CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Human Hair

2.1.1 Chemical composition of human hair

Human hair consists of approximately 65 to 95% proteins, mainly keratin. The remaining constituents are water, lipids, pigment, and trace elements. Twenty one amino acids was found in human hair. Because of the large number of chemical reactions that human hair is subjected to by permanent waves, alkaline straighteners, chemical bleaches, and sunlight exposure, many of these amino acids are converted to amino acid derivatives such as cystine monoxide, cystine dioxide, and cysteic acid. Table 2-1 summarizes results from several sources describing quantitative whole-fiber analyses of these 21 amino acids.

Table 2-1 The amounts of amino acids (μmol/g of dry hair) in whole unaltered human hair.

Amino acid	Reference 1[4]	Reference 2[5]	Other references
1. aspartic acid	444–453	292–578	
2. threonine	648–673	588–714	
3. serine	1,013–1,091	705–1,090	
4. glutamic acid	995–1,036	930–970	
5. proline	646–708	374–694	
6. glycine	463–513	548–560	
7. alanine	362–384	314	
8. half-cystine	1,407–1,512	1,380–1,500	784-1,534[6]

Amino acid	Reference 1[4]	Reference 2[5]	Other references
9. valine	477–513	470	
10. methionine	50–56	47–67	
11. isoleucine	244–255	366	
12. leucine	502–529	489	
13. tyrosine	177–195	121–171	
14. phenylalanine	132–149	151–226	
15. cysteic acid	22–40	-	
16. lysine	206–222	130–212	
17. histidine	64–86	40–77	
18. arginine	499–550	511–620	
19. cysteine	-	41–66	17-70[6]
20. tryptophan	-	20–64	
21. citrulline	-	-	11[7]
% nitrogen as ammonia		15.5–16.9%	

2.1.2 Structures of human hair

Morphologically, human hair contains three and sometimes four different units or structures. At hair surface, hair contains a thick protective covering consisting of layers of flat overlapping scale-like structures called "cuticle". The cuticle layers surround the "cortex", which constitutes a major part of the fiber mass of human hair. The cortex consists of tightly packed spindle-shaped cells, called cortical cells that are aligned along the fiber axis. The cortical cells contain fibrous proteins of hair called macrofilaments that are approximately 0.1-0.4 mm in diameter. Thicker hairs often contain one or more loosely packed porous regions called "medulla", located near the center of the fiber. The fourth unit is the cell membrane complex that glues or binds the cells together and, with other non-keratin components, forms a major pathway for diffusion into the fibers.[1] Each macrofibril consists of intermediate filaments and

matrix, a less organized structure that surrounds the intermediate filaments. These structures are illustrated schematically in Figure 2-1.

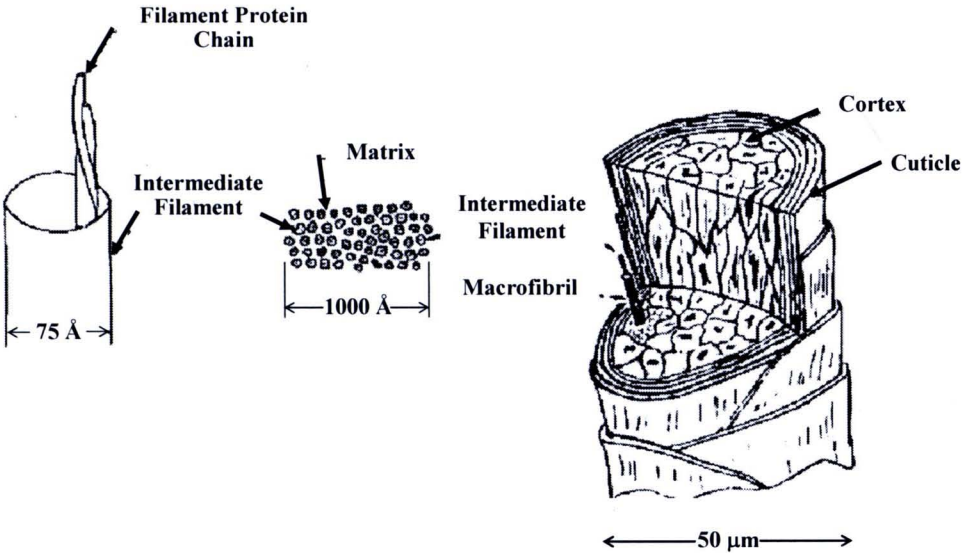


Figure 2-1 Diagram of a human hair including intermediate filament-matrix structures.[1]

The subunits that constitute the intermediate filaments of hairs are polypeptide chains of proteins. Keratin polypeptides are oriented parallel to the longitudinal axis of hair shaft. The polypeptide chains can assume α-helix structure.[8,9] Keratin fiber shows several spacings which are an equatorial spacing (perpendicular to the fiber axis) of 9.8 Å and meridional spacings (parallel to the fiber axis) of 5.1 and 1.5 Å (Figure 2-2).

