Fundamental properties of Afro-American hair as related to their straightening/relaxing behaviour

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Jutta Maria Quadflieg
geb. Bußmann

aus Dortmund

Berichter: Prof. Dr. Hartwig Höcker
Prof. Dr. Franz-Josef Wortmann

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Für Joachim

"The secret of patience: Do something else in the meantime."

Detlev Rossilyo [American philosopher, born 1942]

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Summary

The aim of this thesis is to improve the current understanding of straightening processes in Afro hair by alkaline treatment. It is therefore fundamentally important to gain a more detailed insight into the properties of Afro-American hair.

The geometry of Afro hair has been initially investigated.

- Apparent diameter of Afro-American hair is dependent on gender. An apparent diameter of 73.3 ± 6.2 µm has been found for male, and 59.4 ± 7.6 µm for female Afro-American hair.
- Ellipticity and apparent diameter of pigmented and non-pigmented hair of a single Afro-American hair sample differ significantly.

Furthermore, differences between Caucasian, Asian and Afro hair, with respect to their morphology, chemical composition, and thermal properties, have been evaluated after various treatments such as perming and relaxing, and combination of these treatments.

- Scanning electron microscopy studies, as well as luster measurements, showed no significant changes of the surface after these treatments.
- Amino acid analysis of treated hair leads to the conclusion that dehydro-alanine residues, which are generated upon alkaline relaxing of hair, are quite stable. They are converted with cysteine residues to form lanthionine cross-links. Lanthionine residues occur with a higher frequency after perming treatment of relaxed hair.
- The thermal properties of hair show that relaxer treatments result in a greater loss of helical content in hair than perming treatments. Furthermore, the loss of helical domains is not only dependent of degradation of cystine. The keratin associated proteins of the matrix are strongly affected by relaxing treatments. Subsequent perming leads on the one hand to a further loss of helical domains, on the other hand to an increase of denaturation temperature.

- Caucasian and Asian hair respond to the different treatments in a very similar way. However, Afro hair is considerably more affected by the treatments. This can be traced back to its smaller diameter (56 µm) compared to Caucasian (77 µm) or Asian (84 µm) hair. It is assumed that Afro hair is more rapidly penetrated by alkali, and thus a greater damage of the fiber occurs.

In addition, a detailed study to properties of "simulated" Afro hair has been made. Hair companies sell simulated Afro hair (so-called "Afro hair – kinked"). This hair is actually Asian hair, which has been crimped using steam /29/. Part of this work evaluates the extent to which the properties of this modified Asian hair resemble those of natural Afro hair, so that the results obtained may be realistically transferred.

- Whereas simulated Afro hair behaves chemically and thermally like the other hair samples during perming treatments, it is more significantly affected by relaxing treatments. Furthermore, simulated Afro hair shows more rapid straightening than natural Afro hair does. Hence, the cosmetic research companies are advised against using simulated Afro hair as a model for natural Afro hair.

Besides these topics, the straightening efficacy of modified relaxer creams (pure creams and creams containing added thioles) on Afro hair has been investigated.

- A test method has been developed, using single hairs, to determine the straightening effect of relaxer creams. The time-depending straightening effect can be described by a pseudo first-order kinetics function. The model includes a time lag. This induction period has been associated with the structure of hair. The cuticle act as a natural barrier for chemicals.
- Faster straightening has been measured at pH 12.9 than at pH 12.5. Addition of thioles to the relaxer creams (1 % w/w TGA or cysteine) straightens hair considerably faster than pure relaxer creams do. Addition of TGA causes an even faster straightening than the addition of cysteine. However, the hair becomes softer and is more susceptible to breakage when TGA is used.

A further chapter deals with the chemical and thermal properties of modified relaxed hair.

- The degradation of cystine is dependent on relaxing time, and follows first-order kinetics for Caucasian and Afro hair.
- The straightening effect is mainly based on the cleavage of disulfide bridges which are either reduced to Cysteine (especially in the presence of additional thioles), or form dehydroalanine. Most of the generated dehydroalanine reacts to with cysteine to produce lanthionine. A portion of dehydroalanine remains in the hair after relaxing.
- Formation of lanthionine is not primary requirement for permanent hair straightening.
- The mechanism of the alkaline degradation of cystine is not dependent on pH, but on the compostion of the relaxer creams. Additional thioles cleave cystine even under alkaline conditions and thus reduce the extent to which β-elimination of cystine by alkali takes place. Within the same time pure relaxer creams induce more degradation of cystine than thiole containing creams.
- For straightening effects between 5 and 80 %, a nearly linear relationship between loss of denaturation enthalpy and degree of straightening is found.
- The non-helical domains of the outer area of the fiber (cuticle) are more affected by an alkaline treatment than the non-helical domains of the inner area, which is shown by the change of denaturation temperatue.

The possibility to perm hair after relaxing, and the chemical and thermal changes caused upon perming have been investigated.

- The longer the duration of relaxer cream treatment, and thus the more straightened the hair fibers are, the less effectively the hair can be subsequently permed. The dependence of waveability on a prior obtained straining effect can be mathematically described.

- The additional degree of damage of relaxed hair caused by the permanent waving corresponds to the degree of damage associated with perming treatments alone.
- The greater the extent of cystine reduction that occurs during relaxing treatment, the better is the perm set of hair.

In summary, besides a more rapid straightening of hair, the presence of thioles during the relaxing process leads subsequently to a better waveability of hair. This effect is more significant for TGA compared with cystine as the additional thiole. However, such relaxed hair is softer and has to be handled with the great care.

The last chapter contains results about the swelling of hair at high pH, and diffusion of alkaline solution and thioles (TGA or cysteine) into iodid-dyed hair fiber, which is recorded as a result of the visible color change.

- Swelling of hair follows pseudo-first order kinetics at pH 12.4 and 12.8, and is related to the proportion of broken disulfide bridges. Thiole containing solutions at pH 12.8, and the solution at pH 13.4, do not show a first-order relationship. This is associated with the additional cleavage of disulfide bridges by thioles, and increasing hydrolysis of protein chains by alkali, respectively.
- A sharp front between the colorless outer area and the colored inner area of the fiber was observed, and is attributed to the penetration of alkali. Thiole containing solutions showed two penetration fronts, the first front representing the penetration of alkali and the second front the penetration of thiole.
- Penetration into and swelling of the hair fiber did not always cease at the same time.
- The higher the pH, the faster is the penetration rate.
- The penetration of thioles starts with a time lag, compared to the alkali. The faster the rate of penetration, the shorter is the time lag.

- The diffusion coefficient of thioles is not constant. The diffusion of alkali thus does not show simple Fickian behavior.
- Occasionally, hair fibers showed different penetration rates under similar conditions. This might be attributed to differences in degree of mechanical pre-damage of tips and roots of hair.

List of abbreviations

α	Turnover
a	Long axis of hair fiber cross-section
a	First fitting parameter of polynomial equation
AA	Amino acid
AAA	Amino acid analysis
b	Short axis of hair fiber cross-section
b	Second fitting parameter of polynomial equation
c	Third fitting parameter of polynomial equation
Cys	Cysteine
[CyS-SCy]	Cystine concentration
$CySO_3H$	Cysteic acid
CyS-SCy	Cystine
d	Diameter
D	Diffuse reflection
D	Diffusion coefficient
d_R	Rod diameter
DSC	Differential scanning calorimetry
E	Ellipticity
err.	Error
E_{St}	Straightening effect
E_{ST}	Straightening effect
Fig.	Figure
G_L	Gloss index
GML	General linear model
h	Fitting parameter of exponential algorithm
ΔH_D	Denaturation enthalpy
HP-DSC	High-pressure differential scanning calorimetry

HP-DTA	High-pressure differential thermoanalysis
I	Intensity
k	Reaction rate constant
KAP	Keratin associated proteins
k_S	Effective rate constant of perm set
L	True length of hair fiber
l_a	Extended length after treatment
Lan	Lanthionine
[Lan]	Lanthionine concentration
l_b	Extended length before treatment
L_C	Extent of supercontraction
l_R	Length of the hair ring
Lye	Sodium hydroxide
Lys	Lysine
LysAla	Lysinoalanine
NaOH	Sodium hydroxide = Lye
PW	Permanent wave
r	Radius
R^2	Coefficient of determination
R_{Lan}	Lanthionine rate
RT	Room temperature
s	Standard deviation
s	Distance between the fiber ends of a hair ring
S	Specular reflection
$S_{\%}$	Swelling
s^2	Variance
SDS	Sodium dodecylsulfate
SEM	Scanning electron microscopy
S_P	Perm set

[SS]	Number of intact disulfide bridges
Std. err.	Standard error
t	Treatment time
τ	Characteristic straightening time
Δt	Time lag of straightening
Tab.	Table
TGA	Thioglycolic acid
V	Volume of the hair
WCC	White cell count
$\bar{X}$	Arithmetic Mean
x_t	Penetration depth at time t
$\Delta\Phi$	Difference in power

1. Objectives of the investigation

The structure and chemistry of human head hair have been of great practical interest for a long time. This particularly applies to those working in the toiletries industry in seeking to understand the effects of topically-applied preparations to beautify the individual. This might range from hair shampoos and sprays through to bleaching and dyeing and to systems for re-structuring hair.

However, in the past the main interest in hair science has been directed at the properties of and applications for Caucasian and Asian hair. This has markedly changed during the last decade, with a general increase of investigations directed at Afro hair. There is a need for special formulations namely for this hair type due to its inherent properties: Difficulty of combing, limited styling ability, dryness and brittleness, and low tensile strength. Nevertheless, there is comparatively little detailed knowledge of the basic properties of Afro hair and how these relate to one of the most important ethnic cosmetic treatments, the mechanisms of hair straightening by alkaline creams.

To reduce this gap of knowledge, the general aim of this project was to investigate the shape of Afro hair, to examine for disparities between different hair races and to look in depth into various aspects of the mechanism of hair straightening, also referred to as relaxing.

The first objective of this project was to study the size and shape of hair. The common approach for the analysis of these properties is the investigation of hair cross-sections. Investigations of the dependence of diameter and ellipticity on gender and age of the hair owner were a good starting point for the current investigations. Furthermore, differences between pigmented and non-pigmented hair were of interest.

Three major racial types of hair are known: Afro, Asian and Caucasian hair. The differences between these hair types are particularly related to diameter, geometry, crimp and color. These differences have an influence on the degree of change and damage after a treatment. The quality/condition of the hair types may be studied after various treatments, such as relaxing and perming as well as after a combination of these treatments. The current investigations included the morphology, chemical composition, and thermal properties of hair.

Since long, untreated, natural Afro hair is difficult to obtain, many hair companies sell a substitute for it. This is Asian hair, which has been crimped with steam. Part of this work was to investigate the correspondence between so-called “simulated” and natural Afro hair behaviour and properties, and the extent to which the simulated Afro hair is a suitable alternative for natural Afro hair for experimental purposes.

Great emphasis was placed on the relaxing treatment of hair. Tightly curled hair needs to be relaxed in order to be straightened and to improve its manageability. To gain a better understanding of this process, the changes of the chemical and thermal hair properties with relaxing time and straightening effect were studied. A suitable method to determine the straightening effect was devised.

The current relaxer technology is based on pH conditions which are higher than pH 11. It is known that during the relaxing process part of the disulfide bridges (cystine) in hair are re-formed into a different type of cross-link (lanthionine). This process is called lanthionization. In general, it is assumed that the formation of lanthionine is a primary requirement for the straightening of hair /58/. A closer look was taken at this assumption.

Emphasis was given to investigations of new formulations and methods for hair styling. As most relevant approach, different thioles were added to relaxer creams to investigate their influence on the straightening effect and on the properties of hair after relaxing. Furthermore, it was of great interest to determine to what extent hair could be permed after a relaxing treatment. To curl the hair after a relaxing treatment is the favored way to obtain a desired hair style. The quality of the hair was investigated after the relaxing and subsequently perming treatment. The analysis concerned chemical composition and thermal properties of hair.

The last objective of this project was to investigate the diffusion of alkali into hair as well as its swelling under various conditions. It was of great interest to investigate to what extent α-helcial structures remain in the inner part of the relaxed hair. For this, hair was dyed with iodine. Diffusion of alkaline as well as swelling of hair were recorded microscopically in real time due to the disproportionation reaction of iodine into colorless products under strongly alkaline condition. On the basis of this method, the kinetics of diffusion and swelling were investigated.

2. Introduction

2.1. Human hair

Hair is undoubtedly one of the most important attributes of people in all cultures. Style, length and color changes are influenced by fashion trends. The hair reflects feelings of health and beauty, and thus its properties are of great importance. Therefore, the physics and chemistry of hair have been studied extensively /3, 79/.

2.1.1. Structure of hair

Hair is composed of a type of protein, called keratin, which is different from other proteins because it contains disproportionate amounts of sulfur. It is this sulfur that allows us to perm and straighten hair. Human hair is a keratinized skin addition which grows out of the follicles that are tubular recesses of the skin.

Hair consists of three, sometimes four morphological components /1/. The cuticle – located on the outside – consists of flat, overlapping, tile-like cells that coat the cortex in several layers. The cortex, which constitutes the larger proportion of the hair mass, is composed of oblong, spindle-shaped cells which mutually penetrate each other. Thicker hair often shows a tube-like structure in the center which is called medulla. Cuticular as well as cortical cells are separated by the cell membrane complex which consists of lipids and proteins. The general structure of a keratin fiber is illustrated in Fig. 1 for wool as a keratin fiber.

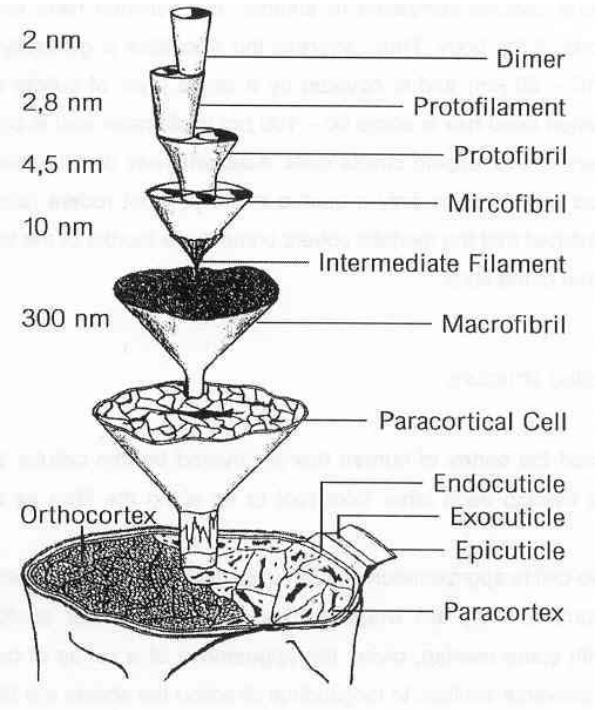

Fig. 1: Schematic diagram of the general structure of keratin fibers (here: wool); adapted from /2/.

2.1.1.1. The cuticle

The cuticle is the outer protective layer of the hair. The cells near the root fit closer at the hair shaft than the cells at the tip because they are mechanically stressed due to their age /3/. Each cuticle has a dimension of approximately 55 x 55 μm^2 and is about 0.5 µm thick. Generally, the cuticle of human hair consists of 5-11 cell layers. The edges of the cuticle are smooth close to the scalp but become rougher along hair length. Also abrasion of sections of the cuticle cells is observed. The longer the hair, the greater is the abrasion of the cuticle. In the worst case, a complete loss of the cuticle is observed, namely at the tip (so called “spliss”).

The individual cuticle cell is composed of several morphological components (Fig. 2). There are three main layers, the A-layer with a high cystine content (> 30 %), the likewise sulfur-rich exocuticle (cystine content > 15 %) and the endocuticle with a low cystine content of approx. 3 %. Between the cuticle cells is a layer which predominantly consists of lipids and membrane proteins. It is called cell membrane complex (CMC) or cellular cement. The proteins of the cuticle are of a predominantly amorphous in nature /7/.

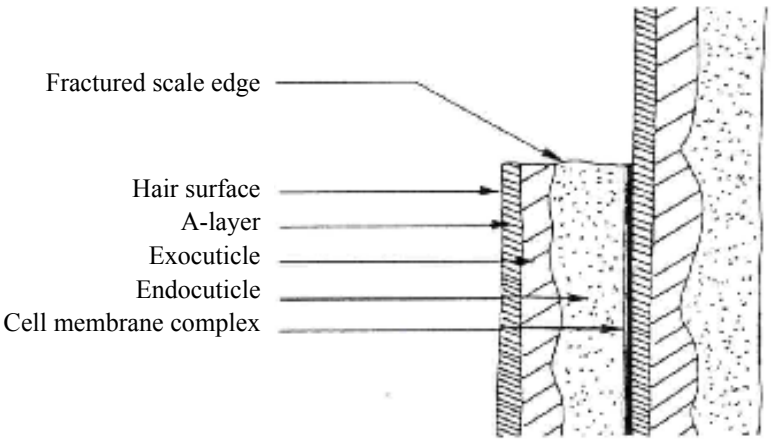

Fig. 2: Schematic diagram of a longitudinal section through the hair surface, and the lamellar substructure of each cuticle cell /4/.

2.1.1.2. The cell membrane complex

The CMC is the vital substance that consists of cell membranes and adhesive material that glues the cuticle and cortical cells together. The CMC contains a lower proportion of sulfur containing amino acids (AAs) compared to other intercellular proteins. The endocuticle and the intercellular proteins form the “non-keratinous regions”.

The non-keratinous regions gain increasing importance in cosmetic science because they are believed to be the primary pathway for the diffusion of ingredients into hair. In addition, during stretching or extension, cuticle separation and damage occur in these regions.

Together, these structures of cell membrane and adhesive material are approximately 0.03 - 0.06 µm thick. A number of sublayers of the CMC have been identified. The most important of these is the central δ-layer /5/. The δ-layer is the intercellular cement. Its proteins are low in cystine (< 2 %) and high in polar AAs. This layer is sandwiched by other layers, sometimes called the inert β-layers. They consist of lipids such as squalene and fatty acids that are rich in palmitic, stearic, and oleic acids.

2.1.1.3. The cortex

The cortex is composed of spindle-shaped cells, which are 1 - 6 µm thick and approximately 100 µm long /4/. The cells are separated by the CMC. The major part of the cortical cells of human hair consists of fibrous structures called macrofibrils or macrofilaments approximately 0.1 to 0.4 µm in diameter. The macrofibrils consist again of fibrous structures which are the intermediate filaments (IF), formerly called microfibrils. A macrofibril contains up to 900 of these 10 nm thick IFs (Fig.1). The IFs are surrounded be the matrix, a less organized structure, which is often referred to as the amorphous region.

The microfibrils are composed of 4 subunits, so-called protofibrils (Fig. 3). Each protofibril has a diameter of 4.5 nm and can be separated into two protofilaments. The 2 nm thick protofilaments are made up of heterodimers which are composed of two protein subunits /6/. The helical domains of these protein chains are approximately 0.001 µm in diameter, including side chains.

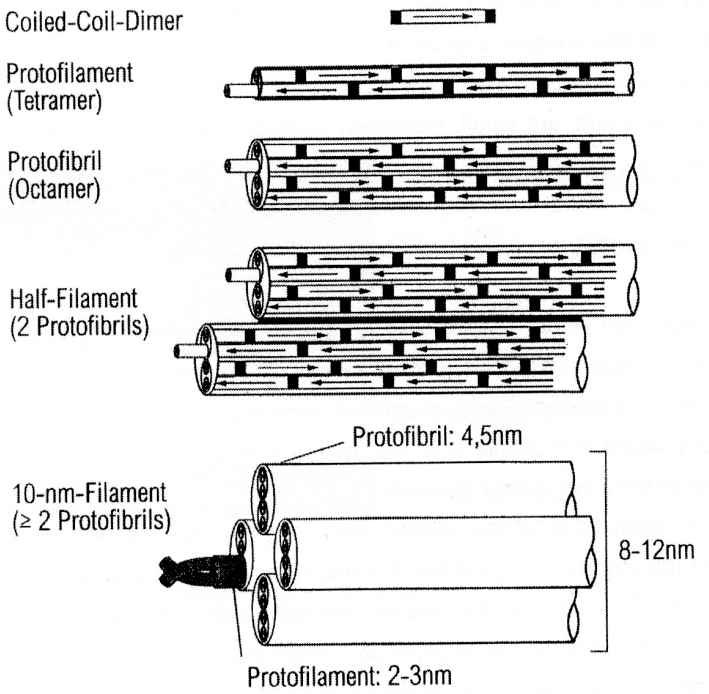

Fig. 3: Model of the composition of the intermediate filament /8/. The white rectangles indicate the coiled coil dimer. The black squares point to the C- and N-terminal amorphous sequences and their overlap upon tetramer formation. Arrows show the direction of the protein chains.

Various estimations of the relative ratio of matrix to IF protein have been made for wool and human hair /9, 10/. Although the relative quantities vary, the matrix-to-IF ratio in human hair is generally greater than 1.

The matrix forms the largest structural unit of the cortex of human hair fibers. It contains the highest concentration of disulfide bonds /11/. The proteins that form the matrix between the Ifs are the keratin associated proteins (KAPs) /77/. In the 1960's these proteins were classified into the three classes "high sulfur", "ultra-

high sulfur" and "glycine/tyrosine rich" proteins. *Rogers et al.* have subdivided these three classes further into 15 distinct KAP multigene families, based on AA homologies and the nature of their repeat structures /78/.

