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MECHANICAL CHARACTERIZATION OF HAIR FIBERS AFTER COSMETIC TREATMENTS

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Metallurgy and Materials Engineering Department

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ABSTRACT

In this study, we analyzed the effects of the combination of chemical hair treatments, so called superpositions. We wanted to clarify several points of how the order of the treatments influences the total damage to the hair.The main combination is between two commercially available and widely used chemical treatments, named Treatment 1 and Treatment 2. Usual complementary treatments are included to represent real life. After each sequence, the mechanical properties of the hair fibers were analyzed by Tensile Test (MTT), Dynamical Mechanical Analysis (DMA) and High-Pressure Differential Scanning Calorimetry (HP-DSC), tests usually used to evaluate hair integrity. The morphology of the cuticle was observed using Scanning Electron Microscopy (SEM). Caucasian natural dark brown hair was used for this study, but tests were carried out on Chinese hair. We have observed that the position of the Treatment 5 in the sequence of a Treatment 1 + Treatment 2 superposition causes little impact on the final physical properties of the fiber (shown by MTT and DMA and further verified by HP-DSC and SEM). There is also evidence that the introduction of Treatment 4 between Treatment 1 and Treatment 2 reduces hair damage.

Keywords: Hair. MTT. DMA. Tensile test. HP-DSC. SEM. Superposition.

RESUMO

Neste estudo, foram analisados os efeitos da combinação de tratamentos capilares, as chamadas superposições. Desejava-se esclarecer de que maneira a ordem destes tratamentos e a inserção de outras aplicações auxiliares podem alterar o dano total à fibra. Isto pode ser usado para o desenvolvimento de novos protocolos de aplicação.Os principais tratamentos combinados foram o Tratamento 1 e o Tratamento 2, ambos tratamentos cosméticos comercialmente disponíveis e amplamente utilizados. Aplicações auxiliares (nomeados Tratamento 4 e Tratemento 5) foram também utilizadas para reproduziar as nuances da vida real. Ao final de cada sequência, as propriedades mecânicas das fibras foram analisadas por Ensaio de Tração (MTT), Análise Mecânica Dinâmica (DMA) e Calorimetria Diferencial de Varredura a Alta Pressão (HP-DSC), os testes usualmente utilizados para avaliar a integridade física do cabelo. O estado da cutícula foi aferido por Microscopia eletrônica de varredura (MEV). O estudo foi baseado em cabelo caucasiano castanho escuro natural, e testes também foram feitos em cabelo chinês. Foi observado que a posição do Tratamento 5 em uma superposição Tratemento 1 + Tratemento 2 é pouco impactante na integridade física final da fibra (mostrado por MTT e DMA e posteriormente verificado também por HP-DSC e MEV). Também houve evidências de que a introdução de um Tratamento 4 entre o Tratamento 1 e o Tratamento 2 reduz a degradação total do cabelo.

Palavras-chave: Cabelo. MTT. DMA. Ensaio de tração. HP-DSC. MEV. Superposição.

LIST OF GRAPHS

LIST OF IMAGES

LIST OF TABLES

SUMMARY

1 Introduction

The rise of the cosmetic industry as an innovative, dynamic and strategic field resulted in part in the development of a vast amount of hair products, each time safer and more affordable. Today, almost nothing seems impossible within hair transformation (both shape and appearance), and consumers are willing to combine more than one change at a time. There are few studies aiming to clarify the impact of the combination of those treatments.

The objective of this study is to analyze, using metrological evaluation techniques, several associations (here named "superpositions") of chemical treatments, hereafter named Treatment 1 and Treatment 2, which are among the most used hair treatments.

The treatment sequences will be applied on Caucasian natural hair, and the fibers will be analyzed regarding their tensile properties using traction (MTT) method, viscoelastic behavior (by DMA technique), as well as their structural integrity (by HP-DSC) and surface morphology (by SEM). Chinese natural hair was also characterized by MTT.

Finally, the tensile results were compared to treated samples from volunteers' head, to confirm that the swatch behavior is close to reality and thus can be used to reproduce it with a high degree of fidelity and allows more freedom in the tests carried out.

2 Literature Review

2.1 Hair and fiber Structure

Hair fibers are divided in two main parts: the root and the shaft. Understanding each part's properties and functions is necessary for any study for product or treatment development.

2.1.1 Root

The root is the living part of the hair and cannot be seen as it is located 4 mm down in the scalp. Its main constituents are represented in Image 1 :

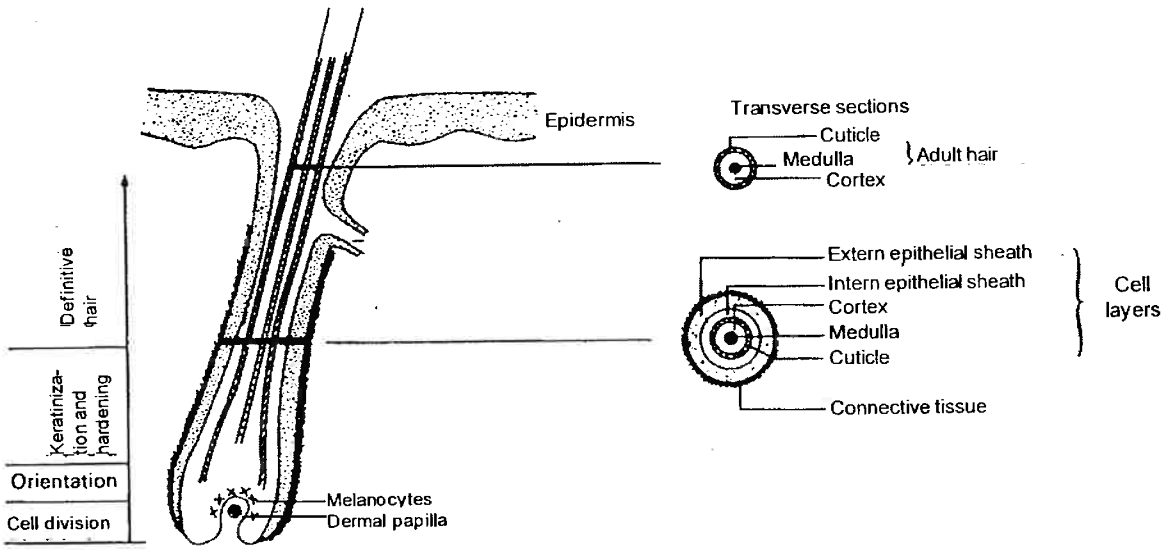

Image 1: Representation of the root parts and the shaft [1]

In the root's base we find the dermal papilla, a cell group originated from connective tissue to which is related hair lifecycle. It is responsible for the size and the form of the shaft (the bigger the dermal papilla, the larger will be). Around the papilla there are two to three cell layers called the capillary bulb, precursor of all cells that move towards the skin. Among them are the melanocytes, which produce the pigments giving hair color. The cellular differentiation forms six concentric ascending cell cylinders, among which three will become the shaft and the others, the internal epithelial sheath.

The cylindrical form of the hair is given by the internal sheath. During multiplication and migration towards the exterior, these cells harden more quickly than the interior cells that will form the shaft, molding the latter.

The capillary cells' multiplication process is followed by high production of a protein called keratin. Gradually, it organizes itself and occupies larger spaces, to the point where the cells are deprived from their nuclei and die. At the moment of leaving the epidermis the fiber is totally formed.

2.1.2 Shaft

The hair shaft has a long cylindrical shape. It constitutes exclusively of dead cells and its subparts are the cuticle, the medulla and the cortex (Image 1).

The external part of the shaft is the cuticle. It's composed by 3 to 10 layers of cells called scales, which are 50 to 70 μm long, 5 to 10 μm wide and less than 1 μm thick. It is colorless and is the main protection against the external degradation elements. Those layers are joined together by intercellular cement that fixes them in the same way as a house roof. The final arrangement is firm and oriented toward the tip (that's why it's easier to slide our fingers or a comb in the root-tip direction along the hair). The cortex protection and the cosmetics' properties of the hair (linked to swelling) depend on the cuticle.

The cortex is the main constituent of the shaft, making up 90 % of its weight. Its cells are all filled with keratin, whose organization gives hair its intrinsic mechanical properties. The keratin macromolecules are long α-helices (Image 3) strongly connected by disulphide bridges (formed by the presence of cysteine). The actual arrangement is more complex and will be discussed with more details later.

The medulla, situated in the center of the shaft, is frequently intermittent in human hair, sometimes absent. Its influence on the physical-chemical properties of the hair is negligible. However, in some animals it may represent more than 50 % of the total diameter and have thermoregulatory functions.

2.1.3 Color

Hair color is given by a protein called melanin, the same that is present in the skin. It is produced by the melanocytes, big star-shaped cells found in the root's base. The melanin grains are produced and then injected in the developing keratinocytes. Although hair contains less than 1 % of melanin, it is responsible for its color throughout the hair fiber's lifecycle.

Melanocytes produce two kinds of melanin: eumelanin and phaeomelanin. The former, more frequent, has a rice grain shape and varies from reddish brown to dark black [2]. The latter has less precise shapes and varies from light blond to reddish. The final color perception will depend on the amount, the proportion and the distribution of both melanin types in the cortex.

Eumelanin comes from tyrosine. Phaeomelanin formation starts the same way, but in the following steps another amino acid intervenes, cysteine (Image 2).

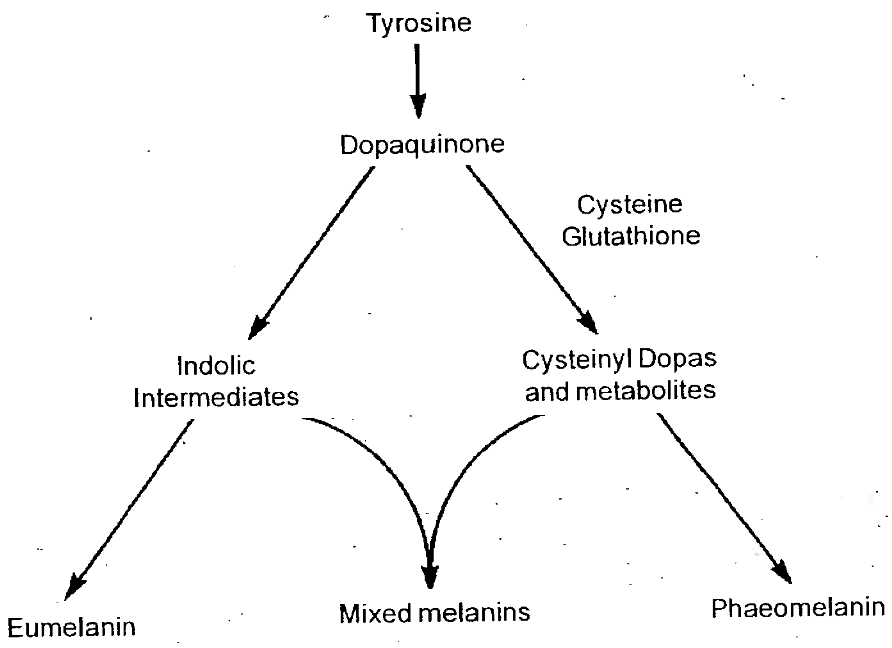

Image 2: Steps of cysteine formation [1]

Just like skin, phaeomelanin-predominant hair (e.g. blond hair) is naturally less protective against the sunlight exposure. The effects are fiber fragilization and color loss.

2.1.4 Molecular structure

A hair fiber of diameter between 50 and 100 µm can support 100 g. Thus, one average head of hair (around 120 thousand fibers) would be able to support 12 tons (if the scalp was strong enough). This remarkable resistance is due the complex organization of the keratin inside the cortex. To better understand it, we must understand the chemical composition of the fibers.

The most abundant elements of the hair are carbon, nitrogen, oxygen, hydrogen and sulphur (Table 1).

Table 1: Chemical elements proportions in human hair [1]

Chemical Element	***Proportion in Hair***
Carbon	45.2 %
Hydrogen	6.6 %
Oxygen	27.9 %
Nitrogen	15.1 %
Sulphur	5.2 %

Those elements are the components of the 18 keratin constituent amino acids. We emphasize the presence of sulphur in cysteine, from which the highly cohesive disulphide bonds are formed.

The keratin macromolecules of non-strained hair are found on a helical form called α-helix or α-keratin. If they are stretched, the keratin chains are broke down and assume a zigzag structure called β-sheet or β-keratin (Image 3). Up to a certain strain limit, this transformation is reversible.

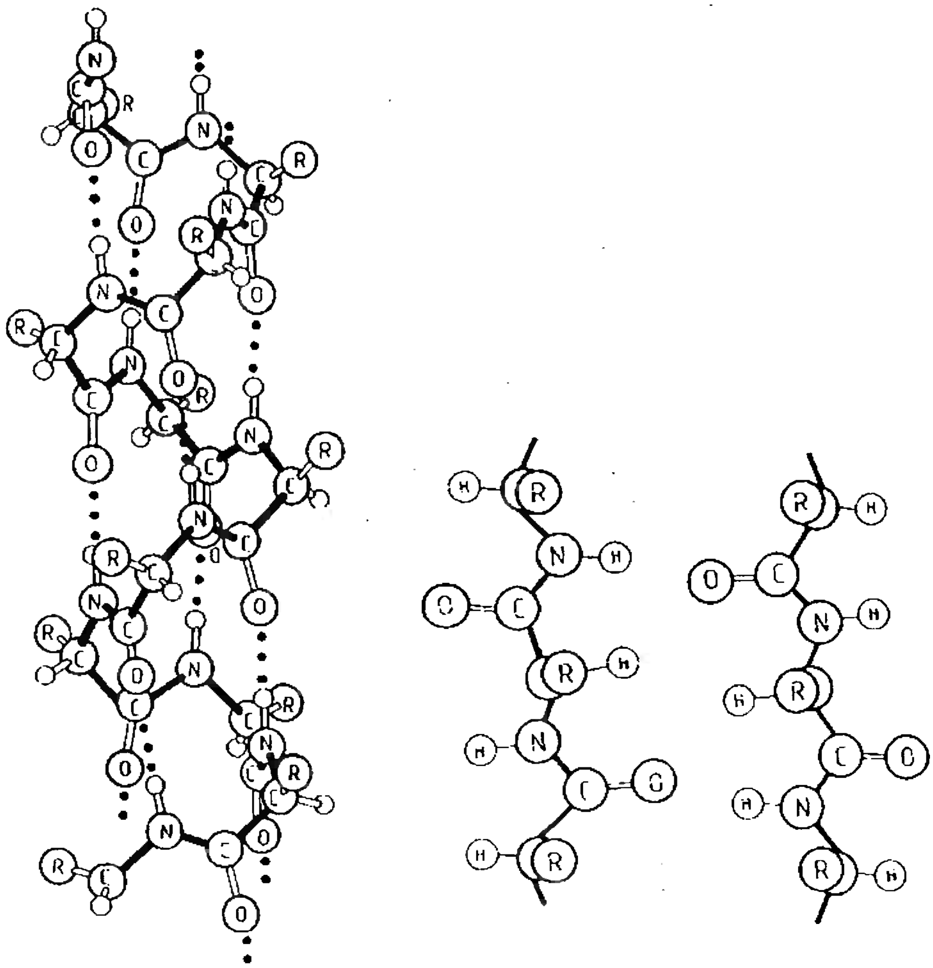

Image 3: α-helix (left) and β-sheet (right) arrangements of keratin molecules [1]

The relative position between the α-helices allows the formation of disulphide bridges (Image 4). That covalent bond, which occurs in a frequency of 1 out of 4 spirals, is extremely solid, but also susceptible to chemical reduction and oxidation, which is the base of form treatments like permanent wave.

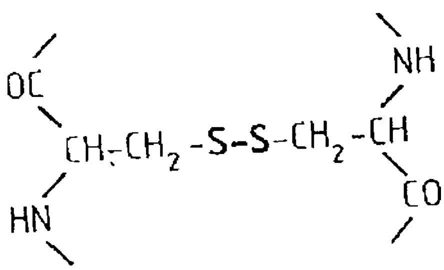

Image 4: Disulphide Bridge formed between two cysteine amino acids

The α-keratin's spirals combine themselves in groups of three or four forming a twisted structure called protofibril (Image 5). These are surrounded by an intercellular cement rich in sulphur and form, in groups of at least nine, the microfibrils. Once again the microfibrils are bounded to the cement to create larger units, the macrofibrils (diameter between 0.1 and 0.2 µm). A group of macrofibrils forms a cortical cell (diameter between 2 and 3 µm), which are visible with the optical microscope in a transverse cut of the fiber.

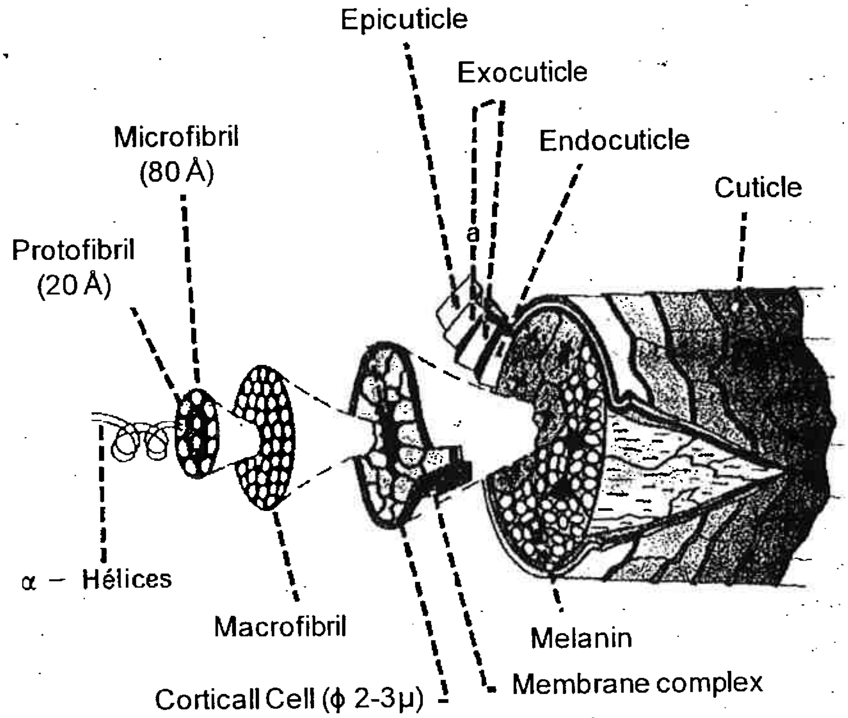

Image 5: Hair fiber structure [1]

2.1.5 Tensile resistance

Hair behavior under mechanical tensile stress is quite particular. Analyzing the graph below, we are able to distinguish three deformation phases:

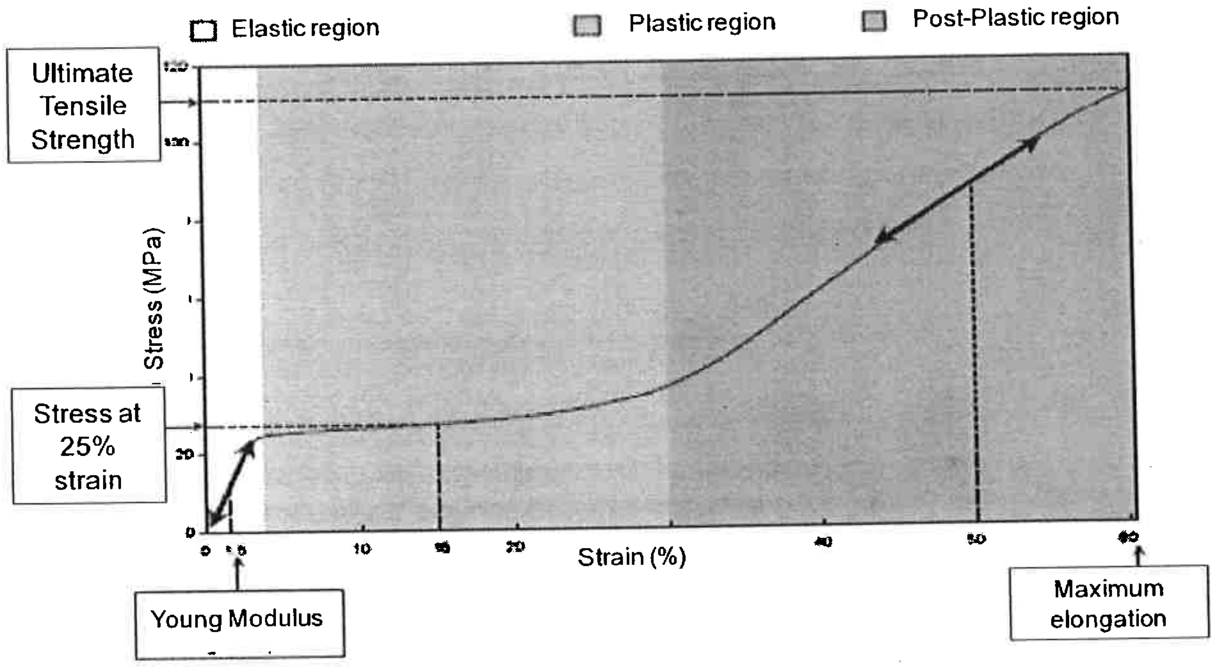

Image 6: Stress-strain curve of a hair fiber showing the three deformation phases

Between 0 % and 2 %, stress is proportional to strain; this part is called Elastic, Pre-Plastic or Hookean region. This behavior is probably due the breaking of hydrogen bonds between the α-helices.

Between 2 % and 30 %, the fiber stretches almost without any supplementary effort, in what is called Plastic Region. This phenomenon is due the transformation of α-keratin into β-keratin. When this phase finishes, around one third of all keratin was transformed.

From 30 % strain on, hair enters the Post-Plastic region. The shape of the tensile-deformation curve is dictated by the β-keratin resistance to stress. The main affected micro-structural bonds are covalent sulphur bonds.

The great mechanical resistance can be confirmed by the tensile parameters. One single non-treated hair fiber can have, in dry conditions, a Young modulus of approximately 3500 MPa, ultimate tensile strength of more than 200 MPa[1] and a total deformation of more than 40 %. A damaged hair can withstand more than 70 % of elongation, but under a much lower stress.

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[1] Values measured in the laboratory tests of this study

2.1.6 Surface properties

The total area of the fibers on a human head (assuming a length of 20 cm) is approximately 6 m^2, spread over more than 100.000 fibers. This explains why it is so difficult to wet all the hair and to optimize the contact between cosmetic products and each fiber. Surface properties depend mainly on the cuticle: its chemical composition makes it hydrophobic. Consequently, it is necessary to add surfactant compounds to most capillary products to facilitate wetting.

Finally, the way different fibers' cuticles interact determinates a wide range of properties as hair order, ease of comb, smoothness of touch, etc.