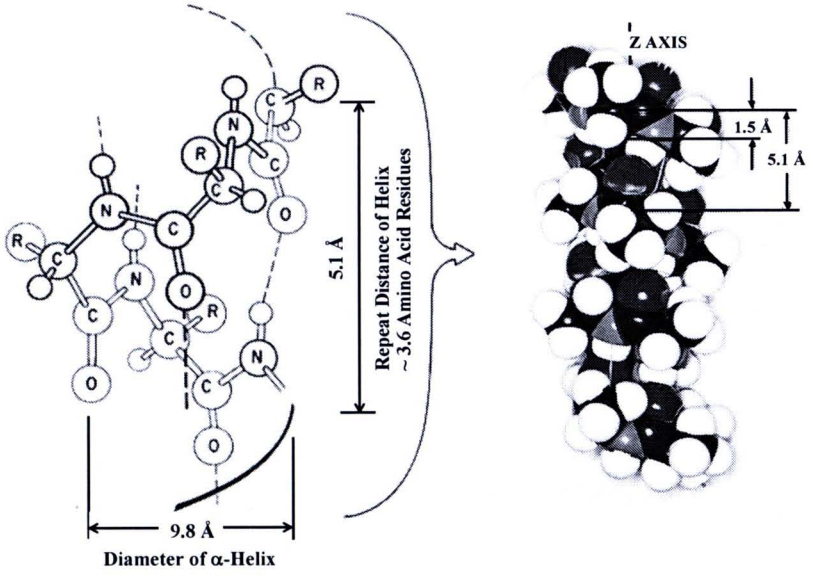


Figure 2-2 Structure of α-helix proposed by Pauling and Corey.[8,9]

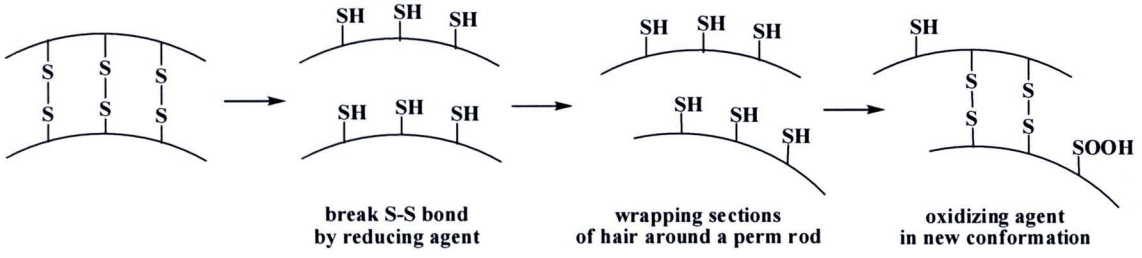
2.1.3 Hair damages

2.1.3.1 Cosmetically-treated hair

Nowadays people tend to visit beauty salon to have their hair pampered by hair stylists. Most of the time it is unavoidable for the hair to be treated with chemicals in lotion, conditioners, and waving-dyeing creams, etc. Hair therefore can be damaged by chemical ingredients from these treatments.

Permanent wave

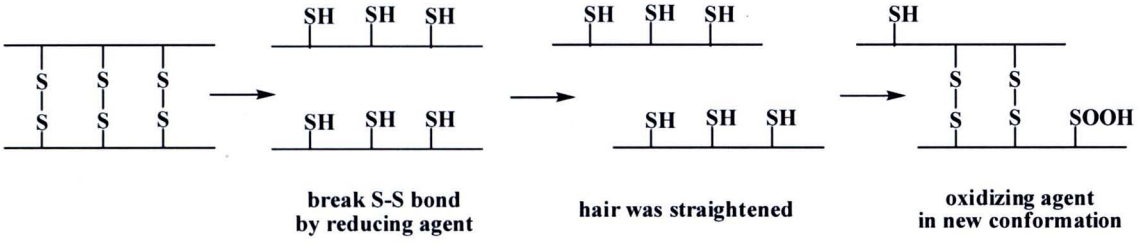
Permanent waving treatment consists of two different processes, the reducing process and the oxidizing process. The first process is intended to act chemically on the covalent disulfide bond. The hairs are wetted with thiol aqueous solution and rolled on curlers so that the imposed deformation of hair is in the shape of curls. In the second process, the curls are set by restoring the initial chemical structure to the fiber. The thiol group of keratin protein, which is produced in the first process, is re-oxidized into the disulfide bond. It is known that an incomplete re-oxidation of the thiol group in keratin protein occurs in the second process.



Scheme 2-1 Mechanism of wave process.

Straightening

Hair straightening, like permanent waving, is an operation in which a permanent deformation of hair is the objective. The same chemical process occurs during straightening as the waving.

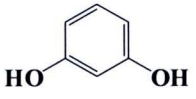
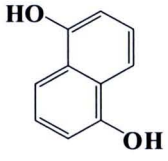
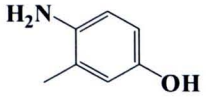
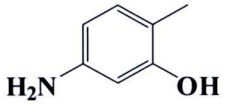
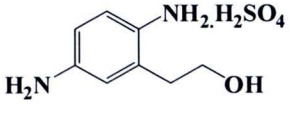
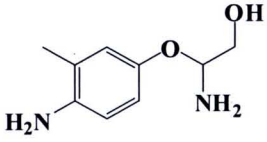


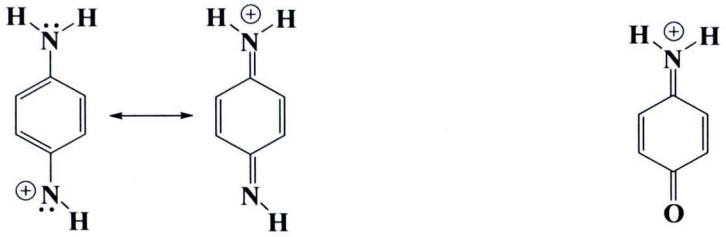
Scheme 2-2 Mechanism of straighten process.

