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Instrumental Analysis of the Human Hair Damaged by Bleaching Treatments[+]

- Focused on ATR FT-IRM -

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Abstract

The physico-chemical characteristics by bleaching treatments were assessed by several instrumental analyses such as surface morphology, chemical structural change, color change as well as tensile strength. The change of morphological characteristic was observed through scanning electron microscope(SEM). The observation of the fine structure on hair surface by SEM showed the bleached hair had much damaged to hair cuticle, and some of cuticle surface were worn away.

To investigate the chemical structural changes in hair keratin, the cross-sections of hair samples were directly analysed using Fourier transform infrared microspectroscopy(FT-IRM). The results showed the cysteic acid S=O band intensity was distinctively increased by performing the bleaching treatment. The cleavage of cystine was appeared to proceed primarily through the sulfur-sulfur (-S-S-) fission whereby cysteic acid was formed as a principal oxidation products. The distribution of amide I band in hair keratin was determined by attenuated total reflectance(ATR) FT-IR mapping image. The results showed that the outer side of hair cortex was more damaged than the inner side of the hair cortex. Also, during chemical bleaching of the hair with alkaline peroxide, the hair was turned to reddish yellow due to the oxidative degradation of eumelanin. This means the eumelanin is more unstable than pheomelanin in chemical oxidation. With bleaching, the tensile strength was also reduced as a results of the chemical oxidation.

Key Words : ATR FT-IRM, damaged hair, bleaching treatment, hydrogen peroxide, cysteic acid

Ⅰ. Introduction

Recently hair cosmetics are becoming of more importance in daily life[1)]. Among them hair bleach is used to lighten the hair color. Hair bleaching has become a regular practice among consumers who desire to follow fashion, care for their appearance and counteract sign of aging. The

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primary purpose of bleaching the hair is done by oxidation of melanins. Hydrogen peroxide is the primary oxidizing agent used in bleaching solutions under alkaline pH condition. The alkaline hydrogen peroxide produces disintegration and dissolution of the melanin granules with subsequent oxidative destruction of the chromophore. Because of the severe reactions required for the destruction of the chromophore of melanin granules, a simultaneous attack of the hair keratin occurs. Although hydrogen peroxide as bleaching agent reacts faster with melanin, oxidation of the disulfide bonds of the matrix and the cuticle does takes place[2]. Hair damage. sometimes, is inevitably created when hair has been exposed to environmental stresses and undergone quite severe treatment. Hair damage refers to the breakdown or removal of structural components or parts of hair that either weaken it or make it more vulnerable to chemical or mechanical breakdown. The cortex, as the main component of the hair, is largely responsible for the mechanical strength of the hair. Chemical hair treatment in particular, such as cold waving or peroxide bleaching, have a harmful effect on the disulfide bonds in the hair keratin of the cortex. The destruction of cross-links has a major influence on the tensile property and the hair structure[3].

Infrared(IR) spectroscopy is widely used in both research and industry as a simple and reliable technique for molecular analysis of hair keratin. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation.

Thus, IR spectroscopy has been widely used for identifying materials of chemical compounds. An IR spectrum provides a specific information for a given molecular component or species. IR intensities are sensitive to changes in molecular structure. However, due to historically tedious sample preparation and data interpretation, IR spectroscopy has been combined other techniques such as light microscope, etc. FT-IRM attached with attenuated total reflectance (ATR) have been conducted by translating a mapping stage a single pixel at a time through the sample area of interest. Especially, the outstanding optical efficiency of the system allows molecular images to be generated from samples, such as fiber and textile.

However, related instrumental analysis such as ATR FT-IRM mapping is not fully examined on physico-chemical characteristics after bleaching the hair. Although the hair represents an important feature of beauty care and is given a great deal of attention, its scientific approach by spectroscopy has been little studied and poorly understood. In this study we carried out to provide beauticians the instrumental analysis methods including ATR FT-IRM image mapping to evaluate effectively hair damage in bleaching treatments.