2.1.1.4. Cross-links in the proteins of hair

There are three types of cross-links in the proteins of hair (Fig. 4):

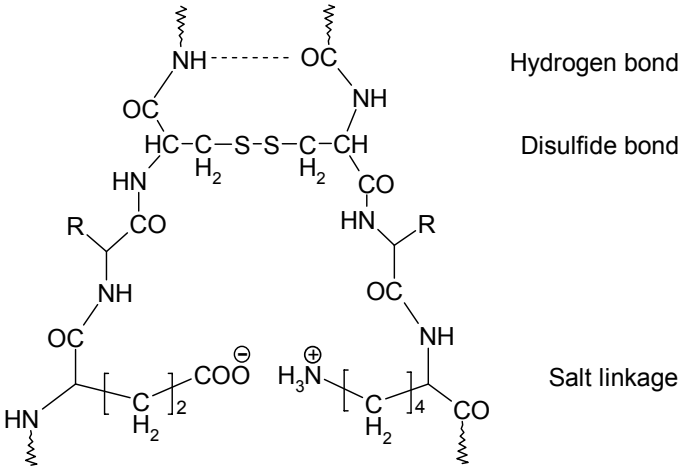

Fig. 4: Cross-links in polypeptides.
~~~ represents the continuous polypeptide chains

- Disulfide bonds

  These are the most important bonds as far as perming and straightening are concerned; these bonds are broken to allow the alteration of the hair shape. The AA cystine (CyS-SCy) forms a link between two adjacent polypeptide chains. The bonds are covalent and can only be altered chemically.

- Salt linkages (also called ionic or electrostatic interactions)

  The proteins of hair contain amino acid residues with basic and acid side groups. The ionic interactions between these groups are much weaker than disulfide linkages and are thus easily broken by weak acids or alkalis.
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- Hydrogen bonds

  These weak bonds arise from the electrostatic attraction between hydrogen atoms and atoms with free electrons (like oxygen or nitrogen). This bond formation occurs within a polypeptide chain or between adjacent protein chains. Although the hydrogen bonds are relatively weak, they are the most frequent interaction in hair. Hydrogen bonds can be broken by water, acids, and bases.

2.1.2. Origin of curls in Afro hair

The quality of the hair of an individual depends on heredity and cannot be changed. Many people believe that Afro hair has completely different characteristics compared to Caucasian hair, so that products must be used in different ways. Afro hair has the tightest curl of any hair type that the hairdresser must deal with. As the hair grows out of the follicle, it hardens and develops the characteristic twists, crimps, and curls of Afro hair (Fig. 5 and 6). It has been suggested that the shape and direction of the hair follicle has an influence on the hair shape. Alternatively, the different sides of the hair could grow with different speeds. But until now, none of these theories has been verified.

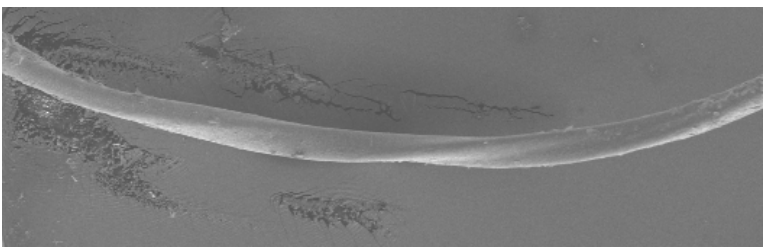


Fig. 5: Crimp in an Afro hair

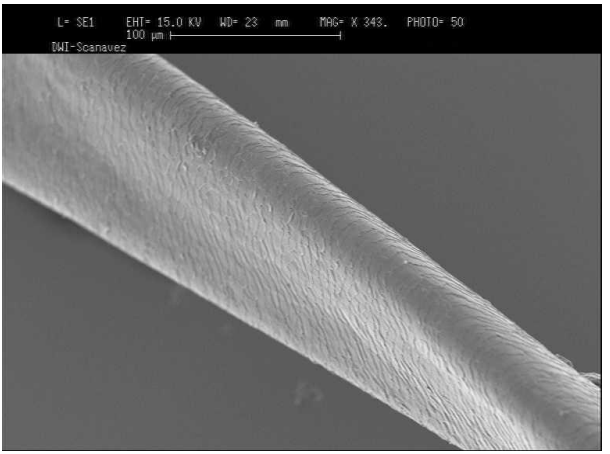


Fig. 6: Torsion in an Afro hair

Hair mainly contains two types of cortex (ortho and para cortex). The ortho-cortex has a less dense structure and lower sulfur content than the para-cortex. For a long time one believed that the para-cortex always lies on the outside of the curve of a wave. But *Phan* /12/ has shown that a bilateral distribution of ortho- and para-cortex cells – like they occur in wool fibers (Fig. 7) – cannot be found in Afro hair or any other hair type. Instead of the bilateral distribution, a statistical distribution of the different cortex cells can be seen when viewing a cross-section of an Afro hair in the Transmission Electron Microscope (TEM).

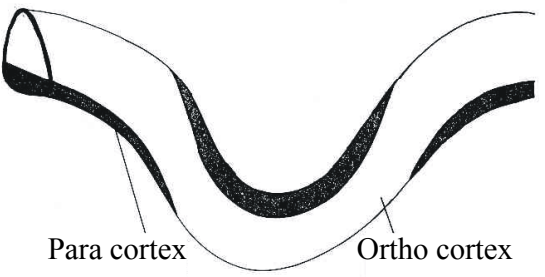


Fig. 7: Ortho and para cortex of a wool fiber. The black areas indicate the para cortex on the inside of the crimp of the hair.

Consequently, the existence of curls cannot be explained by the existence of more disulfide bonds on the inside of the curve.

Until today no verified explanation exists for the origin of curls namely in Afro hair. But new investigation methods will surely give an answer to this question. Recent surveys by synchrotron X-ray micro-diffraction have given information about the keratinization process in human hair follicle /13/. This process can be expected to have an influence on the shape of human hair.

2.2. Hair straightening/relaxing

Hair straightening, like permanent waving, is an operation in which a permanent deformation of hair is the objective. Permanent waving effects a lasting transformation of straight hair into waved hair. Hair straightening does just the opposite, making naturally curly or kinky hair more or less straight.

2.2.1. Thermal straightening – hair pressing

The tight curl of Afro hair limits the number of ways it can be styled. One of the first methods to make Afro hair straigther, and therefore more easy to style, was to straighten it temporarily using heat. In the United States during the second half of the nineteenth century, tin cans were heated in fires and the hair was wrapped around them and stretched /14/.

During the straightening process of hair hydrogen bonds between the polypeptide chains of keratin are broken under the influence of heat in the presence of water. Then, upon rapid cooling, as long as tension is maintained, new hydrogen bonds are formed which keep the hair in straight form. The greater the heat used, the more bonds are broken (pressing combs as shown in Fig. 9 operate between 140 °C and 260 °C). This means that the hair-dresser is

always trying to use the highest possible temperature, which means that permanent damage from heat is likely. This kind of straightening is temporary rather than permanent.

Hair pressing can cause partial straightening but this process causes damage to the surface of the fiber. Extensive loss of cuticle is frequently observed in hair of Negroid origin which has been subjected to this type of treatment, resulting in poor mechanical and fracture behavior /15/.

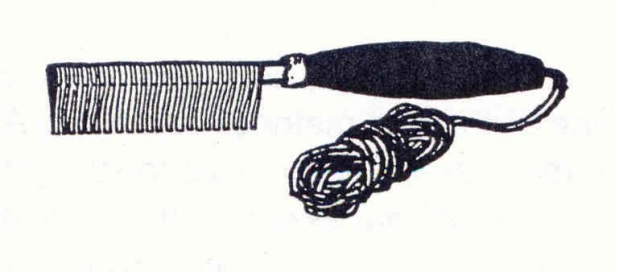


Fig. 9: Hair pressing comb.

2.3.2. Chemical hair straightening

The disadvantage of hair pressing is the temporary nature of the change, because straightening is achieved by breaking and reforming weak hydrogen bonds. There are two ways that curly hair can be chemically altered to become straight: straightening and relaxing. Although both of these methods are permanent, new hair growth must be treated every few months to maintain overall straightness.

2.3.2.1. Differences between hair straightening and relaxing

Many publications apply inconsistent definitions of straightening and relaxing, as if they were the same process. Although the desired result is the same, the

chemical process to achieve it is different. Relaxing produces superior results. The following distinctions can be made between the two processes:

- Straightening is a two-step chemical process involving reduction and oxidation.
- Relaxing is a one-step chemical process applying strong alkali.

2.3.2.2. The chemistry of hair straightening

The way a chemical straightener works is very similar to the permanent waving process. The active ingredient of the straightener is a thiole, mostly ammonium thioglycolate. The straightener creams may contain up to 12 % ammonium thioglycolate, adjusted to a pH of 8.6 to 9.5 depending on the product.

The thiole as reducing agent cleaves the disulfide bond (CyS-SCy) of the keratin For the sake of simplicity Fig. 10 shows a schematic diagram of this reaction (chemical descriptions are given in the chapter 3.2.3.2.). During the reduction phase the hair is mechanically straightened with a comb. The deformation occurs on the molecular level through the sliding of polypeptide chains with respect to each other, leading to changes in their relative positions (Fig. 11).

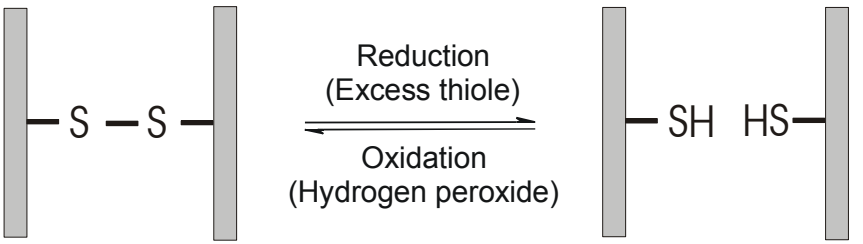


Fig. 10: Schematic diagram of reduction and oxidation of two polypeptide chains which are linked by a disulfide bridge /15a/.

During the process the hair must be kept as straight as possible, and only "heavy" (highly viscous) emulsions can do this. Finally the deformation has to be fixed. The CyS-SCy linkages are reformed by an oxidizing agent, mostly hydrogen peroxide. As a side product, the oxidation leads to the formation of cysteic acid, $CySO_3H$ (Fig. 11).

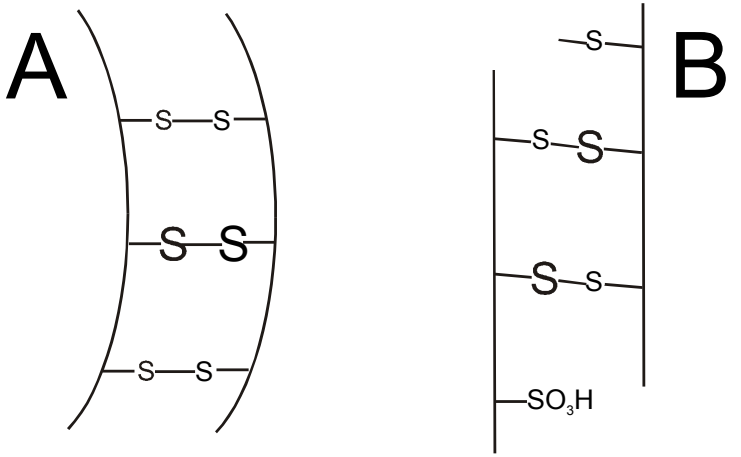


Fig. 11: Relative positions of two polypeptide chains and their disulfide cross links in human hair before (A) and after (B) straightening. As a side reaction the formation of cysteic acid ($-SO_3H$) occurs during the process.

During the reduction stage the hair is fragile. As soon as it is in contact with an active compound, the hair must be handled with great care. This is why, in cold waving, loose rolling is recommended immediately after moistening, with no pulling of the hair. In the case of straightening the situation is different; as soon as the application is started, and then again after the process period is over, the hair needs to be combed out straight. This is the contradiction that embodies the primary difficulty of this operation. To do this correctly and avoid hair damage, great care and extensive experience both with the products as well as with Afro hair are absolutely essential. The risk of hair breakage is very high.

Straighteners based on ammonium thioglycolate are not as popular as the relaxers based on NaOH, because for Afro hair they process more slowly and straightening is not as effective as with relaxers. Straightening is particularly used for Caucasian and Asian hair.

With the current techniques hair straightening methods are still far from perfect. The most recent, successful process of permanent hair straightening applies a hot iron press technique to hair treated with an alkaline solution containing thioglycolic acid and dithiodiglycolic acid /16/.

2.3.2.3. The history of hair relaxing

In the 1930s many hairdressers tried to use reverse heat permanent waving to straighten hair (this was the forerunner of cold permanent waving), but this usually caused unacceptable damage. By about 1940 the modern cold wave, based on ammonium thioglycolate, had been developed and was in general use as a hair straightener. The aqueous formulation of the cold wave lotion was thickened with flour or talc so that the weight would help to straighten the hair. An acceptable process for permanently relaxing the hair had still not been found.