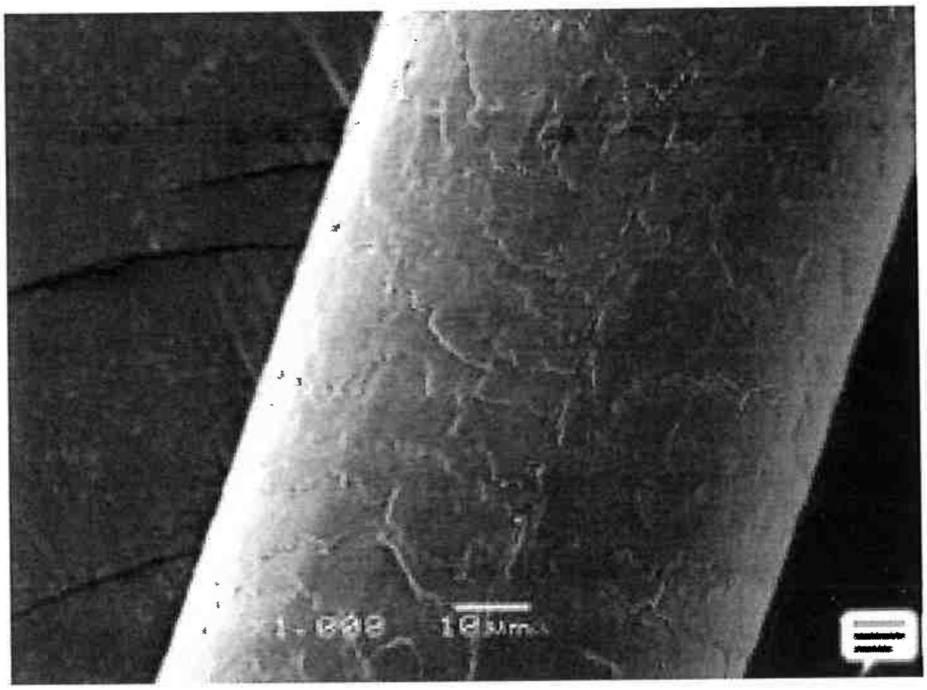

Image 7: SEM image made during the study, showing fiber's cuticle's scales

2.1.7 Interactions with water

Keratin is highly hydrophilic and most of its properties change in the presence of water, thus it is important to understand the behavior in wet conditions.

In low humidity levels (up to 15 %), water molecules attach themselves directly to amino acids lateral chain radicals. Between 15 % and 75 %, water bonds to keratin by hydrogen bonds. Above 75 %, water molecules on the keratin would be self-associated, creating bunches. A hydrogen network is formed, which lowers the potential difference between the α-helices, raising the inter-chain mobility. The effects on the mechanical behavior are:

- *In elastic region*: reduction of Young modulus
- *In plastic region*: reduction of stress plateau
- *In post-plastic region*: increase of maximum deformation, reduction of ultimate tensile strength

The mechanical strength declines and that is confirmed by the tensile parameters. Young modulus of a Caucasian non-treated hair falls, at 80 % relative humidity, to 1500 MPa. At the rupture, 55 % of elongation at 175 MPa, approximately, is observed (severely damaged hair can deform up to 60 %)[2].

Water retention takes place mainly in the intercellular cement, what explains a much larger diameter swell than the longitudinal one. There will also be a bigger separation of cuticle's scales that allows the penetration of bigger molecules into the cortex.

The association of water and other external factors can enhance hair fragilization. Sunlight, which normally attacks melanin pigments, becomes even more harmful when combined with water. The degradation of cuticle results in loss of shine and color.

2.1.8 Types of human hair

There are three main ethnic hair groups, whose physical properties differ: Caucasian, African and Asian.

Asian hair is usually the thickest one, having a diameter near 100 µm under normal conditions, whilst Caucasian stays around 80 µm and African, 50 µm. Regardless the origin, all fibers have an elliptic shape, and the degree of ellipticity (ratio between the biggest and the smallest diameters) is also an important characteristic. Asian and Caucasian hairs are more circular shaped (ratio between 1.3 and 1.6), while African hair is more flattened (ratio can reach 2.0) [3].

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[2] Data found in the laboratory tests of this study

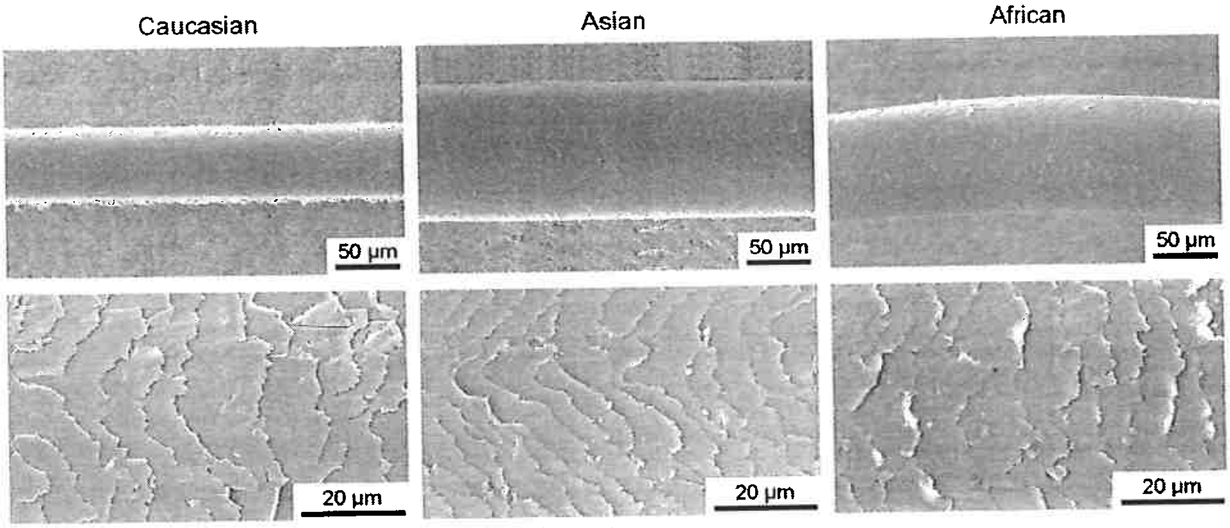

Image 8: SEM images showing shaft and cuticle's particularities of each hair kind

The most noticeable difference between the three hair types is the degree of curliness, which depends on the geometrical shape of the fiber. The plan that contains the bigger diameter may not be constant through the fiber axe, presenting rotations. The higher the rotation frequency, the curlier will be the hair. African hair, besides presenting a high ellipticity, shows strong and numerous rotations (around 90 degrees), thus is the curliest one. The rotation points are weaker, what worsen mechanical resistance properties. Asian hair is generally the most resistant.

Follicles' metabolisms are also different: African hair has the slowest growth, less than 1 cm per month, whilst Asian hair may grow more than 1.3 cm in the same period. Slow grow rate and high fragility makes African hair the most expensive: prices can reach more than 10 thousand Euros per kilogram (Caucasian hair costs around 6 thousand).

2.2 Hair Treatments

Historical and cultural elements are associated to the origin of the great importance dedicated to hair along all the human existence. Today, it is possible to change its shape, its color, its smoothness and almost any other property, thanks to the understanding of fiber's structure and its behavior due to the environment.

2.2.1 Permanent Wave

Permanent wave (or waving) is the technique that allows long lasting changes in the form of the hair. The first attempts in this domain go back to the seventeenth century: in the "Infernal Curling" technique, hair had to be immerged in boiling water for several hours to keep the wig's curls. Later in the twentieth century, new methods were created, but it was not until the 1930's that the first "cold" technique (which don't require the use of heat) was developed.

2.2.1.1 General mechanism

Hair has normally an elastic behavior[3]; it does not keep applied strains (except those too elevated). The basic steps to apply a permanent wave are:

- Render hair fiber plastic
- Apply the desired shape
- Return to elastic state, keeping the applied shape

What fundamentally changes between the permanent wave methods is the way to change hair behavior from elastic to plastic and vice-versa. Within the "Infernal Curling", hydrolysis in boiling water was used. That is possible to produce wigs, but evidently not in a person's head.

In the earliest "hot" permanent wave techniques, plasticity was obtained by a combination of a chemical reductive compound (usually alkaline) and heat. Treatment time fell to just a few minutes, but application temperature remained at almost 100 degrees. Current technique is called "cold" permanent wave. It does not require the use of heat, as the reduction is made through chemical routes at room temperature.

2.2.1.2 Reduction

In the first phase, all cohesive bonds of protein structure are broken: hydrogen bonds, electrostatic attraction and especially the disulphide bridges (step 1 in Image 9). Reductive mixtures are usually alkaline, and contain thiols or mercaptans. They act on sulphur bonds by the chemical reactions described in the Image 9.

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[3] The actual behavior is viscoelastic, but it does not change the fact that is does not keep applied deformations

$$\underset{\text{Keratin}}{K\text{-}S\text{-}S\text{-}K} + \underset{\text{Mercaptan}}{2\ R\text{-}SH} \longrightarrow \underset{\text{Reduced keratin}}{2\ K\text{-}SH} + \underset{\text{Disulphide}}{R\text{-}S\text{-}S\text{-}R} \qquad (1)$$

$$\underset{\text{Reduced keratin}}{2\ K\text{-}SH} + \underset{\text{Oxygen}}{\tfrac{1}{2}\ O_2} \longrightarrow \underset{\text{Reconstituted keratin}}{K\text{-}S\text{-}S\text{-}K} + \underset{\text{Water}}{H_2O} \qquad (2)$$

Image 9: Reduction (1) and oxidation (2) of keratin during a permanent wave treatment [1]

Table 2 shows that some mercaptans are active enough only at elevated pH. Even the lowest values of pK are above 7, what explains the basicity of these mixtures. The most used mercaptan today is thioglycolic acid. It's a colorless liquid, soluble in water and having an unpleasant smell. It is widely used because it is much less irritating and allergenic compared to other options.

Table 2: pK values of different mercaptans [1]

Mercaptan	Chemical formula	pK
Cysteine	$HS\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$	10.8
Dimercaptoadipic Acid	$(CH_2\text{-}CH(SH)\text{-}CO_2H)_2$	10.8
Thioglycolic Acid	$HS\text{-}CH_2\text{-}COOH$	10.4
Thiolactic Acid	$HA\text{-}CH(CH_3)\text{-}COOH$	10.4
Mercaptopropionic Acid	$HS\text{-}CH_2\text{-}CH_2\text{-}COOH$	10.4
Monothioglycerol	$HS\text{-}CH_2\text{-}CHOH\text{-}CH_2OH$	9.3
Thioglycolamide	$HS\text{-}CH_2\text{-}CONH_2$	8.4
Thioglycolhydrazyne	$HS\text{-}CH_2\text{-}CO\text{-}NH\text{-}NH_2$	8.0
Glycol thioglycolate	$HS\text{-}CH_2\text{-}COO\text{-}CH_2\text{-}CH_2OH$	7.8
Glycerol thioglycolate	$HS\text{-}CH_2\text{-}COOCH_2\text{-}CHOH\text{-}CH_2OH$	7.8

2.2.1.3 Oxidation/Fixation

Oxidation (or fixation) step aims to re-form the sulfur bonds previously broken keeping the imposed shape (step 2 in Image 9). Between reduction and neutralizing, fibers are in an extremely weak state and require careful handling.

Neutralization occurs using hydrogen peroxide at acidic pH. It might be found as a "ready to use" solution or as precursors (e.g. sodium perborate or potassium bromate). The reestablishment of disulphide bridges is never complete; consequently hair fibers will always be more fragile after a permanent wave treatment.

2.2.2 Coloring

In the past, changing hair color was a privilege. Science and cosmetology discoveries led to a deep understanding of hair color, resulting in simpler, and more reliable and affordable techniques.

There are three kinds of coloring techniques: temporary, semi-permanent and permanent (or oxidative) coloring. The main difference between them is the durability over time. To allow a certain level of tenacity, they use more or less aggressive formulations, although the application method remains basically unchanged (from "apply by yourself" mixtures to some which require professional preparation). The principle behind color variation is also similar: by fixing pigments to the fiber, we can give it a specific color. The penetration degree of these pigments defines the time the color will last.

2.2.2.1 Temporary coloring

The less durable colorings are usually used to give slight tone variations for some days or one hair wash. The pigments are attached only to the cuticle's exterior, there is no cortex penetration. Natural melanin is not affected, thus after washing hair retakes its original color. It is also the least aggressive technique.

2.2.2.2 Semi-permanent coloring

These colorings bring color changes that last through 4 to 5 washes. The mixtures contain actual pigments, just like temporary colorings, but in this case they end up penetrating the external boundary of the cortex.

2.2.2.3 Permanent coloring

Permanent coloring (or Oxidative coloring) is the most widely used technique today as it is the only one resulting in permanent color changes. The time between two applications is defined by hair growth (the root keeps producing naturally colored fibers), new re-growth at the roots is usually visible after one month.

The technique is called Oxidative because the coloring mixture compounds are not actual pigments, but rather colorless precursors which will be later transformed into colors within the fiber through an oxidation reaction.

These precursors are too big to penetrate the cuticle. To enable penetration, we take advantage of hair swelling properties. The absorption of a large amount of water and the change in pH opens the cuticle's scales, allowing the penetration of bigger molecules. The more alkaline the coloring mixture, the bigger is the swelling capacity. Most coloring lotions contain dissolved precursors in a mixture of an alkaline agent (usually ammonia) and an oxygenated water based mixture. The alkaline environment allows the passage of precursors through the cuticle into the cortex, where they are transformed into colorants under the action of the forming oxygen. Today these colorants have a chemical resistance as good as that of natural melanin.

The main function of the oxidant is to promote the precursors' transformation, but is also causes a slight degradation of melanin. The coloring can then bring a small tone reduction. To reach several tone reductions, a prior hair color removal is necessary.

The coloring mixture also attacks keratin chains as they have lots of accessible active sites. As a result, oxidation coloring weakens hair and changes its surface smoothness.

2.2.3 Straightening

The straightening treatment consists in giving hair a (more or less) lasting straight configuration. The simplest way to do this is by washing the hair: as long as the hair is wet enough, it is kept straight. In spite of the water action on breaking some hydrogen bonds, the effect is mostly due to the weight of water itself (hair absorbs, gets heavier and consequently is self-straightened).

2.2.3.1 Hot comb

The first actual straightening operation is the hot combing and was developed in the late 1800's [4]. That technique allows the breakage of the hydrogen bonds by heat, which gives hair a temporary straight configuration. The longer the fibers are kept away from humidity, the longer the effect will last. Modern flat iron devices

(Image 10) may include simultaneous vapor or cream application, and use temperatures ranging from 150 to 250 °C. Hair fibers are damaged following frequent use of that technique, and alopecia might also be a side effect [4].

Image 10: A modern flat iron device[4]

2.2.3.2 Chemical Relaxing

In order to obtain permanent hair straightening, the disulphide bonds must be affected. That can be done by chemical relaxing: alkaline reducing reagents break the disulphide bonds; then, hair is placed in a straight position; finally, other products are used to reestablish the bonds. The mechanism seems similar to that of permanent waving, but it is actually different; that can be noticed by the fact that if we use a usual thioglycolate waving solution to keep the hair straight instead of curling it, the final acquired configuration will be just temporary, it will not withstand subsequent washes [5]. Thus, the reduction/oxidation steps are not enough to guarantee the straightness of curly hair.

The first main difference is the consistency of the solution. Permanent wave products must be used on hair twisted in rollers, so a liquid formulation is the most suitable. That problem does not exist in straightening (no rollers), and the use of a viscous cream formulation is more appropriate. The cream weight and viscosity can even help keeping the fibers straight without too much physical solicitation (which could lead to breakage).

[4] Source: http://www.virtuallost.com/wp-content/uploads/chapinha-03.jpg

The most used relaxing products are sodium hydroxide, thioglycolic acid (TGA) or Dithiodiglycolic acid (DTDG) based products [6]. Those mixtures have high pH (usually above 13), which requires a careful protection and handling in order not to burn the ears and the scalp. After the reducing agent is applied, hair fibers are kept straight from 10 to 20 minutes [4]. The last step is to wash the product away and bring the pH to normal levels with a neutralizing shampoo.

The chemical mechanisms controlling permanent straightening are not fully understood. It is believed that the first steps are spontaneous uncoiling (radial increase implies a curvature radius reduction) and supercontraction (which locks the fibers in its new configuration) caused by hair swelling and keratin chain transformation [5]. Furthermore, it is possible that both factors would be associated and correlated to the efficiency of the treatment; their kinetics would also affect the final result. It is also believed that the cysteine cleavage and the formation of new lanthionine cross-links would be necessary, but that effect might be only a collateral effect of the two previously mentioned mechanisms (spontaneous straightening and supercontraction) [5].

2.2.3.3 Hybrid techniques

The most recent techniques combine the relaxing agents and the hot combing. Hair is hot-combed still with the relaxing cream, and a final shampoo wash may follow (in the well known "Brazilian Straightening", the hot-comb is the last step; the consumer shall not wash her hair for 3 days, though). Besides the combination of both two straightening factors, it was shown that high temperatures change the formation and concentration of cysteine residues during the reduction, heat treatment and oxidation steps, leading to a better cross-link recovery [6]. With more disulphide bonds recovered, hair integrity is preserved.

2.2.4 Damage to the fiber

There are several natural elements that weaken the hair: mechanical stress (combing), wetting/drying and sunlight, especially UV radiation. The combination of two or more elements might be even more aggressive [7]. For example, a humid atmosphere increases the damage caused by UV radiation to keratin chains, affecting, irreversibly, the mechanical resistance of the hair. To avoid that, capillary sunlight protection products were developed and tested [8].

However, what really affect hair integrity are cosmetic products. Even a simple wash with neutral shampoo tears off cuticle's scales. The most severe treatments are form treatments, followed by hair color removal and oxidation coloring.

Each treatment's effects were already extensively studied and quantified by several methods: dynamic mechanical analysis (DMA) [9], differential scanning calorimetry [10] and tensile test [11]. The cuticle has been analyzed by Scanning Electronic Microscopy. Image 11 compares two fibers with different degradation levels: the one on the left did not suffer any chemical treatment and shows a healthy cuticle structure: parallel scales, equally spaced, not separated and covering the entire cortex. On the other hand, the one on the right was highly damaged: we notice loss of scales, layers irregularities and cortex exposure.

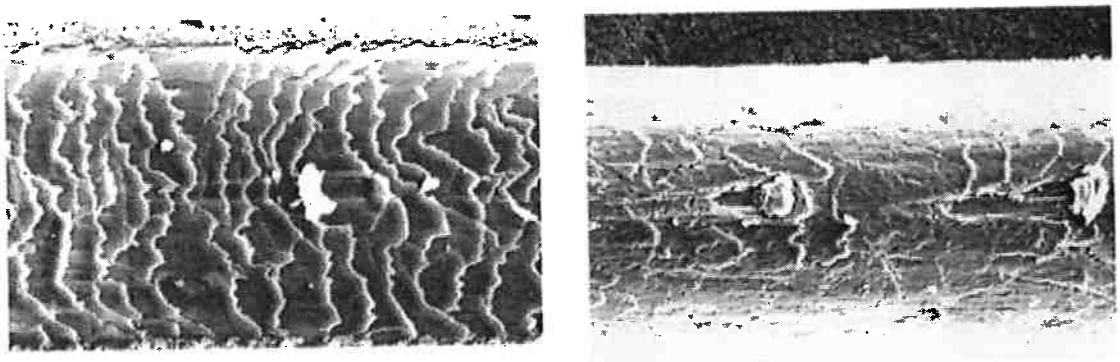


Image 11: SEM images of an untreated hair fiber (left) and a severely damaged one (right) [3]

Until now, little attention has been given to the combination of chemical treatments, so called superpositions. The toxicological issues of applying two treatments in a day are being reduced and even disappearing as the products become less aggressive and formulations get more efficient. Within this framework, it is important that the treatments be compatible, besides fulfilling its primary function. The whole group must be efficient and harmless to the final user.

3 Materials and methods

3.1 Hair and products used

This study was based on swatch evaluation. A swatch consists of thousands of hair fibers crimped at one end (Image 12); each study may use dozens of swatches, which cannot be provided from a single human head. In order to create a batch of sometimes more than one hundred swatches, hair from different people but having similar properties (porosity, color, ethnic origin, integrity) are combined, homogenized and finally crimped into swatches of different lengths (from 15 to 30 cm).

Image 12: Hair swatches

Two types of hair were used during the study: Caucasian brown natural hair (tone 5) and Chinese natural dark hair (tone 2). The first one was used for the majority of the analysis; the second one had the tensile properties evaluated and compared to those of the Caucasian hair.

Three cosmetic treatments were tested, Treatment 1, Treatment 2 and Treatment 3 (hereafter called T1, T2 and T3). They are all commercially available products, widely used.

3.2 Complementary procedures

Two complementary procedures were used, in order to approach the sequences to the real life damages of hair. They will be called hereon "T4" and "T5". In order to apply T5 to the fibers, it was necessary to remove some hair from the swatch and transfer them to a special support.

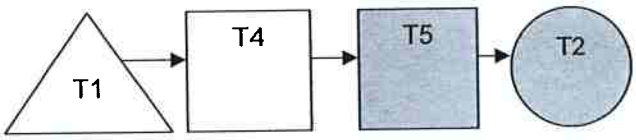
3.2.1 Treatment combination sequences

Several treatment sequences were tested and analyzed, in order to determine the combination least damaging to the hair. The sequences are represented with the schema below (Table 3), where:

- The shape indicates the nature of treatment:
  - Triangle: corresponds to T1
  - Circle: correspond to either T2 or T3
  - Square: corresponds to either T4 or T5
- The color indicates the application support of the referred treatment:
  - Light gray: corresponds to a treatment made on swatch
  - Dark gray: corresponds to a treatment made to fibers on the special support

In the example below, the hair fibers have an initial T1. Then a T4 is applied. These two first treatments are carried out on a swatch. Fibers are then transferred to the support for the T5. Afterwards, they receive a T2.

Table 3 : Example of a superposition sequence



3.3 Evaluation techniques

3.3.1 DMA (Dynamic Mechanical Analysis)

The Dynamic Mechanical Analysis is an analytical method dedicated to studying the mechanical properties of viscoelastic materials, those which have both elastic and plastic behavior. This technique is able to measure several physical properties, among which the Young modulus, the loss factor and the glass transition temperature.

3.3.1.1 Physical Principles

The method involves the application of an oscillatory deformation and measuring the dynamic force and the resulting displacement. The phase shift and the ratio of the amplitudes of waves allow the calculation of the intrinsic properties of the material.

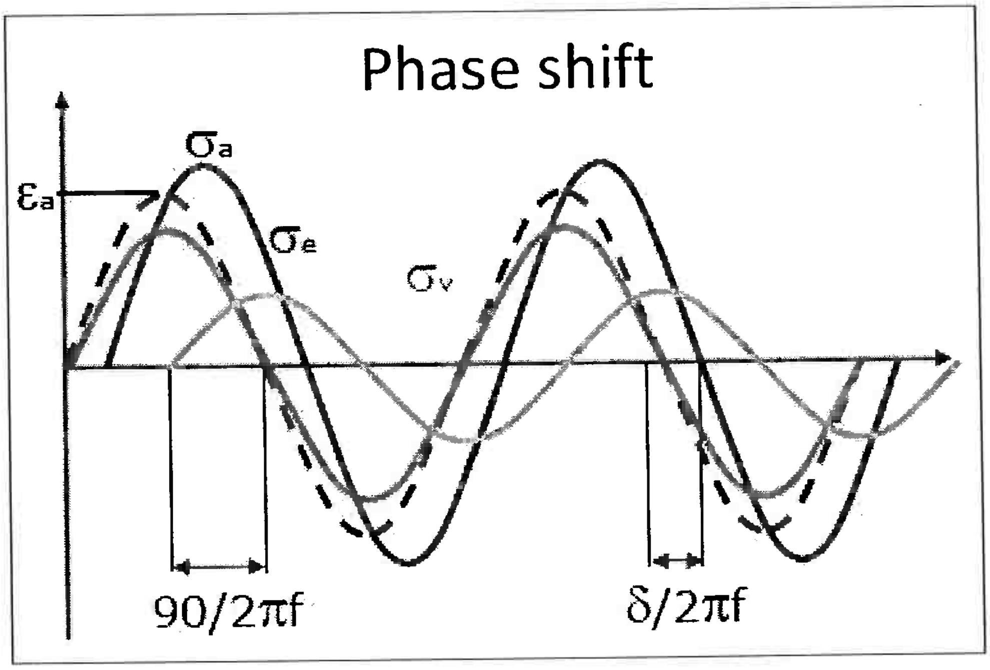


Image 13: Representation of the phase shift between an applied deformation (dotted black), the resulting stress of an elastic material (In phase), of a viscous fluid (90° delay) and of a viscoelastic material

In the case of tensile stresses, there are some requirements to ensure reliable results. First of all, the size of the sample must meet certain criteria to ensure the absence of other types of solicitations (e.g. torsion). In the case of cylindrical samples, the length must be greater than twice the diameter. In practice, for the hair, with a diameter around 60 microns and length of several centimeters, this is widely respected.