II. Experimental

1. Materials

Hair samples were collected from virgin hair obtained from Korean girl aged 20 years. Tresses of virgin dark brown hair, approximately 4 g and 20 cm long, were prepared and grey hair removed so as to obtain more uniform results. The collected hair samples were wetted and massaged with 1% Triton X-100 from top to bottom for 1 minute. The tresses were throughly

rinsed under 35℃ tap water and soaked in deionized water overnight. Finally, the hair samples were dried at room temperature for 24 hr.

2. Instrumental Analysis

Electron microscope analysis was performed using Hitachi S-4700 SEM. The samples were coated with gold using Hitachi E-1030 ion coater for 20 min, operating at a voltage of 10kV (x2,000). The cross-sectional intensity of the cross-sectioned hair samples were measured using Jasco IMV-4000 IR imaging system and HW-1 multi-angle slicer. The cross-sections were cut to a thickness of 5 micrometer using microtome and measured in transmission with 8 cm^{-1}. A spectroscopic images were recorded by two dimensional intensity with X-Y stage for each wave length in the spectrum. The brighter area on a map represents region of higher IR absorbance at the corresponding wavelength of the image.

The change in hair color was compared by X-rite Spectrophotometer SP-64 with 4 mm target window. The reflectance spectra were recorded values from CIE $L^*a^*b^*$ system. The CIE, or Commission International de l'Eclairage (translated as the International Commission on Illumination), is the body responsible for international recommendations for photometry and colorimetry. The of L^*, a^*, and b^* values were obtained from 2° observer angle and tungsten light source. An average 5 readings were calculated for each hair sample.

The physical properties of the hair were measured by Sun Scientific Rheometer model COMPAC-100 II with adaptor No 21, operating at 10 mm/min constant speed. The measurement of tensile strength was carried out under room temperature 23℃ and relative humidity 45%.

III. Results and Discussion

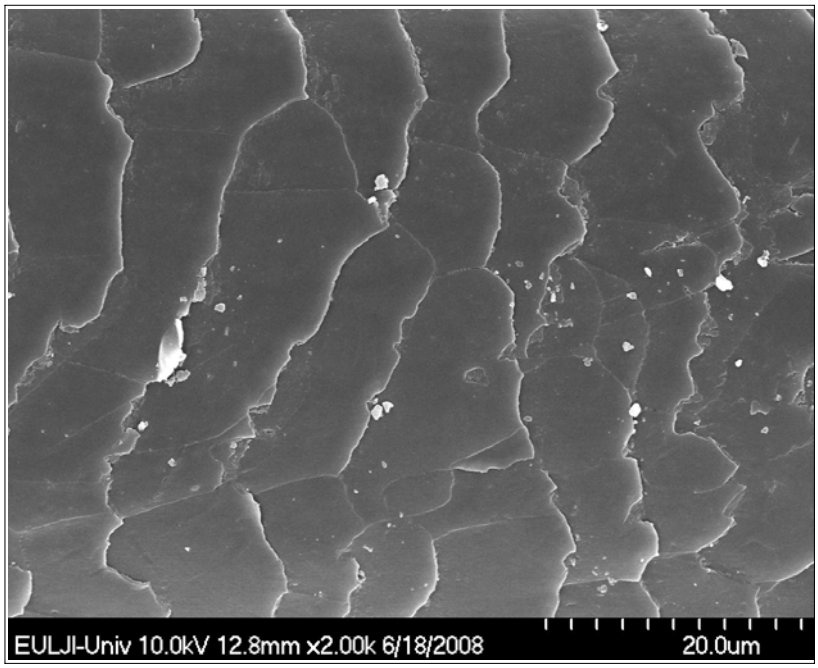
1. Morphological characteristic

The SEM pictures of bleached hair and control samples were shown in <Figure 1>. The SEM picture of control sample shows a normal overlapping intact cuticle and scales which can be clearly defined.