Dyeing

The classification of human hair dyes can divide as four groups such as permanent or oxidation dyes, semi-permanent dyes, temporary dyes or color rinses, and other dyes. Oxidation dyes are often referred to permanent hair dyes and are the most important of the commercial hair dyes. Permanent hair dyes generally consist of oxidation dye precursors such as difunctional *ortho-* or *para*-diamines or aminophenols (as shown in Table 2-2) that are capable of oxidized by hydrogen peroxide to diiminium or quinoniminium ions as active intermediates (Figure 2-3) of the process. These active intermediates then reacts inside the hair with oxidation dye couplers such as substituted resorcinols or meta-phenylenediamines (Table 2-2) to color compound. Oxidation dye couplers usually produce little or no color, but in the presence of oxidation dye precursors and oxidizing agent, they modify the color formed by the precursor.[1]

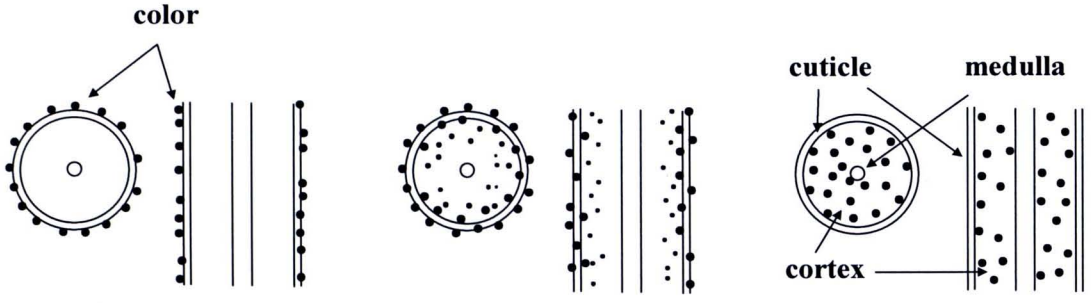
Table 2-2 Some oxidation dye precursors and oxidation dye couplers.

Oxidation dye precursors	Oxidation dye couplers
 **p-phenylenediamine**	 **Resorcinol**
 **1,4 diamino phenoxyethanol HCl**	 **1,5-naphthalenediol**
 **4-amino-*m*-cresol**	 **4-amino-2-hydroxytoluene**
 **Hydroxyethyl-*p*-phenylenediamine sulfate**	 **2,4-diamino-5-methyl phenoxyethanol**



Diiminium ion of *p*-phenylenediamine **Quinoniminium ion of *p*-aminophenol**

Figure 2-3 Structure of diiminium ion of *p*-phenylenediamine and quinoniminium ion of *p*-aminophenol.



(a) Outermost layer of cuticle (epicuticle) (Temporary hair dyeing)

(b) Parts of cuticle and cortex (Semi-permanent hair dyeing)

(c) Cortex (Permanent hair dyeing)

Scheme 2-3 Mechanisms by which different hair dyeing act: (a) temporary hair dyeing, (b) semi-permanent hair dyeing, and (c) permanent hair dyeing.

2.1.3.2 Sunlight-exposed hair

The amino acids of the cuticle are altered to a greater extent than those of the cortex because the outer layers of the fiber receive higher intensities of radiation. Hair protein degradation by light radiation was shown to occur primarily in the wavelength region of 254 to 400 nm or UV. A recent work by Hoting and Zimmermann[10] shows that the proteins of the cuticle are degraded by UV-A (320-400 nm) and UV-B (290-320 nm). This reaction is similar to the oxidative damage to proteins and mitochondrial decay associated with aging.[11]

2.1.4 Hair product containing chitosan and its derivatives

Over the past few decades, polymers have become increasingly important component in the cosmetic industry. They are used as primary ingredients or adjuncts in hair products. They have been used to hair conditioner to improve the substantivity of other ingredients to hair, to improve emulsion stability, and to improve combing.

The types of bond between the polymer ingredient and hair fiber are valence bonds (ionic and covalent bonds), hydrogen bonds, dispersion forces (Van der Waals attractions). The primary valence bonds include ionic and covalent bonds which are the strongest binding forces. They generally have bond energies of approximately 50 to 200 kcal/mole.[12] Hydrogen bonds are the next strongest binding forces, with bond energies generally of the order of 4 to 10 kcal/mol.[12] These bonds are important to the binding of polymers containing polyalcohol or polyamide units to hair. Dispersion forces or Van der Waals attractions have bond energies generally of the order of 1 kcal/mole.[13] These attractive forces are short range and act only between the molecule surfaces. Therefore, the total strength of Van der Waals force increases with molecular surface area i.e. increasing molecular size. In polymers, these can approach the valence bonds strength.[1] A typical conditioner consists of cationic surfactant, fatty alcohols, silicones and water. Table 2-3 displays the chemical structure and functions of the major conditioner ingredients.