Next, we must ensure that the sample remains in linear behavior for the entire range of deformation. If we take a large dynamic deformation, the material will enter the plastic zone, where the measures are no longer relevant. However, if this deformation is too small, the measured values are not easily identifiable from the background noise.

Finally, the change in the rigidity of the sample should remain in the measurable range of the device, which depends on the frequency used during the test. This property depends on the size of the sample.

In addition to the dynamic deformation, a prior static deformation can be applied to the sample before the start of the sinusoidal stress.

3.3.1.2 Parameters studied

- *Real/Storage Modulus*

The storage modulus represented by E' [MPa], is a measure of the material's ability to resist and recover from a deformation. It is different from the Young modulus measured in tensile test, as the latter represents the behavior in stress / strain at low non-dynamic strains (< 2 %). The storage modulus (as well as other parameters out of DMA) depends on temperature and frequency and decreases in the presence of moisture.

- *Imaginary / Loss Modulus*

The loss modulus E" [MPa] is a viscous property and indicates the tendency of the material to flow and dissipate energy.

- *Damping factor*

The damping factor is a measure of the damping properties of the material. It can be measured in two ways: either by the tangent of the phase shift between the imposed and measured waves (method actually used by the DMA analyzer) or by the ratio E"/E' (the methods find the same value). This amortization is related to the energy loss associated with friction and intra-molecular rearrangements. The damping factor increases with humidity.

- *Loss Angle*

This is the angle whose tangent is the damping factor. Physically, it is the angle between the vectors of the storage modulus / loss modulus, or the phase shift between the applied strain and resultant stress.

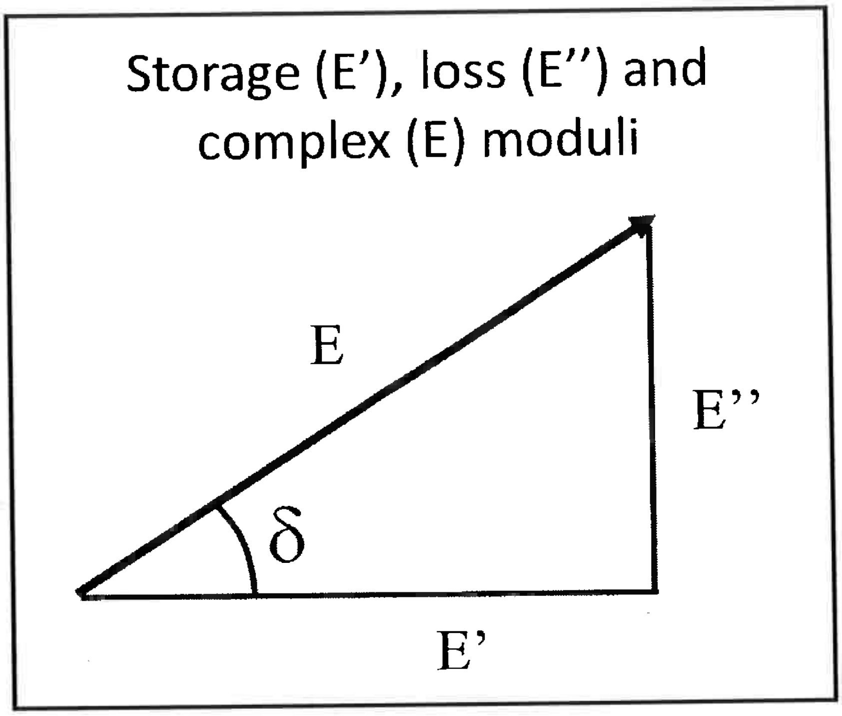


Image 14: Vector representation of the complex, loss and storage moduli

3.3.1.3 Procedure

The procedure is similar to those already described in the literature [9].

Hair samples were prepared and then fixed in the DMA analyzer, at room temperature and humidity (25 degrees, 55 % relative humidity). The fiber diameter had already been taken, to enable the machine to convert force to stress. Only one diameter value could be inserted at a time, so we decided to use the wet diameter, which is larger than the dry diameter[5]. The sinusoidal deformation was then applied during 3 minutes. In that instant, the hair sample was immerged in distilled water, and the stress kept for more 2 minutes. Value acquisitions (E', E'', δ) were made every 4 seconds, and it was decided to calculate the mean value using the 10 values just before immersion (for the dry phase) and the 10 last values (for the wet phase). These periods were the more stable along the time.

For each treatment evaluation, 10 samples were analyzed, and the mean and standard deviation calculated for the results. Usually, the more treatments a swatch was submitted to, the bigger was the dispersion (as the fibers suffered different damage levels according to their physical position).

[5] The first DMA and MTT results showed that the wet parameters enabled a better differentiation between treatments, what motivated the abandon of dry analysis further on.

3.3.2 MTT (Miniature Tensile Test)

The Miniature Tensile Test (MTT) was used to determine the mechanical tensile properties of the hair until the rupture.

3.3.2.1 Physical Principles

The samples are hair fibers fixed in each end by brass crimps. One end is attached to the cassette containing the samples, and the other end is pulled at constant speed by the machine. Imposed resistance is then measured, and by combining it with the displacement we obtain the classical stress-strain curve previously described (Image 6). The diameters of the fibers are required to go from strength (physical quantity actually measured) to stress. They had been previously measured by using a laser scan with an accuracy of 1 µm.

Tensile test shows how water impact depends on hair integrity. Making a dry analysis (fibers are in a controlled atmosphere of low relative humidity) on damaged hair, the results are not easily distinguishable: the gaps between Young modulus (and any other parameter) of differently treated fibers are very small, and do not allow drawing conclusions considering the standard deviation.

However, when the same samples were submitted to a wet test (the fibers are immerged for 30 minutes before the test and remains for all its duration) one will notice larger gaps between the treatments. Water can penetrate deeper into the fibers whose cuticles and cortex were subjected to reduction and oxidation, so its impact will be stronger. The mechanical strength will be more affected and consequently the difference for the Young modulus (and other parameters) will be higher.

3.3.2.2 Parameters studied

Several parameters can be studied with tensile test. In this study, we were rather interested on:

- *Elastic/Young modulus (MPa):* the coefficient that characterizes the inherent stiffness of the material during reversible deformation phase (for the hair, usually up to 5 % strain). In a tensile test, it is calculated as the slope of the Strain vs Stress graph between 0 and 5 % strain.

- *Stress at 15 % strain (MPa):* the stress in the plastic phase (plastic deformation plateau)
- *Ultimate tensile strength (MPa):* stress at rupture
- *Maximum elongation (%):* strain at rupture

These parameters can be graphically viewed in the Image 6.

3.3.2.3 Procedure

The procedure was similar to those described in the literature [8].

The first tests were made both in dry (55 % of humidity) and wet (80 % humidity) conditions, but the dry results showed very little difference between natural and treated hair, in comparison to the wet tests. Hence, we decided to continue the study under wet conditions only.

To evaluate the tensile properties of a swatch (that had previously passed a sequence of treatments), 40 samples were prepared. The two extremities of the samples are put inside small cylindrical brass crimps (Image 15), which are flattened and then firmly holds the fiber. The machine actually grips the metallic crimp and pulls it.

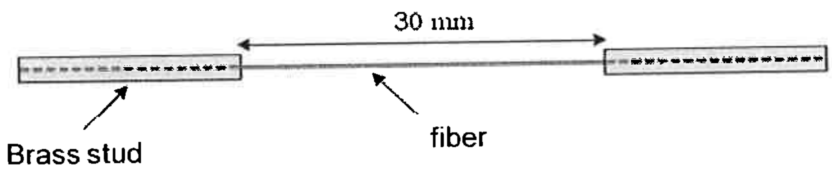


Image 15: A sample used in tensile test

Initially, the fiber diameter was measured, and then the fiber went to the actual tensile tester, where it was stretched until rupture. The stress-strain graph was calculated for each fiber, and it was necessary to verify them one by one to discard the aberrant graphs (usually due to bad sample preparation or anomalous fibers). The software automatically detects the three regions of the graph and calculates the parameters of interest mentioned above.

3.3.3 HP-DSC (High Pressure Differential Scanning Calorimetry)

In the HP-DSC analysis, we measure the integrity of keratin α-helices inside the fiber. Like any protein, keratin undergoes a denaturation process when subjected to high temperatures.

3.3.3.1 Physical Principles and procedure

Hair fibers are first soaked in deionized water for one night. They are then dried and cut into fragments of less than 1 mm and placed (about 10 mg for one analysis) in a sample carrier with deionized water. For each treatment sequence, 5 analyses were made.

The sample is then heated from 25 to 200 degrees at a constant rate of 10 degrees per minute. Heat rate required to maintain these conditions is measured. This forms a Heat Flux vs Temperature plot, which can be analyzed to find the denaturation temperature and enthalpy variation.

3.3.3.2 Parameters Studied

For each test, a concavity on the DSC curve indicates the endothermic denaturation of keratin (Image 16). The main properties of interest are the temperature at which this happens (specifically, the temperature of the extreme point of the concavity) and the area of the concavity, which represents the enthalpy of transformation. A reduction of these two properties indicates a more damaged cortex.

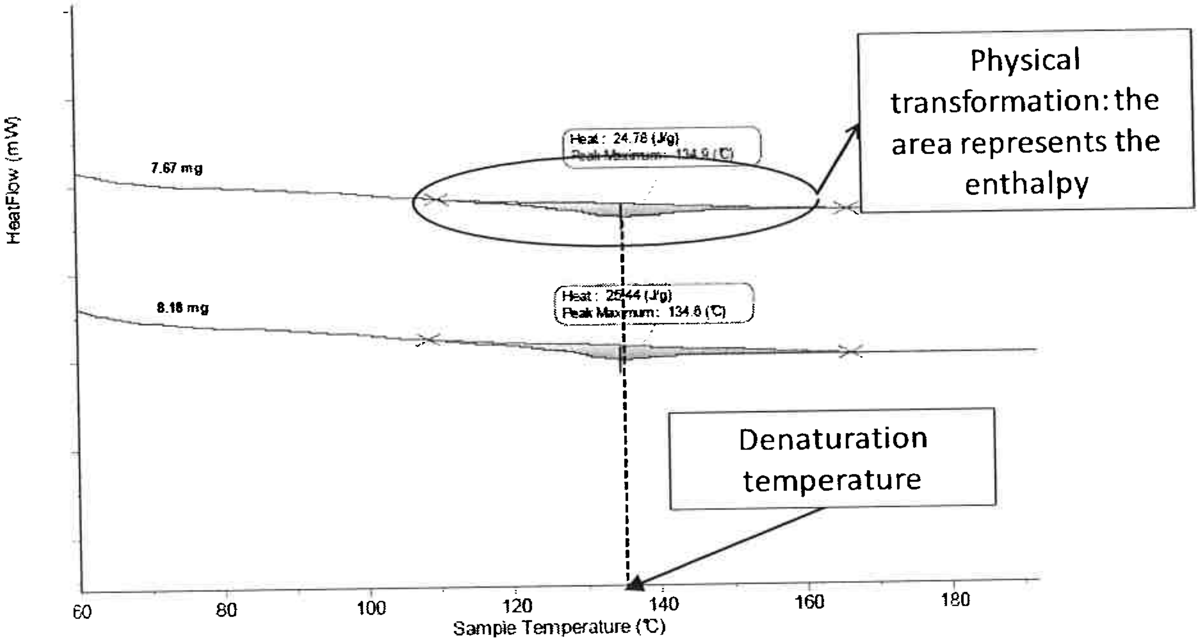


Image 16: HP-DSC thermmograme showing keratin denaturation

3.3.4 SEM (Scanning Electron Microscopy)

3.3.4.1 Physical principles

The Scanning Electron Microscopy is an analysis technique based on the interaction between the material's surface and an electron beam. The beam is usually produced by heating a tungsten filament until it emits electrons. The beam is next focused by a sequence of lenses before reaching the sample.

The interaction between the electron beam and the sample is not limited to the surface. There is actually a teardrop-shaped penetration volume where the atoms are excited by the electrons, generating a multitude of responses, which are collected by several sensors and analyzed to provide information.

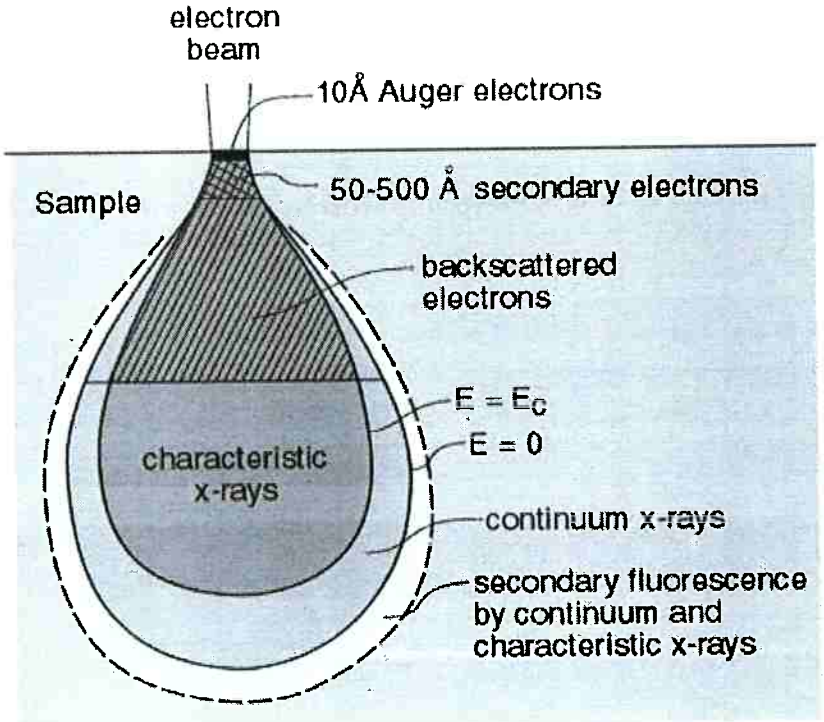


Image 17: Teardrop-shaped interaction volume of electron beam and sample, showing the penetration of each type of analysis[6]

The most pertinent information is the one from secondary electrons, backscattered electrons, Auger electrons and characteristic X-rays. In this study, the SEM technique was used to visualize the surface of fibers, so only secondary electrons analysis was used.

[6] Source : http://www4.nau.edu/microanalysis/Microprobe-SEM/Signals.html

3.3.4.2 Procedure and studied parameters

Hair is a non-conductive material, meaning that prior to the analysis; the samples must be metalized in order to prevent charge accumulation in the surface. An ultrathin gold coating layer is deposited by high vacuum evaporation. The electrons are now free to leave the sample.

The scanning electron microscopy allows the visualization of hair surface. It allows the analysis of the surface of the cuticle, on several levels:

- Presence of cortex exposure (loss of scales)
- Regularity of scales distance and borders conditions
- Separation of the scales
- Presence of product deposit

For each analysis, 10 microscope images are taken. For each one a degradation rating is established (from 0 to 5), and average is the overall degradation level of the hair.

However, a qualitative surface comparison between two treatments is also desirable, because the average rating does not take into account the nature of the damage, which can be very important, depending on the products tested.

4 Results and discussion

In the beginning of the study, the initial sequences were established, prepared and analyzed. All the following tests were developed following the results and conclusions drawn in the previous test sequences. That is why all the study's results are presented in chronological order, instead of been presented by test or treatment type. The results are based on limited sample sizes. Statistics were not possible. Hence results here are best described as trends.

4.1 First base sequences: effect of single treatment and combination of treatments on the mechanical behavior of the hair

The procedures applied in each treatment are described in section 3.2. Due to the complexity of the sequences, some symbols are used in this dissertation (section 3.2.1) and are here reproduced again:

- The <u>shape</u> indicates the nature of treatment:
  - Triangle: corresponds to T1
  - Circle: correspond to either T2 or T3
  - Square: corresponds to either T4 or T5
- The <u>color</u> indicates the application support of the referred treatment:
  - Light gray: corresponds to a treatment made on swatch
  - Dark gray: treatment made to fibers on the special support

4.1.1 Objectives

These initial sequences (Table 4) were conducted to evaluate the degradation of mechanical properties for each single treatment (sequences T00, T01 and T02) and after simple combinations of T1 and T2 or T3 (sequences S01 and S02). We also tested the impact of adding T5 between those treatments (generating sequences S11 and S12). The evaluation methods used were MTT and DMA. The comparison between S01/S11 and S02/12 sequences shows the impact of adding T5. Finally, long sequences S31/32 represent what happens after two rounds of T1+T2/T3.

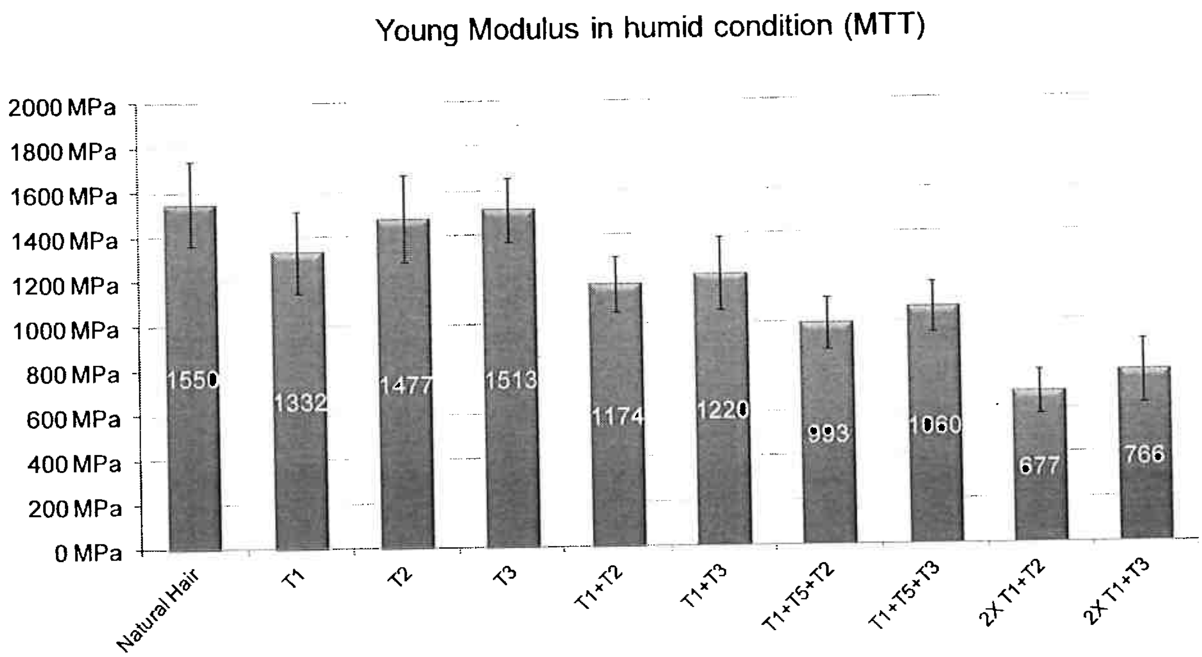
Table 4: Details of the first tested sequences

Sequence Code	Treatments sequence	Details
T00	T1	T1 treated hair
T01 / T02	T2 T3	T2/T3 treated hair
S01 / S02	T1 → T2/T3	T1+T2/T3 in sequence
S11 / S12	T1 → T5 → T2/T3	T1+T5+T2/T3 sequence
S31 / S32	T1 → T2/T3 → T5 → T2/T3 → T5 → T1 → T2/T3	Double superposition of T1+T2/T3

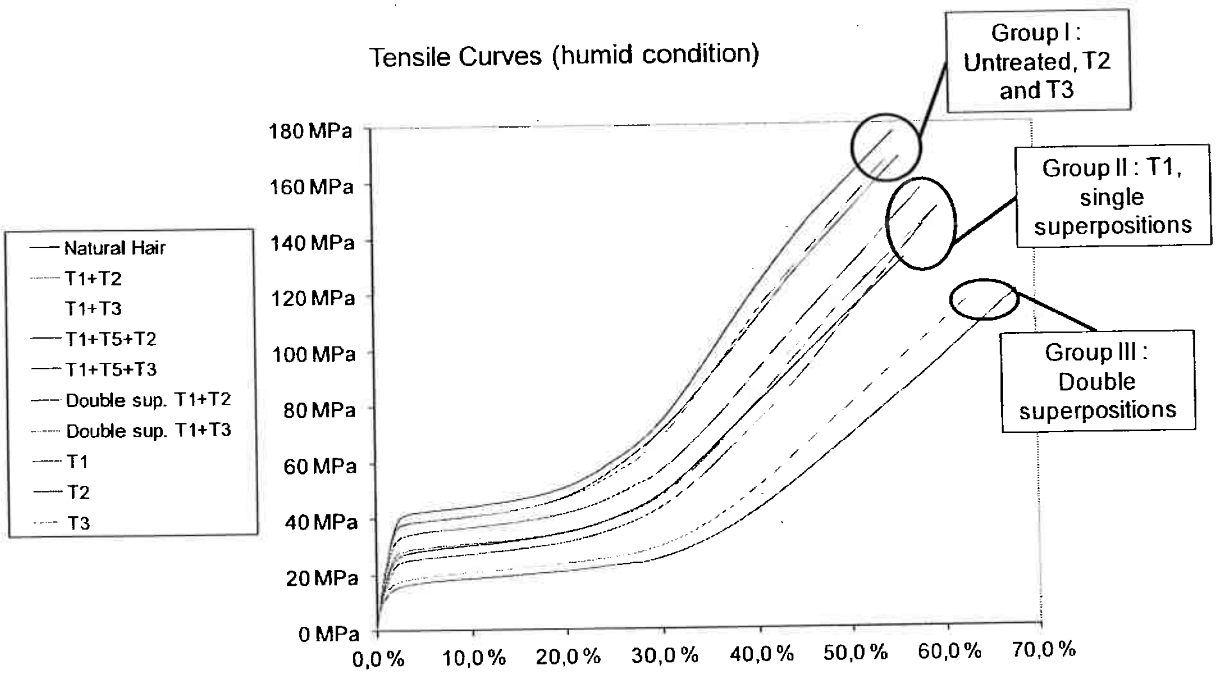
4.1.2 Results

Comment: The measurements under dry condition did not show differences among the distinct treatments and hence are not shown here. From the next chapter on, only analyses under humid conditions were performed.

