea, kaolin, magnesium stearate, ammonium chloride, diethylhexyl

sodium sulfosuccinate, vp/va copolymer, polydecene, sodium metasilicate, magnesium peroxide, EDTA, titanium dioxide, cyamopsis tetragonoloba gum, sodium carboxymethyl starch, sodium benzoate.

NOTES

Chapter One: Introduction

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- ⁶ Tanada, N., Kashimura, S., Kageura, M., Hara, K., "Practical GC/MS Analysis of Oxidative Dye Components in Hair Fiber as a Forensic Investigative Procedure," *Journal of Forensic Sciences*, Vol. 44, No. 2, 1999, pp. 292-296.
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- ¹³ Tanada, N., Kageura, M., Hara, K., Hieda, Y., Takamoto, M., Kashimura, S., "Demonstration of Oxidative Dyes on Human Hair," *Forensic Science International*, Vol. 64, 1994, pp.1-8.

Chapter 2: Instrumentation

- ¹⁴ Olson, L.A., "Color Comparison in Questioned Document Examination Using Microspectrophotometry," *Journal of Forensic Sciences*, Vol. 31, No. 4, Oct.1986, pp. 1330-1340.
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- ¹⁷ Euring, M.B., "Visible Microscopial Spectrophotometry in the Forensic Sciences," *Forensic Science Handbook*, Vol. I, 2nd ed. (New Jersey: Prentice Hall, 2002), pp. 322-387.

Chapter 4: Results and Discussion

- ¹⁸ Corbett, J.F., "Chemistry of Hair Colorant Processes – Science as an Aid to Formulation and development." *Journal of the Society of Cosmetic Chemists*, Vol. 35, 1984, pp. 297-310.
- ¹⁹ Corbett, J.F., "Hair Coloring Processes." *Cosmetics & Toiletries*, Vol. 106, 1991, pp. 53-57.

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