In the mid 1950s, extensive research finally resulted in the development of special emulsions which made safe and effective hair relaxation a reality. The active ingredient, incorporated into a heavy cream, was sodium hydroxide (NaOH). These early products were strongly alkaline and required the use of a protective cream which was applied to the scalp and around the entire hairline. This prevented the alkaline chemicals of the relaxer from coming into contact with the skin. This protective cream was known as a “base”, and application of the base to the scalp was known as basing. Another ten years passed before further noteworthy improvements were made for hair relaxer products. A “no base” relaxer was introduced so that the application of a base cream was no

longer required. This reduced the application time and made relaxing more pleasant. These relaxers contain extra conditioning agents and skin coolers to lessen possible scalp irritation.

2.3.2.4. The chemistry of hair relaxing

The most popular and widely used relaxers for Afro hair are still based on NaOH (in the USA the common name for NaOH is “Lye”). Since it is well known that it can damage the hair, many products claim that they do not contain lye. Marketing experts perceive that hairdressers will be attracted to such “No-Lye” products. However, these just contain alternative hydroxides – calcium, potassium, lithium or guanidine hydroxides – and are still damaging the hair. The pH of a relaxer may range from 11 to as high as 14. Generally, the pH is in the region of 12-13.

The advantages of NaOH relaxers are:

- Fast processing time (the more NaOH a relaxer formation contains, the shorter the processing time)
- Good smooth relaxation
- High efficacy with reduced tendency of the hair to re-curl on shampooing

However, the relaxer treatment also has various disadvantages:

- Cleavage of CyS-SCy (disulfide bridge), formation of lanthionine (Lan; monosulfide bridge) and hydrolysis of proteins inducing pronounced hair damage
- Irritation of the scalp
- Contact with the eyes may lead to blindness
- Hair cannot be permed further on, dyeing and bleaching are only partly possible

Relaxers are available in formulations of different strength to be suitable for various hair types. The amount of NaOH present in relaxers varies between 1.8 to 2.5 % by weight.

The chemistry of hair relaxing differs from the chemistry of hair straightening. During the relaxing process both disulfide bridges and protein chains are broken. In contrast to the straightening process in which disulfide bonds are reformed by oxidation, Lan is formed during the alkaline treatment, which is an irreversible step. Fig. 12 shows a schematic diagram of the formation of Lan, which is a monosulfide bridge between the protein chains. The detailed reaction is discussed in chapter 3.2.3.2.

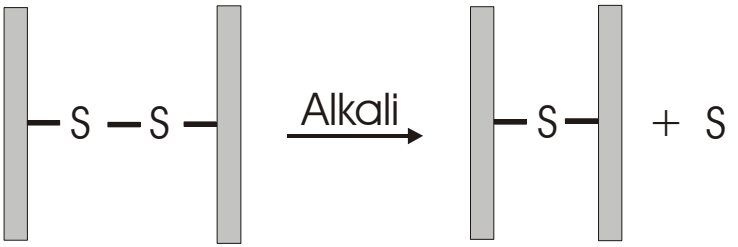


Fig. 12: Schematic diagram of the formation of lanthionine (monosulfide bridge) from CyS-SCy (disulfide bridge) during an alkaline treatment.

Furthermore, the alkaline relaxing treatment also hydrolyses protein chains which build up the backbone of the hair fibers. This step is irreversible, too, and causes strong damage to hair.

3. Results and discussion

3.1. Investigations of hair cross-sections

3.1.1. Introduction

Form and size of hair is of great interest. Publications on this topic date back to the beginning of the last century /17, 18/. As a general rule, human hair has an elliptical shape. Only Afro hair sometimes shows a triangular shape /19/. The apparent diameter of hair affects various single fiber as well as collective properties, including body, hair volume, combability, waveability, luster and styling set /20, 21/.

Fig. 13 shows a typical cross-section of Afro hair in the light microscope. To characterize cross-sections of fibers, very thin cuts are investigated using light microscopy. The lengths of the long and short axes of at least 100 fibers of each sample are determined. These values are averaged and used for calculation of ellipticity E and apparent (equivalent) diameter. The ellipticity is calculated as follows:

$$E = \frac{a}{b} \tag{3.1}$$

Where a represents the long axis and b the short axis.

Shape of hair is genetically fixed. The intensively crimped Negroid hair possesses the most distinct ellipticity (1.6 – 1.9) followed by lightly waved Caucasian hair (1.3 – 1.5). Cross-sections of Oriental or Asian hair are nearly round (1.1 – 1.3) /22, 23/. The natural ellipticity has even been used for anthropological investigations /23, 24/.

Fig. 13: Cross-sections of Afro hair.
a: long axis, *b*: short axis

The apparent diameter *d*, which is the diameter of a circle with the same area, is calculated by the following equation assuming an elliptical shape of hair:

$$d = \sqrt{ab} \tag{3.2}$$

Hairs of Caucasian people reach an average apparent diameter of about 60 µm and they are ca. 25 % thinner than hairs of Negroid people (~ 75 µm). The apparent diameter of Asian hair is even thicker with around 90 µm /22, 25/.

3.1.2. Influences of age and gender

Ellipticity and apparent diameter of 12 hair samples were investigated. The origins of these samples are known. The results were correlated with age (between 4 and 91) and gender (7 samples of female and 5 samples of male origin) of the hair donors. The results are shown in Tab. 1 and are summarized in Figs. 14 and 15.

Tab.1: Results of the cross-section investigations of Afro hair (± Standard deviation, number of cross-sections: 100).

Gender	Age in years	Ellipticity	Apparent diameter in µm
Female	6	1.83 ± 0.23	51.5 ± 9.0
	11	1.63 ± 0.31	62.2 ± 12.6
	41	1.91 ± 0.27	70.4 ± 11.4
	50	1.71 ± 0.18	59.5 ± 8.3
	51	1.82 ± 0.24	56.5 ± 7.9
	84	1.68 ± 0.24	61.5 ± 11.4
	90	1.66 ± 0.23	53.1 ± 10.1
Overall Mean		1.75 ± 0.26	59.4 ± 7.6
Male	17	1.92 ± 0.30	76.2 ± 13.7
	18	1.84 ± 0.21	78.9 ± 9.3
	23	1.64 ± 0.18	73.2 ± 11.6
	41	1.94 ± 0.23	73.3 ± 8.9
	41	1.69 ± 0.23	84.5 ± 15.2
	43	1.74 ± 0.18	63.2 ± 6.6
Overall Mean		1.80 ± 0.23	75.1 ± 6.2

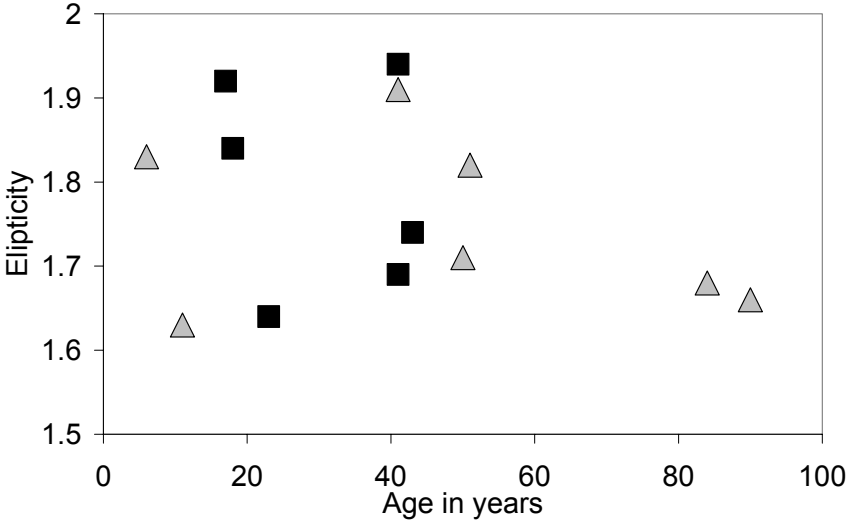


Fig. 14: Ellipticity of Afro hair samples in relation to age and gender.
■ = male, △ = female

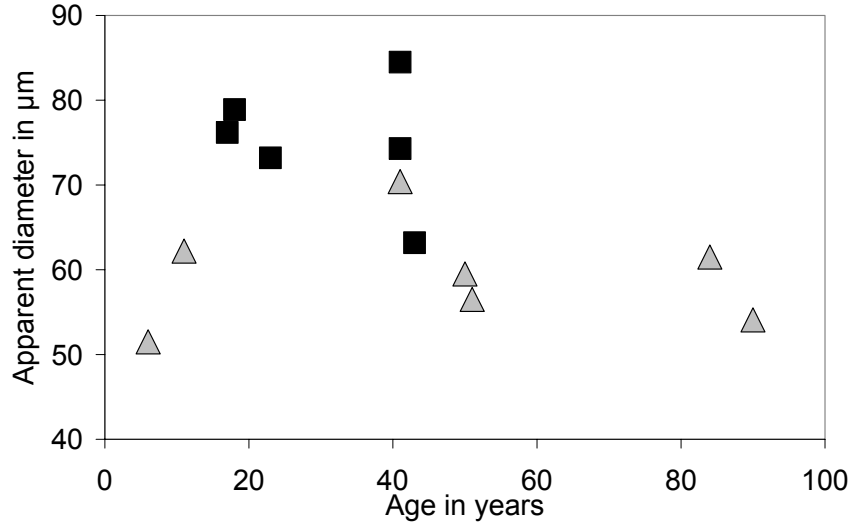


Fig. 15: Apparent diameter of Afro hair samples in relation to age and gender.
■ = male, △ = female

To test the parameters ellipticity and apparent diameter for their mutual dependencies as well as on age and gender, statistical tests are done. Since gender signifies a category, age is a continuous variable, and ellipticity and apparent diameter are assumed to be normally distributed, a general linear model is used for testing (c.f. chapter 4.4.5.).

The statistical test shows that ellipticity shows no dependency on age, gender and apparent diameter. Furthermore, the apparent diameter is independent of age. But in contrast to this, the apparent diameter is dependent on gender.

The t-test for independent samples (eq. 6.7) is used to determine the degree of independence. The test evaluates the significant differences in means between the apparent diameter of males and females. The t-test for independent samples shows that on the 99 %-level the apparent diameter of male (with 73.3 µm) and female (with 59.4 µm) hair differs significantly. A box & whisker plot of the values for the apparent diameter depending on gender is given in Fig. 16:

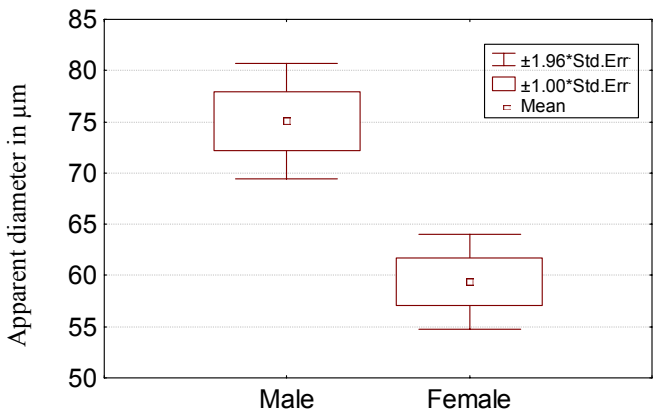


Fig. 16: Apparent diameters of male (n = 6) and female (n = 7) Afro hairs in the form of a box & whisker plot.
1.00*Std.Err. = standard error (c.f. chapter 4.4.4)
1.96*Std.Err. = expectation range for the 95 % confidence limits.
Numer of cross sections in each individual case is n = 100.

3.1.3. Comparison of pigmented and non-pigmented Afro hair

For one of the available samples a comparison of pigmented (black) and non-pigmented (gray) hair was possible. The proportion of gray hairs of this sample is about 40 %. The results for ellipticity and apparent diameter of this individual hair sample are given in Tab. 2:

Tab. 2: Cross-sectional parameter values of pigmented and non-pigmented hairs from an individual grey Afro hair sample.

		Pigmented hair	Non-pigmented hair
Ellipticity	Mean	1.72	1.55
	Number of cases	61	37
	Standard error	0.02	0.04
Apparent diameter	Mean	56.0	51.1
	Number of cases	61	37
	Standard error	1.4	1.3

The t-test for independent samples shows that on the 99 %-level the ellipticity of pigmented and non-pigmented hairs of this sample differ significantly. The apparent diameters of pigmented and non-pigmented hairs of this sample differ significantly on the 98 %-level.

Graphical summaries for these results for ellipticity and apparent diameter depending on hair color are shown in Figs. 17 and 18.

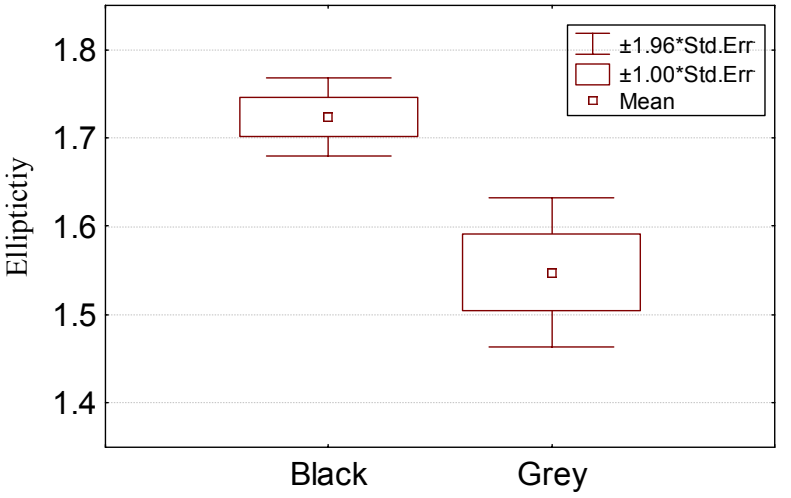


Fig. 17: Ellipticity of black and grey hair of an individual Afro hair sample.

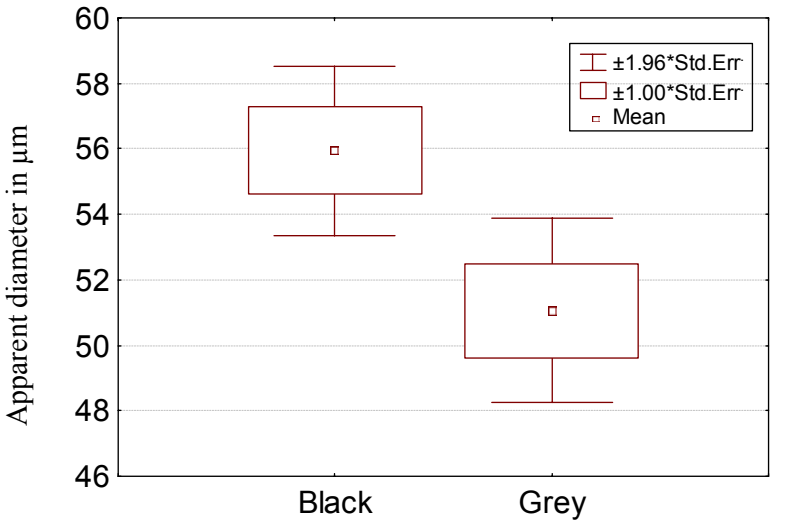


Fig. 18: Apparent diameter of black and grey hair of an individual Afro hair sample.

3.2. Comparison of Afro, Asian and Caucasian hair

Three major racial types of hair are known: Afro, Asian and Caucasian hair. Tab. 3 shows the percentage of each of the three racial groups globally and in the USA population.

Tab. 3: Percentage of the three major racial groups globally and in the USA population.

	% of earth's population /26/	% of USA population /27/
Caucasoid	56	86.9
Negroid	10	11.5
Asiatic	34	< 1.6

The differences between these hair types are particularly related to diameter, geometry, crimp and color (Fig. 19).

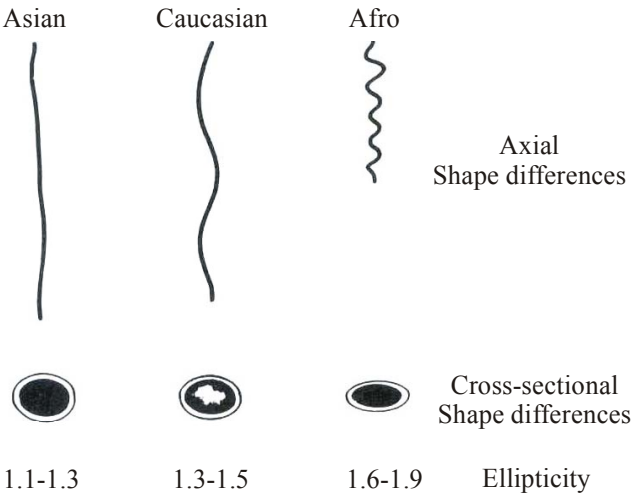


Fig. 19: Specific properties of hairs of different racial origins /28/.

These differences have an influence on the degree of damage after a chemical treatment. Crimp (curvature) is perhaps the most important fiber characteristic for styling, combing, and other aspects of hair behavior. Permanent waving and hair relaxers primarily change fiber curvature to either a curlier or a straighter form. When curvature is low (the hair is straight) other fiber properties play a more important role for hair behavior. However, when curvature is high (hair is very curly), this fiber property dominates other fiber properties and controls hair performance. In the following discussion the chemical and thermal properties of the hair types are compared after various treatments.

3.2.1. Characterization of hair samples