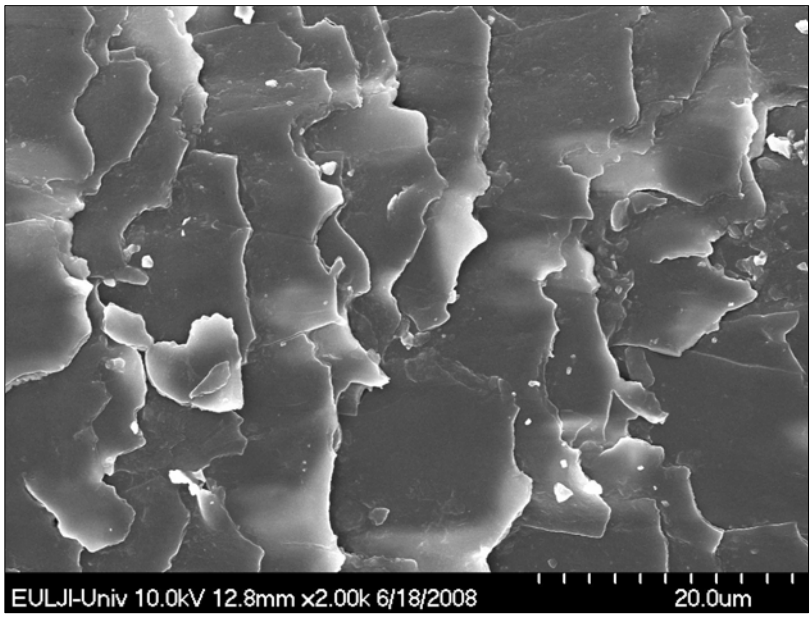
<Figure 2> shows the bleached hair which they treated to hydrogen peroxide for 30 minutes. As shown in <Figure 2>, the scales of bleached hair appeared to be rougher and bulkier because of side reaction during bleaching process[4~5]. The resulting bleached hair was rougher with some particles deposited on the hair surface, and some lifting cuticles were observed on the hair surface. This cuticle erosion leads to surface roughness and loss of sheen[6]. This may be caused by the attack of hydrogen peroxide to disulfide linkage.

2. ATR FT-IRM Imaging System

IR absorption positions are generally presented as either wavenumbers or wavelengths. Wavenumber defines the number of waves per unit length. Thus, wavenumbers are directly proportional to frequency. IR absorption information is generally presented in the form of a spectrum with wavenumber(cm^{-1}). The total number of observed absorption bands is generally different from the total number of fundamental vibrations. The major types of molecular vibrations are stretching and bending. Infrared radiation is absorbed and the associated energy is converted into these type of motions. In simple terms, IR spectra are obtained by detecting changes in transmittance (or absorption) intensity as a function of frequency. Most commercial instruments separate and measure IR



<Fig. 1> SEM picture of virgin hair (x2,000).



<Fig. 2> SEM picture of bleached hair (x2,000).

radiation using Fourier transform(FT) spectrometers. FT-IR stands for Fourier transform Infrared, the preferred method of IR spectroscopy. FT spectrometers have recently replaced dispersive instruments for most applications due to their superior speed and sensitivity. They have greatly extended the capabilities of IR spectroscopy and have been applied to many areas that are very difficult or nearly impossible to analyze by dispersive instruments. Instead of viewing each components frequency sequentially, as in a dispersive IR spectrometer, all frequencies are examined simultaneously in FT-IR spectroscopy.

FT-IR spectroscopy detects the vibration characteristics of chemical functional groups in a sample. In FT-IR spectroscopy, IR radiation is passed through a sample. Some of the IR radiation is absorbed by the sample and some of it is passed through. The resulting spectrum represents the molecular absorption and transmission.