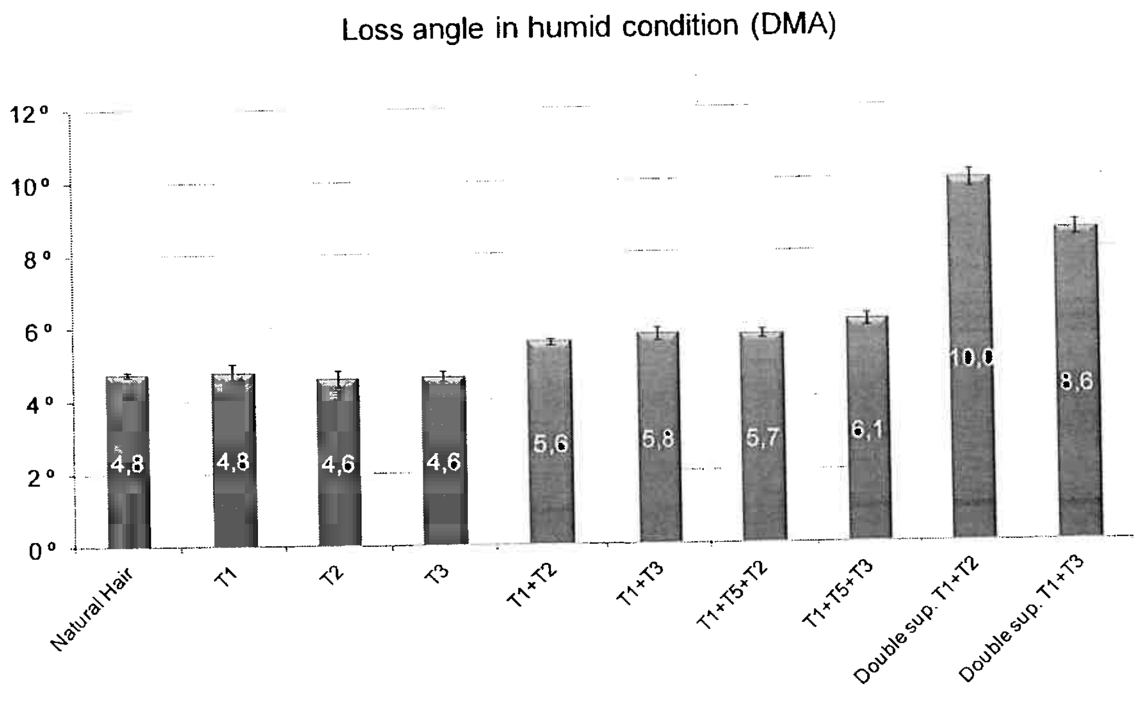
Graph 1, Graph 2 and Graph 3 present the Young modulus, the stress-strain curves and loss angle for the initial sequences. All tests were performed under constant humid condition (80 % of relative humidity) (Table 4).



Graph 1 : Young Modulus in humid condition of the first sequences

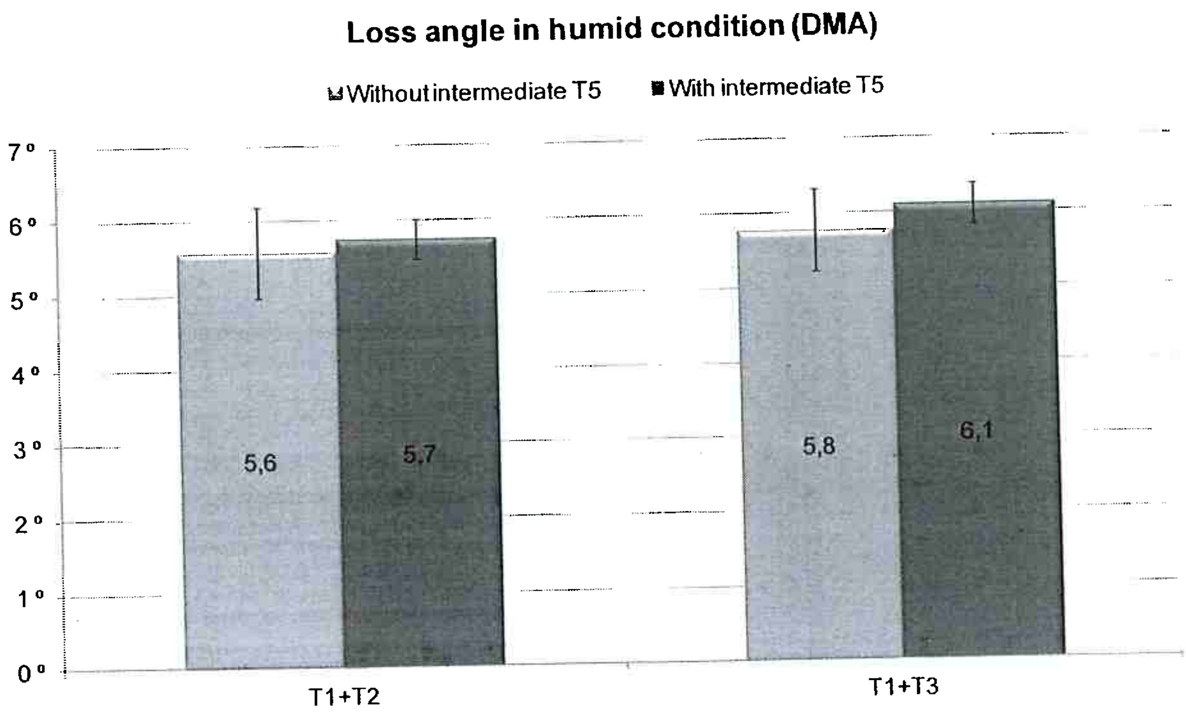


Graph 2: Tensile curves in humid condition of the first sequences, showing the three groups of similar fragilization

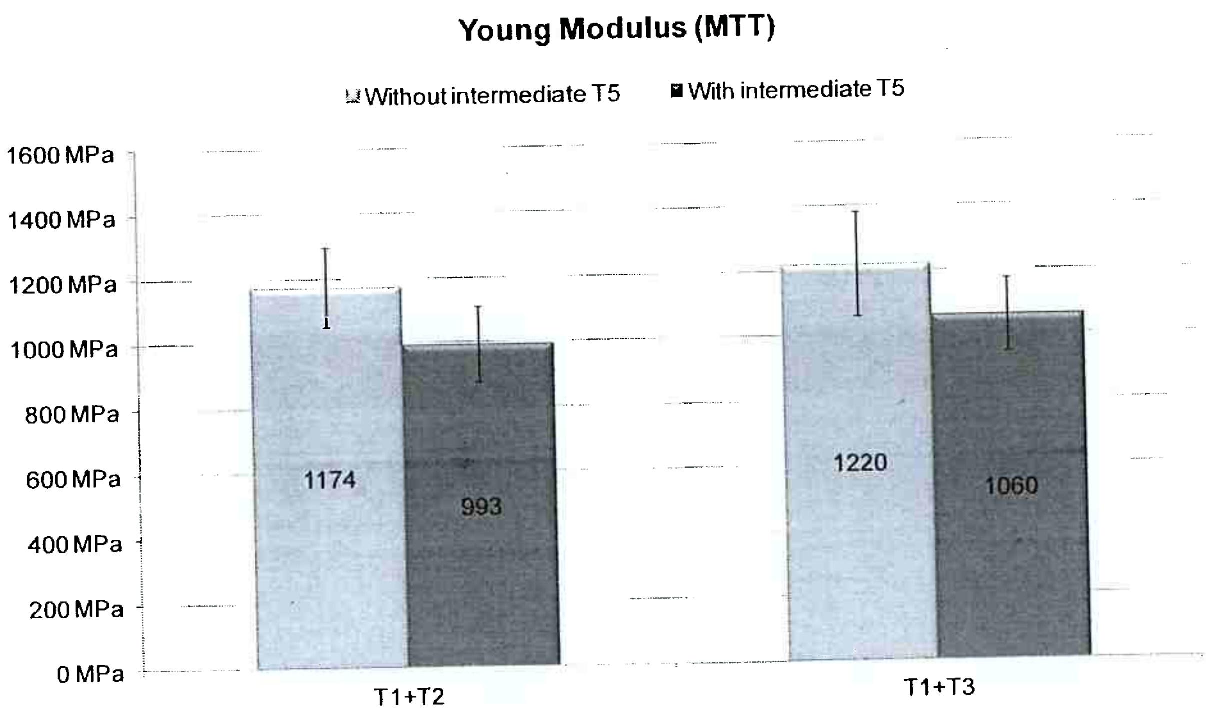


Graph 3: Loss angle in humid condition of the first sequences, also showing the division in three groups of similar fragilization

Graph 4 and Graph 5 show the impact on the mechanical behavior, observed by DMA and MTT, after adding T5 between the treatments.



Graph 4: Impact of T5 between treatments in loss angle (humid condition)



Graph 5: Impact of T5 between treatments in Young Modulus (humid condition)

4.1.3 Discussion

Using the Young Modulus measured by MTT (Graph 1) it is possible to classify the sequences of treatments according to their impact on the mechanical properties of the hair, due to different degradation processes (less damaged first):

1. Natural hair
2. T2 and T3 treated hair (T01 and T02)
3. T1 treated hair (T00)
4. T1+T2 and T1+T3 (S01 and S02)
5. T1+T5+T2 or T3 (S11 and S12)
6. Double superpositions (S31 and S32)

The stress-strain curves show rather a separation into three groups (Graph 2):

- Group I: natural and T2/T3 (less damaged)
- Group II: T1 and simple superpositions (moderately damaged)
- Group III: double superpositions (severely damaged)

The division into three groups was also observed in the DMA results, specifically, in loss angle (Graph 3). However, in this case, T1 treated hair is as aggressive as T2 and T3.

The sequences T1+T5+T2/T3 were slightly more aggressive than those without T5, according to results from both MTT (on all parameters; in the Graph 5 we show the example of Young Modulus) and DMA (idem; in the Graph 4 we show the example of loss angle).

There was no significant difference between T2 and T3, except for the double superpositions, where T2 has shown to be more aggressive (Graph 3).

4.1.4 Interpretation

The MTT results showed damage levels consistent with current knowledge (T2/T3 types of treatment are less aggressive than T1, and the combination of both raises damage). However, superpositions of treatments are much more severe, specially the double ones.

It was also noted that the presence of T5 between T1 and T2/T3 fragilized slightly more than its absence. The origin of this difference could be based on one or more of the following assumptions:

I. Applications in the special support would be more aggressive because of the substrate exposition to a large excess of chemicals.

II. Residues of T1 may combine with T2 or T3 increasing the total amount of degradation.

III. T5 would also bring extra amount of degradation to the fibers. In order to make the two sequences (S0X and S1X) comparable, we should include T5 at the end of S01/S02.

To verify each statement, new sequences were developed and tested. The results are explained in the next section.

4.2 Complementary analysis

4.2.1 Objectives

The sequences presented in Table 5 aim to test the assumptions mentioned at the end of the previous section (4.1.4).

By analyzing the sequences S01SHPE (Table 5) and his counterpart S02SHPE (both different from S01SH / S02SH just by the fact that they are applied in the special support) we can analyze the hypothesis I. This will also be possible by comparing to S01EC and S01ECPE (yet the only difference is support for applying T2/T3) and its counterparts S02EC / S02ECPE.

The sequences S01SH / S02SH are in fact the same S01 and S02 after the introduction of T4 between T1 and T2/T3. This will test the hypothesis II.

Finally, by making the comparison between S01/02 and S01/02EC, we will test the effect of T5 at the end of the sequence, the hypothesis III.

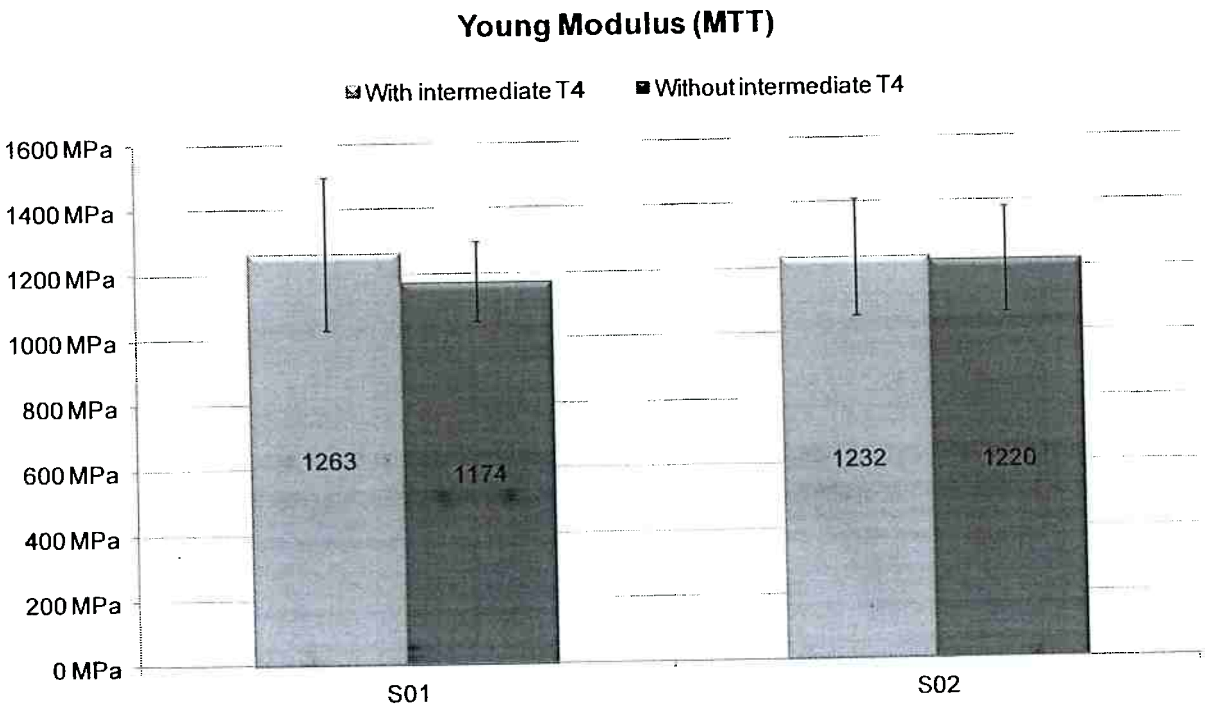
Table 5: Complementary sequences tested to test the three hypotheses

Sequence Code	Treatments sequence	Details
S01/02 (old)	T1 → T2	Already tested
S01SH S02SH	T1 → T4 → T2	Compare with S01/02: test II
S01SHPE S02SHPE	T1 → T4 → T2	Compare with S01/02SH : test I
S01EC S02EC	T1 → T2 → T5	Compare with S01/02: test III
S01ECPE S02ECPE	T1 → T2 → T5	Compare with S01/02EC: test I
S11 (old) S12 (old)	T1 → T5 → T2	Compare with S01/02ECPE: test impact of T5

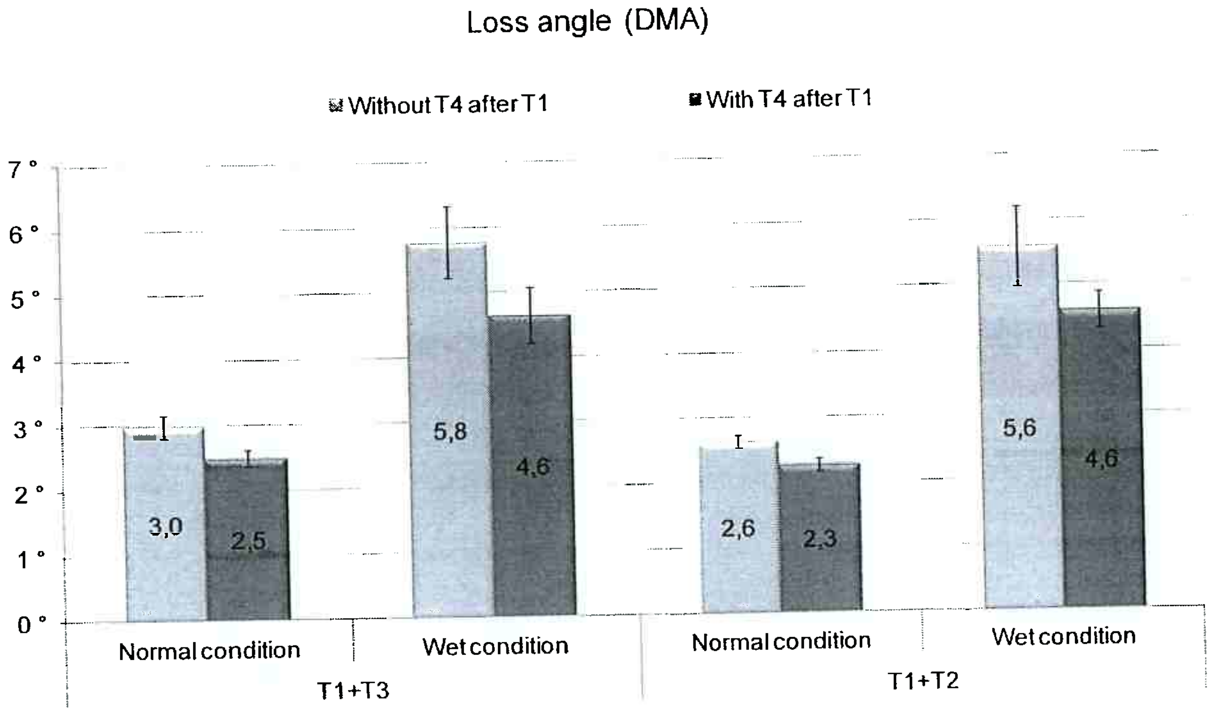
4.2.2 Results

With this new set of tests, we were able to identify the impact of T4 between T1 and T2/T3.

Graph 6 and Graph 7 show the impact of T4 between T1 and T2/T3 in MTT and DMA parameters.

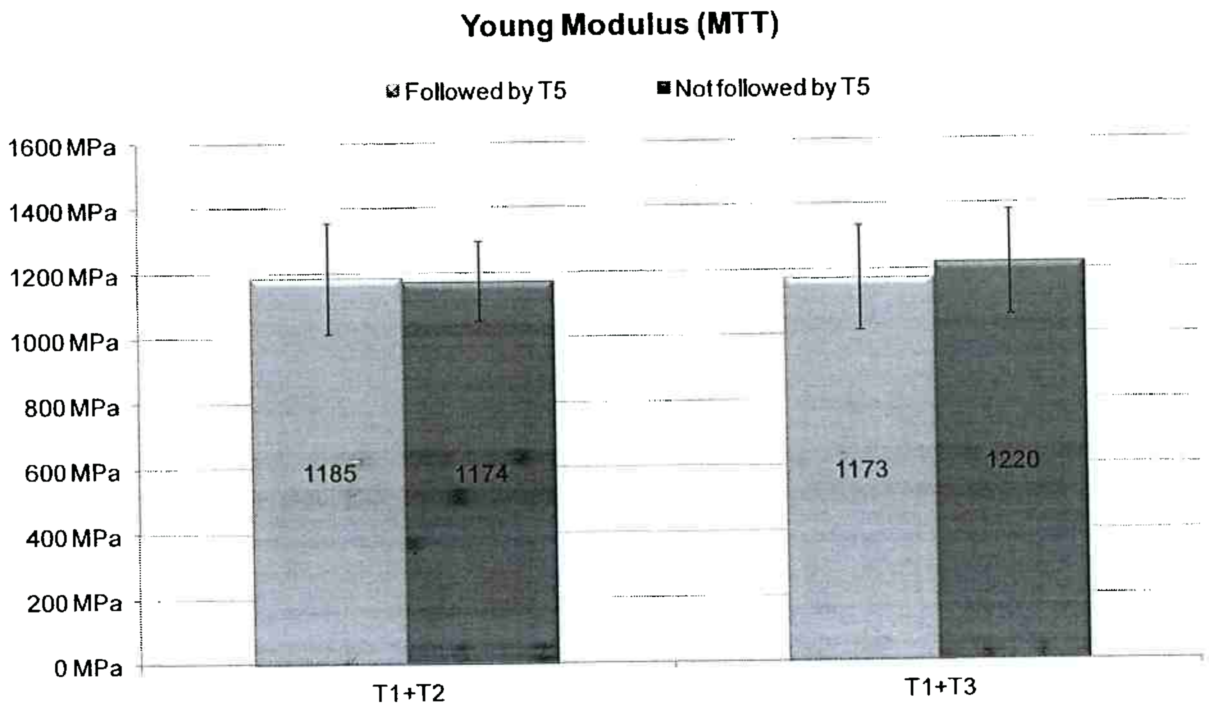


Graph 6: Impact of T4 on the Young Modulus of a T1+T2/T3 superposition

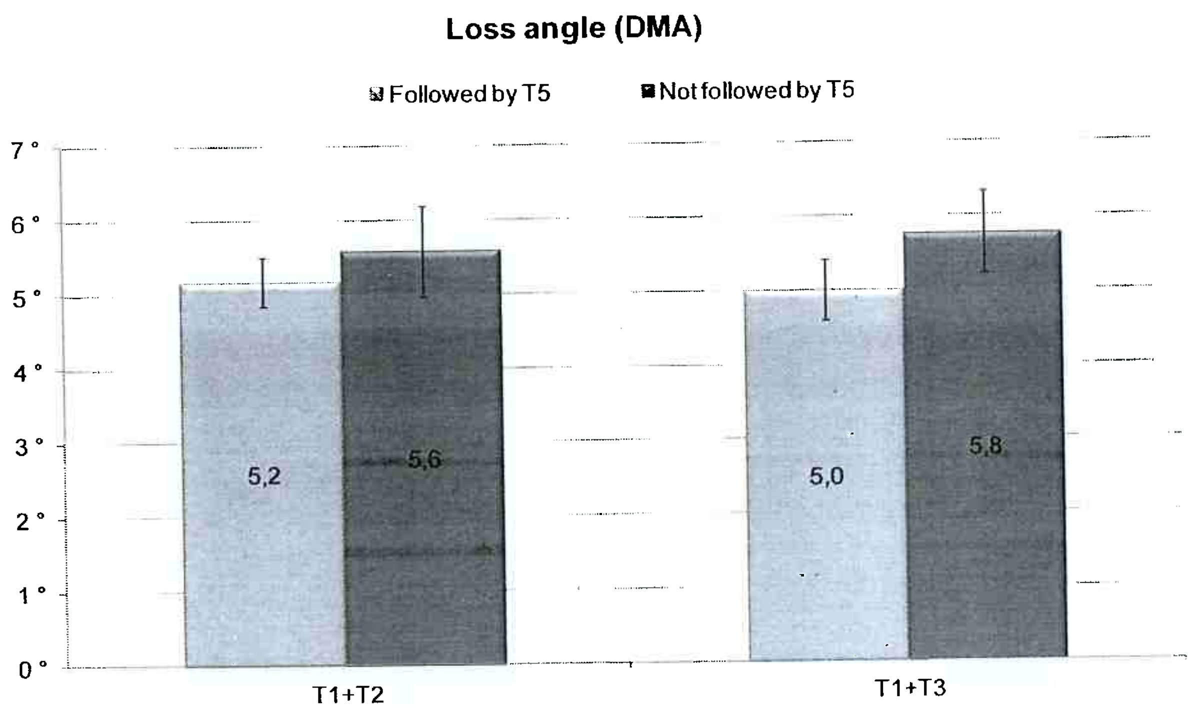


Graph 7: Impact of T4 on the Loss angle of a T1+T2/T3 superposition

Graph 8 and Graph 9 show the impact of adding T5 after a T1+T2/T3 superposition in MTT and DMA parameters.