Besides the three major racial hair types - Afro, Asian, and Caucasian - natural and so-called simulated Afro hair have been investigated. In practice it is in fact very difficult to obtain longer length of untreated Afro hair for testing. For this reason hair companies sell simulated Afro hair. This hair is actually Asian hair which has been crimped using steam /29/. This method inevitably imposes damage to the hair /30/. Part of this work evaluates the extent to which this modified Asian hair has properties comparable to natural Afro hair, so that results obtained are realistically transferable.

The Caucasian, Asian, and simulated Afro hair used in this study are mixed source samples. In contrast, the natural Afro hair samples originates in each case from a single source. This means that results obtained for mixed head hair samples reflect the typical properties of the particular racial group. In contrast, the results for natural Afro hair indicate the properties of only a particular sample. Results of light microscopic investigations of hair cross-sections are summarized in Figs. 20 (Ellipticity) and 21 (Apparent hair diameter), respectively.

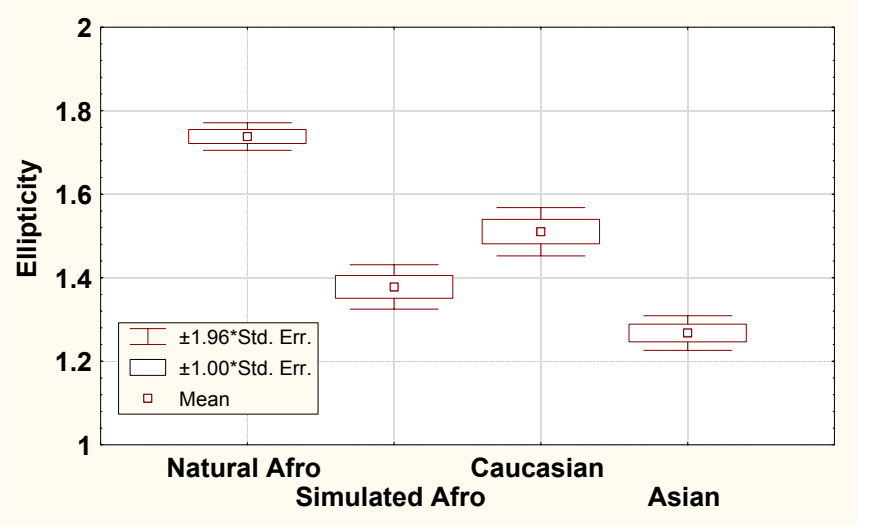


Fig. 20: Ellipticities of different ethnic hair samples.

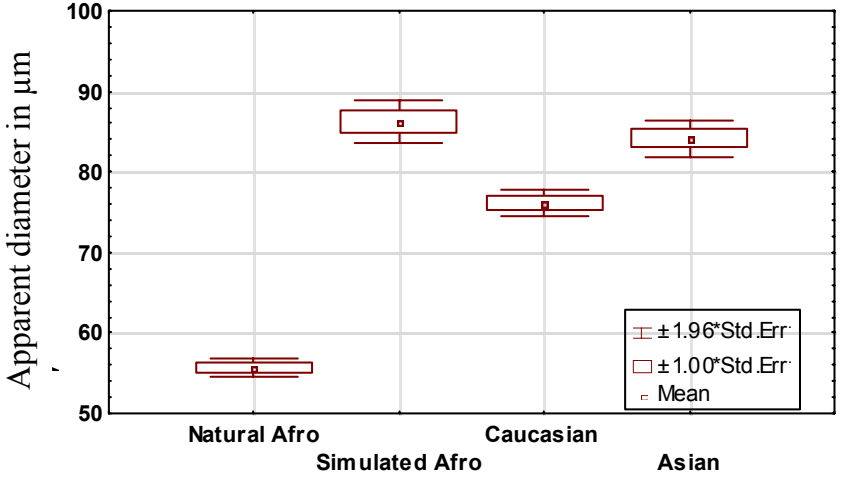


Fig. 21: Apparent diameters of different ethnic hair samples.

The measured values for ellipticity of all natural hair types correspond with literature data /28/. As expected the simulated Afro hair (1.39) has a degree of ellipticity similar to that of Asian hair (1.30). While the diameter of the Asian and simulated Afro hair (82 and 86 µm), and the Caucasian hair (76 µm) agree with literature data /25/, the natural Afro hair (55 µm) has a small diameter compared with the reference (~ 75 µm) /25/. However, this measured diameter fits with a typical diameter for an African female as shown in the prior chapter (Fig. 16).

3.2.2. Hair treatments

To investigate differences between the hair types, the hair samples were treated as shown in Tab. 4. Every treatment was made under specific conditions which were not changed during the test series.

Tab. 4: Hair treatments

Abbreviation	Treatment
Untreated	Untreated hair
PW	Permanent wave (treatment with an aqueous solution of ammonium thioglycolate followed by a peroxide treatment)
No-Lye	Relaxing treatment with a relaxer cream, which contains no sodium hydroxide (NaOH = Lye), pH 12.9
No-Lye + PW	Treated with the No-Lye cream, followed by a permanent wave
NaOH	Relaxing treatment with a NaOH containing relaxer cream, pH 12.5
NaOH + PW	Treated with the NaOH-cream, followed by a permanent wave

3.2.3. Quality of hair samples after treatment

In this work four different methods of analysis are employed to characterize the hair and the degree of damage after different treatments. Morphology, chemical composition and thermal properties are investigated.

3.2.3.1. Surface quality of the hair

The morphology of the hair was investigated by using Scanning Electron Microscopy (SEM) to obtain a visual impression of the surface. Additionally, gloss measurements were made to obtain an impression of the roughness of the surface.

3.2.3.1.1. Scanning Electron Microscopy

The surface quality of the hair was investigated by using the knot test. For this purpose a single hair is simply knotted (Fig. 22).

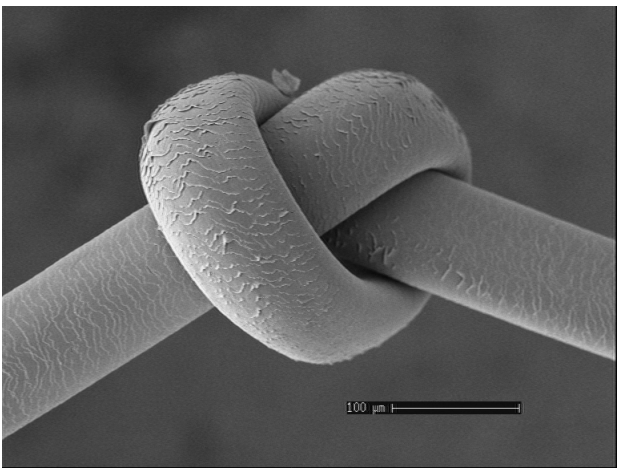


Fig. 22: Knot in an undamaged Caucasian hair in the SEM
Because of the tensile strain, namely on the outside of the knot, damages become readily apparent.

At the outer region of the knot strong tensile strains are imposed on the scales of the cuticle. Scales which protrude from the surface ("fir cone" effect) suggest a high level of damage as shown in Fig 23. In contrast to this, undamaged hair shows a generally smooth surface (see Fig. 22). At least ten hairs per sample were investigated by SEM /31/.

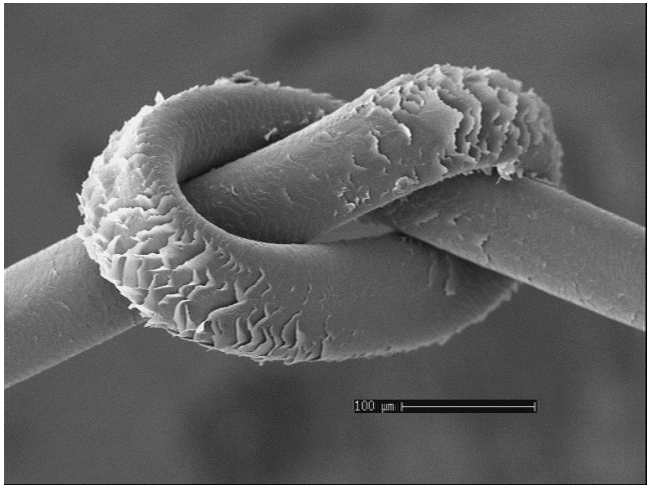


Fig. 23: Typical hair damage ("fir cone" effect) of an Caucasian hair induced by a strong PW or bleaching.

The examination of the different ethnic hairs after the various treatments according to Tab. 4 suggested no significant changes compared to untreated hair. The scales showed no "fir cone" effect. The only damage which was observed was the appearance of small cracks in the cuticle, especially at the points which were more stretched – for example at the outer side of the knot (Fig. 24). This is prominent on hair which was treated with the relaxer creams plus the PW. If the cuticle splits, very often the whole cuticle fractures so that the cortex is exposed (Fig. 25). The fracture of the cuticle is associated with a reduction of adhesion in the cuticle-cortex interface. This observation may have a bearing on styling and combing of hair.

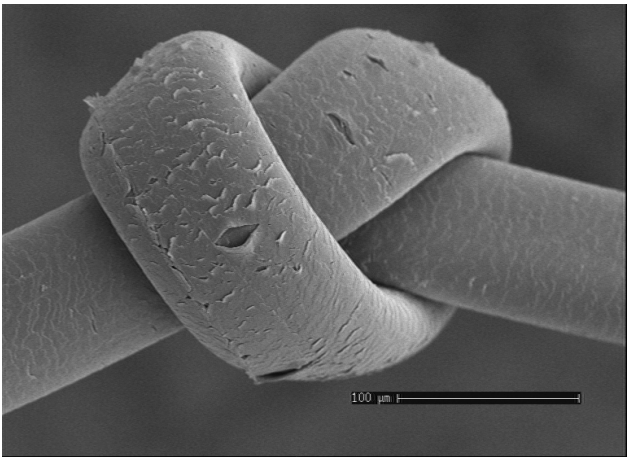


Fig. 24: Damaged Caucasian hair after a relaxer plus PW treatment.

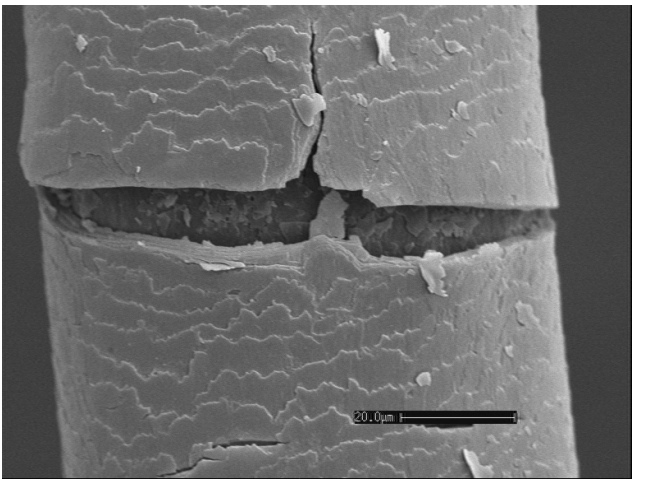


Fig. 25: Fracture of the cuticle of an Caucasian hair after a relaxing and subsequent perming treatment.

3.2.3.1.2. Gloss measurements

Determing hair gloss by measuring the intensity distribution of reflected laser light on individual hairs gives an extremely good correlation with the subjective perception of hair luster. Moreover, this technique is particularly effective due to high accuracy and reproducibility /32/.

When a light beam hits the surface of the hair, a first fraction of light S is specularely reflected (Fig. 26). A second, principal fraction of light D is diffusely scattered and reflected at and near the fiber surface, namely at surface roughnesses, at the various interfaces between the cuticle cell layers of human hair, the interface of cuticle and cortex, and at optical imperfections of the cortex, such as voids and inclusion /33/. If the surface of the hair is rough and uneven then the amount of specular reflection is low, and the scattered amount is correspondingly higher.

The definition of hair gloss (eq. 3.3) is based on the hypothesis that gloss results from the contrast between specular reflection and the overall light reflection (Fig. 27). A gloss index (G_L) is determined which corresponds to the percentage ratio of the specular reflections (S) to the total reflected light (S + D). A higher gloss index corresponds to glossier looking hair.

$$G_L = \frac{S}{S+D} 100\% \qquad (3.3)$$

Ten single hairs of a sample were measured at different positions. Fig. 28 shows the gloss index with a standard error of a 95 % level of significance for the treated Caucasian and Asian hair.

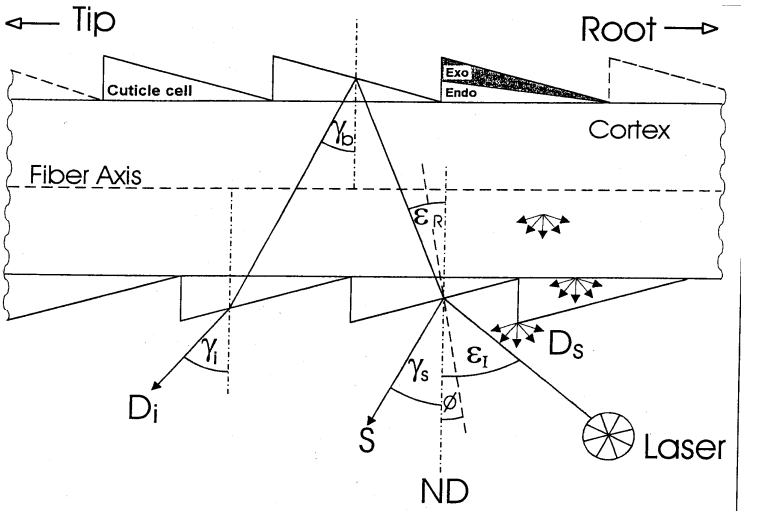


Fig. 26: Principles of light reflection and backward scattering on and in a hair fiber /34/.
S is specularly reflected light, D is diffusely scattered and reflected light, ND is the normal direction with respect to the fiber axis and in the horizontal plane.

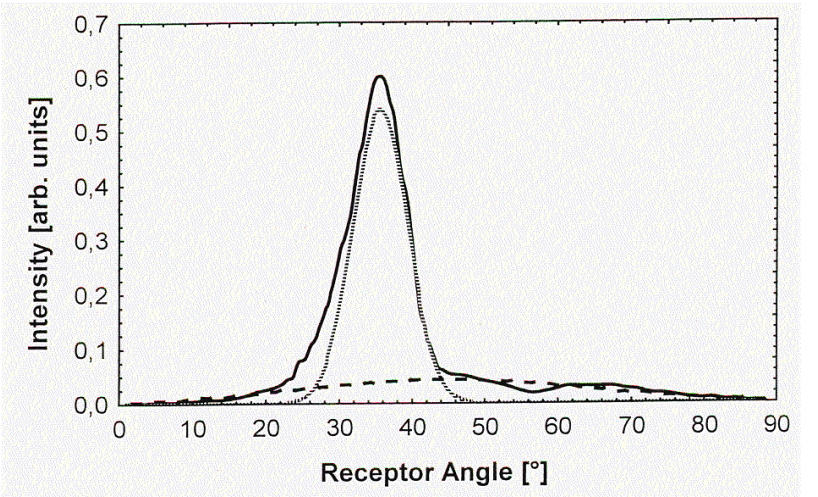


Fig. 27: Gonio-photometric-curve data (——) for an Asian hair /34/.
Distributions for specularly (····) and diffusely (---) reflected light, as fitted to the curve.

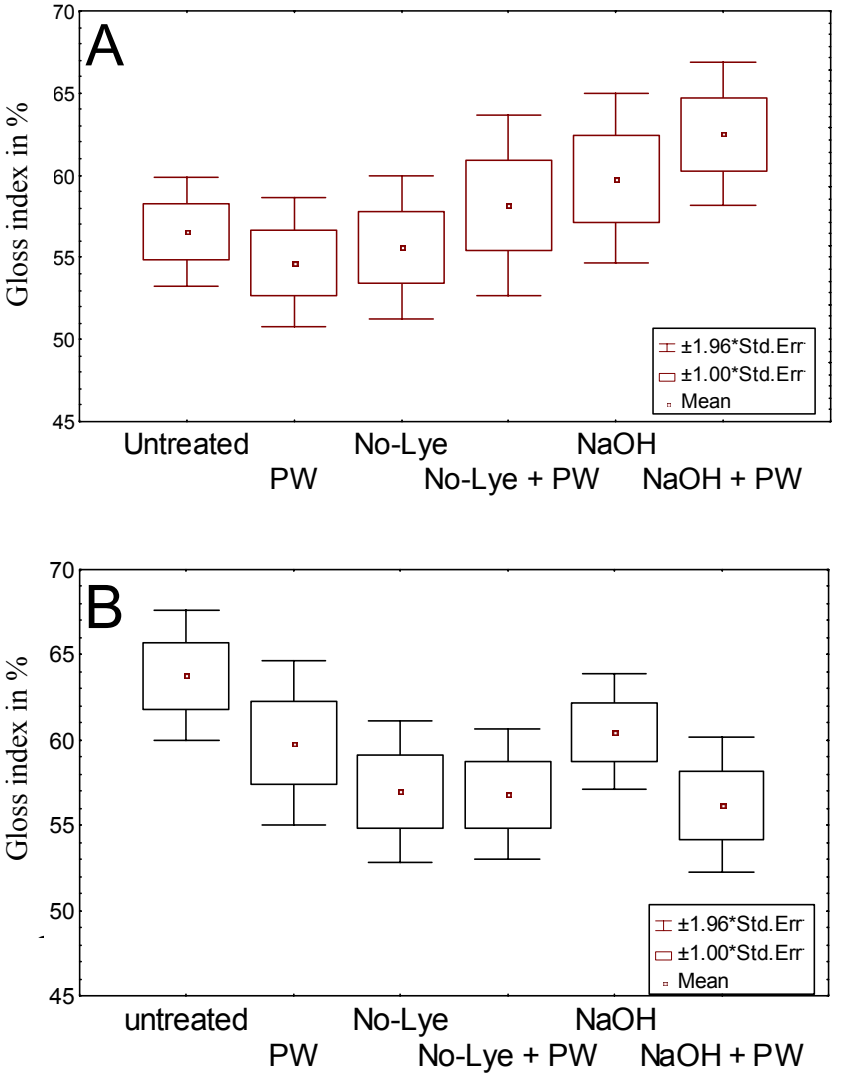


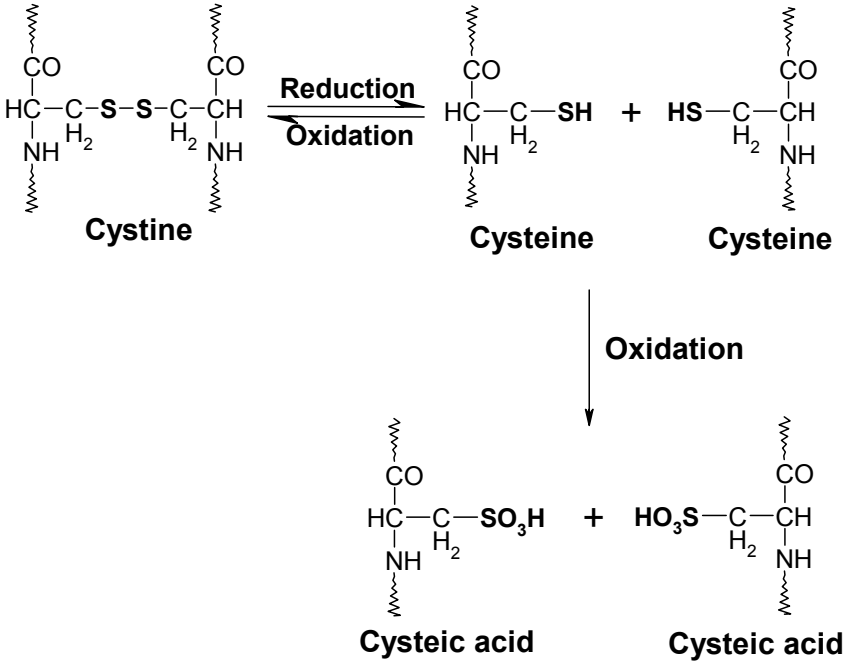
Fig. 28: Box & whisker plot of the gloss indices of Caucasian and Asian hair before and after different treatments.
A: Caucasian hair
B: Asian hair

The different treatments have no significant influence on the gloss of the hairs. As was shown by SEM, the roughness of the hair surface, assessed visually, does not change during the treatments. However, gloss is largely determined by the roughness of the hairs. Since the roughness does not change after treatment, the gloss index is not significantly altered, too. Therefore, the objective luster measurement corroborates the SEM investigations.

Since the different treatments according Tab. 4 on Caucasian and Asian hair showed no influence on the gloss of hair, it was assumed there no different gloss indexes would be found for simulated and natural Afro hair. Thus, it was decides to stand aside for cost's benefit to do measurements on these hair types.

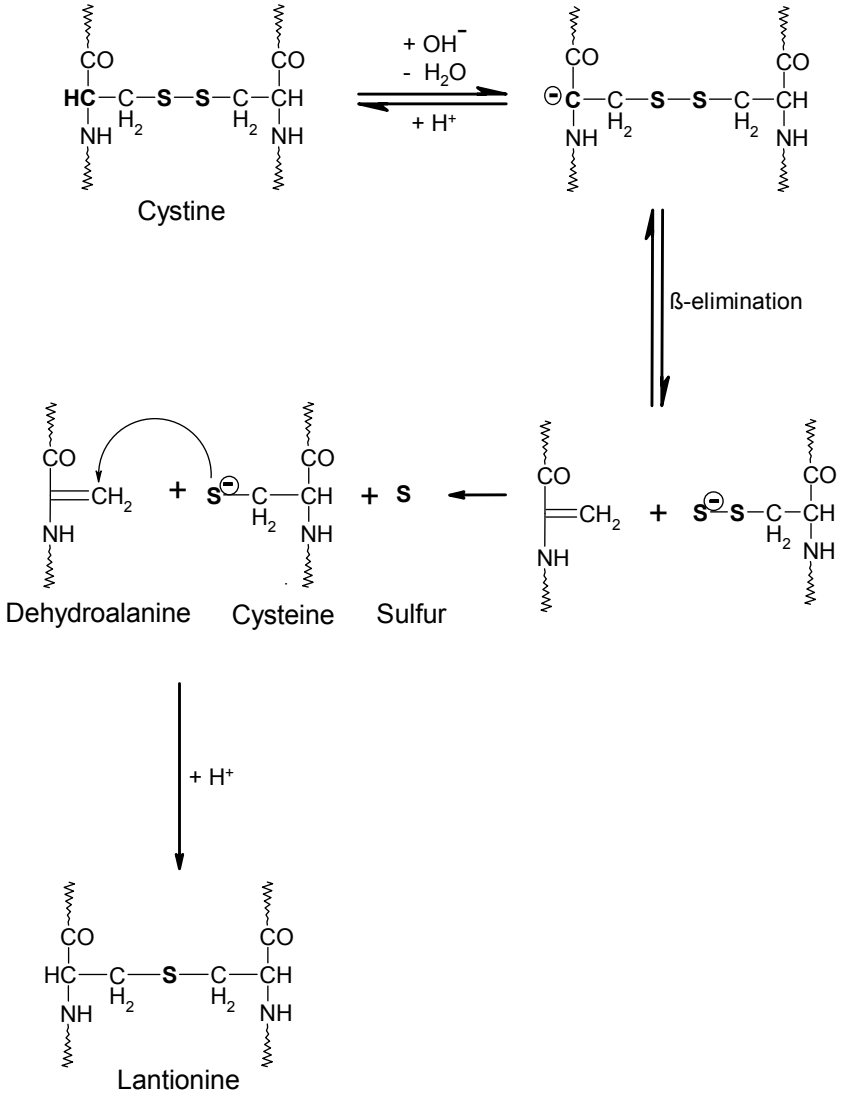
3.2.3.2. Amino acid composition

Dry hair consists of 90 to 95 % proteins. 19-20 different amino acids (AA) can be found after an acid hydrolysis /3/ according to the method of *Moore* and *Stein* /35/. A change in the amino acid composition indicates hair damage, especially changes of the amounts of cysteic acid ($CySO_3H$), cystine (CyS-SCy) and lanthionine (Lan). During hydrolysis the pre-existing cysteine (Cys) is oxidized to CyS-SCy. Damages caused by oxidizing agents (such as used during a permanent wave or bleaching treatment) are indicated by an increase in $CySO_3H$ /28/, originating from CyS-SCy. CyS-SCy in a protein chain is oxidized into two residues of $CySO_3H$ (Scheme 1).



Scheme 1: Formation of cysteic acid via cysteine from cystine during PW /24/.

Damages caused by an alkaline treatment (like a relaxing treatment) are detected by changes of Lan, which also originates from CyS-SCy (Scheme 2). In the first step, CyS-SCy reacts to form dehydroalanine residues and Cys in a β-elimination reaction. In a second step dehydroalanine and Cys are able to react with each other to form Lan /36, 37/. This reaction is supported by kinetic calculations /38/. One CyS-SCy residue can generate, at most, one Lan residue as shown in Scheme 2.

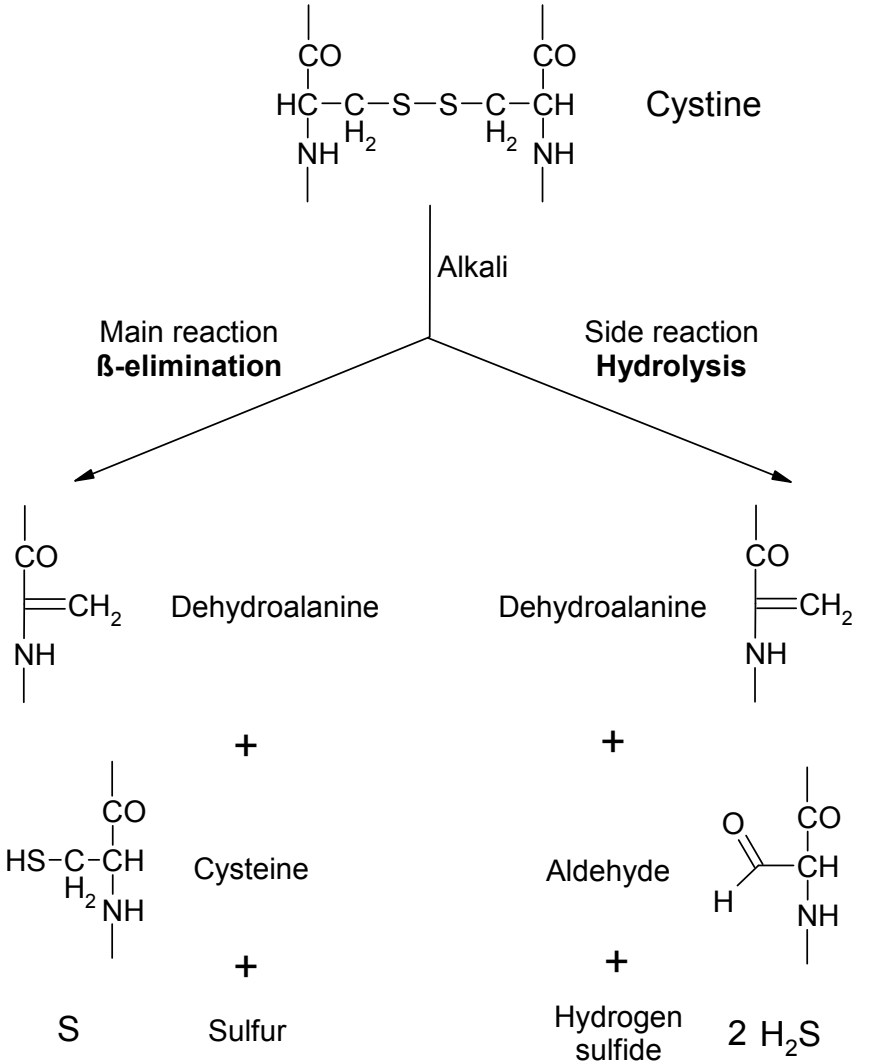


Scheme 2: Reaction of cystine into lanthione and sulfur caused by an alkali treatment /45, 46/.

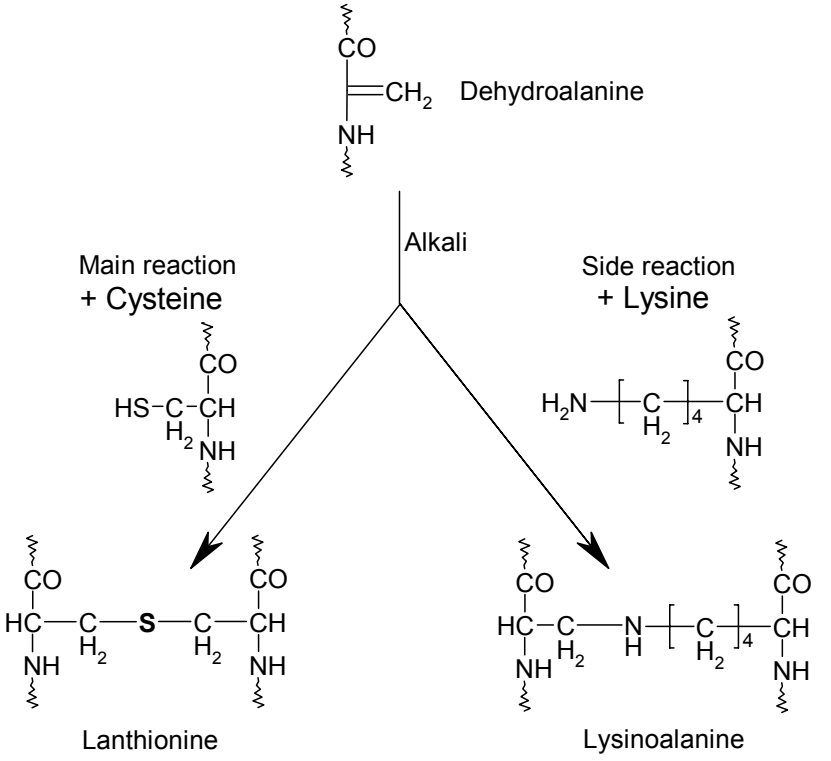
Besides this main reaction, side reactions occur during an alkali treatment. The most important reactions are the hydrolysis of CyS-SCy /39, 40/ as shown in Scheme 3 and the formation of lysinoalanine (LysAla) /30, 41, 42, 43/ as shown in Scheme 4.

Hydrolysis of CyS-SCy produces dehydroalanine, together with an aldehyde and hydrogen sulfide (Scheme 3). This reaction competes with the β-elimination, which produces dehydroalanine, sulfur, and Cys. The side reaction is mainly initiated after long treatment times (ca. 30 min) /44/.