The role of FT-IR spectroscopy in the analysis and characterization of biological products has increased in recent years. FT-IR spectroscopy also offers many opportunities to study such structures, from the point of view of tissue health and from consideration of various treatments, both medical and cosmetic. During recent years much of the focus has been on tissue health, in particular for detection of cancerous and precancerous material, in both organ and skin tissue. However, maybe perceived more frivolous, there are the cosmetology aspects of skin and hair condition. At least two aspects of hair can be considered, the actual condition of hair, as it relates to general health and environmental or chemical damage, and the impact of beauty aids and treatments, such as permanent wave, hair coloring, and hair grooming products. ATR is a perfect tool for studying the molecular chemistry of hair. Virtually all of the characteristics and chemistry of hair are reflected in the surface of the hair fibres, and by nature this is the mode of measurement provided by ATR. By the use of ATR FT-IR spectroscopy, it is possible to perform spatial analysis while preserving the hair structure. Conventional FT-IR and microscopy have made it possible to create the visualization for the distribution map of chemical bonds. Recent advances have allowed this technology to be extended further by using ATR FT-IRM. The power of ATR FT-IRM lies in its ability to obtain molecular information on a microscopic scale. The technique is a combination of conventional FT-IR spectroscopy and optical microscopy. The advantage of this technique is that it is nondestructive, requires no sample extraction or purification, and provides information about -S-S- groups through oxidation, which is impossible to record using infrared spectroscopy.

The identity and distribution of chemical components within a sample can be determined by ATR mapping. The utility of ATR, one of the most popular sampling technique is very useful to analysis the surface of a samples. The ATR technique allows the surface molecular analysis of a sample that can not be studied in conventional KBr window method. To collect an ATR FT-IRM mapping image, the sample is first positioned in the center of the field of view of the microscope by moving X-Y stage.

The health and condition of a person of hair is reflected in the conventional FT-IR spectroscopy[7]. Hair damage caused by bleaching or exposure to a harmful environment is observed as oxidation process. The damage in hair was observed on the two-dimensional ATR FT-IRM map. Panayiotou et al[8] suggested that FT-IRM could be a useful tool to study hair. FT-IRM

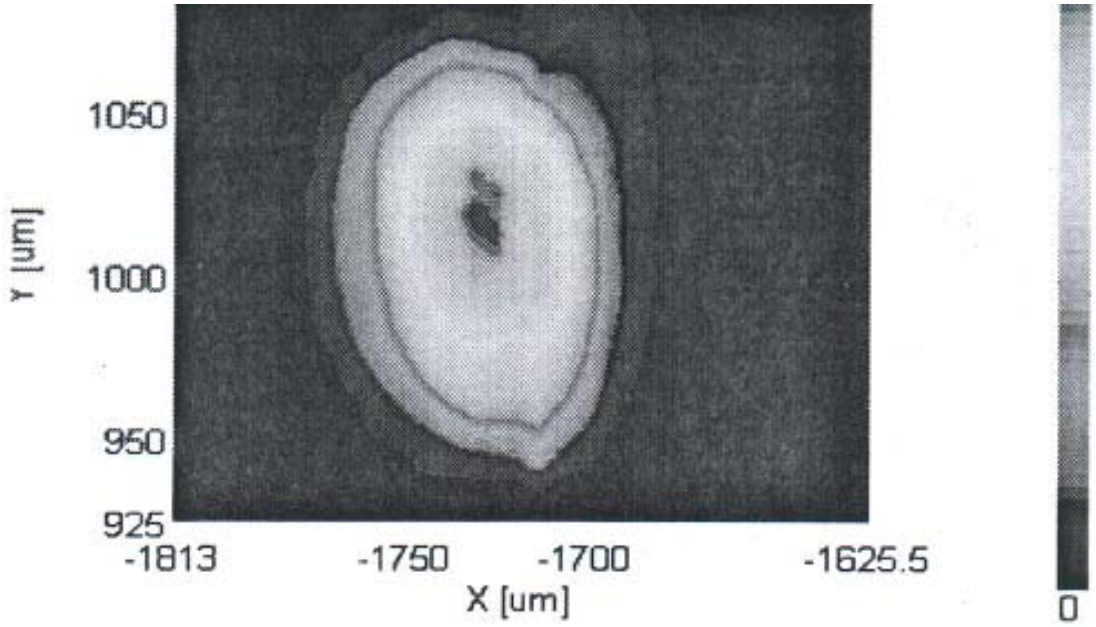
was used to investigate the chemical nature of biological tissues[9], particularly hair. It is generally accepted that the damage of hair protein proceeds *via* oxidation of cystine following the S-S scission yields to cysteic acid. ATR with FT-IRM was used to directly estimate the damage to hair measuring the increase of the S=O band as a result of oxidation of the S-S link in cystine[10].