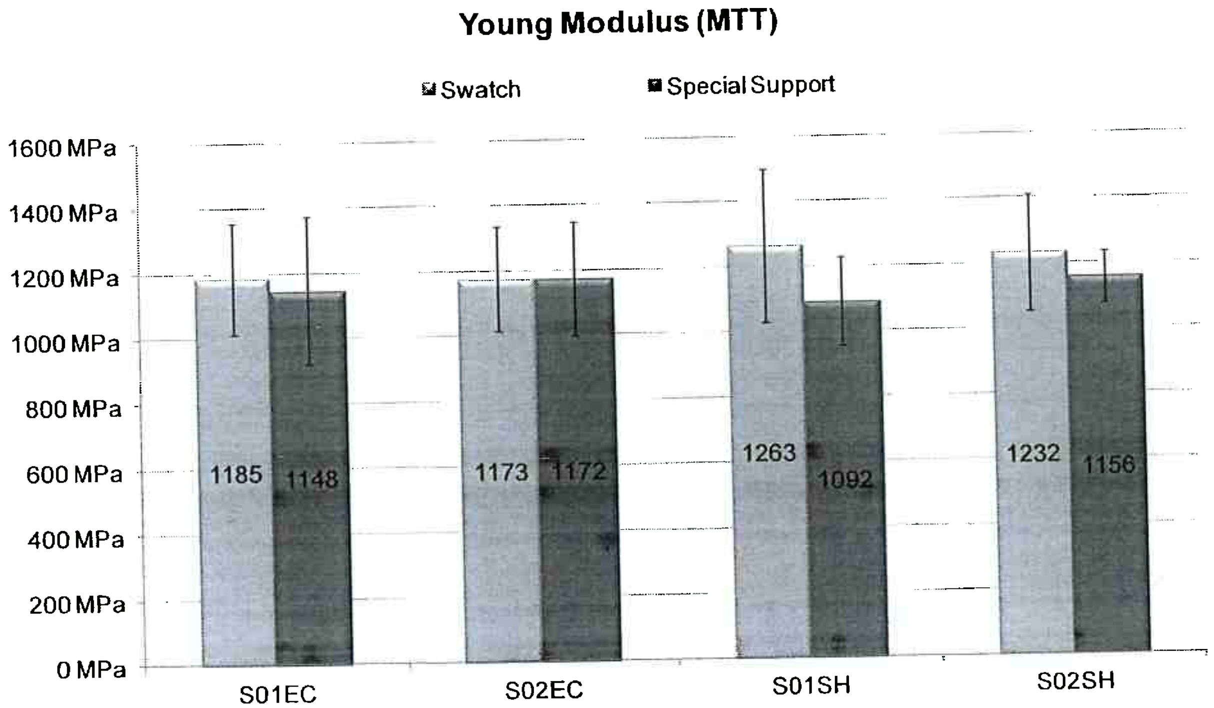


Graph 8: Impact of a terminal T5 in the Young Modulus of a T1+T2/T3 superposition

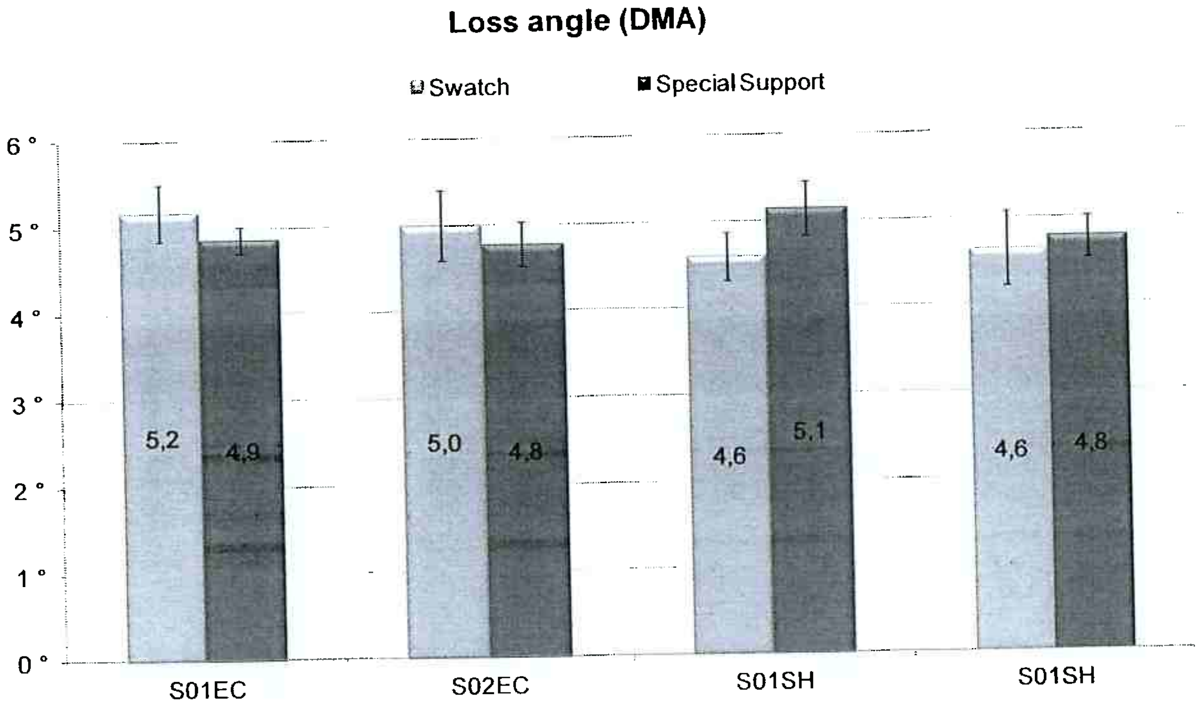


Graph 9: Impact of a terminal T5 in the Loss angle of a T1+T2/T3 superposition

Graph 10 and Graph 11 show the impact of T2/T3 application support (swatch or special support) in MTT and DMA parameters.



Graph 10: Impact of the application support in the Young Modulus of a T1+T2/T3 superposition



Graph 11: Impact of the application support in the Loss angle of a T1+T2/T3 superposition

4.2.3 Discussion

The MTT results showed no difference after the introduction of T4 between T1 and T2 or T3 (Graph 6). However, the DMA analysis (Graph 7) suggests that it would limit the damage to the hair, as a strong reduction of loss angle.

The introduction of a terminal T5 after the T1+T2 and T1+T3 superpositions did not significantly increase the level of degradation (Graph 8 and Graph 9).

The application of T2 and T3 in the special support did not much affect the mechanical properties of the hair (Graph 10 and Graph 11). However, there is a slight tendency for a drop in values, indicating that it is more aggressive.

4.2.4 Interpretation

Hair damage seems to be limited by T4 after T1, as shown by DMA results, so that procedure will now be maintained for all new superpositions (including those where the treatments are separated by T5). T1 residues probably combine themselves with T2 or T3 increasing the hair damage. Other mechanisms may also occur within the hair structure, for example a decrease in the scale separation or the acceleration of keratin recovery.

The introduction of T5 at the end of the sequence did not significantly increase hair damage. Additional literature searches were conducted to validate the consistency of this result. They confirmed that dark brown hair is quite resistant for T5 in the conditions it was applied.

Finally, we confirmed that T2/T3 application on special support would not be much more aggressive than the normal one on swatch. From here on, we will only compare sequences in which the treatments are performed on the same support.

Until now, there was no difference between T2 and T3 in MTT and DMA parameters (except for the loss angle of double superpositions). Consequently, it was decided to continue the study only with T2.

4.3 First results' validation

4.3.1 Objectives

Having a better understanding of the sources of hair damage after the complementary analysis, we wanted to validate the initial results with the sequences below:

Table 6: Sequences used to validate the first results

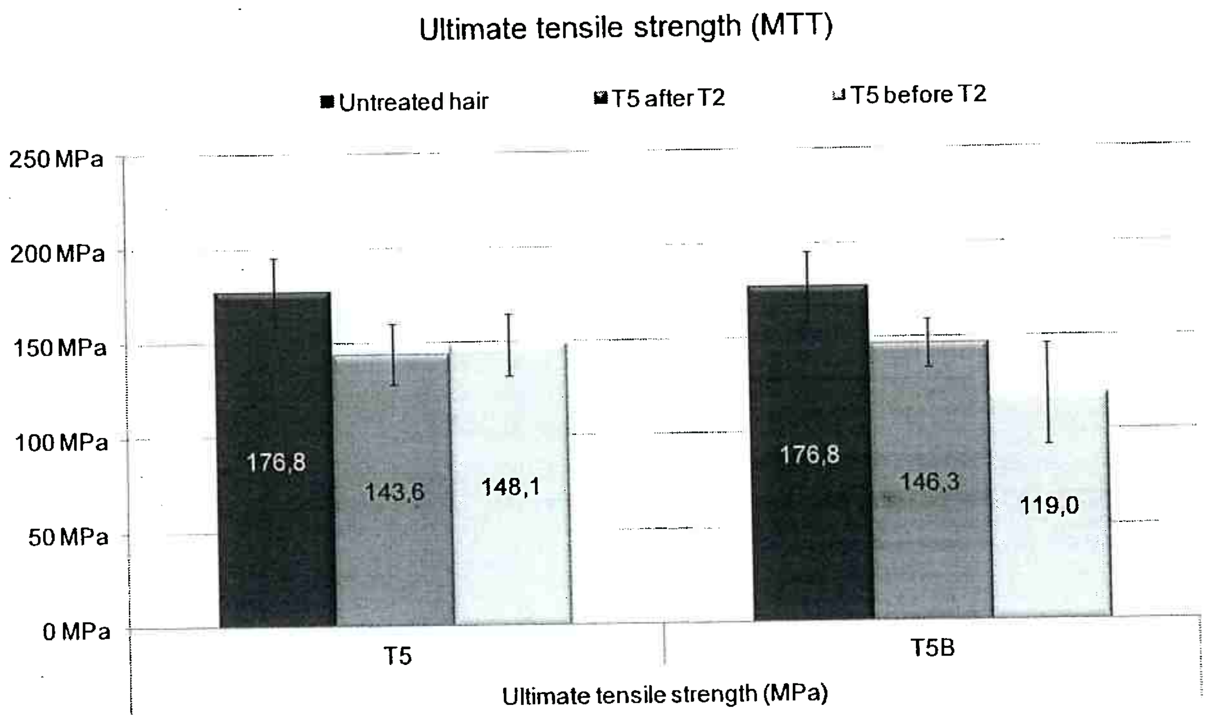
Sequence Code	Treatments sequence
Sequence 1	T1 → T4 → T2 → T5
Sequence 3	T1 → T4 → T5 → T2
Sequence 2	T1 → T4 → T2 → T5B
Sequence 4	T1 → T4 → T5B → T2

These are actually the initial sequences after the inclusion of the findings of the complementary tests. The sources of damage are now equivalent (T1 carried out on a swatch, T5 and T2 carried out on special supports) and T4 after T1 limits the combination between its residues and the following chemical treatments.

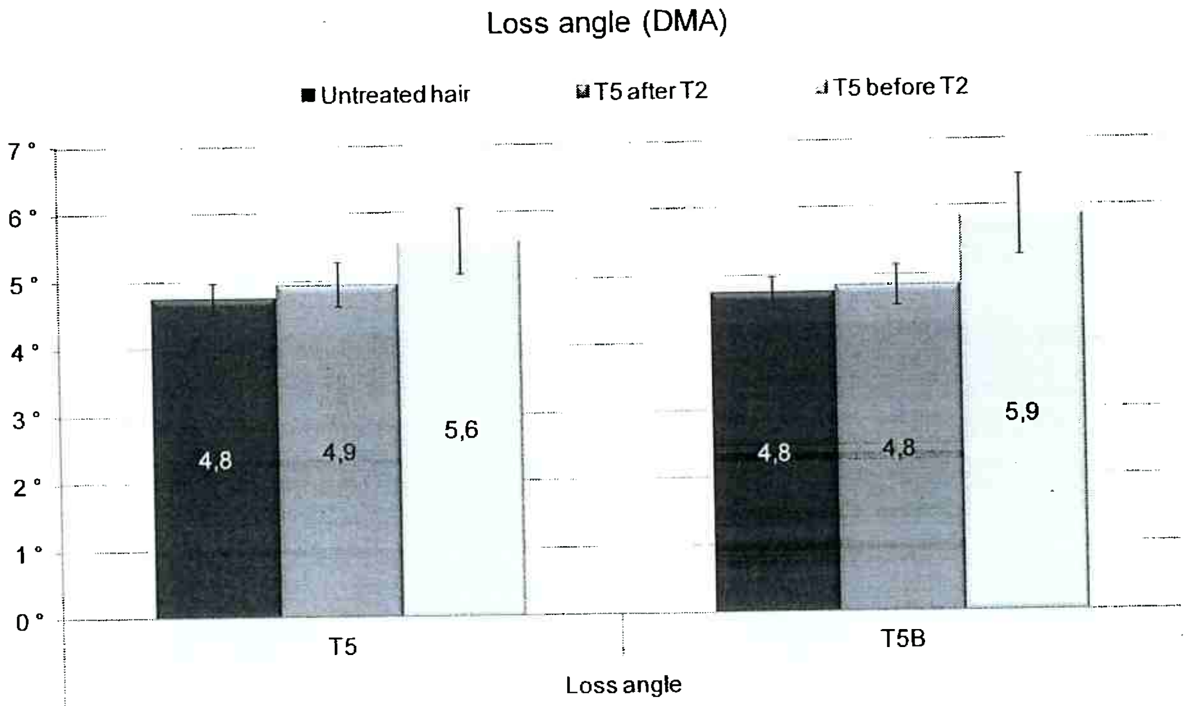
We also wanted to test the impact of changing the conditions of T5, adding sequences with the chemical treatment T5B.

4.3.2 Results

Graph 12 and Graph 13 show the impact of T5 position on the results of MTT and DMA analyses:

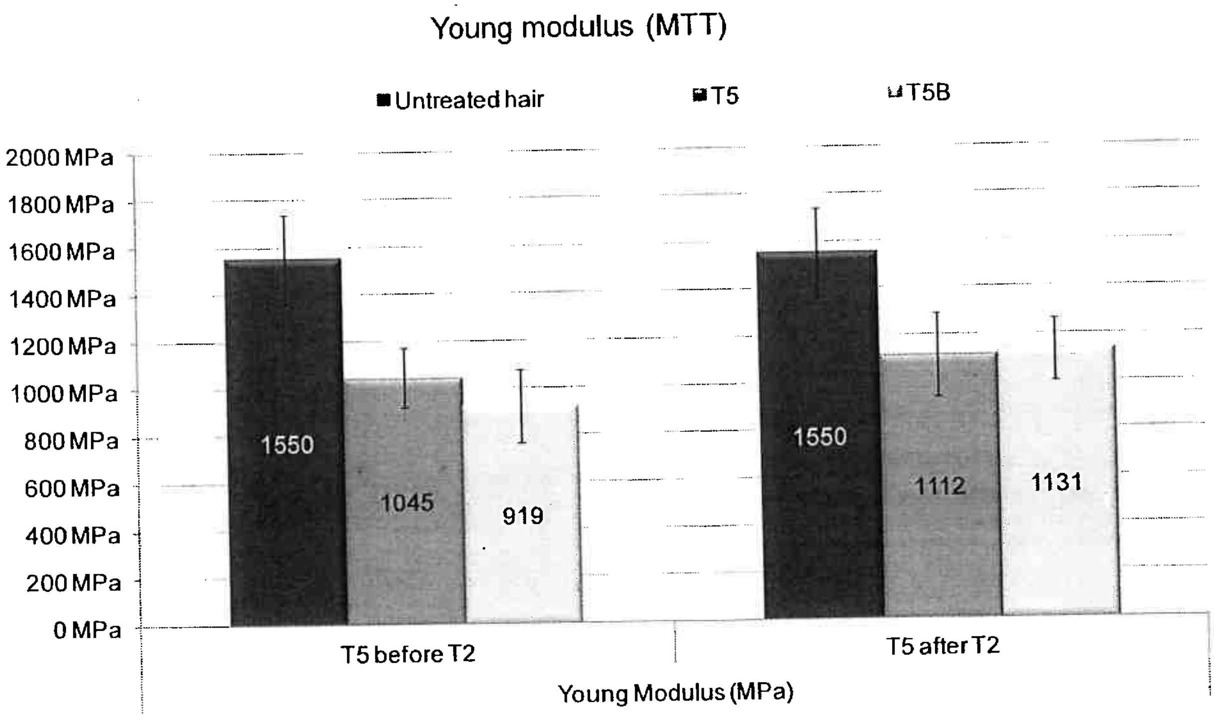


Graph 12: Impact of T5 position in the Ultimate Tensile Strength of a T1+T2 superposition

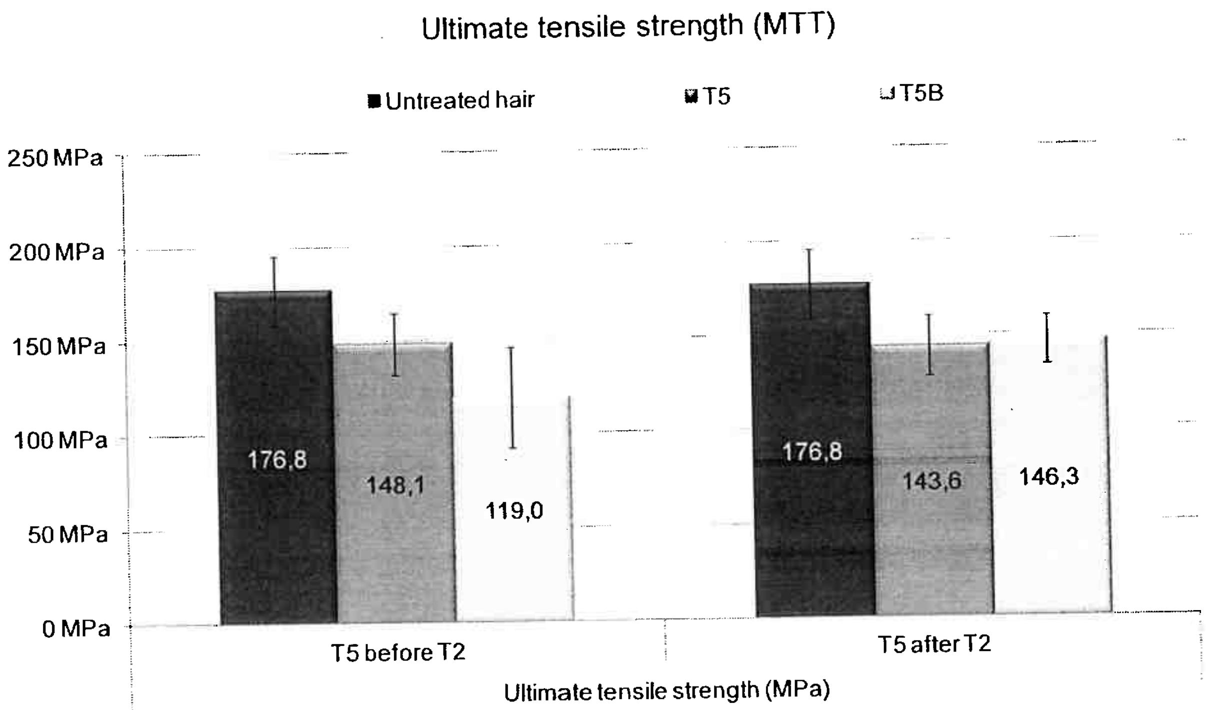


Graph 13: Impact of T5 position in the Loss angle of a T1+T2 superposition

Graph 14 and Graph 15 show the effect of substituting T5 for T5B on MTT results:



Graph 14: Impact of substituting T5 for T5B in the Young modulus of a T1+T2 superposition



Graph 15: Impact of substituting T5 for T5B in the Ultimate Tensile Strength of a T1+T2 superposition

4.3.3 Discussion

The MTT and DMA results show that:

A. Concerning the position of T5/T5B in the treatment sequence (Graph 12 and Graph 13): placing it before T2 is slightly more aggressive than if it is placed after, both for T5 and T5B. The difference is slightly higher for T5B.

B. Concerning the substitution of T5 for T5B (Graph 14 and Graph 15): when T5B is placed before T2, we do notice a damage increase compared to T5, which does not happen when it is placed after T2.

4.3.4 Interpretation

According to previous studies, T5/T5B effect is more significant on an already damaged fiber, so we might have expected that placing them at the end (Sequences 1 and 2) would be more aggressive to the hair. To verify this, it would be necessary to make mechanical tests before and after T5 and T5B.

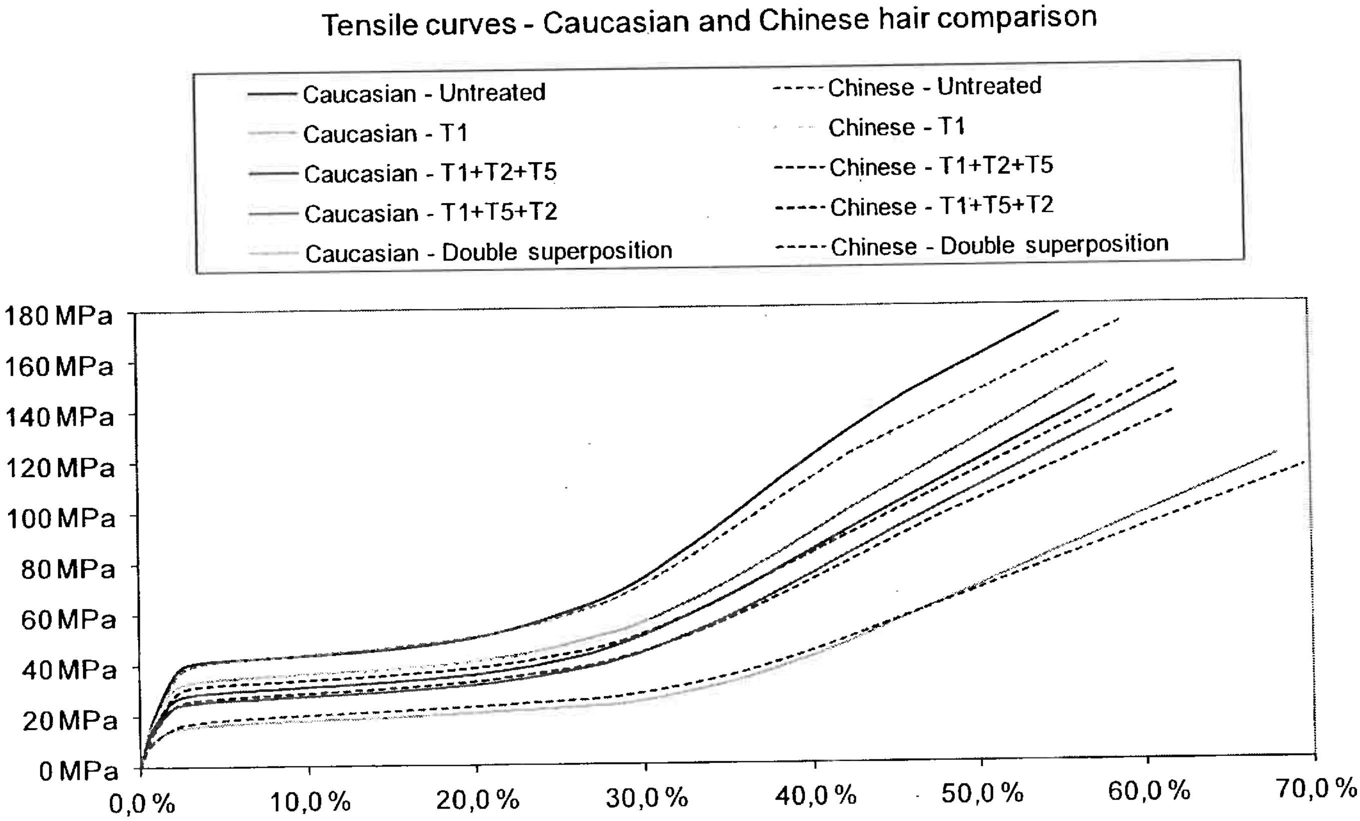
We actually noticed that sequences having T5/T5B after T2 (Sequences 1 and 2) were less aggressive than those having it between treatments (Sequences 3 and 4), especially when for T5B. Total damage is the sum of each treatment's damage; given that T1 is applied in the exactly same conditions for all sequences, it is likely that the difference is due T2. The Sequences 3 and 4 were applied on more sensitized hair than those of the Sequences 1 and 2, so the former resulted in a more extensive degradation. This difference might be quite large, which could explain the observation A.