The formation of LysAla competes with the formation of Lan (Scheme 4). LysAla is formed by reaction of dehydroalanine with lysine (Lys) which is also found in hair keratin. Dehydroalanine cannot be determined by amino acid analysis (AAA) because it decomposes during the acid treatment of AAA. LysAla is formed just in small quantities. Since it has an experimentally absolutely unacceptable retention time in AAA, it was not analysed for the current investigations. The results for the most relevant AAs - such as CyS-SCy, Lan, and $CySO_3H$ - are given in Fig. 29-30 (CyS-SCy), 31-32 (Lan) and 33-34 ($CySO_3H$).



Scheme 3: Competing reactions of cystine with alkali.
Main reaction: β-elimination
Side reaction: Hydrolysis



Scheme 4: Reaction of dehydroalanine with different AA residues during alkaline treatment.
Main reaction: With cysteine into lanthionine
Side reaction: With lysine into lysinoalanine

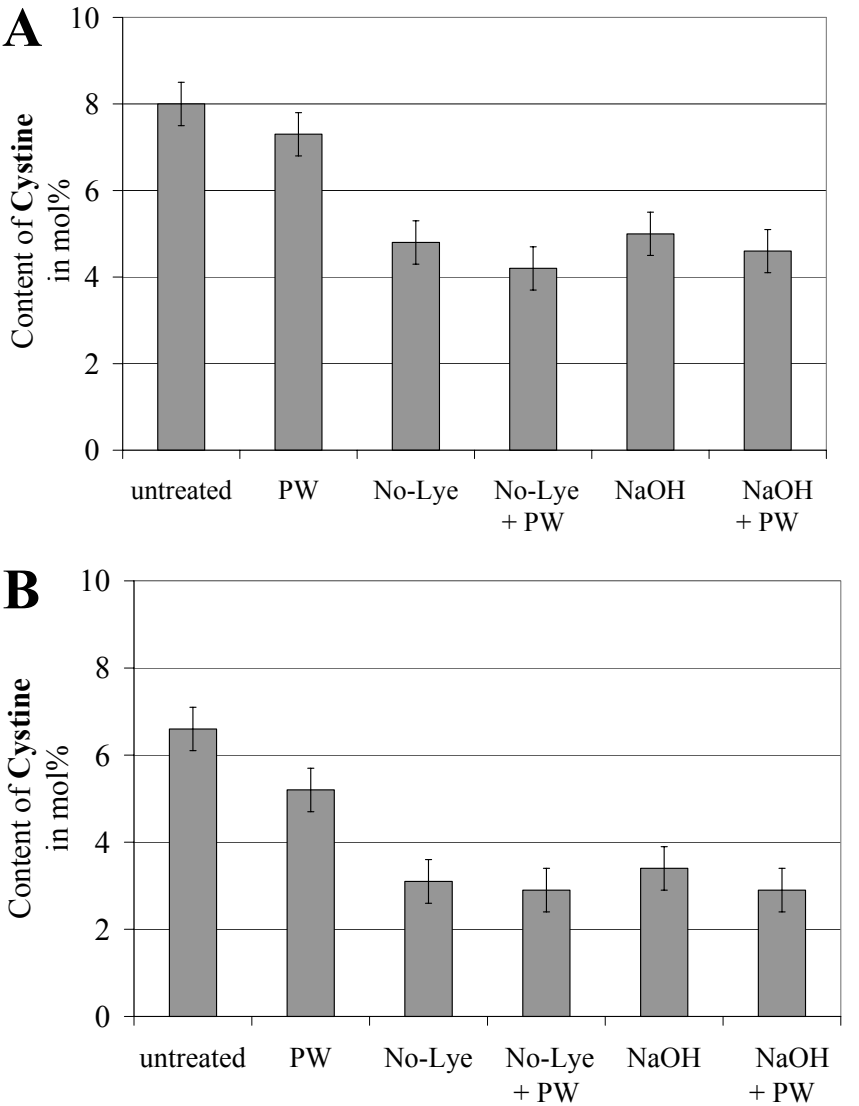


Fig. 29: Content of cystine in natural and simulated Afro hair samples

The error bars represent the maximum and minimum value for this AA.
A: Natural Afro hair
B: Simulated Afro hair

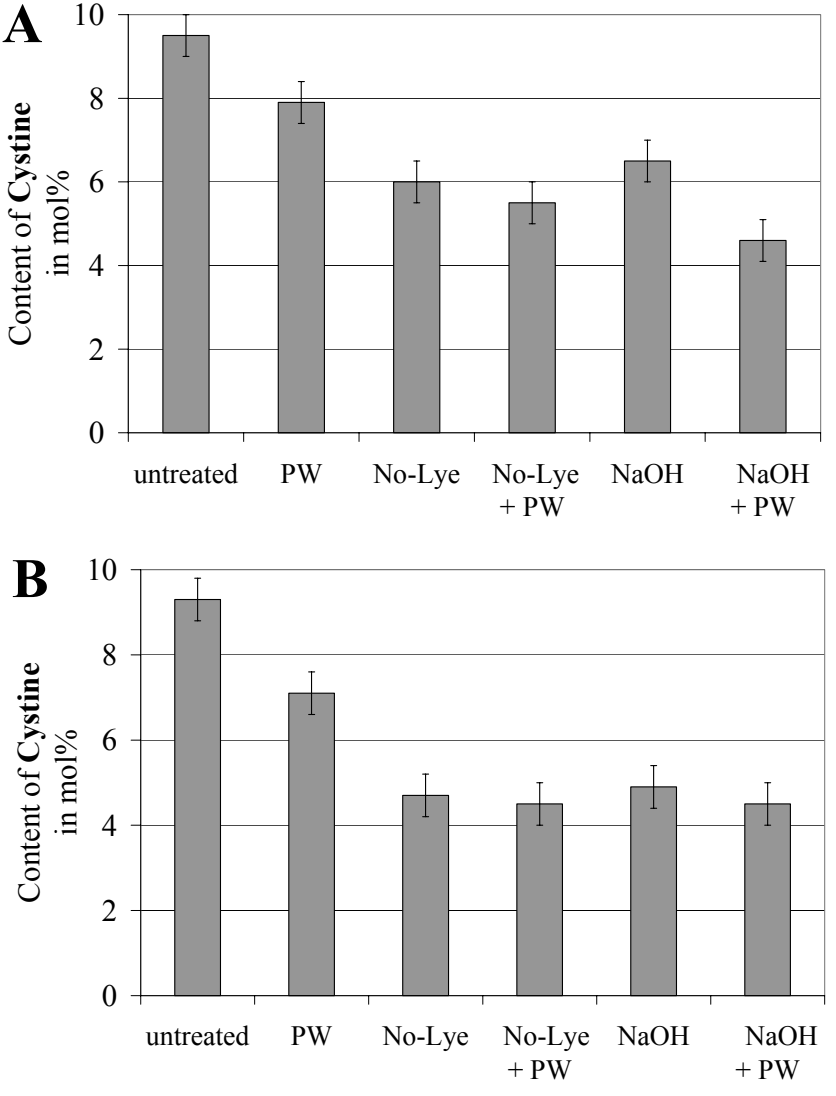


Fig. 30: Content of cystine in Caucasian and Asian hair sample.
The error bars represent the maximum and minimum value for this AA.
A: Caucasian hair
B: Asian hair

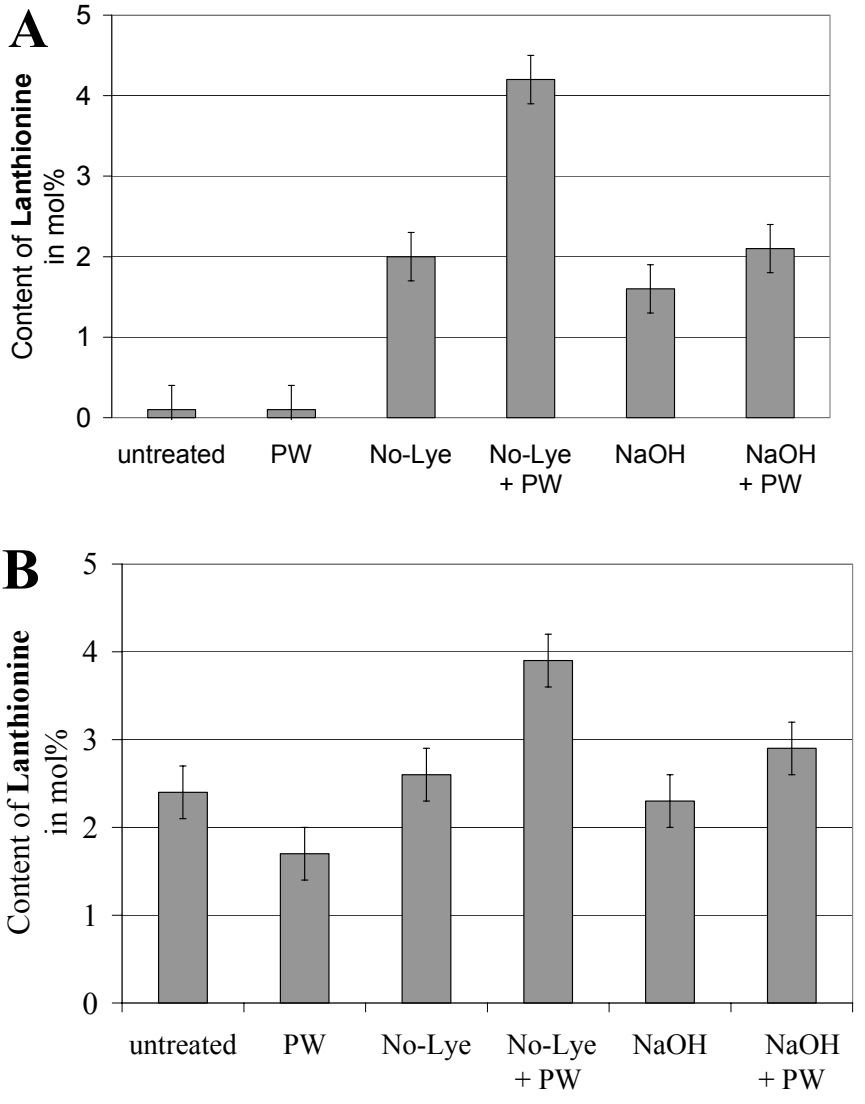


Fig. 31: Content of lanthionine in natural and simulated Afro hair samples
The error bars represent the maximum and minimum value for this AA.
A: Natural Afro hair
B: Simulated Afro hair

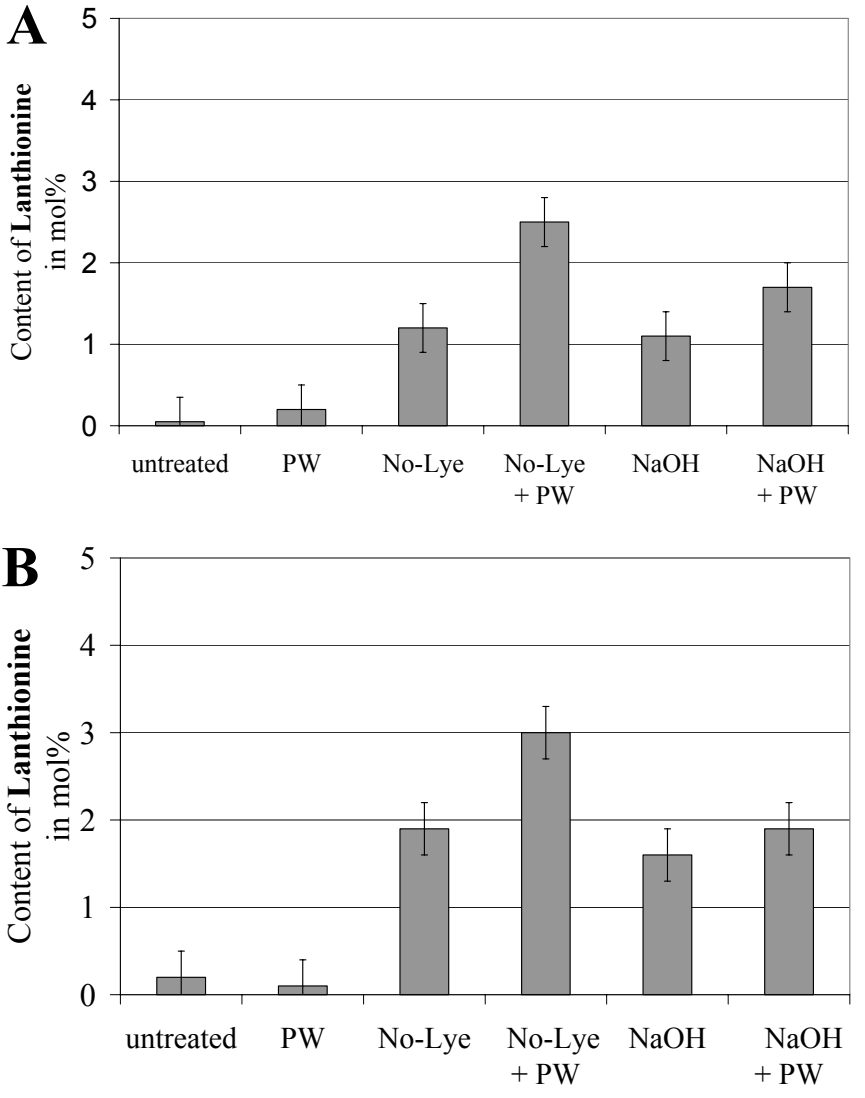


Fig. 32: Content of lanthionine in Caucasian and Asian hair samples
The error bars represent the maximum and minimum value for this AA.
A: Caucasian hair
B: Asian hair

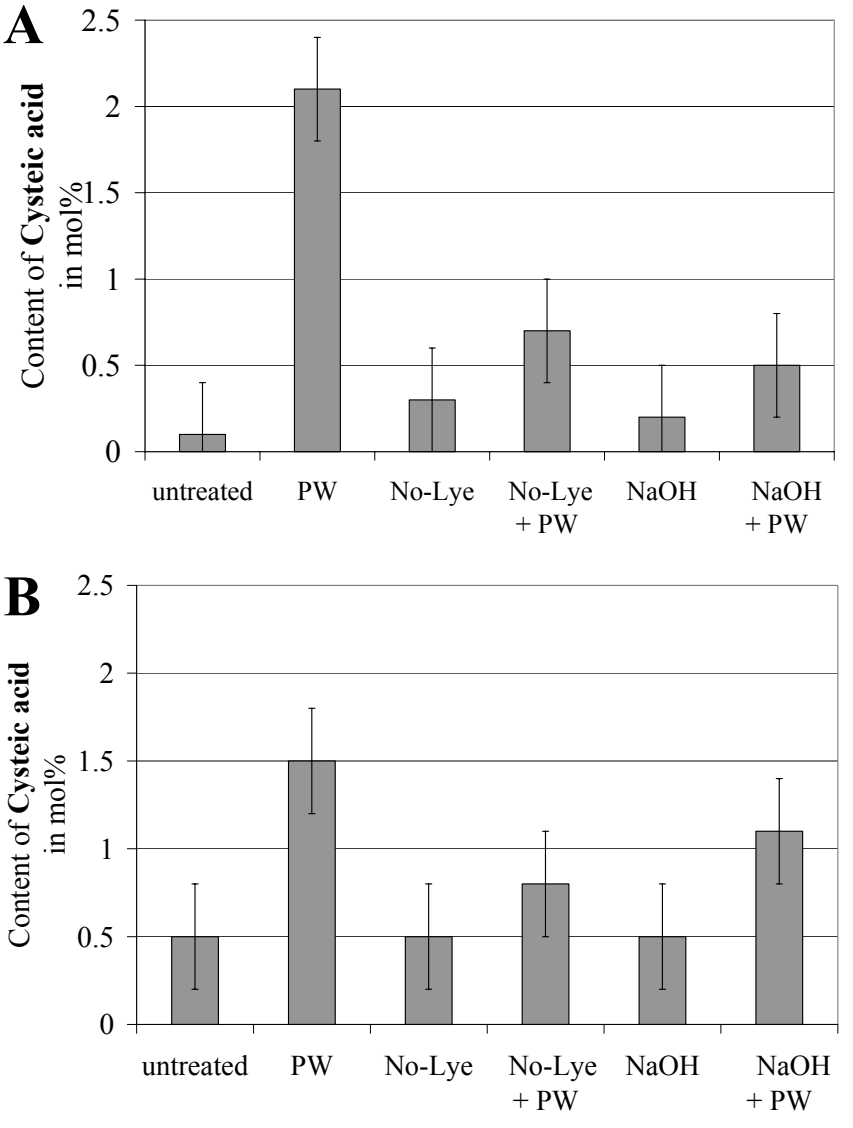


Fig. 33: Content of cysteic acid in natural and simulated Afro hair samples
The error bars represent the maximum and minimum value for this AA.
A: Natural Afro hair
B: Simulated Afro hair

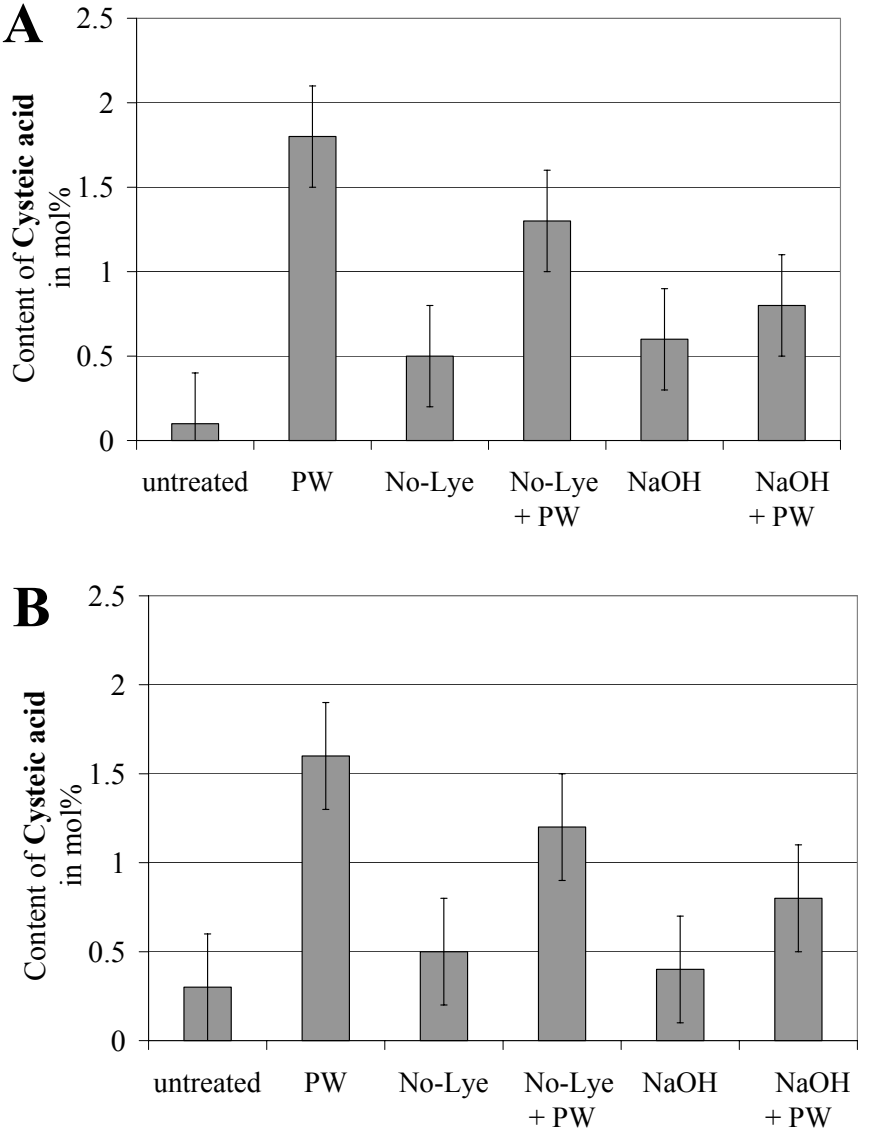


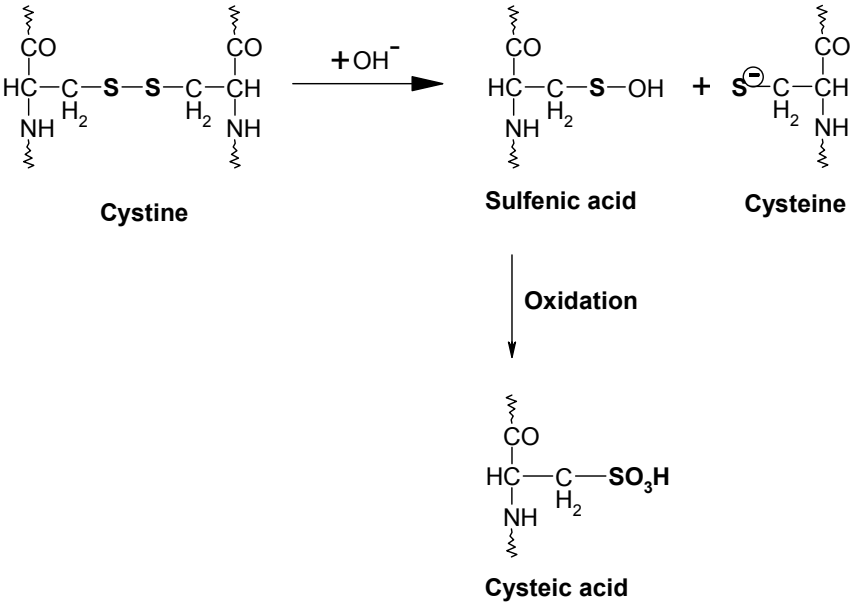
Fig. 34: Content of cysteic acid in Caucasian and Asian hair samples
The error bars represent the maximum and minimum value for this AA.
A: Caucasian hair
B: Asian hair

The amino acid content of the untreated natural hair samples corresponds to literature values /47/. Only the simulated Afro hair (crimped Asian hair /29/) shows differences in the AA compostion. In comparison to the Asian hair the CyS-SCy content is low (30 % less), and the Lan content is significantly higher (up to 20 times higher than in the other samples). These differences are attributed to the steam treatment to crimp the hair /30/.

As expected, the CyS-SCy content (Figs. 29-30) of the various ethnic hair samples (natural Afro, Asian, and Caucasian hair) decreases after waving (PW) by about 0.5 - 1.5 mol%. This is a significant change because the error of this method is ± 0.3 mol%. Some CyS-SCy is oxidized into $CySO_3H$ (via Cys or cystine oxides), as verified through amino acid analysis (AAA) (Figs. 33-34). The $CySO_3H$ content increases by about 1.0 – 2.0 mol%. This is a surprisingly large rise in $CySO_3H$. This effect might be due to an excess of peroxide, either resulting from the liquor to hair ratio used or from the long time of exposure. Additional Lan (Figs. 31-32) is not generated because the reducing solution is not sufficiently alkaline (pH 8.8). The simulated Afro hair behaves chemically as the other hair samples during PW.

The relaxer creams (No-Lye and NaOH) have an entirely different influence on the AA content than the perming treatment. As a result of the high alkalinity of the creams (pH 12.9 and 12.5) a high proportion of CyS-SCy reacts to form dehydroalanine and Cys. This Cys and dehydroalanine may subsequently form Lan. The natural hair samples lose between 40 to 50 % of their original disulfide bridges (corresponding to 3 – 4 mol% of CyS-SCy) as seen in Figs. 29-30. The newly formed Lan stabilizes the hair by the formation of intermolecular sulfide bridges. Around 1.5 - 2.0 mol% of Lan are generated. Because just half of the lost CyS-SCy reappears in the AA composition as Lan, one may assume that the other half reacts either with Lys to form LysAla, or the formation of Lan does not proceed to completion and the reaction remains at the stage of dehydro-alanine formation.

A small increase in $CySO_3H$ (+ 0.2 - 0.4 mol%) is measured after the relaxing process for all types of hair. Beside the other mentioned reaction, alkali reacts with CyS-SCy to form small amounts of sulfenic acid which is easily oxidized by air during the AAA to form $CySO_3H$ (Scheme 5).



Scheme 5: Side reaction of Cys into sulfenic acid and Cys caused by an alkali treatment /45, 46/.

The simulated Afro hair behaves differently during the relaxing treatment compared to the other hair samples. Although the CyS-SCy content decreases by around 60 % (from 6.8 mol% to 2.9 mol%) no additional Lan is detected. The explanation is the same as above. After cleavage of the CyS-SCy S-S-bonds by the alkali, the polypeptide chains are no longer fixed in their position and the reacting partners may drift apart. The chain residues are not able to react with each other. They either remain as dehydroalanine and Cys or react with another partner (like dehydroalanine with Lys to form LysAla).

A subsequent perming treatment of relaxed hair (No-Lye + PW, NaOH + PW) leads to a small decrease of the CyS-SCy content compared to just relaxed hair (decrease of 0.2 - 0.5 mol% CyS-SCy). This decrease corresponds approximately to the loss of CyS-SCy during perming of untreated hair. In contrast to the CyS-SCy, the Lan content nearly doubles (rise from 1.5 - 2.0 mol% up to 2.5 - 3.2 mol%), particularly in the case of the No-Lye + PW treated hair. The difference between the two relaxer creams relates to their different pH values. The increase in Lan can be explained as follows. During the reduction step of the waving treatment a high amount of Cys is formed. If one assumes that the hypothesis of the remaining dehydroalanine during the relaxing process is correct, this remaining dehydroalanine reacts now with the large supply of Cys formed during the waving process to produce Lan. The amount of $CySO_3H$ of relaxed hair increases after a perming treatment. But the increase is not as high as in the case of perming untreated hair. This can be attributed to the lower amount of CyS-SCy which is available during the reduction step of perming. During relaxing, part of the CyS-SCy has already reacted to form Lan. Thus, less $CySO_3H$ can be formed.

The relaxed and subsequently waved simulated Afro hair shows a similar change in the AA composition. However, because of its pre-history of steaming, the level of CyS-SCy is lower, Lan higher.

Overall, the different ethnic hair samples respond to the treatments in very similar ways. The natural Afro hair reacts more strongly to the treatments. This can be reasonably explained by its smaller diameter (55 µm) compared to the Asian (82 µm) and Caucasian hair (76 µm). The reagents may penetrate the fiber faster, therefore, the concentration of chemicals is higher in the fiber and the damage of the fiber is stronger.

3.2.3.3. Thermal properties

The method of thermal analysis contains a multiplicity of analytic techniques to measure the properties of materials or their changes with temperature and time /48/. By using these methods many physical processes, like crystallization, melting, desorption or phase conversions and many chemical reactions may be investigated /49/.

Calorimetry in which a sample is subjected to a temperature-time-power-compensated-program is called scanning calorimetry. Differential calorimetry measures two systems simultaneously. One system contains the test sample**,** the other system the reference sample. Both samples are heated simultaneously. The difference in power, $\Delta\Phi$, is measured between the heating element in contact with the test sample and the heating element in contact with the reference sample. The amount of heat absorbed or released is determined by integration of the heat flow difference curve over time, and corresponds to the peak area (Fig. 35) /50/.

Keratin fibers - like hair – are composite systems, which are physically and chemically heterogeneous. From the point of view of elastics properties the important difference in these compounds is based on their α-helical domains or amorphous (matrix) character. This has lead to the two-phase filament-matrix model for keratin fibers, originally proposed by *Feughelman* /51/. *Spei* et al. /52/ used conventional DSC to investigate the melting behavior of different, dried keratins. The endothermic “melting peak” at 240 °C signifies the change from α-helix into an unordered structure. *Crighton and Hole* /53/ developed a special form of high-pressure differential thermoanalysis (HP-DTA) to determine the denaturation of α-keratins in water. Thereby “the melting point” shifts to a temperature range between 130 and 155 °C.

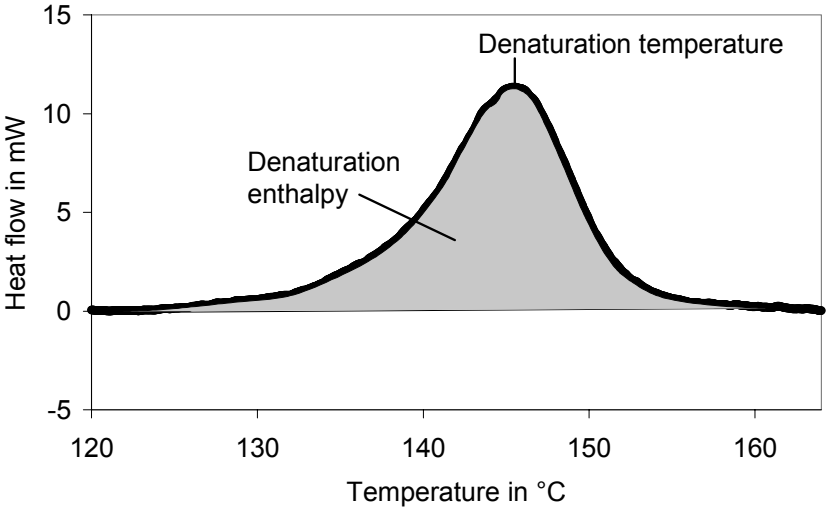


Fig. 35: HP-DSC diagram (black line) of relaxed Caucasian hair in water. The highest point of the black curve represents the denaturation temperature, the grey shaded area beneath the curve (integration of the heat flow curve over time) the denaturation enthalpy.

In this work a combination of both methods has been used in so-called High-Pressure Differential Scanning Calorimetry (HP-DSC). For this commercial, pressure resistant capsules are used for the measurement of the hair moistened by water /54/. Denaturation enthalpy and temperature for the different treated hair types are given in Figs. 36-37 (enthalpy) and 38-39 (temperature).

Denaturation enthalpy represents the helical content of hair. The greater the denaturation enthalpy, the higher is the helical content of hair. Denaturation temperature reflects the influence of treatments to the matrix of hair.