The chemical method of assaying possible chemical damage to hair is done by determining bond breakage, especially, the breaking of disulfide bonds. Hair damage can be defined as an decrease in disulfide bonds. <Figure 3> shows the distribution mapping of amide I band of cross-sectioned normal hair. As shown in <Figure 3>, a major advantage of ATR FT-IRM is its ability to make chemical mappings of the analysed sample, in which the light area on the map means the band intensities. An automated X-Y mapping stage allows to be linearly scanned. The X, Y units [um] of the axis mean the relatively distance on the stage.

The amide bands have been used to interpretate the molecular structure of hair keratin. In general, the spectrum of hair keratin appeared the three characteristic bands(amide I, II, and III) at absorptions approximately 1650 cm^{-1}, 1560 cm^{-1} and 1240 cm^{-1} respectively.

Especially, amide I band at 1650 cm^{-1} is useful to analyze hair keratin. The amide I band(1650 cm^{-1}) intensity of cortex was much higher than that of cuticle layer[11~12]. However, the amide I band distribution was not found in medulla layer.

To investigate the influence of the bleaching treatments on hair keratin, the structure of cross-sections of bleached hair was directly analyzed

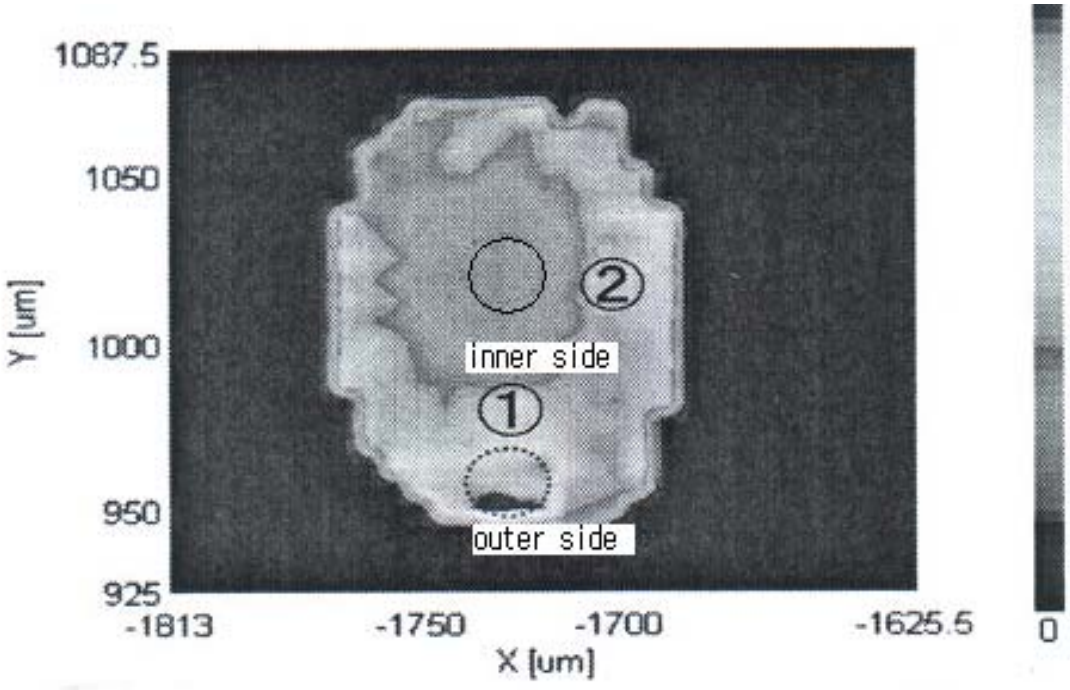


<Fig. 3> ATR FT-IRM images of cross-section of normal hair.
The images are mapped out by integrated absorption intensity of amide I band.