4.4 Chinese hair analysis

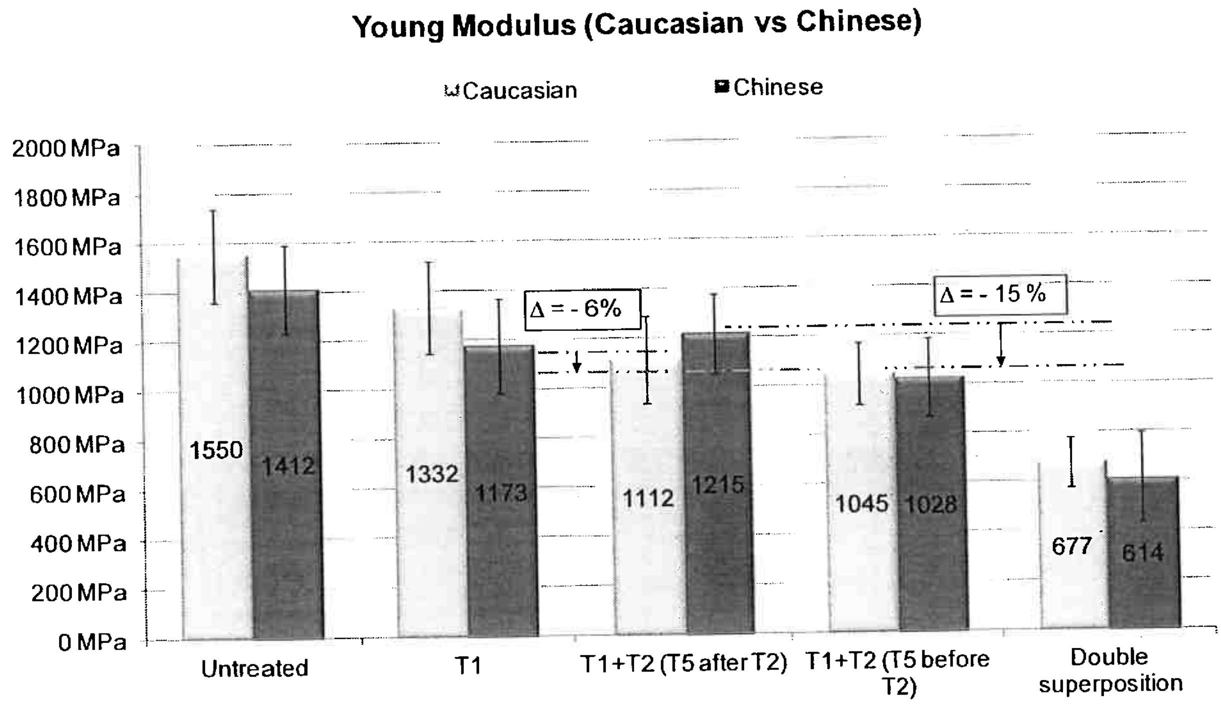
4.4.1 Objectives

To complete the study, we repeated Sequences 1 and 3 of the previous section (Table 6) and the double superpositions S31 of Table 4 in Chinese natural dark hair. We also analyzed untreated hair and T1 and T2 treated hair. The aim is to discover whether we will see similar behavior in a hair with different morphology. Chinese hair is for example less elliptical, what implies a diverse area distribution relative to the volume (which may change the absorption of compounds).

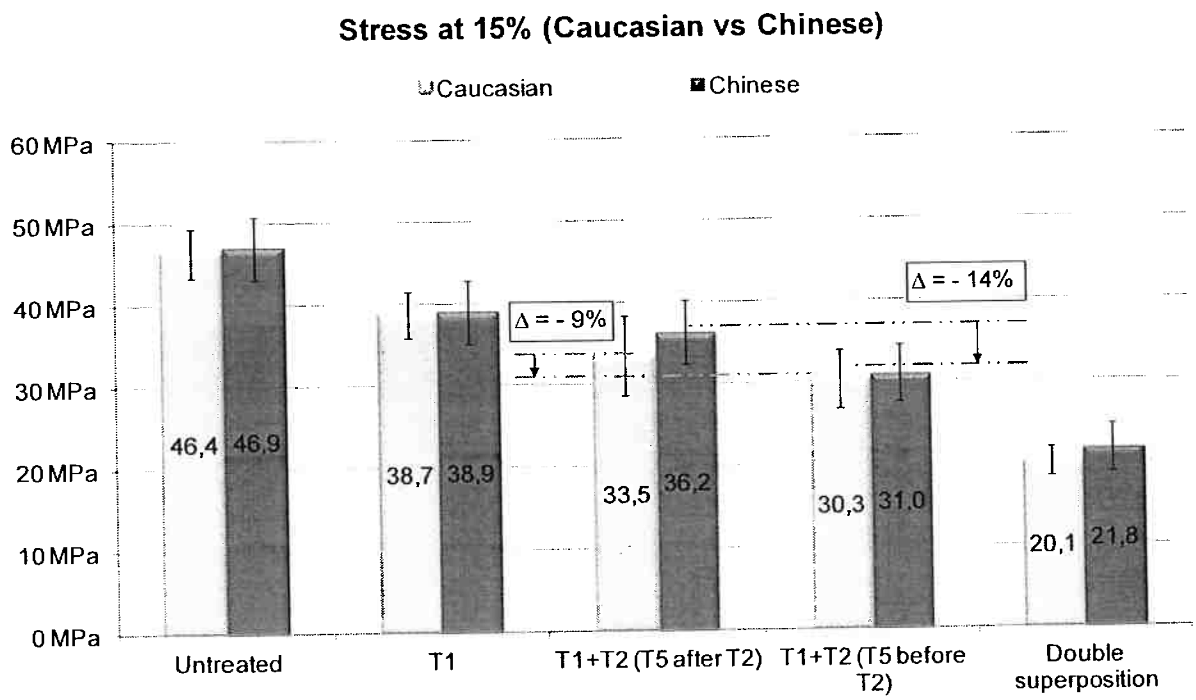
4.4.2 Results



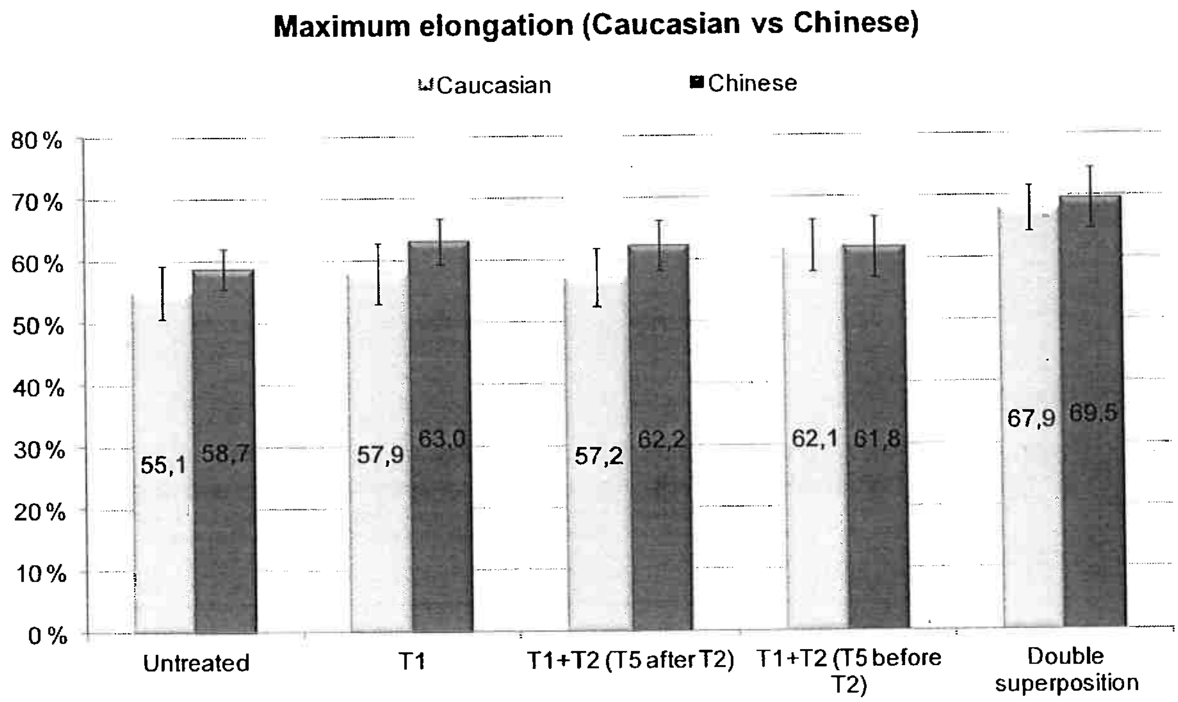
Graph 16: Tensile curves of different treatment combinations in Chinese (dotted) and Caucasian (continuous) hair



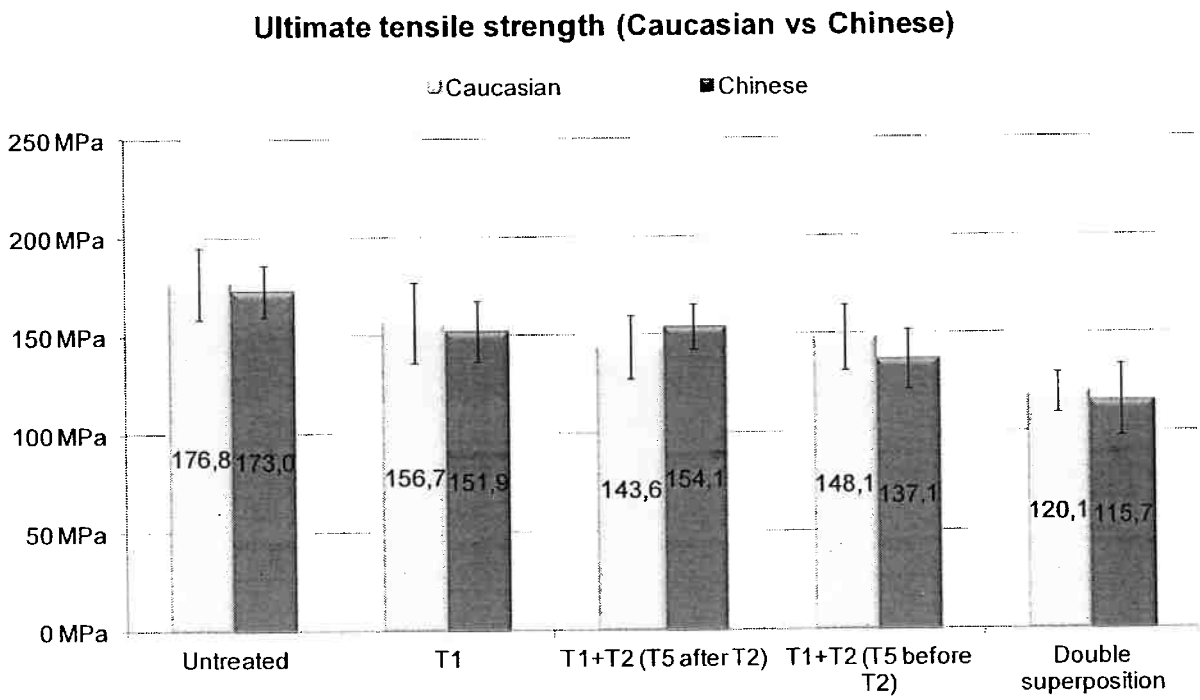
Graph 17: Young modulus of the two hair kinds and impact of T5 position



Graph 18: Stress at 15 % strain of the two hair kinds and impact of T5 position



Graph 19: Maximum elongation of the two hair kinds



Graph 20: Ultimate tensile strength of the two hair kinds

4.4.3 Discussion

The tensile behavior was similar for Chinese and Caucasian hair up to 25-30 % strain (Graph 16): we find close and parallel stress-strain curves. From 30 % strain on, the curves intersect and diverge; at higher deformations, Caucasian hair shows larger tensile strength, related to higher mechanical resistance.

It is not possible to say that the two hair types are different concerning the Young Modulus (Graph 17). Although for natural and T1 treated hair, Caucasian seems to be stronger, the opposite happens for the simple and double superpositions.

In the plastic region (from 5 % to about 20 % strain), the stress at 15 % does not allow neither the identification of a stronger candidate (Graph 18).

Concerning the tensile rupture, Chinese hair tends to deform to a slightly larger extent than Caucasian (Graph 19). On the other hand, the ultimate tensile strength is not necessarily different (Graph 20).

4.4.4 Interpretation

For the Chinese hair, placing T5 before T2 led again to more hair damage, and the difference (compared to placing it at the end) was bigger than for Caucasian hair (Graph 17 and Graph 18).

The tensile behavior of the two types of hair seems to differ only after high deformations. The Young Modulus suggests that the hydrogen bonds (whose rupture is responsible for deformations up to 5 %) are equally strong in both cases. Then the transformation of α-keratin in β starts at a similar point (as indicated by the stresses at 15 % and 25 % strain). However, this similarity gradually disappears as the fiber approaches the break. It is interesting to note that at this point, the deformation of Chinese hair is a little higher at a comparatively lower stress. The explanation could be based on how the macromolecules of keratin are arranged within the cortex's matrix.

4.5 HP-DSC Results

4.5.1 Objectives

HP-DSC was the first technique selected to complement the study of the hair's physical integrity. It allows the calculation of keratin denaturation temperature and enthalpy. This is clearly a different approach of evaluating hair integrity, as MTT and DMA are both based on fibers' response to mechanical stress. If the results of HP-

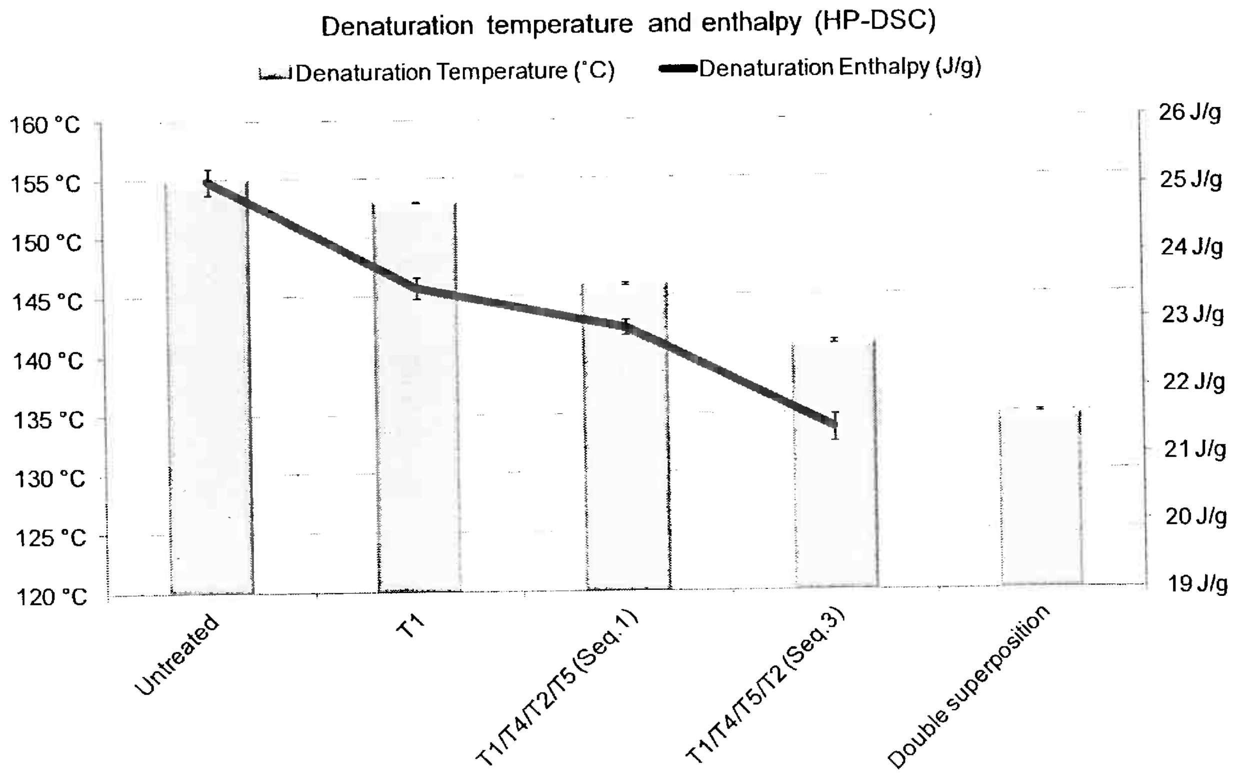
DSC go in the same direction of those of MTT and DMA, we would have a good indicator of the validity of the results.

The analyses were done on Caucasian natural dark brown hair (the same as for most tests), and with the following sequences:

a) Untreated and T1 treated hair (T00, Table 4)
b) T1+T4+T5+T2 (Sequence 3, Table 6)
c) T1+T4+T2+T5 (Sequence 1, Table 6)
d) Double Superposition (S31, Table 4)

4.5.2 Results

The chart below shows the results for the denaturation temperature of the sequences. Lower temperatures are related to a more damaged hair. The sequence having T5 before T2 (b) is once again more aggressive than the one having it after T2 (c), but the difference is much more significant now (Graph 21).



Graph 21: Denaturation temperature and enthalpy for each tested chemical treatment or sequence

4.5.3 Discussion and Interpretation

The keratin helices in better condition were those of natural hair, closely followed by those of T1 treated hair (Graph 21).

The fibers of the superposition having T5 after T2 are more difficult to denature (temperature of 146 °C) than those with T5 before T2 (141 °C), reflecting a more resistant structure at the molecular level (intermolecular bonds more difficult to break).

The analysis of the enthalpy of the transformation (Graph 21) shows the same tendency, except for the double superposition. The enthalpies are ordered exactly in the same way (from more to less damaged hair), but the differences between two consecutive elements are smaller. The value of the double superposition enthalpy was not established due to an insufficient sample size (not enough fibers available to test). Although, the calculation of the area under the curves (enthalpies) is not as precise as the determination of the temperatures (as it depends on a good establishment of the baseline), so the conclusions were preferably made on the temperature results.

4.6 SEM Results

4.6.1 Objectives

The SEM was selected to analyze the surface conditions after the superposition. We were interested in knowing if the cuticle degradation would also be in agreement with the cortex results (MTT+DMA+HP-DSC). The fibers having T5 of Table 6 (Sequences 1 and 3) were observed.

4.6.2 Results

Image 18 shows the most representative photo (from a total of 10) of the cuticle condition for Sequence 3 and Sequence 1, as well as a photo of a untreated healthy hair.

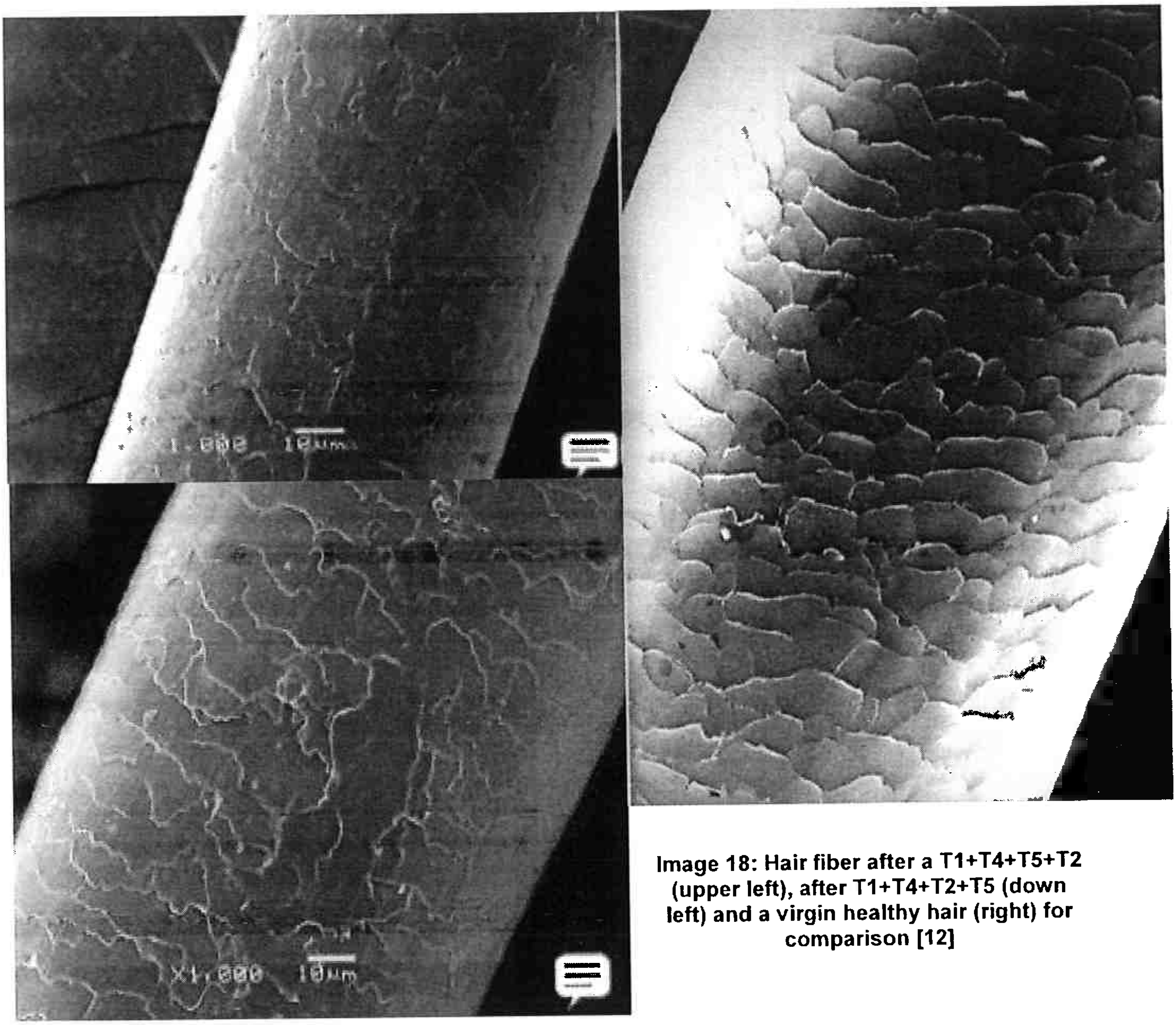


Image 18: Hair fiber after a T1+T4+T5+T2 (upper left), after T1+T4+T2+T5 (down left) and a virgin healthy hair (right) for comparison [12]

4.6.3 Discussion and Interpretation

The pictures in Image 18 confirm that both fibers were also externally damaged.