Chitosan and its derivatives have been introduced in hair-care products, especially the hair conditioners for a few decades. Several studied have been revealed in patent application. In 1985, Lang *et al.*[14] invented treatment cream for hair and skin containing macromolecular glyceryl-chitosan. In 1986, Maresch *et al.*[15] invented film formers in hair-treatment agents. In 1990, Omura *et al.*[16] invented a hair cosmetic without any stickiness, excellent in touch and brush ability, by blending a chitosan derivative which was prepared by reacting chitosan with polysaccharides. In 1996, Satou and Go[17] produced a hair setting agent containing *N*-(3-carboxypropanoyl)-6-*O*-(carboxymethyl)chitosan, 6-*O*-(carboxymethyl)chitosan or 6-*O*-(carboxymethyl)chitin. In 2001, Nishimoto and Toda[18] obtained the composition containing water soluble chitosan such as hydroxypropylchitosan, succinyl chitosan, succinylated carboxymethyl chitosan, and chitosan-pyrrolidone-carboxylate and a cationic polymer such as chlorinated *O*-[2-hydroxy-3-(trimethylammonio)propyl]hydroxyethyl cellulose. In

2003, Yamamoto and Taniuchi[19] invented hair dyeing agents that were based on chitosan and/or chitosan derivatives mixed with oxidation dyes. The dyes showed good properties in terms of dyeing technique and hair care. In 2004, Shimogaki *et al.*[20] provided a gray hair-preventing/improving agent comprises a sulfated polysaccharide and/or the salts of chitin-chitosan. In 2005, Krause *et al.*[21] invented hair treatment containing *N*-hydroxyalkyl-*O*-benzyl chitosan. In 2007, Liu[22] disclosed a new detergent, prepared by mixing basic cleaning material with functional additive such as water-soluble chitosan, chitosan hydrochloride, chitosan citrate, and chitosan lactate.

Additionally, these inventors find ways of modifying chitin and chitosan by physical blending or chemical reactions in order to improve the solubility of chitin and chitosan in each type of hair-care products. This is because chitosan is difficult to manipulate because of the solubility problems in neutral water and bases which explained in section 2.2.

From all above literature reviewed, there are few studies emphasizing on the positive charge contents in TMC and HTACC and their effects on hair when used in the form of leave-on conditioner formula.

Table 2-3 Chemical structure and functions of ingredients in hair conditioner.

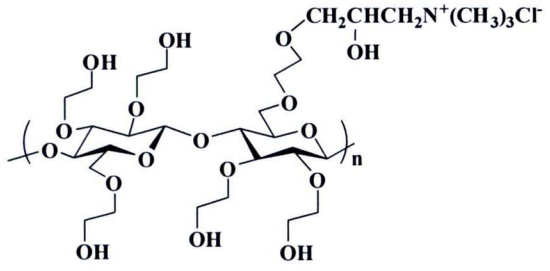
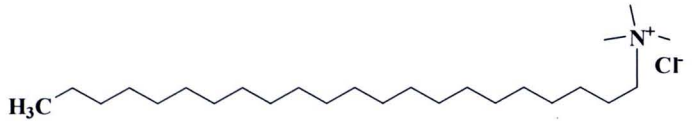
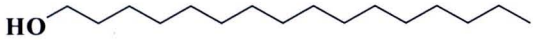
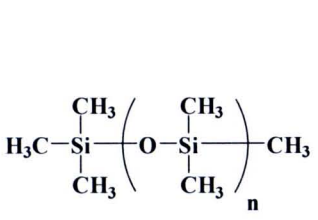
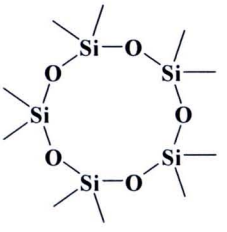
Ingredient	Chemical Structure	Functions
Cationic surfactants	 **Quaternized hydroxyethyl cellulose (polyquaternium-10)**	Lubricant and static control agent
	 **Behentrimonium chloride (BTMAC)**	
	 **Stearamidopropyl dimethylamine**	

Table 2-3 *continued*

Ingredient	Chemical Structure	Functions
Fatty alcohols	 **Cetyl alcohol**  **Stearyl alcohol**	Lubricant and moisturizer
Silicones	 **Dimethicone**  **Cyclopentasiloxane**	Lubricant
Water		

2.1.5 Hair analysis

Traditionally, Analysis of human hair and/or analysis of surface hair were performed by using many techniques such as high performance liquid chromatography (HPLC),[23-25] gas chromatography/mass spectroscopy (GC/MS),[26-30] time-of-flight secondary ion mass spectroscopy (ToF-SIMS),[31,32] Raman spectroscopy,[33-36] and DNA analysis.[37] Limitations of these techniques are complicated sample preparation, large amounts of sample area for analysis, long scanning analysis times as well as sample destruction in order to obtain good spectral quality. In order to overcome these limitations, the FT-IR with a microscope attachment was developed.