without isolating the inner side and outer side of cortex, using ATR FT-IRM image mapping. Normalization of ATR FT-IRM of keratin fibre was carried out based on the C-H band at 1450 cm^{-1} in which the peak was not influenced by chemical treatment. The cysteic acid content of the hair sample was compared by estimating the ratio of the peak of the S=O band.

<Figure 4> shows the ratio of the peak height 1076 cm^{-1}/ 1450 cm^{-1} in bleached hair. As shown in <Figure 4>, the peak height of the S=O band (1076cm^{-1}) was measured and ratioed with methylene band (1450 cm^{-1}). The cysteic acid band intensity at 1076 cm^{-1} assigned to oxidized form was increased, indicating that some of the hair keratin changed to the oxidized form by performing the bleaching treatment.

The lighter area of the map means the damage area to the sectioned hair. Methylene band at 1450 cm^{-1} was measured as an internal reference, which was chemically unchanged during oxidation process. The ratio of S=O/ methylene band is therefore an indication of the degree of oxidation of hair fibers. The higher the ratio, the worse the degradation. In <Figure 5>, the FT-IR spectrum suggests that the outer side of the hair cortex exhibits much higher oxidized than that of inner side of hair cortex. Oxidation of the amino acid cystine to cysteic acid can occur in hair, resulting in an increase of the S=O stretching absorbance. The arrow at 1076 cm^{-1} indicates that the S=O band assigned to oxidized form of hair. The spectrum shows the increase of the S=O symmetric cysteic acid stretch due to the bleaching process and the spectral differences due to the oxidation of cystine

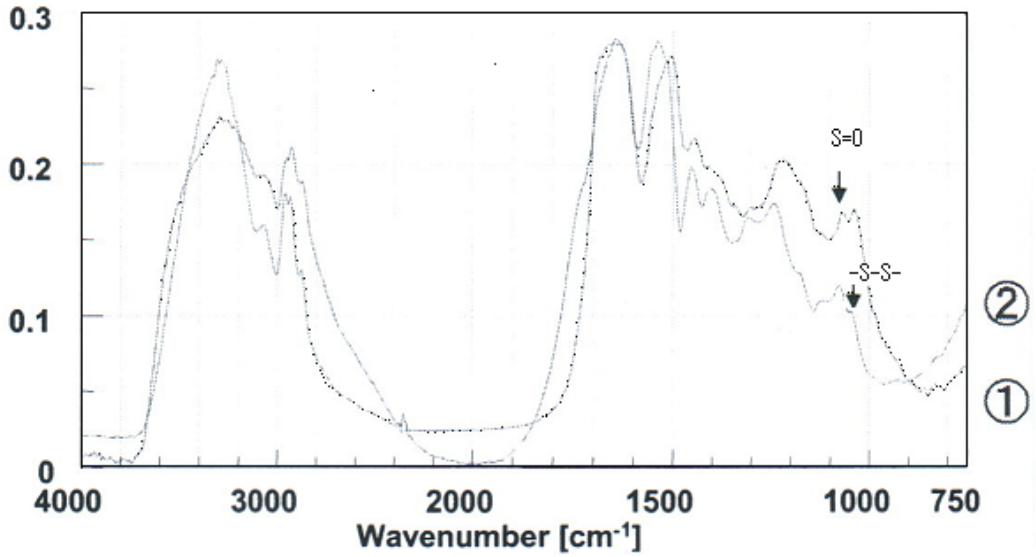


<Fig. 4> ATR FT-IR images of cross-section of bleached hair. The images are mapped out by the ratio S=O/methylene band (① : outer side of cortex, ② : inner side of cortex).

to cysteic acid. When the hair was treated with a bleach solution, significant damage is imposed on the hair and an increase in cysteic acid results.