- The distances between the scales are irregular
- The scales' edges are broken, raised and separated
- The cortex is exposed

However, there is no significant difference between the sequences. This is demonstrated by equal degradation levels (2.5 in a scale of 5 for both sequences). Indeed, if we take a look at each group of 10 images, we notice a big heterogeneity which makes the comparison quite subjective. However the representative chosen pictures (Image 18) indicates that T5 causes a similar damage if placed before or after T2.

4.7 Evaluation and comparison of hair samples from volunteers' heads

4.7.1 Objectives

The analysis of real people's hair is very important to complement any study based on swatches, especially when it leads to products at the end of the development process. The treatment sequences and conditions must be, as far as possible, reproduced on volunteers. This exposes the limitation of this type of analysis: it is extremely hard to find a large group of people with the same hair conditions, and even if we do find it is unlikely they are exposed to the environmental conditions (especially over a large period of time); samples cannot be taken with the same regularity as in laboratory, and they are frequently too small to allow several tests (sometimes the fibers are even too short for some techniques). Finally, people are not necessarily available or willing to participate in these studies, fearing that their hair will be permanently damaged (which is not true).

Verification of similar behaviors would provide strong validation of the results and allow the continuation of the study with swatches, which is much less limiting than involving real people. If there are differences between the results, it is advisable to stop the procedures for a moment and determine the possible sources of the discrepancy.

For this study, it would have been ideal for our comparison to have models having received T1+T2 and also with T1+T5+T2. Unfortunately, only volunteers having received the same sequence were available.

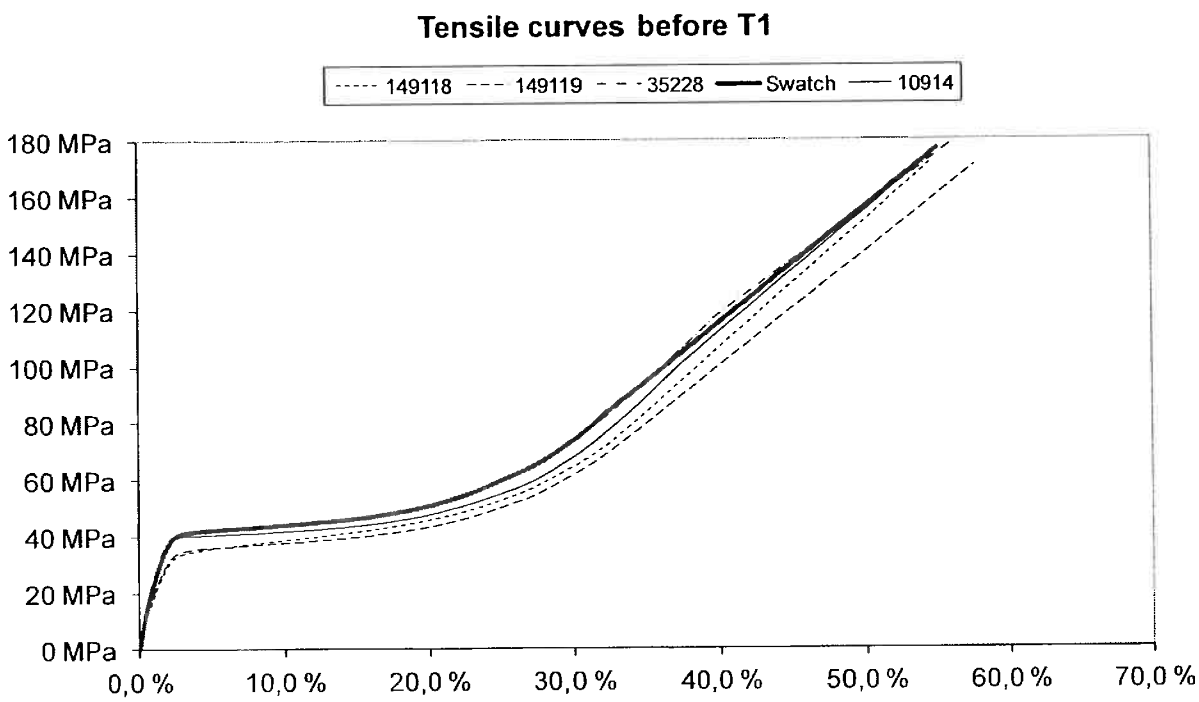
An MTT study on the hair from four volunteers having undergone T2 and T1 was carried out under wet conditions. Table 7 shows the details of the applications. There are differences with respect to swatch applications: chemical treatments similar to T1 but not identical were used. The application conditions for T2 were also slightly different. These were probably the adaptations the hairdresser had to make in order to customize the treatments based on the hairdresser's evaluation of the initial physical integrity of each volunteer's hair.

Table 7: Details of the applications on each volunteer's hair and on swatch

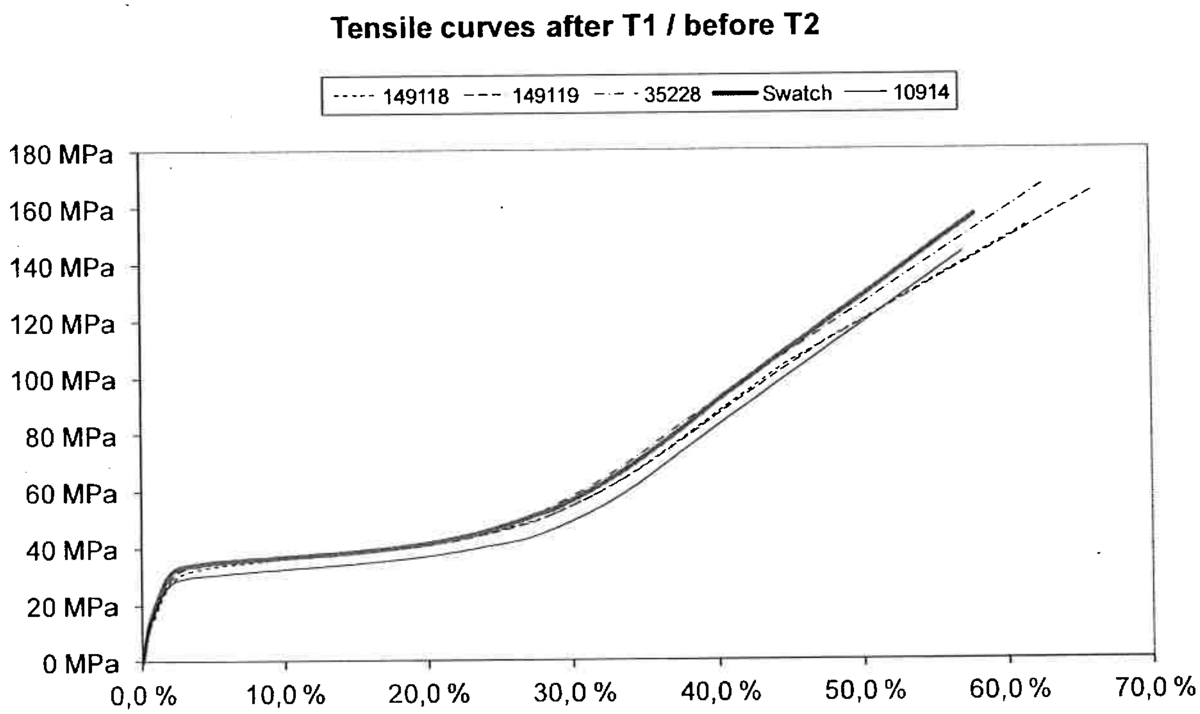
Volunteer	*Initial hair condition*	*Chemical Treatment*	*Chemical Treatment*
Swatch	untreated	T1	T2
149118	treated	Very Similar to T1	Very Similar to T2
149119	untreated	Similar to T1	Very Similar to T2
35228	treated	Very Similar to T1	Similar to T2
10914	treated	Very Similar to T1	Similar to T2

4.7.2 Results

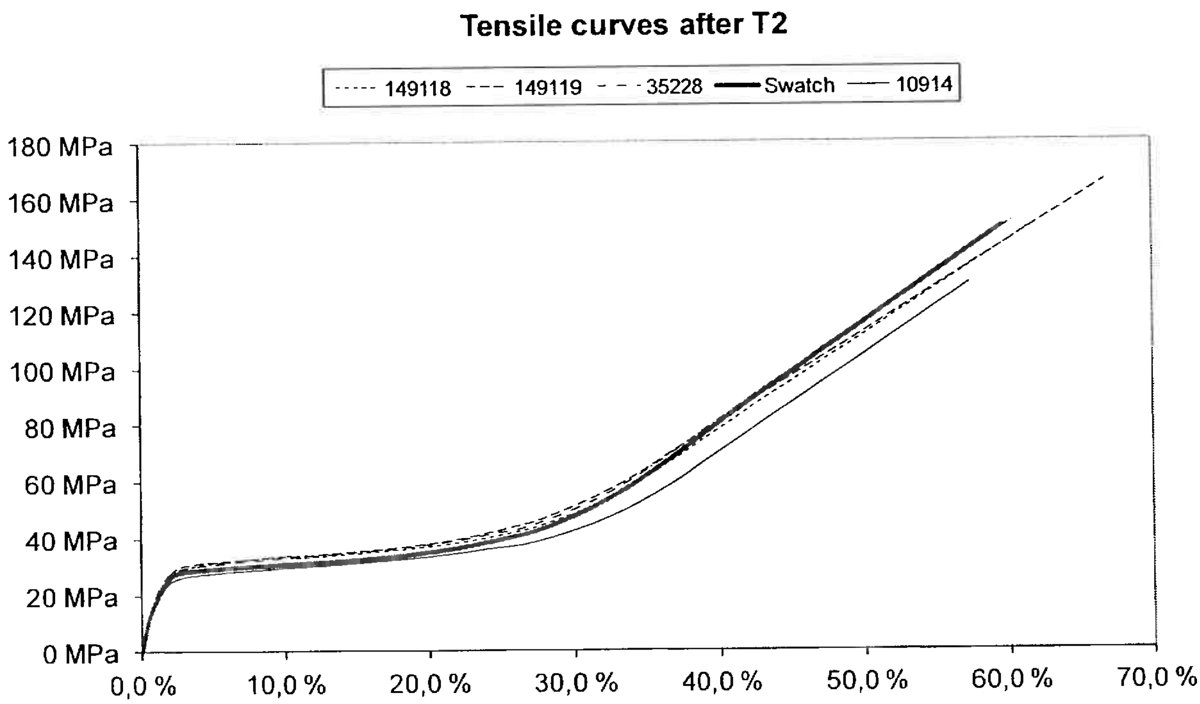
The first three graphs show the tensile curves of the hair originating from each volunteer and of the swatch, at three stages: initial state (before T1), just after T1 application and after T2 application. Next, we show the absolute and the relative (the parameter divided by its initial value) variation of one parameter of the elastic phase (Young Modulus), one of the plastic phase (stress at 15 % strain) and one of the post-plastic phase (ultimate tensile strength) at the same stage.



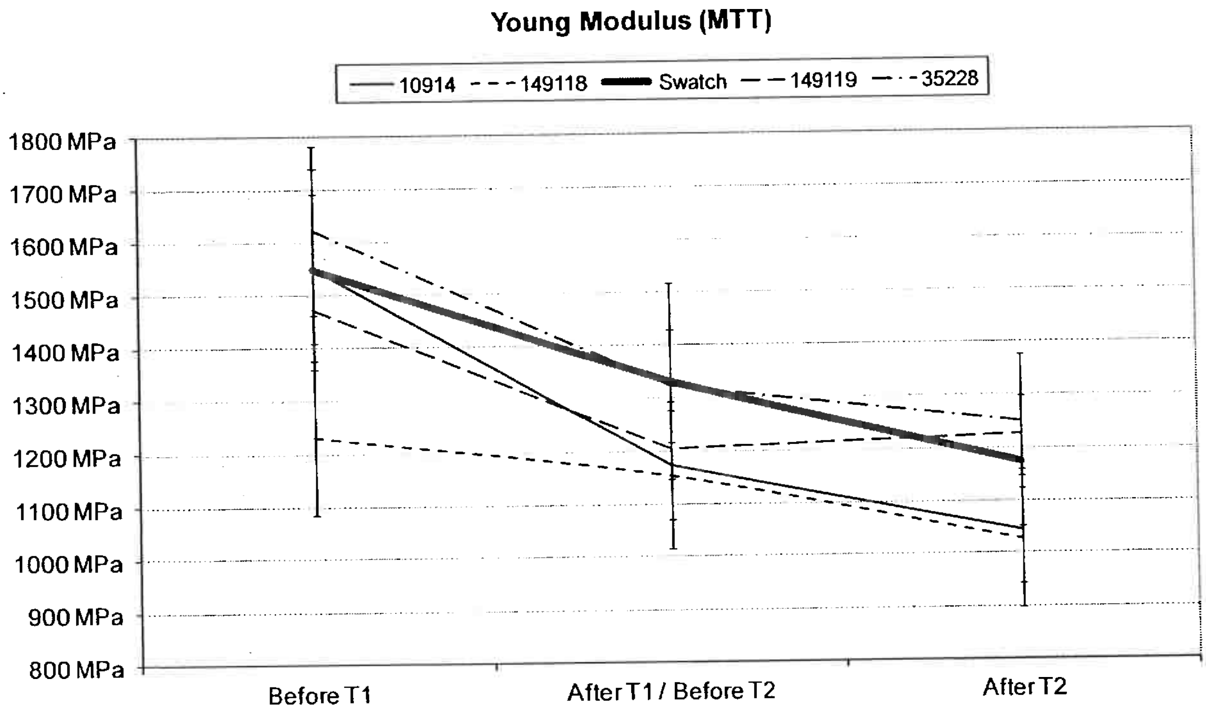
Graph 22: Tensile curves before T1 application



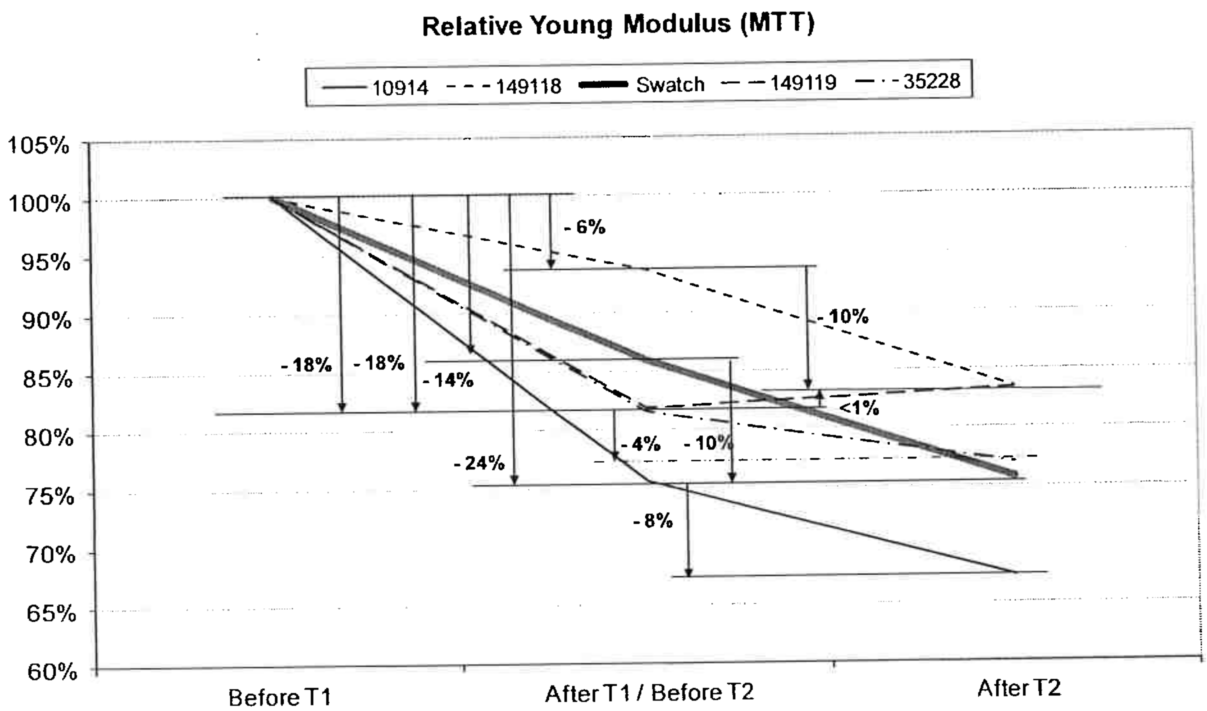
Graph 23: Tensile curves after T1 application and before T2



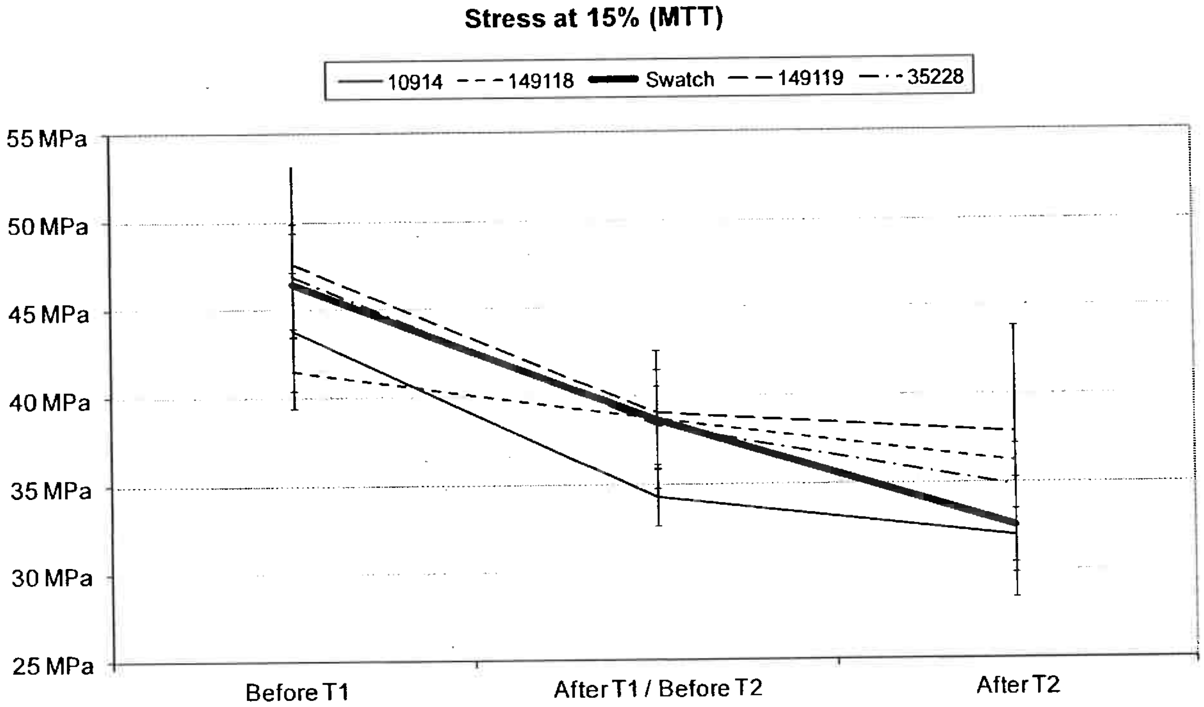
Graph 24: Tensile curves after T2 application



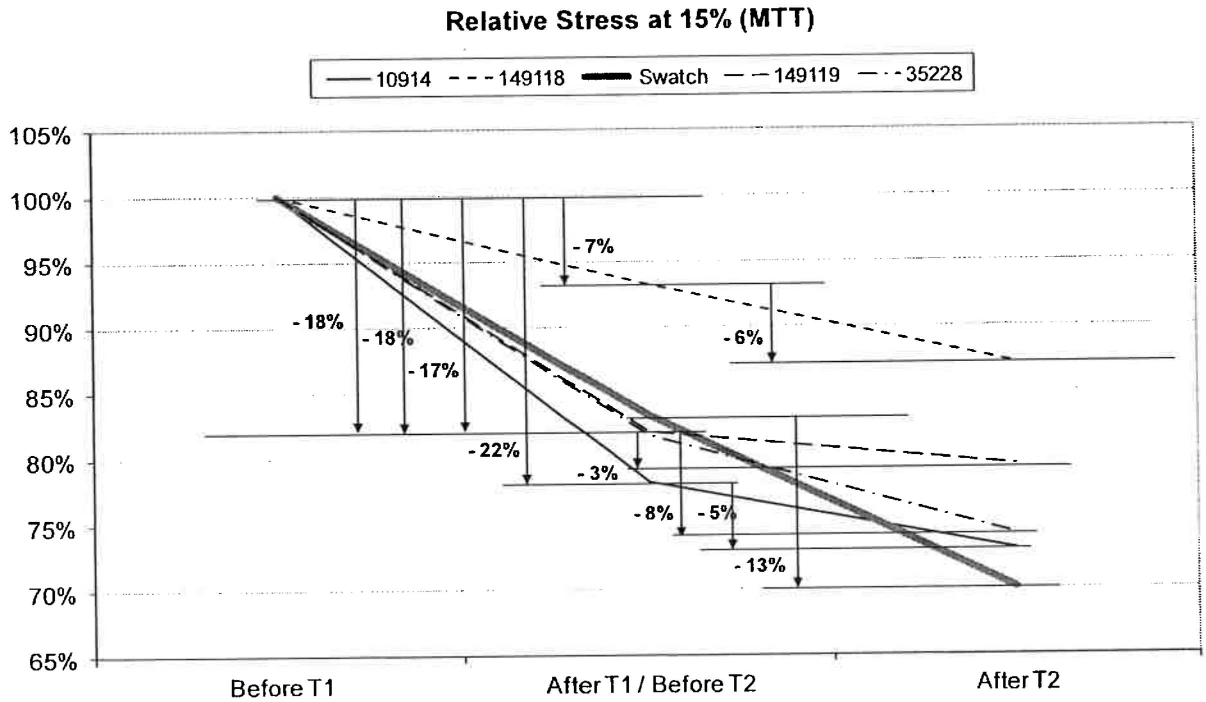
Graph 25: Young modulus absolute value evolution with treatments' applications



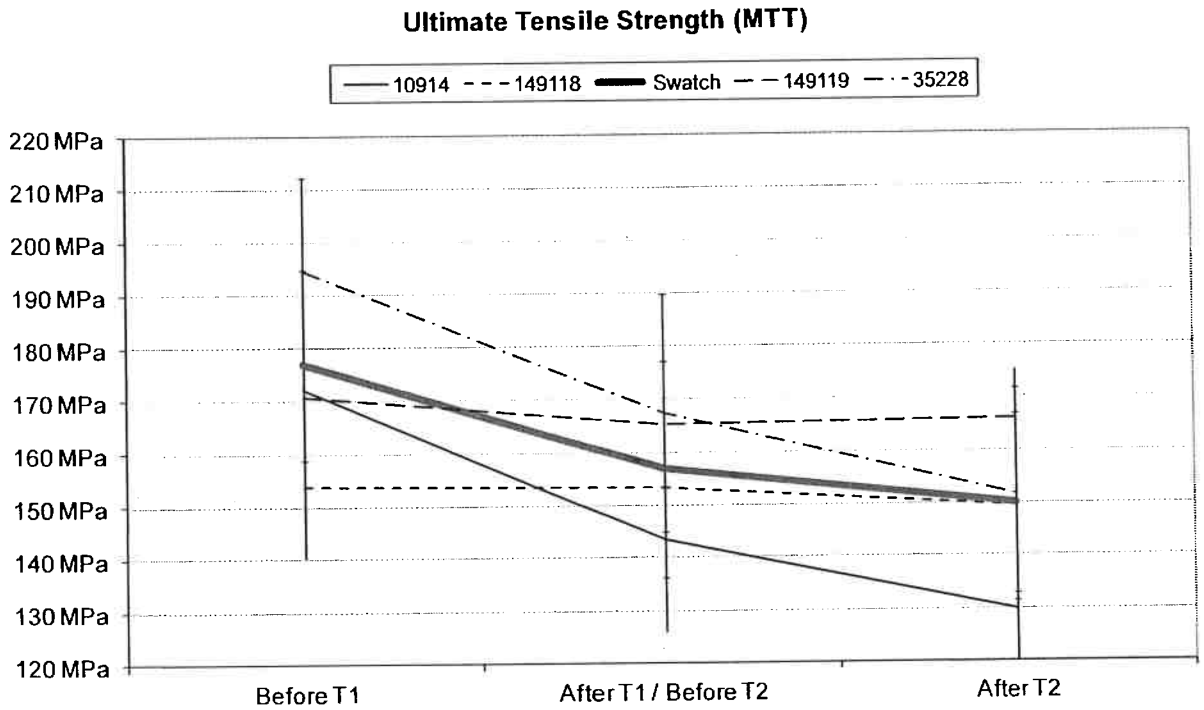
Graph 26: Young modulus relative value evolution with treatments' applications