2.1.5.1 ATR FT-IR for material surface analysis

Fourier transform infrared (FT-IR) spectroscopy is a technique based on the determination of absorption of infrared light due to energy resonance with vibrational motions of functional molecular groups, this technique has been applied as an analytical technique in different fields such as geology, material science, polymer science, and many others.

Recently, It was suggested that IR spectroscopy was one of the powerful technique for the forensic analysis of human hair.[38] This is because this method is

sensitive to the presences of chemical functional groups in a hair sample. It can provide rapid and specific chemical information at the molecular level associated with the nature of human hair and its composition.

FT-IR sampling technique is generally a transmission technique using KBr pellets.[39] The infrared beam is directly passed through the sample. Since the color of hair sample is usually dark, transmission technique cannot be used. Also a sample thicker than 20 μm cannot be analyzed. Reflectance sampling techniques is therefore utilized. The IR beam is bounced off the sample instead of passing through it. In the conventional attenuated total reflectance (ATR) technique, a good contact between the sample and internal reflection element (IRE) is required.[40]

ATR occurs when a sample is brought into contact with an IRE that has a higher refractive index than the sample and is transparent through the mid-infrared radiation. In general, the IRE configuration includes variable-angle hemispherical crystal with single reflection and multiple reflection planar crystal. The IRE is used in internal reflection spectroscopy for establishing the conditions necessary to obtain internal reflection spectra of materials. Germanium (Ge) is a typical IRE with a refractive index of 4.0, which has significantly higher depth of penetration than that of a ZnSe IRE with a refractive index of 2.4. Table 2-4 shows optical properties of some infrared transmitting materials.[41]

Table 2-4 Typical properties of selected optical materials used in internal reflection spectroscopy.[41]

Material	Penetration depth (μm)	Mean refractive index	Property
Germanium	2.0-12	4.0	good for fine depths of penetrations
Silicon	1.1-6.5	3.5	hard, high resistivity, high-temperature applications
Cadmium telluride	1.0-23	2.64	expensive, relatively inert, can be used in aqueous studies
Zinc selenide	0.5-15	2.4	expensive, water-insoluble, toxic when used with acids
Diamond	1-3.8, 5.9-100	2.4	hard

The penetration depth is the distance from the interface of materials where the evanescent filed strength decays to 1/e (roughly 13%) of its values at the interface. The penetration depth is given by the following equation:[41]

$$d_p = \frac{1}{2\pi\nu n_1 (\sin^2\theta - (n_2/n_1)^2)^{1/2}} \qquad (2.1)$$

where θ is the angle of incidence, ν is the frequency of the infrared radiation and n_1, n_2 are the reflective index of the IRE and a sample. By altering the angle of incidence while ensuring that it remains above the critical angle of the IRE, the depth of penetration can be varied and qualitative depth profiles of a sample can be obtained. In this study, ATR-FTIR was used for identifying functional groups on the surface of the films. Sampling depth of characterization was 1-2 μm.[42]

ATR FT-IR spectroscopy is a surface sensitive technique. However, it has several limitations. One of them is an optical contact between the sample and IRE. The larger the air gap, the smaller the spectral intensity. If an air gap is large enough, the spectrum cannot be obtained. To solve this problem, high pressure is applied at the sample against the IRE. Nevertheless, it has to be handled very carefully to avoid a damage of brittle IRE by an excessive pressure.

Homemade slide-on Ge IRE accessory

The homemade slide-on Ge μIRE was developed by Associate Professor Sanong Egkasit, from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University. The angle of incidence from the objective microscope is varied from 15.6° to 35.5°. To eliminate interference from the internal reflection associated with the radiation having an angle of incidence smaller than the critical angle, an opaque circular adhesive tape is placed on the center of the hemispherical dome. In such manner, ATR FT-IR spectra of a small sample or a small area can be acquired.

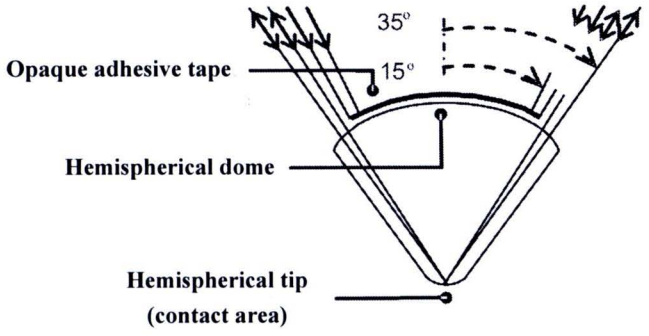


Figure 2-4 The infrared radiation tracing within the Ge μIRE.

2.1.5.2 Tensile testing of hair samples by miniature tensile tester (MTT) and laser scanning micrometer (LSM)

In this study, hair samples were tested for their tensile properties by miniature tensile tester (MTT) and laser scanning micrometer (LSM). The parameters such as elastic modulus (N/m^2; Pa), plateau load (gmf/sq.μm), break extension (%strain), and break load (gmf/sq.μm) are reported.