The denaturation enthalpy of the investigated untreated hair is between 18 and 22 J/g (Figs. 36-37). Caucasian hair has the highest, and simulated Afro hair the lowest value for denaturation enthalpy. A reduction in enthalpy of between 2 and 5 J/g is measured after perming for all treatments, corresponding to a reduction in the helical content.

Both relaxer treatments (NaOH and No-Lye) lead to a stronger decrease of enthalpy than just the perming treatment. But, whereas Caucasian and Asian hair retain more than 60 % of their initial helical content, natural and simulated Afro hair lose around 60 %. No-Lye treatment causes a stronger reduction of denaturation enthalpy than NaOH treatment. This can be traced back to the higher pH of No-Lye cream and thus higher concentration of the active ingredient, the alkali.

When relaxed hair is subsequently permed, the denaturation enthalpy decreases again. Especially for natural and simulated Afro hair a very low helical content is detected after No-Lye + PW treatment. Caucasian and Asian hair still retain around 50 % of their initial helical content after this treatment.

Generally, Caucasian and Asian hair are less affected by relaxer creams than the two Afro hair samples. The reasons are the same as mentioned in the previous chapter. Because of its small diameter, the natural Afro hair may be more rapidly penetrated by alkali, and thus a greater damage of this sample occurs. The simulated Afro hair is more affected by the alkali because of its pre-damage by steaming.

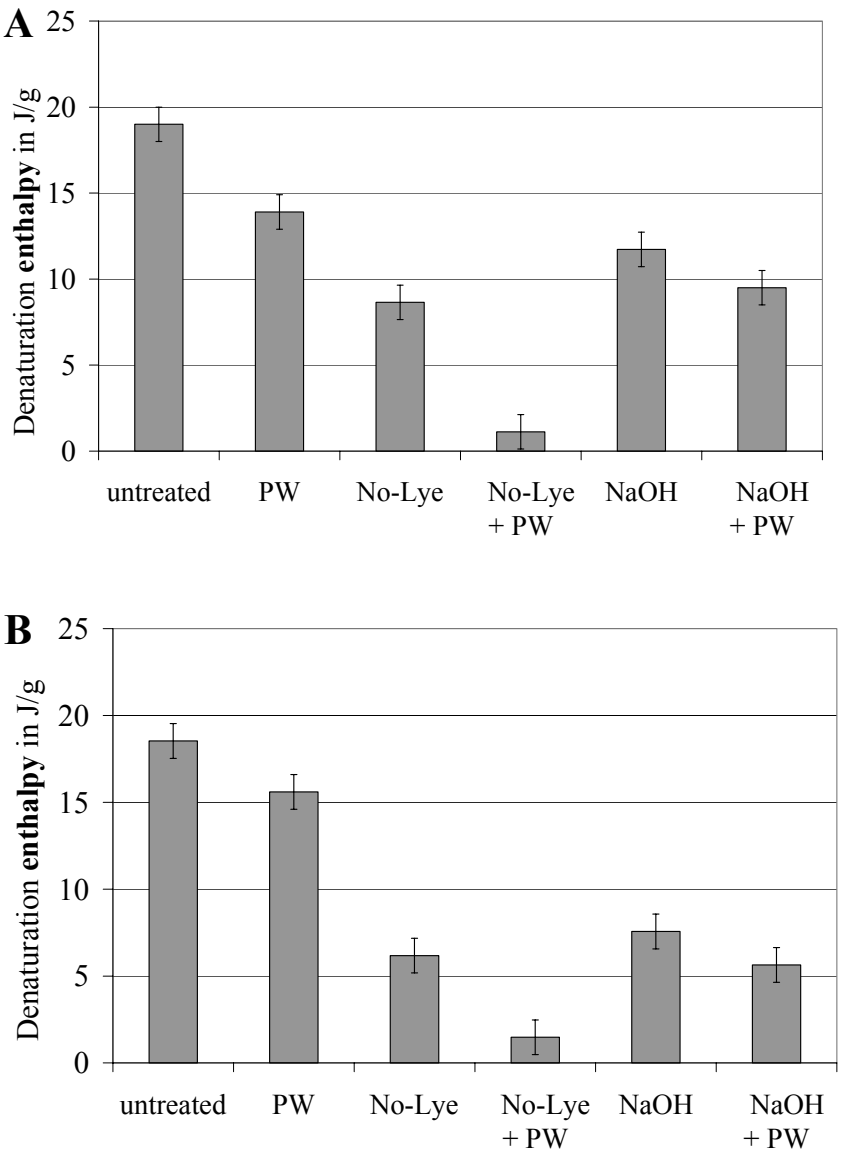


Fig. 36: Denaturation enthalpy of natural and simulated Afro hair samples
The error bars represent the standard deviation (n = 5).
A: Natural Afro hair
B: Simulated Afro hair

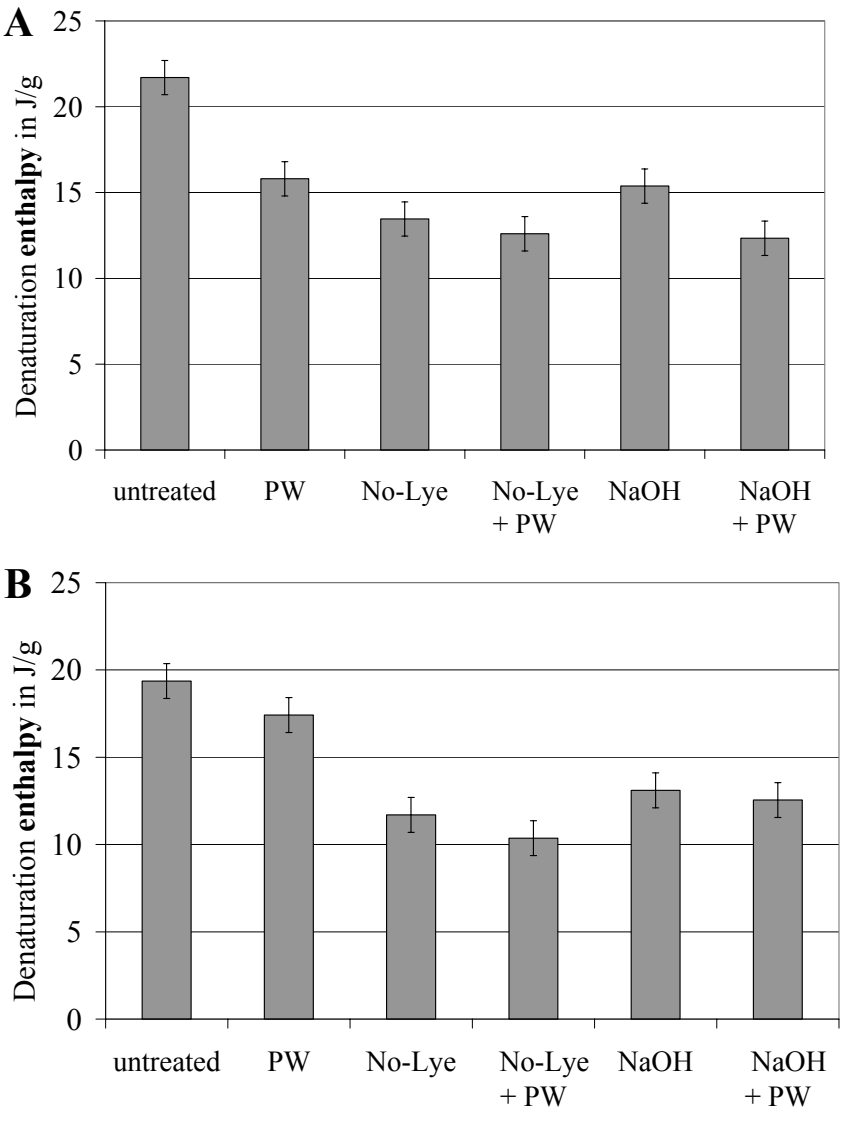


Fig. 37: Denaturation enthalpy of Caucasian and Asian hair samples
The error bars represent the standard deviation (n = 5).
A: Caucasian hair
B: Asian hair

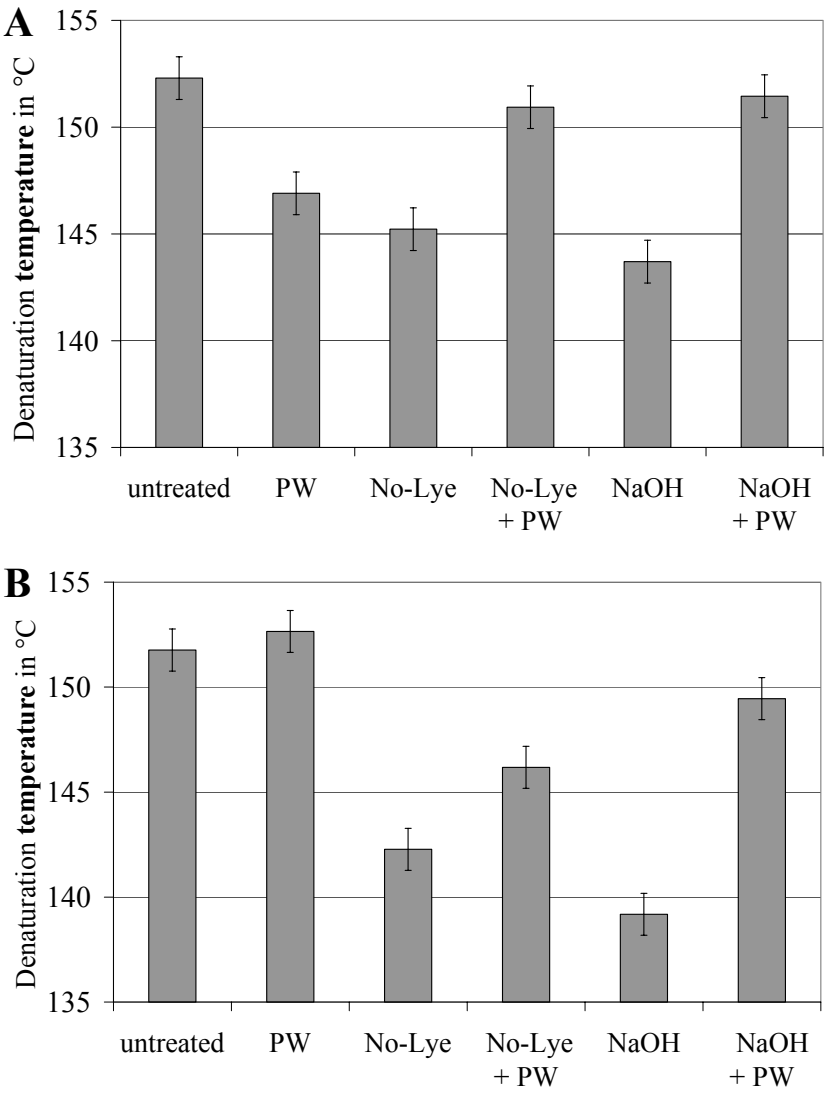


Fig. 38: Denaturation temperature of natural and simulated Afro hair samples
The error bars represent the standard deviation (n = 5).
A: Natural Afro hair
B: Simulated Afro hair

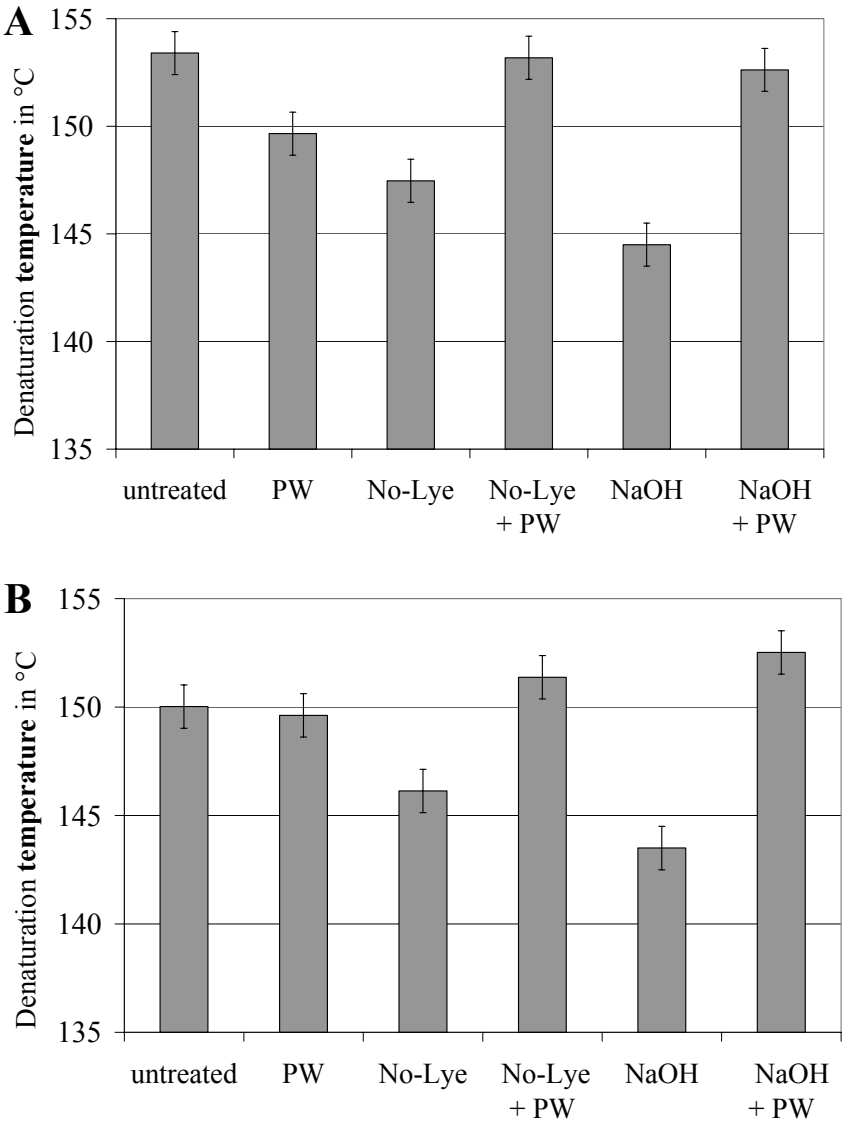


Fig. 39: Denaturation temperature of Caucasian and Asian hair samples
The error bars represent the standard deviation (n = 5).
A: Caucasian hair
B: Asian hair

A comparison of denaturation enthalpy and CyS-SCy content for the same treatments shows that the treatments have different strong effects on each of these properties. For example, Asian hair loses about 60 % of its initial helical content after the relaxing treatments, whereas the CyS-SCy content decreases by approximately 50 % for the same treatments. For both types of Afro hair just a small residual denaturation enthalpy is detected after the No-Lye + PW treatment (~ 10 % of the initial value). In contrast, the CyS-SCy content for both hair types is around 50 % of the initial content for this treatment. Thus, loss of helical structure is not directly related to degradation of CyS-SCy.

The denaturation temperature of untreated hair is found to be around 152 ± 2 °C for all hair types (Figs. 38-39). A lower temperature is detected for natural Afro hair and Caucasian hair after perming treatment of virgin hair (~ 4 °C), whereas denaturation temperature of Asian and simulated Afro (steamed, crimped Asian) hair is largely unchanged after the perming treatment. This indicates a higher resistance of Asian hair against reduction agents or a higher reforming of disulfide bridges in the matrix.

All hair types show a significant decrease in the denaturation temperature after the relaxing treatments. Since the denaturation temperature is a measure of the condition of the hair matrix, the matrix around the helical domains is altered, becoming weaker after relaxing treatments. The matrix proteins (KAPs) contain the highest concentration of disulfide bonds in hair, most of which are probably intrachain bonds /11/. The decreases of denaturation temperature (Figs. 38-39) and of CyS-SCy content (Figs. 29-30) suggest that the KAPs are strongly affected by the relaxing treatment.

When the relaxed hair is subsequently permed, a significant increase in the denaturation temperature is observed for all hair types. The denaturation

temperature for all natural hair samples reaches approximately the same value as for untreated hair. Thus, a strengthening of the matrix is effected by perming treatment. This could be attributed to the formation of Lan as described in the prior chapter. The additional bridges within the protein fibers support the protein fibers of the matrix.

The denaturation temperature of simulated Afro hair also increases after perming of relaxed hair. However, the measured temperature does not reach the value of “untreated” simulated Afro hair as the natural hair samples. The AAA has shown that “untreated” simulated Afro hair already has a chemically changed initial state compared to the natural hair samples. The CyS-SCy content is lower and the Lan content is much higher. Lan is a chemical end product which does not react anymore. Since less amount of CyS-SCy is available, simulated Afro hair cannot form new bonds to the same extent as the natural hair samples. Therefore, the denaturation temperature after relaxing and subsequent perming treatment is not as high as for its “untreated” state.

3.3. Evaluation of hair straightening efficacy

One of the most important quality aspects of relaxer creams is at what speed the product decurls hair. Apart from tests in hair saloons on hair *in vivo*, tests are made with curly hair in the laboratory. As a rule hair for such tests is purchased from companies who trade in hair. But these companies usually only sell so-called "Afro Hair – Natural Hair Kinked". This hair is obviously Asian hair, curled by steam as discussed above. Natural Afro hair is very difficult to obtain, most often not through commercial but rather through private sources. In what follows a comparison will be made between this simulated "Afro" hair and natural Afro hair with respect to their decurling behavior, as well as the influence of different relaxer creams on natural Afro hair.

3.3.1. Relaxer creams

In this work two commercial relaxer creams were used. These creams contain different ingredients to reach the high pH levels:

- pH 12.5, relaxer cream with sodium hydroxide (NaOH = lye) as alkali (in what follows referred to as "NaOH relaxer cream")
- pH 12.9, relaxer cream with calcium hydroxide and guanidine carbonate as alkali (No-Lye relaxer cream)

3.3.2. Treatments

The two different relaxer creams are used for the comparison of the simulated versus the natural Afro hair. In addition to the classical relaxer creams (NaOH and No-Lye), a combination of relaxer cream plus a small amount of thiole (thioglycolic acid or cysteine) was investigated. Thioglycolic acid (TGA) is the typical commercial reduction reagent for a permanent wave treatment. It is responsible for the cleavage of the disulfide bridges (Fig. 10). Cysteine (Cys) is also able to cleave disulfide bonds but it is easily oxidized by air oxygen in

aqueous alkaline solutions to form CyS-SCy /55/. The influences of these two thioles on the relaxing process are studied for natural Afro hair. The following list gives the investigated treatments and their abbreviations used in this work.

- NaOH
  Relaxing treatment with NaOH relaxer cream, pH 12.5
- NaOH + TGA
  Relaxing treatment with NaOH relaxer cream + 1 % w/w TGA, pH 12.5
- NaOH + Cys
  Relaxing treatment with NaOH relaxer cream + 1 % w/w L-Cysteine, pH 12.5
- No-Lye
  Relaxing treatment with No-Lye relaxer cream, pH 12.9
- No-Lye + TGA
  Relaxing treatment with No-Lye relaxer cream + 1 % w/w TGA, pH 12.9
- No-Lye + Cys
  Relaxing treatment with No-Lye relaxer cream + 1 % w/w L-Cysteine, pH 12.9

3.3.3. Procedure for single hair relaxing

To determine the extent of decurling caused by relaxer creams, a suitable test method of practical relevance had to be developed. Since only small amounts of each natural Afro hair sample were available, the new test had to be based on single hairs. The following procedure was found to be suitable.

Single hairs are mechanically straightened and fixed on a glass plate. They are then treated with a relaxer cream. After treatment the hair is removed from the plate and wetted in warm water in its unfixed state. As a result of wetting the influence of secondary cross-links (salt linkages and hydrogen bonds) is removed and the permanent component of straightening is observed. Finally the

effective length (l_a) of the dried hair is determined (Fig. 40). To determine the initial length (l_b) the hair is not treated but only wetted and dried. The length between the fixations represents the true length of the hair fiber (L).

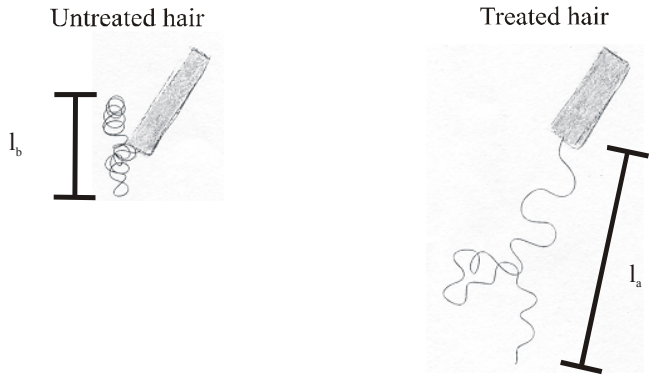


Fig 40: Determination of the effective length of a single hair fiber before (l_b) as well as after treatment (l_a).
The hair is fixed on one end.

The effect of straightening, described by the variable $E_{St}(t)$ in %, is calculated as the ratio of the difference between the effective length after treatment of a hair fiber (l_a) and the effective length of an untreated hair fiber (l_b) to the difference between the true length of the hair fiber (L) and the effective length of an untreated hair fiber (l_b) as given in eq. 3.4.

$$E_{St}(t) = \frac{l_a - l_b}{L - l_b} 100\,\% \qquad (3.4)$$

3.3.4. Results of single hair relaxing

3.3.4.1. Comparison of simulated and natural Afro hair

To photograph the treated hair, two examples are illustrated in Fig. 41. The photos show simulated and natural Afro hair that were treated 3 and 15 min with NaOH, after the final wetting. The difference between these two hair samples is clearly visible. At all times simulated Afro hair is more effectively straightened than natural Afro hair. The results for the straightening effect with treatment time are summarized in Fig. 42 for both the NaOH and the No-Lye treatment.