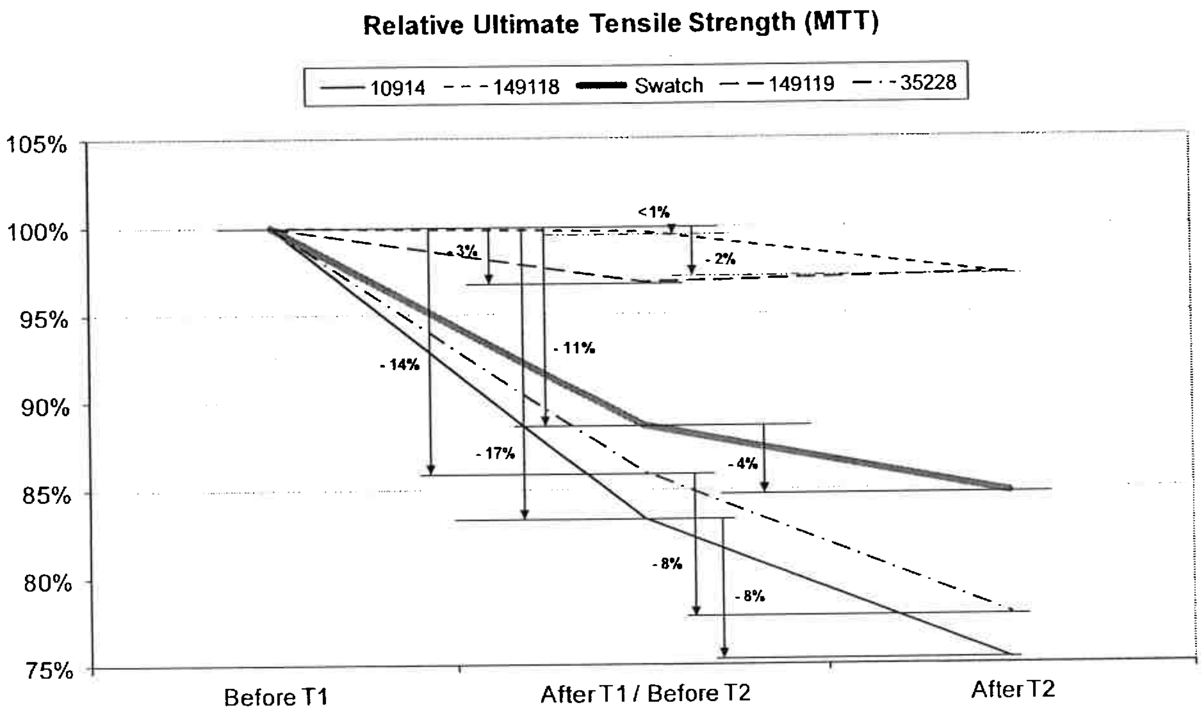
Graph 27: Stress at 15 % strain absolute value evolution with treatments' applications



Graph 28: Stress at 15 % strain relative value evolution with treatments' applications



Graph 29: Ultimate tensile strength absolute value evolution with treatments' applications



Graph 30: Ultimate tensile strength relative value evolution with treatments' applications

4.7.3 Discussion and Interpretation

Graph 22 (hair's initial condition) shows a similar behavior among the volunteers' hair and the swatches. However, the curves are not very close as the initial state of the fibers originated from the volunteers was already sensitized, while the swatches were untreated. The curves are parallel and maintain a constant small difference up to 35 % strain, from that point on, they get slightly separated.

After T1 application, we present an approximation of the curves (Graph 23); the parallelism gives place to a superposed condition up to 35 % strain (except for 10914). Again from this point on, they diverged continuously, and the volunteers' hair end up by breaking up at larger deformations (except again for 10914).

After T2 (Graph 24), the curves are similar throughout the entire deformation process. However, model 10914 presents lower strength from 30 % strain on and model 149119 has the deformation at break larger than the others.

Analyses of the individual parameters show similar results. In the elastic region, this is emphasized by the evolution of the relative Young modulus (Graph 26). T1 damages more than T2, as we had already remarked. The swatch and three volunteers show similar decreases (-14 %, -18 %, -18 % and -24 %), only 149118 is away from the trend. This is probably due an already fragilized initial state, which required a very weak treatment in order not to destroy the fibers.

There are similar variations in plastic phase (shown by the stress at 15 % strain, Graph 28) and post-Plastic (showed by the ultimate tensile strength in Graph 30). Especially for the stress at 15 %, four out of five curves (swatch, 149119, 35228 and 10914) are almost superposed (Graph 27) and keep the parallelism along the treatments. For the ultimate tensile strength (Graph 29), we note the proximity especially with three curves (swatch, 35228 and 10914).

MTT results give good indications that the behavior of swatch hair and samples from volunteers is quite close. Obviously, since we have studied only four people's hair, it is important to broaden the sample population or trying to find volunteers with similar initial hair conditions.

It is important to extend the study to volunteers having passed a T1+T5+T2 sequence. This would validate the other half of the swatch results.

5 Conclusions

In this study, we analyzed the impact of chemical treatments combination on the physical integrity of the hair, both internally (cortex) and externally (cuticle). The main evaluation techniques used were MTT and DMA, which were complemented by HP-DSC and SEM. Caucasian dark brown natural hair was the basis of the study, but some sequences were also applied on Chinese dark natural hair. The tested products were the commercially available cosmetic treatments T1, T2 and T3. However, during the study, the results observed for T2 and T3 were always similar, thus only T2 was thoroughly tested. Two complementary procedures, T4 and T5, were applied in between the treatments in order to approach the sequences to real life's damage.

The MTT and DMA results showed that the damage due to each single treatment depends on the immediate previous history of the hair. For example, mechanical results suggest that a superposition of T1 and T2 with an intermediate T5 step does not reduce the total damage. Surface analysis show a similar trend and HP-DSC results even indicate that making those two treatments in sequence (without T5) would reduce sensitization of the hair (to be confirmed by statistical tests). It is very interesting to note that the same sequences of treatments on the Chinese hair gave the same conclusions regarding the tensile tests.

The final part of the study deals with the difference between the results from controlled experiments on hair swatches and volunteer hair samples tests. Fortunately, we are able to confirm the similarity for the superposition T1+T2. The complete validation would also require the confirmation for volunteer hair samples treated with a T1+T5+T2.

Other interesting findings have emerged. For example, we noticed a possible beneficial effect of the introduction of T4 between T1 and T2. Several hypotheses are put forward. Even if it would be necessary to confirm one of those, the remarkable results showed by DMA might motivate the inclusion of T4 in a real life superposition protocol.

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7 Appendix: raw data

The complete results from the tensile tests and DMA analysis are shown below (all the parameters are represented). "CV" corresponds to the Coefficient of Variation, the ratio between the standard deviation and the mean.

7.1 MTT

Table 8: Complete results of MTT at dry condition

		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
Untreated Hair	Mean	4136	3497	125	140	44,9	213
	Standard deviation	1036	491	6	7	6,0	18
	CV	25%	14%	5%	5%	13%	8%
T00 (T1)	Mean	4233	3466	118	131	47,9	206
	Standard deviation	1026	331	6	7	4,8	15
	CV	24%	10%	5%	5%	10%	7%
T01 (T2)	Mean	3963	3641	125	138	45,1	210
	Standard deviation	1058	324	8	9	4,4	20
	CV	27%	9%	7%	6%	10%	10%
T02 (T3)	Mean	4009	3491	124	136	45,8	212
	Standard deviation	1061	370	5	6	4,6	18
	CV	26%	11%	4%	4%	10%	9%
S01 (T1+T2)	Mean	4474	3572	117	125	49,4	198
	Standard deviation	1105	310	4	5	4,9	15
	CV	25%	9%	3%	4%	10%	7%
S02 (T1+T3)	Mean	4084	3528	116	127	49,0	198
	Standard deviation	1097	348	6	8	5,9	16
	CV	27%	10%	5%	6%	12%	8%

Table 9: MTT results at wet conditions for the first tested sequences

		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
T00 (T1)	Mean	4195	1332	39	47	57,9	157
	Standard deviation	1271	185	3	4	4,9	20
	CV	30%	14%	7%	8%	9%	13%
T01 (T2)	Mean	4282	1477	43	56	55,7	168
	Standard deviation	1171	197	4	6	4,2	18
	CV	27%	13%	9%	10%	7%	11%
T02 (T3)	Mean	4440	1513	43	55	54,7	169
	Standard deviation	1396	145	3	4	4,7	17
	CV	31%	10%	7%	8%	9%	10%
S01 (T1+T2)	Mean	4420	1174	33	39	59,6	150
	Standard deviation	1359	123	3	3	4,5	17
	CV	31%	10%	8%	8%	7%	11%
S02 (T1+T3)	Mean	4628	1220	34	42	61,9	153
	Standard deviation	1010	161	3	4	6,8	17
	CV	22%	13%	9%	10%	11%	11%
S11 (T1+T5+T2)	Mean	4826	993	30	37	56,1	132
	Standard deviation	1109	117	2	3	7,1	25
	CV	23%	12%	7%	8%	13%	19%
S12 (T1+T5+T3)	Mean	4689	1060	32	39	59,6	150
	Standard deviation	1342	114	2	3	4,6	21
	CV	29%	11%	7%	8%	8%	14%
S31 (double superposition)	Mean	5005	677	20	23	67,9	120
	Standard deviation	1417	98	2	2	3,7	10
	CV	28%	14%	9%	9%	5%	8%
S32 (double superposition)	Mean	4948	766	22	26	63,5	119
	Standard deviation	979	142	2	3	6,9	21
	CV	20%	19%	9%	10%	11%	18%

Table 10: MTT results at wet conditions for the complementary sequences

		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
S01SH (T1+T4+T2)	Mean	4857	1263	35	43	60,7	160
	Standard deviation	1329	235	3	6	5,5	13
	CV	27%	19%	8%	15%	9%	8%
S02SH (T1+T4+T3)	Mean	4529	1232	35	43	60,2	154
	Standard deviation	1151	177	4	4	6,2	24
	CV	25%	14%	10%	10%	10%	15%
S01SHPE (T1+T4+T2)	Mean	4812	1092	32	39	59,5	148
	Standard deviation	1308	136	3	4	5,4	20
	CV	27%	12%	10%	9%	9%	14%
S02SHPE (T1+T4+T3)	Mean	5098	1156	34	42	59,5	154
	Standard deviation	1397	80	3	3	4,0	17
	CV	27%	7%	8%	8%	7%	11%
S01EC (T1+T2+T5)	Mean	4858	1185	33	41	59,5	153
	Standard deviation	1406	170	3	4	3,7	12
	CV	29%	14%	9%	9%	6%	8%
S02EC (T1+T3+T5)	Mean	4863	1173	34	42	58,3	150
	Standard deviation	2184	161	5	6	5,2	20
	CV	45%	14%	15%	14%	9%	13%
S01ECPE (T1+T2+T5)	Mean	4615	1148	33	40	56,4	146
	Standard deviation	1373	227	4	4	6,8	22
	CV	30%	20%	12%	10%	12%	15%
S02ECPE (T1+T3+T5)	Mean	5030	1172	35	44	57,1	149
	Standard deviation	1541	174	4	5	5,1	21
	CV	31%	15%	10%	11%	9%	14%

Table 11 : MTT results at wet conditions for the validation sequences

		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
Sequence 1 - T1+T4+T2+T5	Mean	4604	1112	34	41	57,2	144
	Standard deviation	1316	175	5	5	4,6	16
	CV	29%	16%	15%	13%	8%	11%
Sequence 2 - T1+T4+T2+T5B	Mean	4711	1131	32	41	59,1	146
	Standard deviation	1342	133	3	4	4,0	13
	CV	28%	12%	8%	10%	7%	9%
Sequence 3 - T1+T4+T5+T2	Mean	4437	1045	30	37	62,1	148
	Standard deviation	977	127	4	4	4,2	17
	CV	22%	12%	12%	11%	7%	11%
Sequence 4 - T1+T4+T5B+T2	Mean	4653	919	26	32	57,0	119
	Standard deviation	1355	154	2	3	15,6	27
	CV	29%	17%	10%	10%	27%	23%

Table 12: MTT results at wet conditions for the models' hair

Sample		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
149118 - before T1	Mean	6619	1231	41	52	56,5	154
	Standard Deviation	1123	146	2	3	3,9	14
	CV	17%	12%	5%	5%	7%	9%
149118 - after T1	Mean	6959	1154	39	46	61,7	153
	Standard Deviation	1136	139	4	4	4,7	14
	CV	16%	12%	10%	10%	8%	9%
149118 - after T1 and T2	Mean	6682	1028	36	43	61,4	149
	Standard Deviation	1333	130	8	8	5,3	19
	CV	20%	13%	21%	19%	9%	13%

Sample		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
149119 - before T1	Mean	4878	1472	48	59	57,7	171
	Standard Deviation	728	156	6	6	6,1	20
	CV	15%	11%	12%	11%	11%	12%
149119 - after T1	Mean	4981	1204	39	47	66,0	165
	Standard Deviation	702	96	1	2	4,8	5
	CV	14%	8%	3%	4%	7%	3%
149119 - after T1 and T2	Mean	4653	1226	38	46	66,7	166
	Standard Deviation	1131	122	2	2	4,9	9
	CV	24%	10%	4%	5%	7%	6%

Sample		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
35228 - before T1	Mean	3613	1623	47	60	60,4	195
	Standard Deviation	662	160	3	4	5,4	18
	CV	18%	10%	6%	7%	9%	9%
35228 - after T2	Mean	4416	1323	38	47	62,6	167
	Standard Deviation	710	106	2	3	5,7	23
	CV	16%	8%	6%	6%	9%	13%
35228 - after T1 and T2	Mean	4048	1250	35	42	60,3	152
	Standard Deviation	649	127	2	4	6,4	20
	CV	16%	10%	7%	9%	11%	13%

Sample		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
10914 - before T1	Mean	3809	1551	44	55	54,7	172
	Standard Deviation	776	141	3	4	5,0	22
	CV	20%	9%	8%	8%	9%	13%
10914 - after T1	Mean	3992	1173	34	41	57,0	144
	Standard Deviation	627	103	2	2	5,0	18
	CV	16%	9%	5%	5%	9%	12%
10914 - after T1 and T2	Mean	3860	1044	32	37	57,3	130
	Standard Deviation	559	101	2	2	5,7	22
	CV	14%	10%	5%	6%	10%	17%

Sample		Cross section (µm²)	Elastic modulus (MPa)	Stress at 15% (MPa)	Stress at 25% (MPa)	Break extension (%)	Break stress (MPa)
swatch - before T1	Mean	4907	1550	46	59	55,1	177
	Standard Deviation	1295	190	3	4	4,3	18
	CV	26%	12%	6%	7%	8%	10%
swatch - after T1	Mean	4195	1332	39	47	57,9	157
	Standard Deviation	1271	185	3	4	4,9	20
	CV	30%	14%	7%	8%	9%	13%
swatch - after T1 and T2	Mean	4420	1174	33	39	59,6	150
	Standard Deviation	1359	123	3	3	4,5	17
	CV	31%	10%	8%	8%	7%	11%

7.2 DMA

Table 13: DMA results for the first tested sequences

		Complex Modulus (MPa)		Storage Modulus (MPa)		Loss Modulus (MPa)		Loss Angle (°)	
		dry	humid	dry	humid	dry	humid	dry	humid
T00 (T1)	Mean	3796,4	1638,8	3792,1	1633,1	179,4	136,7	2,7	4,8
	Standard Deviation	277,4	146,3	277,7	146,2	10,6	8,8	0,2	0,3
	CV	7%	9%	7%	9%	6%	6%	9%	5%
T01 (T2)	Mean	4174,5	1620,9	4171,5	1615,6	159,2	129,7	2,2	4,6
	Standard Deviation	670,8	339,5	670,5	338,6	27,4	28,4	0,2	0,5
	CV	16%	21%	16%	21%	17%	22%	10%	10%
T02 (T3)	Mean	4378,7	1932,0	4374,4	1925,7	194,0	154,2	2,5	4,6
	Standard Deviation	377,1	278,5	377,0	279,0	12,7	8,1	0,1	0,6
	CV	9%	14%	9%	14%	7%	5%	5%	12%
Untreated Hair	Mean	4165,2	1826,0	4161,5	1819,7	173,4	151,0	2,4	4,8
	Standard Deviation	285,1	189,9	284,9	189,5	10,8	15,4	0,1	0,2
	CV	7%	10%	7%	10%	6%	10%	3%	5%
S01 (T1+T2)	Mean	4010,3	1421,7	4006,0	1415,0	184,9	136,6	2,6	5,6
	Standard Deviation	360,9	187,1	360,6	187,4	16,6	10,6	0,1	0,6
	CV	9%	13%	9%	13%	9%	8%	3%	11%
S02 (T1+T3)	Mean	3570,9	1361,8	3566,1	1354,9	185,2	136,4	3,0	5,8
	Standard Deviation	518,7	212,6	518,2	211,7	23,7	23,2	0,2	0,5
	CV	15%	16%	15%	16%	13%	17%	6%	9%
S11 (T1+T5+T2)	Mean	3884,0	1306,7	3879,8	1300,2	181,4	130,6	2,7	5,7
	Standard Deviation	507,0	162,2	506,5	161,4	23,4	17,1	0,1	0,3
	CV	13%	12%	13%	12%	13%	13%	5%	5%
S12 (T1+T5+T3)	Mean	3886,8	1291,4	3881,7	1284,3	199,0	133,3	2,9	6,1
	Standard Deviation	628,4	312,9	628,0	312,9	27,4	17,8	0,2	0,9
	CV	16%	24%	16%	24%	14%	13%	6%	14%
S31 (double superposition)	Mean	3878,1	755,0	3872,6	743,7	207,4	130,1	3,1	10,0
	Standard Deviation	319,4	103,8	319,0	104,3	22,8	8,8	0,2	0,9
	CV	8%	14%	8%	14%	11%	7%	8%	9%
S32 (double superposition)	Mean	4171,6	958,5	4168,1	947,8	224,8	142,1	3,1	8,6
	Standard Deviation	574,9	146,1	574,4	145,4	32,7	21,9	0,2	0,9
	CV	14%	15%	14%	15%	15%	15%	6%	11%

Table 14: DMA results for the complementary results

		Complex Modulus (MPa)		Storage Modulus (MPa)		Loss Modulus (MPa)		Loss Angle (°)	
		dry	humid	dry	humid	dry	humid	dry	humid
S01SH (T1+T4+T2)	Mean	4494,2	1580,7	4490,6	1575,6	177,9	126,3	2,3	4,6
	Standard Deviation	601,0	190,0	600,5	189,4	24,8	17,5	0,1	0,3
	CV	13%	12%	13%	12%	14%	14%	4%	6%
S02SH (T1+T4+T3)	Mean	4161,9	1403,2	4157,9	1398,6	181,8	112,9	2,5	4,6
	Standard Deviation	269,7	146,5	269,6	146,5	10,6	10,7	0,1	0,4
	CV	6%	10%	6%	10%	6%	9%	5%	9%
S01SHPE (T1+T4+T2)	Mean	4354,6	1355,3	4351,0	1349,8	175,0	121,1	2,3	5,1
	Standard Deviation	269,6	82,2	268,8	82,1	12,6	8,0	0,1	0,3
	CV	6%	6%	6%	6%	7%	7%	4%	6%
S02SHPE (T1+T4+T3)	Mean	4605,9	1601,1	4601,1	1592,8	208,7	132,8	2,6	4,8
	Standard Deviation	260,6	182,8	259,9	182,5	22,6	10,1	0,1	0,2
	CV	6%	11%	6%	11%	11%	8%	6%	5%
S01EC (T1+T2+T5)	Mean	4607,2	1572,1	4603,3	1565,8	190,5	140,8	2,4	5,2
	Standard Deviation	369,4	213,4	369,2	213,2	17,1	12,1	0,2	0,3
	CV	8%	14%	8%	14%	9%	9%	7%	6%
S02EC (T1+T3+T5)	Mean	4440,9	1664,6	4435,7	1658,3	213,7	143,9	2,8	5,0
	Standard Deviation	400,5	199,4	399,8	198,4	31,5	21,6	0,3	0,4
	CV	9%	12%	9%	12%	15%	15%	9%	8%
S01ECPE (T1+T2+T5)	Mean	4518,7	1597,2	4515,3	1591,5	175,8	135,1	2,2	4,9
	Standard Deviation	217,8	101,4	217,5	101,3	13,8	6,5	0,1	0,2
	CV	5%	5%	5%	6%	8%	5%	4%	3%
S02ECPE (T1+T3+T5)	Mean	4268,0	1521,1	4264,2	1515,9	180,6	125,9	2,4	4,8
	Standard Deviation	400,5	190,0	400,2	189,8	19,2	11,2	0,2	0,3
	CV	9%	12%	9%	13%	11%	9%	7%	5%

Table 15: DMA results for the validation sequences

		Complex Modulus (MPa)		Storage Modulus (MPa)		Loss Modulus (MPa)		Loss Angle (°)	
		dry	humid	dry	humid	dry	humid	dry	humid
Sequence 1 - T1+T4+T2+T5	Mean	4709,2	1477,0	4706,0	1471,6	173,3	125,2	2,1	4,9
	Standard Deviation	317,1	131,6	316,7	131,3	29,0	11,1	0,3	0,3
	CV	7%	9%	7%	9%	17%	9%	14%	5%
Sequence 2 - T1+T4+T2+T5B	Mean	4682,4	1602,7	4679,0	1597,0	176,7	135,3	2,2	4,8
	Standard Deviation	707,7	326,1	707,2	325,1	27,2	27,0	0,1	0,3
	CV	15%	20%	15%	20%	15%	20%	6%	6%
Sequence 3 - T1+T4+T5+T2	Mean	4907,1	1303,3	4904,0	1296,9	174,7	128,0	2,1	5,6
	Standard Deviation	387,2	167,4	387,2	166,8	14,0	17,3	0,2	0,5
	CV	8%	13%	8%	13%	8%	14%	9%	9%
Sequence 4 - T1+T4+T5B+T2	Mean	4586,9	1149,2	4583,8	1143,2	169,6	117,3	2,1	5,9
	Standard Deviation	202,2	86,5	202,2	86,7	8,0	11,0	0,1	0,6
	CV	4%	8%	4%	8%	5%	9%	6%	10%