2.1.5.3 Hair texture analysis

Hair texture in terms of smoothness or slippery feel was determined by measuring the frictional force of hair surface. The friction resistance to the relative motion of two surfaces is proportional to the force, which presses the surfaces together, as well as the roughness of the surfaces. The results can help claim about the effectiveness of hair care products. The area of friction loops (g.mm) is reported.

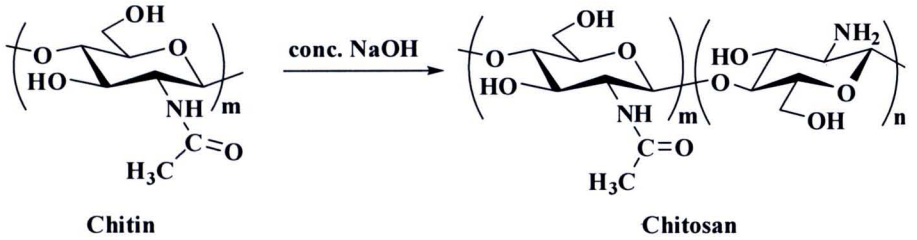
2.1.5.4 Wet combing test by Instron 5564 tensile test tester

This technique was used for measuring the frictional forces generated upon combing a hair tress with a fine toothed comb representing ease of combing and hair detangling. Instrumentally, the ease of combing can be measured by monitoring the frictional forces that result as the hair passes through the comb. These combing forces are measured as a function of the distance traveled along the hair tress. The parameters such as maximum combing force (gmf), average combing force (gmf) and combing energy (mJ) can be used to evaluate hair-care products. The distinctions of hair-care products are easier to detect during wet combing experiments.

2.2 Chitosan and Charged Derivatives

2.2.1 Chitosan

Chitosan was prepared by alkaline *N*-deacetylation of chitin, which is the second most abundant polysaccharide found on earth next to cellulose. Chitosan is a natural polysaccharide consisting primarily of the repeating unit of 2-amino-2-deoxy-D-glucose (GlcN) with a small amount of 2-acetyl-2-deoxy-D-glucose (GlcNAc) unit. Chemical structures of chitin and chitosan are shown in Scheme 2-4. The amount of GlcN unit in chitosan is generally referred to the percentage degree of deacetylation or $\%DD$, which influencing its physical, chemical properties as well as biological activities. Various techniques can be used for determination of $\%DD$ such as IR,[43,44] NMR,[45] colloidal titration method,[46] UV Spectrophotometry,[47] and pH-potentiometric titration.[48]



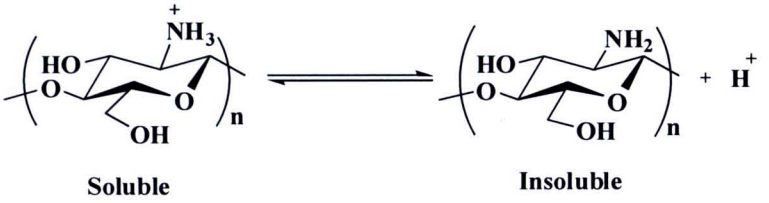
Chitin **Chitosan**

Scheme 2-4 Structures of chitin and chitosan.

As a natural renewable resource, chitosan has a number of unique properties including antimicrobial activity, non-toxicity, and biodegradability. These attract

scientific and industrial interest in such fields as cosmetics, biotechnology, pharmaceutics, wastewater treatment, agriculture, food science, and textiles. Due to its reactive amino and hydroxyl groups, chemical modification of chitosan to achieve its derivatives is used to expand its application.

Chitosan dissolves in dilute organic acids, but is insoluble in neutral water. The pKa value of the primary amino groups in chitosan is determined to be around 6.5[49-51] (Scheme 2-5); it precipitates above this pH due to lossing its cationic nature. The application of chitosan is thus limited owing to the insolubility in neutral or high pH region.

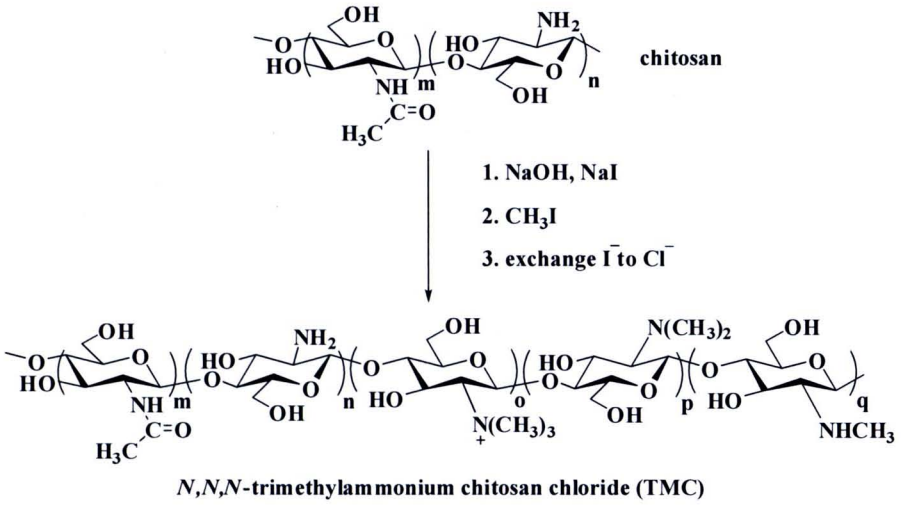


Scheme 2-5 Soluble and insoluble form of chitosan in water.