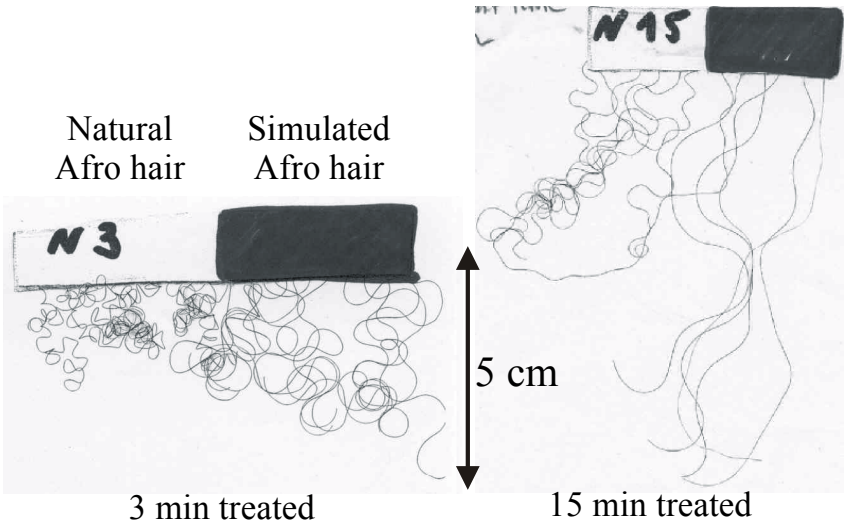


Fig. 41: Photos of simulated and natural Afro hair treated for 3 min and 15min, respectively, with NaOH.

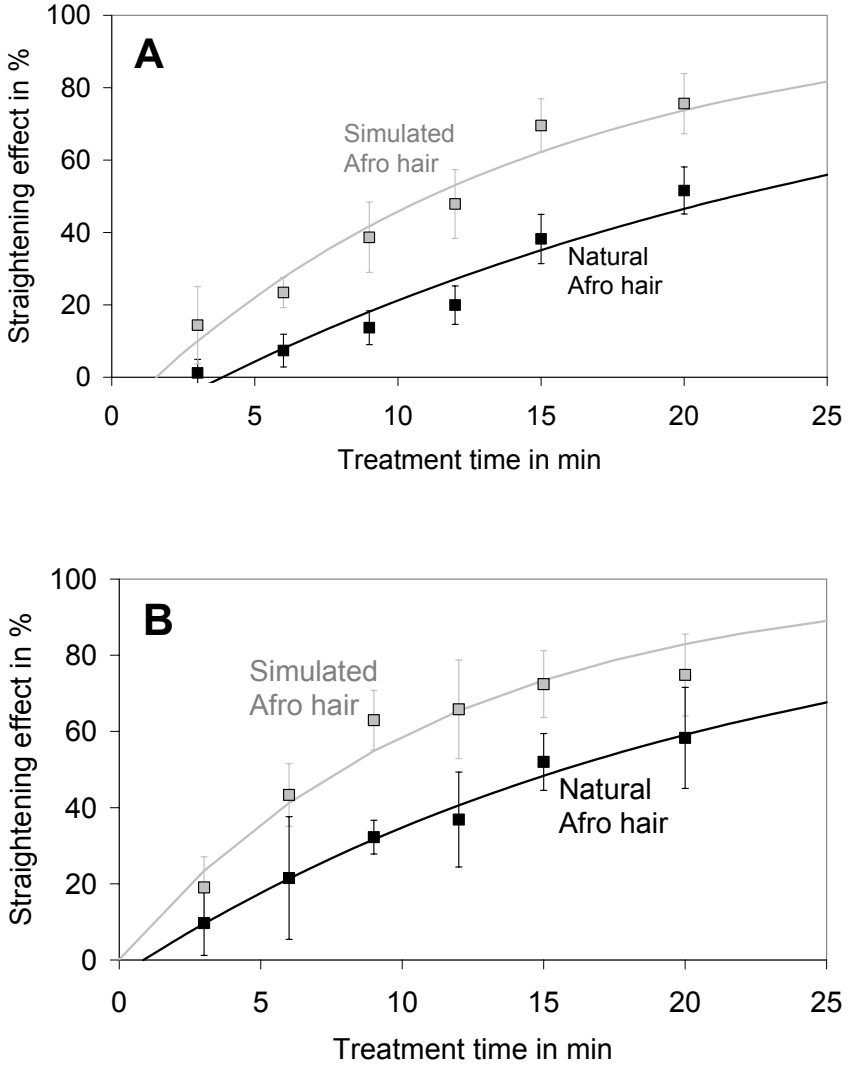


Fig 42: The straightening effect of the relaxer creams (NaOH and No-Lye) for simulated and natural Afro hair versus treatment time. The error bars represent the standard deviation (n = 8).
A: NaOH treatment
B: No-Lye treatment

As expected, the straightening effect increases with relaxing time.

The experimental data for the dependence of straightening on treatment time have been fitted by eq. 3.5, which corresponds to a pseudo first-order kinetics function. The equation contains a theoretical time lag, Δt, to take into consideration that the straightening effect E_{St} shows an induction period, in that the straightening is very slow at the beginning of the treatment. This time lag can be explained by the structure of the hair. Initially, the alkali has to pass the cuticle, a natural barrier of the hair.

$$E_{St}(t) = E_{St}^{\max}\left(1 - e^{-\left(\frac{t-\Delta t}{\tau}\right)}\right) \tag{3.5}$$

Parameter $E_{St}^{\max}$ stands for the maximum value the straightening effect can reach. Values greater than 100 % would mean a lengthening of the hair fiber. Assuming from the inspection of Fig. 42 that straightening always approaches completeness (100 %), eq. 3.5 simplifies:

$$E_{St}(t) = \left(1 - e^{-\left(\frac{t-\Delta t}{\tau}\right)}\right)100\% \tag{3.6}$$

Longer treatment times lead to dissolution of hair fibers so that the theoretical straightening effect of 100 % cannot be checked practically.

τ is called the characteristic straightening time. The smaller τ, the faster the straightening takes place. When (t-Δt) is equal to τ, the effect of straightening has reached a value of (1-1/e), which is 63.2 %.

Tab. 5 gives an account of the calculated parameters which are fitted to the experimental obtained data of the straightening experiments with natural and

simulated Afro hair using eq. 3.6. The smaller the characteristic straightening time, τ, the faster the straightening occurs.

Tab. 5: Calculated time lag, Δt, characteristic straightening time, τ ($\pm$ standard error) of the fitted curves for simulated and natural Afro hair (n = 8) using eq. 3.6.

Type of hair	Treatment	Δt in min	τ in min
Simulated Afro hair	NaOH	1.6 ± 0.6	14 ± 4
	No-Lye	0.0 ± 0.2	11 ± 2
Natural Afro hair	NaOH	3.9 ± 0.5	26 ± 7
	No-Lye	0.9 ± 0.2	21 ± 4

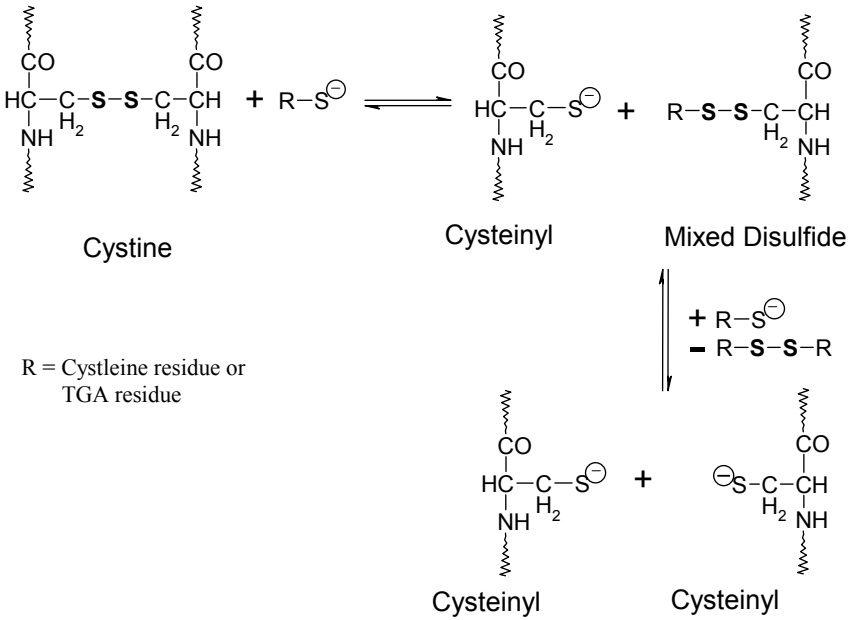
No-Lye causes a faster straightening of both hair types than NaOH, which is expressed by the characteristic straightening time, τ. It is always smaller for No-Lye treatments than for NaOH treatments. This can be explained by the higher pH of the No-Lye cream and thus a higher concentration of reacting agent - the alkali. This effect is also expressed by the time lag. Because of the higher concentration of alkali in the No-Lye cream, the alkali is able to pass the cuticle faster. The calculated values of Δt confirm this. The time lag is much larger for NaOH treatments.

Furthermore, it turned out that in both treatments simulated Afro hair shows faster straightening than natural Afro hair does. This is expressed by the characteristic straightening time, τ, as well as by the time lag, Δt. τ is approximately two times smaller for simulated Afro hair. When simulated Afro hair is treated with alkali, nearly no time lag is observed. This can be attributed to the pre-damage of the simulated Afro hair.

Since simulated Afro hair had previously been steam treated, the structure is damaged and it is not possible to compare simulated with natural Afro hair. Due to this results, the cosmetic research and companies are advised against using simulated Afro hair as being a relevant model for natural Afro hair.

3.3.4.2. Comparison of different treatments of natural Afro hair

In the second test series the straightening effect of different thioles added to the relaxer creams was investigated. By this, a second chemical reaction will take place during relaxing. Besides the cleavage of the disulfide bonds by alkali (and subsequent formation of Lan), a breakage of the disulfide bonds by the thioles occurs. Scheme 6 shows this reaction.



Scheme 6: Cleavage of the disulfide cross-link (cystine) between two protein chains through the formation of cysteine.
R-S-S-R = Dithio diglycolic acid, when R = TGA residue
= Cystine, when R = cysteine residue
〰 = Protein chain

The complete reduction of CyS-SCy is a two-step mechanism. Besides Cys as main product, mixed disulfide can be formed containing TGA and cysteine residues. The break-down of disulfide bonds leads to flexibility of the protein chains and to softening of the whole hair. Thus, it is to be expected that addition of thioles leads to faster straightening. The results are demonstrated in Fig. 43. The curves are fitted again by the eq. 3.6. The calculated parameters are given in Tab. 6.

Tab. 6: Calculated time lag, Δt, characteristic straightening time, τ (± standard error) of the curves in Fig. 43 for differently treated natural Afro hair samples using eq. 3.6 for fitting (Average of 8 single treated hairs per relaxing time and experiment).

Treatment	Δt in min	τ in min
NaOH	3.9 ± 0.5	26 ± 7
NaOH + TGA	3.3 ± 0.7	13 ± 2
NaOH + Cys	4.0 ± 0.6	19 ± 3
No-Lye	0.9 ± 0.2	21 ± 4
No-Lye + TGA	0.8 (fixed)	7 ± 2
No-Lye + Cys	0.8 ± 0.3	10 ± 3

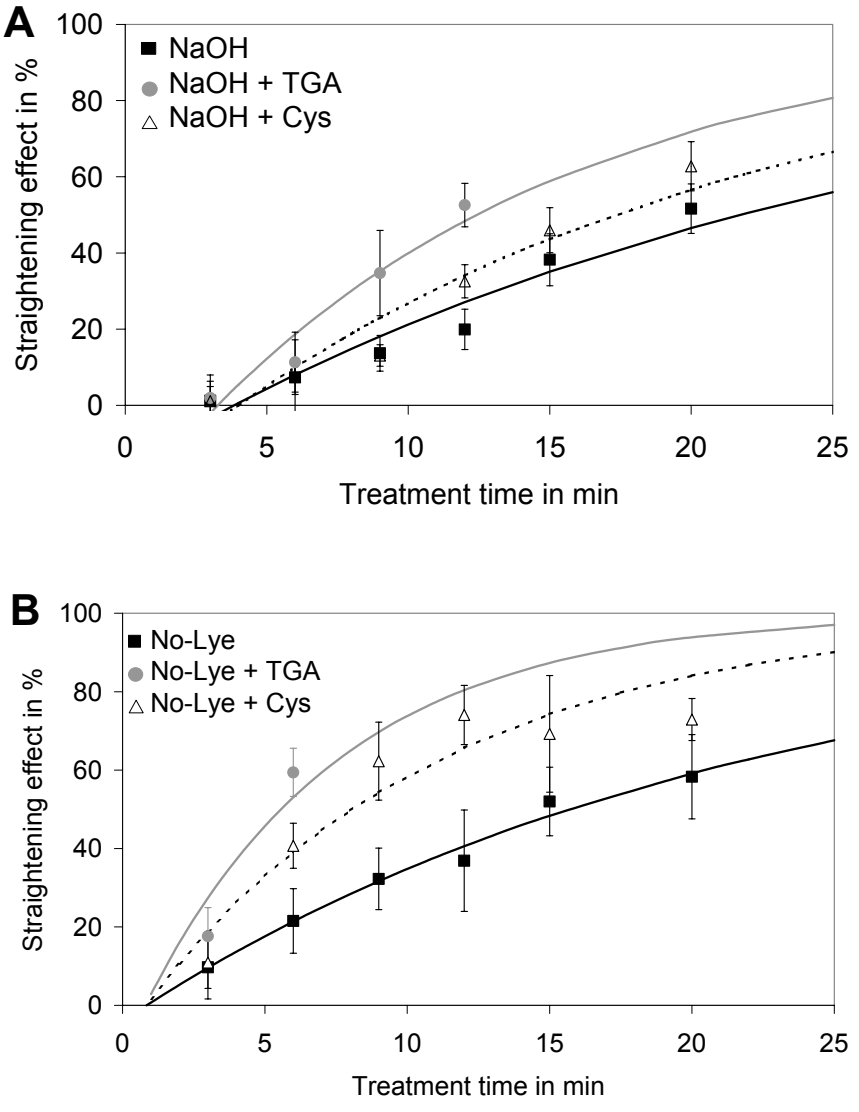


Fig. 43: Straightening effect (natural Afro hair) of different relaxer creams (with and without TGA or cysteine) versus relaxing time. The error bars represent the standard deviation (n = 8).
A: NaOH treatment
B: No-Lye treatment

Indeed, the results show that the presence of additional thioles (1 % w/w thiole) straightens hair considerably faster than pure relaxer creams do. Whereas NaOH treatment causes a straightening effect of 50 % after 20 min, NaOH + Cys treatment straightens hair up to 60 %.

Addition of TGA causes an even faster straightening than the addition of cysteine. NaOH + TGA treatment causes a straightening effect of 50 % after 11 min. The same effect is reached by cysteine containing cream after 16 min and without thiole after 20 min (comparative data of No-Lye series for straightening effect of 50 %: plus TGA in 5 min; plus Cys in 7.5 min, without thiole in 16 min).

It was not possible to measure the straightening effect of the relaxer creams + TGA for longer treatment times since hair becomes very soft and weak upon this treatment. During rinsing under running water the hair stretched longitudinally (sometimes more than 200 %) and broke afterwards. This phenomenon also occurred occasionally for hair which was treated with relaxer creams + Cys, though for longer treatment times (15 – 20 min). The stretching indicates that the amount of internal linkages of hair has been diminished to a large extent. It corroborates the above mentioned proposition of the additional cleavage of disulfide bonds by added thioles. Since only values for two treatment times (3 and 6 min) were able to determine for the treatment of No-Lye + TGA, it is not possible to calculate the complete set of parameters for this treatment. That is why an additional parameter had to be fixed. The values for time lag for the different NaOH treatments as well as for the No-Lye treatment are always very similar. Thus, on the basis of lag times of the No-Lye series, the time lag of the No-Lye + TGA treatment was fixed to 0.8 min. A reliable value for τ was thus determined.

3.4. Study of hair relaxing

Natural Afro hair cannot be obtained commercially. Hair shops only sell simulated Afro hair. Thus, natural Afro hair has to be obtained through more or less private contacts, which proves to be very difficult for large amounts of hair. To avoid the general problems of the availability of Afro hair, Caucasian hair has been used for those treatments which are afterwards investigated chemically. The comparison of natural Afro hair and Caucasian hair has shown that they react chemically in a very similar way.

Two kinds of comparisons were made for the following part of the investigations. First, a NaOH treatment of Caucasian and natural Afro hair was investigated. Second, the chemical effects of different relaxer treatments (see chapter 3.3.3.) on Caucasian hair were analyzed.

3.4.1. Kinetics of cystine degradation

Klibanov et al. /56/ have shown that proteins undergo heat-induced β-elimination of CyS-SCy residues in the pH range from 4 to 8. The time course of this process closely follows first-order kinetics. During an alkali treatment of keratins the β-elimination in CyS-SCy is presumed to be the first reaction step, too (c.f. Scheme 2). With the help of AAA the degradation of CyS-SCy can be examined for its kinetics.

The amount of CyS-SCy in Afro and Caucasian relaxed hair depends on the time of relaxing (NaOH treatment) as shown in Fig. 44. For each treatment time a new experiment was carried out. Thus, the obtained data are not received from a continuous reaction but from a stop-flow reaction. This statement is valid for all the following experiments.

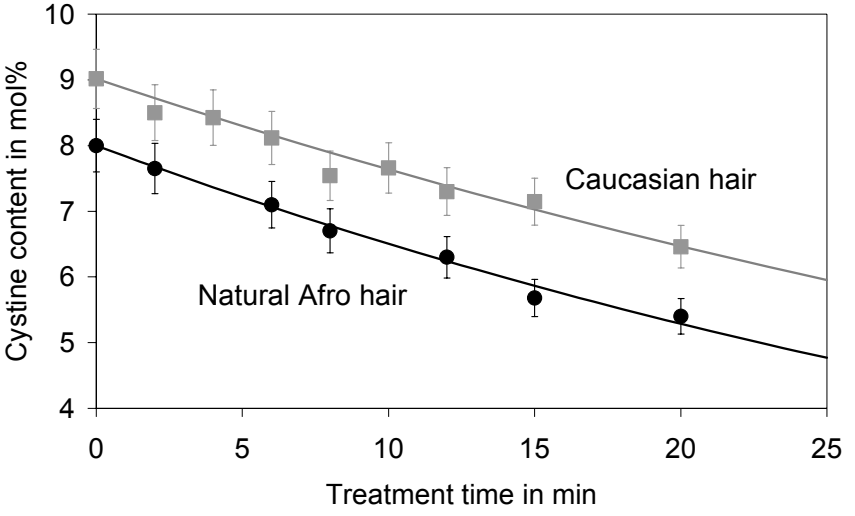


Fig. 44: Time dependent degradation of CyS-SCy in Afro (●) and Caucasian (■) hair during treatment with NaOH relaxer cream at 21 °C. The mean value of two AAAs for each relaxing time is shown. The error bars give the minimum and maximum value.

A reaction of first-order kinetics is described by eq. 3.7; with $[CyS\text{-}SCy]_0$ as the initial CyS-SCy concentration, and $[CyS\text{-}SCy]_t$ the amount of CyS-SCy at time t.

k is the rate constant.

$$-\frac{d[CyS-SCy]_t}{dt} = k[CyS-SCy]_t \qquad (3.7)$$

Integration results in:

$$\ln\frac{[CyS-SCy]_t}{[CyS-SCy]_0} = -kt \qquad (3.8)$$