3. Color measurement

The change of hair color is popular in beauty salon. However, Korean hair is highly pigmented, higher bleaching is needed to achieve some of the fashionable colors. The primary purpose of bleaching the hair is to lighten the hair, which is done by oxidation of melanins. Hydrogen peroxide is the primary oxidizing agent with ammonia solution. The ammonia also opens up the hair cuticles, while the peroxide under alkaline condition destroy the some of melanins. There are two types of melanin, the brownish black eumelanin and the less prevalent reddish yellow pheomelanin.



<Fig. 5> FT-IR spectrum of outer side of cortex vs inner side of cortex in bleached hair. (①: outer side of cortex, ②: inner side of cortex).

<Table 1> The changes of hair color according to bleaching treatments

sample / parameter	virgin hair	bleached hair (number of treatment)			
		1 times	2 times	3 times	4 times
L*	20.4	24.16	28.27	31.32	34.31
a*	1.11	6.21	8.70	9.75	11.02
b*	0.68	7.49	11.59	14.45	18.88

The naturally occurring colors are the result of a mixture of two types of melanins occurring in the cortex region of the hair[13]. <Table 1> shows the results obtained from hair samples used in this works. CIE $L^*a^*b^*$ is based on the opponent colors vision, which says that two colors cannot be both green and red at the same time, nor blue and yellow at the same time. When a color is expressed in CIE $L^*a^*b^*$, L^* defines lightness, a^* denotes the red/green values and b^* the yellow/blue value. As shown in <Table 1>, the color plotting diagrams for $L^*a^*b^*$, a color movement in the +a direction depicts a shift toward red. Along the b^* axis, +b movement represents a shift toward yellow. This means that the pheomelanin is predominant in human hair after bleaching, and it is more chemically stable than eumelanin under oxidation condition.

4. Tensile strength

Although mechanical properties such as tensile strength and post yield modulus are extremely important features affecting hair morphology, the hair fibre surface properties are equally important to characterize the hair cuticle scales. Fibre tensile properties can be mainly to quantify the hair fibre internal damages. Although mechanical properties such as tensile strength and post yield modulus are extremely important features affecting hair morphology, the hair fibre surface properties are equally important to characterize the hair cuticle scales. Fibre tensile properties can be mainly to quantify the hair fibre internal damages.

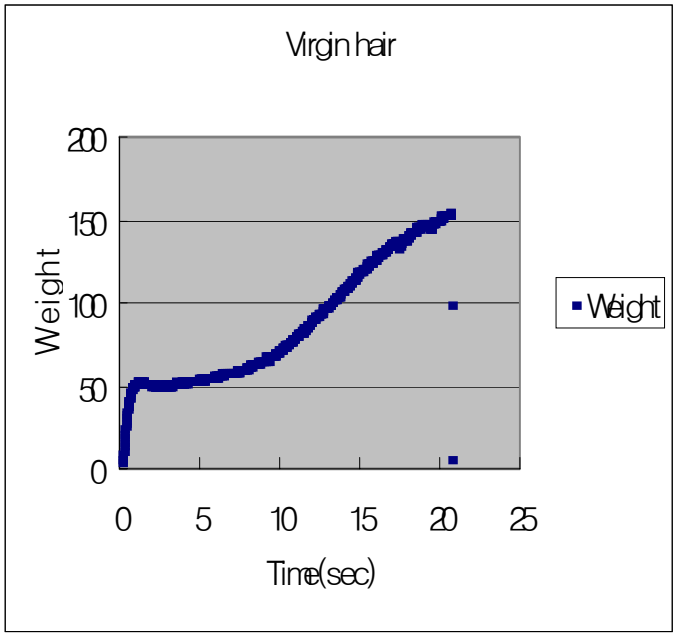
In regard to texture changes, studies of both virgin and bleached hair have shown that exposure to the hydrogen peroxide has deleterious effects on appearance and texture[14]. In chemically treated hair, there is even more of a chance that further damage will occur to the structuring and chemically disoriented hair shaft. Loss in tensile strength can be shown by the use of rheometer.