2.2.2 Charged derivatives of chitosan

An effective way to improve the solubility of chitosan is to introduce charged functional groups to the native chitosan. Chitosan has both reactive amino and hydroxyl groups, which can react as versatile functional groups for chemical modification under mild reaction conditions. This research is interested in the positively charged functional groups, i.e. TMC and HTACC, which are potentially hair-coating materials because in a normal environment human hair is negatively charged. The following related research publications have been reported on chemical modification of chitosan especially at the amino and/or hydroxyl groups to preduce charged derivatives.

Methylation at the amino groups was explored by many researchers.[2,52-54] The resulting product is *N,N,N*-trimethylammonium chitosan chloride (TMC) (Scheme 2-6). The presence of positive charges along the polymer chain helps increase its solubility in water.



Scheme 2-6 Synthesis of *N,N,N*-trimethylammonium chitosan chloride (TMC) from chitosan.

In 1998, Sieval *et al.*[2] synthesized TMCs and studied their solubility comparing to the native chitosan. The product yield and degree of quaternization ($\%DQ$) could be controlled by means of the number of methylation steps, the duration of each reaction step and the amount of methyl iodide. $\%DQ$ was calculated using the following equation:

$$\%DQ = \left[\left(\int N^{+}(CH_3)_3 \right) / \left(\int NHCOC\underline{H}_3 \right) \times 3/9 \right] \times 100 \qquad (2.2)$$

where $\int N^{+}(CH_3)_3$ is the integral of the 9 H's on the three methyl groups (9 H's) attached to the quaternary ammonium atom. $\int NHCOC\underline{H}_3$ is the integral of methyl protons from acetamides of GlcNAc unit. A two-step reaction gave products with high degrees of quaternization (40-80%). A three-step reaction procedure yielded products with a higher degree of quaternization > 80%, but with substantially decreased water-solubility. This was because it also resulted in methylation at the hydroxyl group (*O*-methylation), which decreased the number of –OH groups along the chitosan chain, resulting in the decrease of solubility.

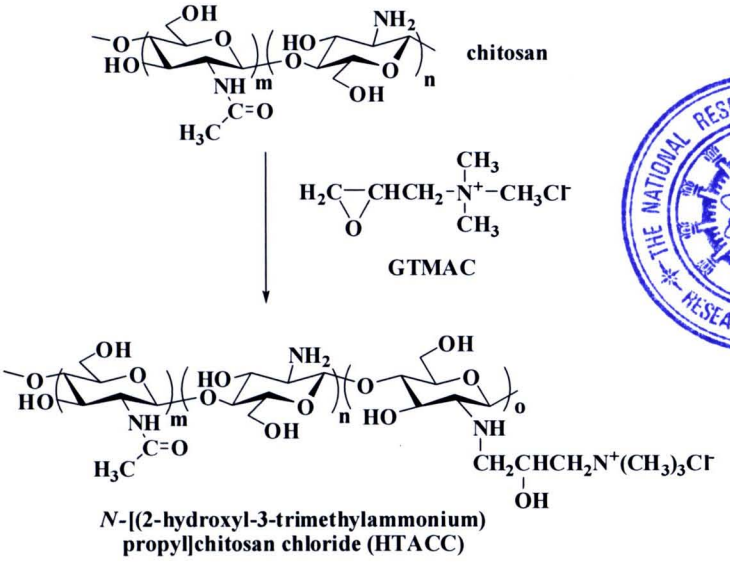
In 2008, Runarsson *et al.*[53] suggested a new synthesis of TMC by using DMF/H_2O mixture as a reaction solvent. The reaction was performed without the aid of a catalyst (i.e. sodium iodide). $\%DQ$ was calculated using the following equation:

$$\%DQ = \left[\left(\int N^{+}(CH_3)_3 \right) / \left(\int H-2',3,4,5,6,6' \right) \times 6/9 \right] \times 100 \qquad (2.3)$$

where $\int N^{+}(CH_3)_3$ is the integral of the 9 H's on the three methyl groups (9 H's) attached to the quaternary ammonium atom. $\int H-2',3,4,5,6,6'$ is the integral corresponding to the H-2′,3,4,5,6 and 6′ protons. This reaction condition significantly reduced *O*-methylation. They obtained TMCs with degree of quaternization between 81 and 88% without any *O*-methylation.

Additionally, positively charged chitosan was prepared by grafting glycidyltrimethyl ammonium chloride (GTMAC), a molecule carrying an ammonium group, on the chitosan chain via epoxide ring opening by the amino groups of chitosan.

In 2000, Seong *et al.*[3] synthesized *N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride (HTACC), using a reaction of GTMAC (Scheme 2-7) and chitosan. The complete substitution of NH_2 in chitosan with GTMAC was achieved when the reaction was performed at 80°C for 18 h with a 4:1 mole ratio of GTMAC to $-NH_2$ in the presence of acetic acid. HTACC showed superior antimicrobial activity to chitosan due to the quaternary ammonium group from the substitution of NH_2 in chitosan with GTMAC. They were applied to the cotton fabrics.



Scheme 2-7 Synthesis of *N*-[(2-hydroxyl-3-trimethylammonium)propyl]chitosan chloride (HTACC) from chitosan.

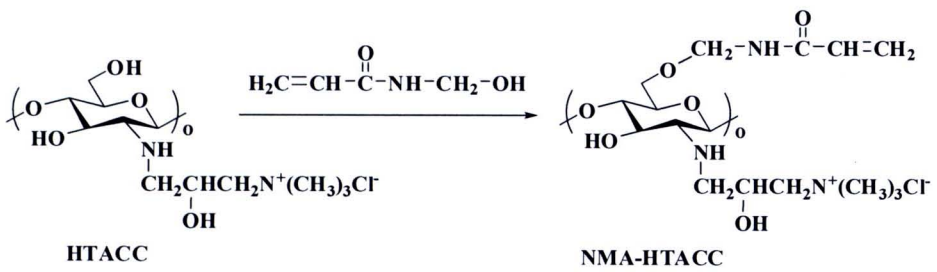
In 2006, Cho *et al.*[55] synthesized HTACC in order to facilitate its use as a novel material for biomedical applications. Varying the molar ratio of GTMAC to chitosan

from 3:1 to 6:1 produced HTACCs with $\%DQ$ that ranged from 56% to 74%. $\%DQ$ was determined based on the conductivity and calculated using the following equation:

$$\%DQ = \frac{1.7 \times 10^{-5} \times V_{AgNO_3}}{\left(\frac{W_w - (1.7 \times 10^{-5} \times V_{AgNO_3} \times m_{GTMAC})}{(m_G \times DD) + m_{AG}(1 - DD)} \right) \times DD} \times 100 \quad (2.4)$$

where 1.7×10^{-5} corresponds to the number of moles of $AgNO_3$ in 1 mL of solution. W_w is the weight of HTACC in 100 mL (0.1 g). m_{GTMAC} is the molecular weight of GTMAC (151 g/mol). m_G is the molecular weight of glucosamine (161 g/mol). m_{AG} is the molecular weight of *N*-acetyl-glucosamine (203 g/mol). The DD of chitosan is 0.92. The HTACC with the highest $\%DQ$ was soluble in water up to concentrations of 25 g/dL at room temperature.

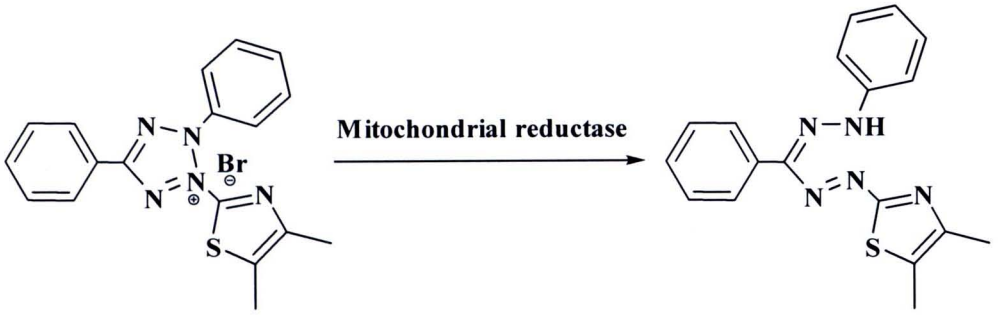
In 2004, Lim *et al.*[56] synthesized a fiber-reactive chitosan derivative in two steps from low molecular weight chitosan and low degree of acetylation. First, HTACC was prepared as presented in Scheme 2-7. Second, this derivative was further modified by reacting with *N*-methylolacrylamide (NMA), which can be covalently bonded to cellulose textile fibers having nucleophilic groups under alkaline conditions. The prepared fiber-reactive chitosan derivative, *O*-acrylamidomethyl-HTACC (NMA–HTACC) is shown in Scheme 2-8. The NMA–HTACC showed complete bacterial reduction within 20 min at the concentration of 10 ppm, when contacted with *Staphylococcus aureus* and *Escherichia coli* [1.5-2.5 × 10^5 colony forming units per milliliter (CFU/mL)].



Scheme 2-8 Synthesis of NMA–HTACC.

2.3 MTT Reduction Assay

An alternative method that was originally developed as a rapid assay for growth and survival of mammalian lymphoma cells is based on the transformation and colorimetric quantification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay that described by Mosmann in 1983.[57] MTT assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and thereby form non-water-soluble violet formazan crystals within the cell (Scheme 2-5) which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of the cells by the addition of a detergent results in the liberation of the crystals that are solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The color can then be quantified using a simple colorimetric assay, such as a UV spectrophotometer.



Tetrazolium salt, yellow **Formazan, violet**

Scheme 2-9 Reduction of the MTT tetrazolium salt to formazan.[57]

The MTT assay is used in many studies to evaluate the viability of different cells because this test is fast. Many samples can be examined at the same time and many replications of each sample can be performed simultaneously.