This experiment was carried out to assess the loss in hair strength caused by bleaching. The disulfide bonds of cystine are responsible for the mechanical strength of hair. Hair passes through three phases while being extended under constant loading forces. The first phase, called the Hookean region, is characterized by reversible extension. Hydrogen bonds are believed to be disturbed in this region. The second phase, the yield region, is a partially reversible transformation in which covalent and salt bonds are likely to be disturbed. Finally, the third phase leading to the breaking point is the post yield region. The measurement of the longitudinal mechanical properties of hair is frequently applied to assess the hair damage. The most common parameter used to quantity damage is the total working force required to break a single hair strand. The damaged hair fiber shows a much higher elastic modulus, which indicates the hair is intrinsically different than the undamaged one.

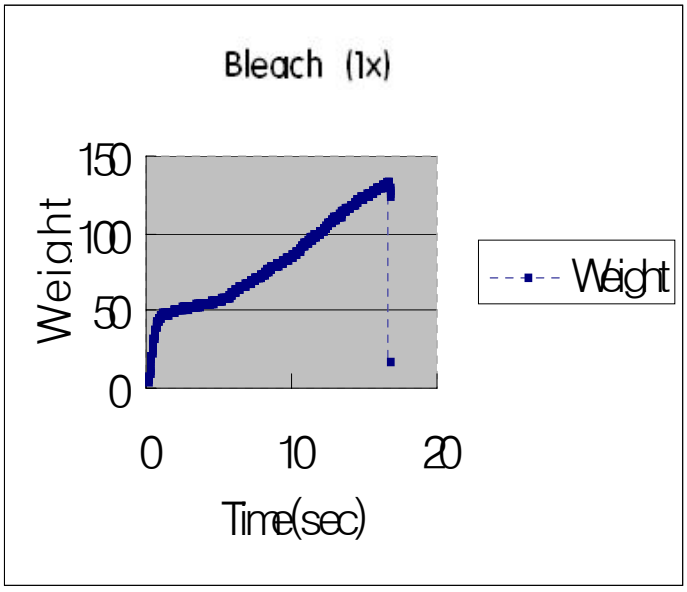
As demonstrated in <Figure 6> and <Figure 7> there is significant difference of tensile strength reductions for bleached hair. The tensile strength of normal hair was around 150g, while bleached hair was definitely decreased in tensile strength. This means the oxidative damage resulted in the decrease the working force required to break a hair fibre.

Ⅳ. Conclusion

In this paper, four different methods were applied to evaluate the damage with respect to the surface morphology, chemical structural change, color change as well as tensile strength.



<Fig. 6> The tensile strength of normal hair.



<Fig. 7> The tensile strength of bleached hair.

SEM had been applied to examine the surface morphology of bleached hair in order to evaluate the change of morphological characteristic of hair surface. The observation of the hair surface by SEM showed the bleached hair appeared to be rougher and its scale was diminished after undergoing the bleaching treatments. The bleached hair showed the lifting of the cuticle scales.

To investigate the chemical structural change in hair, the cross-sections of hair samples were directly analysed using ATR with FT-IRM. The ATR FT-IRM was useful to provide the molecular image mapping of damaged hair compared to SEM. Chemical bleach with alkaline peroxide turned the hair reddish yellow due to he oxidative degradation of eumelanin. This means the eumelanin is more unstable than pheomelanin in chemical oxidation. With bleaching, the tensile strength was also reduced as a results of the chemical oxidation. With respect to the tensile strength property, the results obtained indicates that the breaking load of bleached hair decreases dramatically after bleaching.

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