A plot of ln ($[CyS\text{-}SCy]_t/[CyS\text{-}SCy]_0$) against t yields a straight line, in case the degradation of CyS-SCy follows first-order kinetics (Fig. 45). The slope of the straight line gives the rate constant as -k.

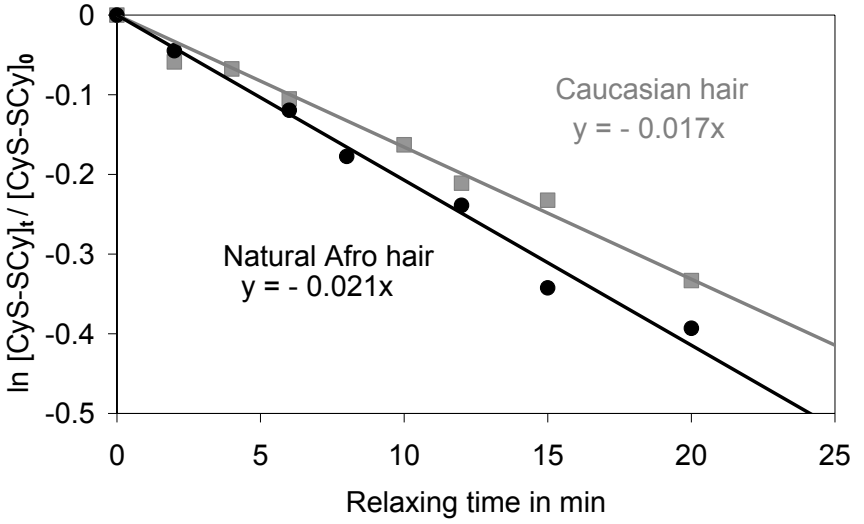


Fig. 45: Time dependent degradation of CyS-SCy (standardized to initial amount of CyS-SCy) in Afro (●) and Caucasian (■) hair during treatment with NaOH relaxer cream at 21 °C after integration. The lines shown are the regression lines corresponding to the first-order model. The average values of Fig. 45 are used for calculation (coefficient of determination $R^2 \geq 0.98$).

Fig. 45 shows that the degradation of CyS-SCy in both hair types follows first-order kinetics. Thus, the same kind of alkali reaction takes place in both hair types. But the two hair samples possess different rate constants. The CyS-SCy of Afro hair (k_{Afro} = 0.021 min^{-1}) is converted faster than for Caucasian hair ($k_{Caucasian}$ = 0.017 min^{-1}). This could be a result of variances for the hair of different races or to the smaller diameter of Afro hair (Fig. 21), which probably allows a faster penetration of the hair by alkali.

Since both hair types follow first-order kinetics, Caucasian hair can be used for further chemical investigations instead of Afro hair. One has to keep in mind, however, that the obtained results for Caucasian hair in the following chapters cannot be directly transferred to Afro hair but they give an impression of the degree of the efficacy of treatments and the extent of hair damage.

3.4.2. Quality of the hair after modified relaxer treatments

To investigate the chemical influence of the additional thioles in the relaxing process, the hair samples were subjected to AAA. Furthermore, the changes of hair structure caused by alkali and thioles were investigated by DSC.

3.4.2.1. Amino acid composition

20 different AA were detected and determined after an acid hydrolysis of the untreated and treated hair samples. Despite the addition of TGA, no mixed disulfides or other derivates of TGA were found. The concentration of the additional TGA seems to be too small. Therefore, one can assume that the additional Cys in the relaxer cream also plays no significant role for the AA composition. Cys itself is not determined in the AAA because it is oxidized by air into CyS-SCy during the course of the AAA.

Tab. 7 summarizes the AA contents of the untreated and 12 min treated hair samples. CyS-SCy and Lan are typed in bold since they are indicators of chemical damage. It is shown that the content of the other AAs does not differ significantly upon the different treatments. A matter of particular interest are the changes of CyS-SCy and Lan depending on treatment time, as shown in Fig. 46 (cystine content) and 47 (lanthionine content).

Tab. 7: Amino acid content in mol% of untreated and treated (12 min relaxed) Caucasian hair samples. CyS-SCy and Lan are the only AAs which change significantly.

Treatment / Amino acid	Untreated	NaOH	NaOH + TGA	NaOH + Cys	No-Lye	No-Lye + TGA	No-Lye + Cys
Cysteic acid	0.5	0.5	0.4	0.4	0.5	0.4	0.4
Aspartic acid + asparagine	5.8	5.9	6.4	6.3	6.2	6.1	6.0
Threonine	7.7	8.3	7.7	7.8	7.7	7.7	7.7
Serine	10.1	10.3	9.7	10.8	10.7	10.4	10.0
Glutamic acid + glutamine	13.8	13.4	13.8	14.0	13.8	14.0	13.6
Proline	8.7	9.0	8.0	7.9	8.5	8.7	9.0
Glycine	6.8	6.7	6.9	7.0	7.2	6.8	7.0
Alanine	4.8	5.1	5.1	5.0	5.0	5.1	4.8
Valine	6.4	6.6	6.3	6.5	6.2	6.6	6.4
Cystine	**9.0**	**7.3**	**8.2**	**7.7**	**6.6**	**7.4**	**7.2**
Methionine	0.5	0.5	0.6	0.4	0.5	0.4	0.5
Isoleucine	3.5	3.3	3.4	3.5	3.4	3.3	3.4
Leucine	7.5	7.2	7.8	8.2	7.5	7.7	7.9
Tyrosine	2.1	2.3	2.1	1.7	1.9	2.0	2.1
Phenylalanine	1.9	1.9	2.1	1.8	2.0	1.9	1.9
Ornithine	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Lysine	2.6	2.7	2.7	2.7	2.7	2.7	2.8
Histidine	0.9	0.8	0.8	0.8	0.9	0.9	0.8
Arginine	7.2	7.5	7.3	6.4	7.1	6.9	7.0
Lanthionine	**0.1**	**0.6**	**0.5**	**0.8**	**1.2**	**0.9**	**1.3**

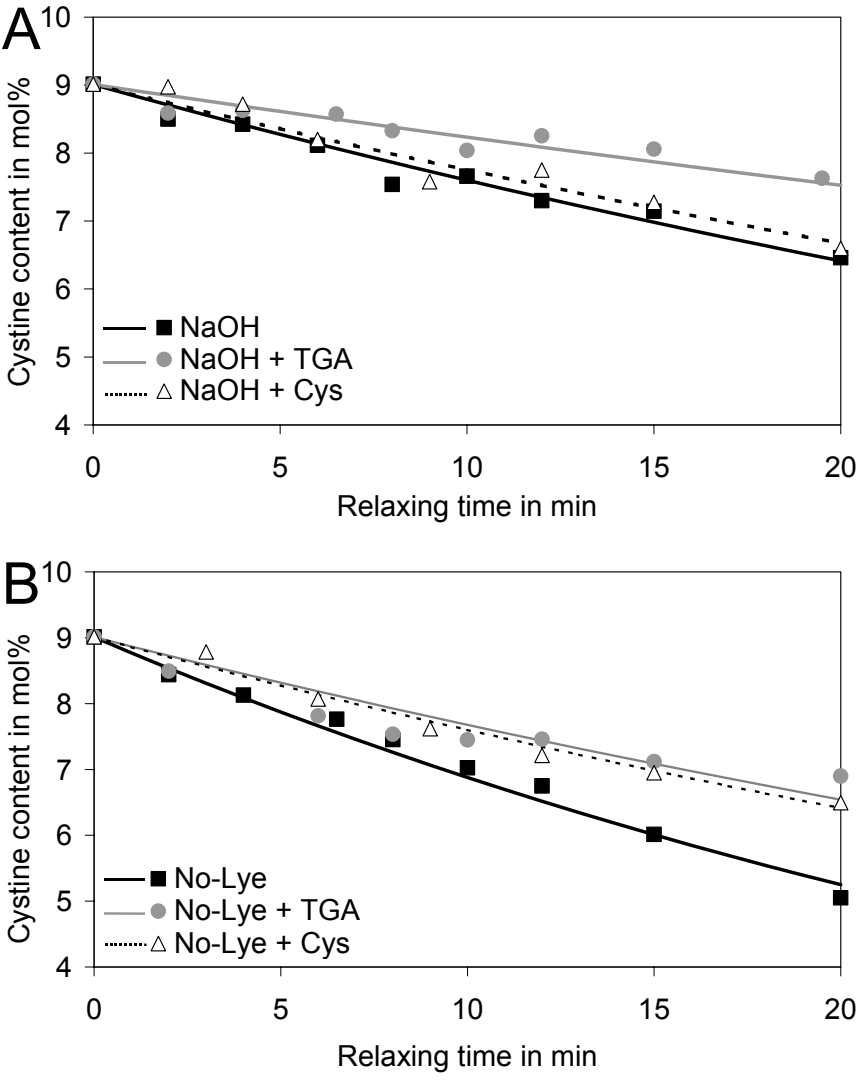


Fig. 46: Cystine content versus treatment time.
A: NaOH treatments
B: No-Lye treatments
The points are fitted using eq. 3.8.

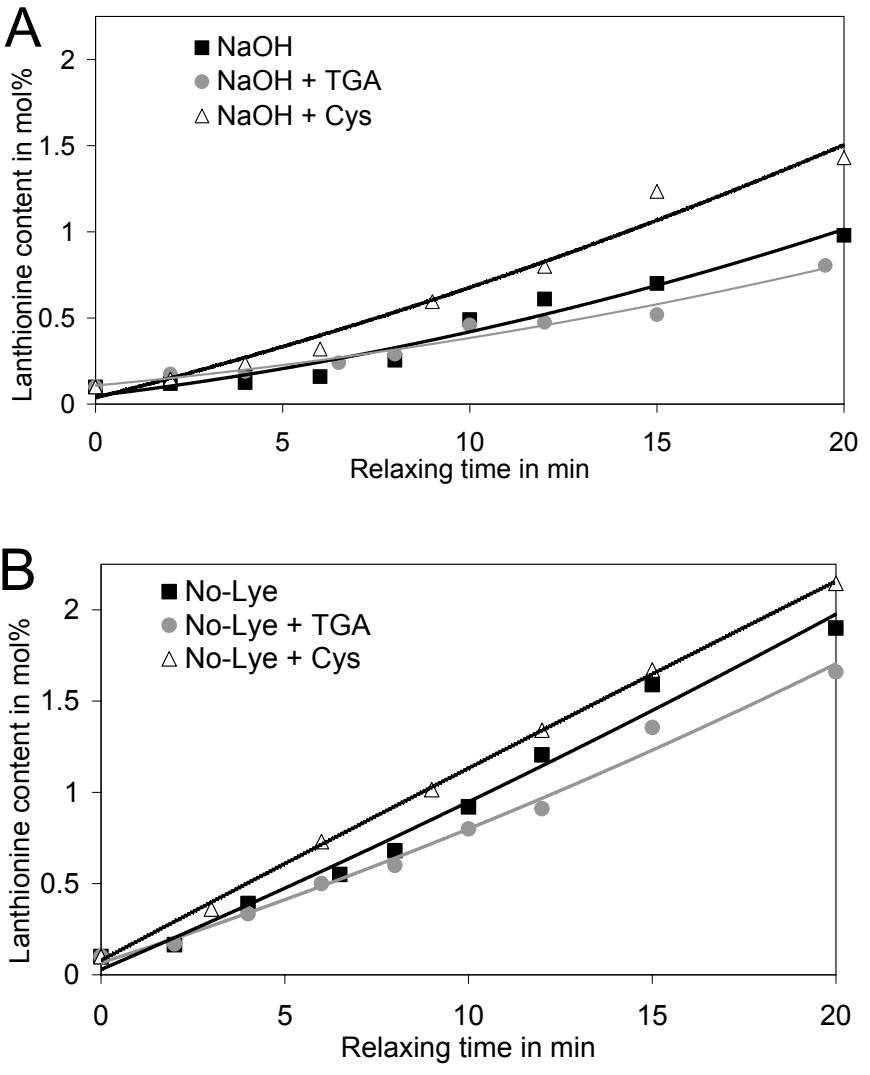
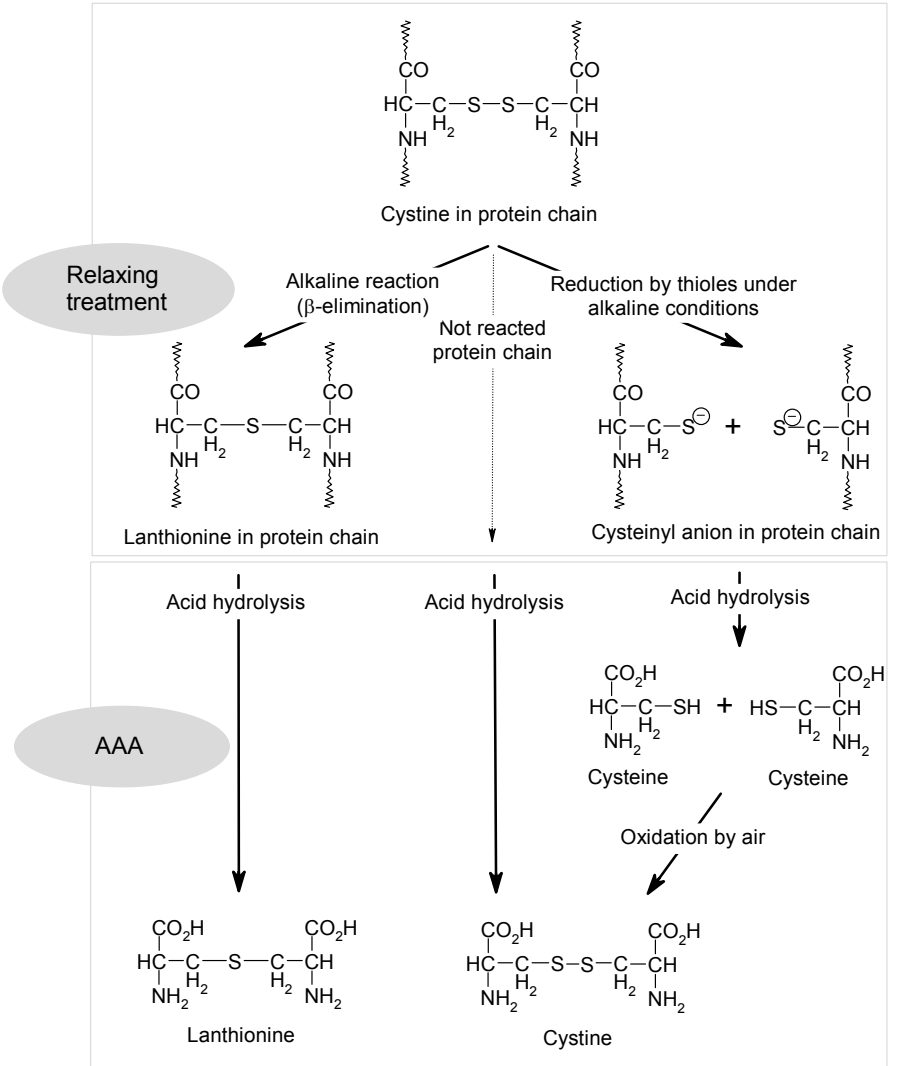


Fig. 47: Lanthionine content versus treatment time
A: NaOH treatments
B: No-Lye treatments
As guide for the eye, the data are emphasized by fitted curves using a polynomial Equation of second power.

As a basic principle, No-Lye treatments degrade CyS-SCy and generate Lan faster than NaOH treatments. These results point at a greater damage in hair and can be explained by the higher pH of the No-Lye-cream compared to the NaOH-cream.

Within both series the same ranking can be observed. Relaxer creams without any thiole (NaOH and No-Lye) show the lowest amount of CyS-SCy, followed by relaxer creams with additional Cys. The largest amount of CyS-SCy was detected in samples which were treated with relaxer creams + TGA. It appears that the alkali and the thioles compete for CyS-SCy. The thioles cleave CyS-SCy into Cys. Thus, less β-elimination by alkali takes place. Since Cys is air-oxidized into CyS-SCy in the course of the acid AAA (Scheme 7), the amount of detected CyS-SCy is high.

On the assumption that the formed Cys is completely oxidized into CyS-SCy, the difference of the initial CyS-SCy content and the detected CyS-SCy content equals the amount of CyS-SCy which is degraded by alkali. Since TGA is a stronger reducing agent than Cys, it gives a higher “protection” of the CyS-SCy against β-elimination by alkali. Thus, more CyS-SCy is found for samples, which are treated with relaxer creams + TGA. According to that, pure relaxer creams provoke more degradation of CyS-SCy than thiole containing creams.



Scheme 7: Chemical reactions of cystine and its products during relaxing treatment and AAA.

The degradation curves of CyS-SCy in Fig. 46 can be fitted by eq. 3.8. Thus, the degradation of CyS-SCy follows first-order kinetics for all treatments. This corroborates the prior statement that the difference between initial and determined CyS-SCy content reflects the amount of CyS-SCy which is degraded by alkali. If other parameters had influenced the CyS-SCy determination, the degradation would not follow first-order kinetic. Tab. 8 shows the calculated first-order rate constant, k.

Tab. 8: First-order rate constants, k, ± standard error of the different treatments using eq. 3.8.

Treatment	k in min^{-1}
NaOH	0.017 ± 0.004
NaOH + TGA	0.009 ± 0.004
NaOH + Cys	0.015 ± 0.005
No-Lye	0.027 ± 0.004
No-Lye + TGA	0.016 ± 0.009
No-Lye + Cys	0.017 ± 0.004

Another ranking can be observed for the Lanthionine content. The data show that the amount of Lan formed is not directly related to the degradation of CyS-SCy by alkali under these conditions.

The lowest Lan content is obtained by a treatment of relaxer creams + TGA, followed by a treatment of pure relaxer creams. Samples treated with relaxer creams + Cys show the highest amount of Lan. Probably, the added free Cys catches immediately the emerged dehydroalanine and forms Lan. Thus, most of the generated dehydroalanine is reacting to Lan. The side reaction – the formation of lysinoalanine – would be suppressed.

Arai et al. /57/ showed that after the reaction of wool with aqueous KCN the sum of CyS-SCy and Lan stays constant over time from the start. This suggests that all of the Lan originates from CyS-SCy residues and that the degraded CyS-SCy is completely transferred into Lan. This behavior could not be observed in this work. Fig. 48 shows the change of the sum of CyS-SCy plus Lan content over time for different treatments. The values of the fitted curves of Fig. 46 (CyS-SCy vs. t) and 47 (Lan vs. t) are used. The curve of the CyS-SCy content is calculated using eq. 3.8. The values for k are taken from Tab. 8. The Lan curves are fitted by a polynomial equation of second power:

$$y = ax^2 + bx + c \tag{3.9}$$

The fitted values of *a*, *b*, and *c* are given in Tab. 9.

The sum of CyS-SCy plus Lan decrease with treatment time and rapidly approaches equilibrium. The sum is thus not constant as Arai found in his experiments. Therefore, beside the main reaction – the formation of Lan – side reactions definitely have occurred. Products of possible side reactions (c.f. Schemes 3 and 4) were not determined within this work. At equilibrium the amount of formed Lan corresponds to the amount of degraded CyS-SCy by alkali. The complete degraded CyS-SCy has formed Lan at this time. Side reactions are totally suppressed.

Tab. 9: Calculated parameters of a polynomial equation of second power (eq. 3.9) for different treatments of the lanthionine data versus t.

Treatment	*a*	*b*	*c*
NaOH	0.0011	0.0259	0.0485
NaOH + TGA	0.0008	0.0190	0.1079
NaOH + Cys	0.0009	0.0545	0.0377
No-Lye	0.0005	0.0864	0.0288
No-Lye + TGA	0.0001	0.1067	0.0786
No-Lye + Cys	0.0009	0.0649	0.0656

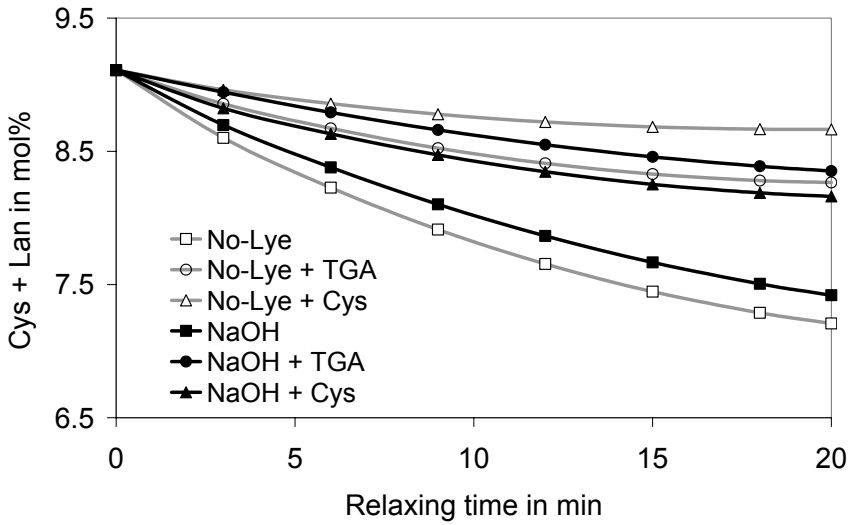


Fig. 48: The change of cystine content plus lanthionine contents versus relaxing time for different treatments.
The curves are calculated by resolving eq. 3.8 and 3.9 to t, equating them and using the values of Tab. 8 and 9.

In 1969 *Robson et al*. postulated that when fibers are set in boiling water, a new cross-linkage, namely, either Lan or lysinoalanine is formed for every SS-bond undergoing cleavage. Thus, the sum of Lan and lysinoalanine corresponds to the amount of degraded CyS-SCy /30/. Lysinoalanine was not determined in this work. But if this behavior applies to an alkaline treatment as well, the percentage of CyS-SCy which forms Lan should be constant in relation to degraded CyS-SCy. This percentage is called lanthionine ratio, R_{Lan}, and is defined by eq. 3.10; with $[Lan]_0$, $[CyS\text{-}SCy]_0$ being the initial amounts of Lan and CyS-SCy, and $[Lan]_t$, $[CyS\text{-}SCy]_t$ the amount of Lan and CyS-SCy at time t:

$$R_{Lan} = \frac{[Lan]_t - [Lan]_0}{[CyS-SCy]_0 - [CyS-SCy]_t} 100\% \qquad (3.10)$$

Fig. 49 shows the lanthionine ratio versus time. For calculating the lanthionine ratio the fitted values of Lan and CyS-SCy content have been used as outlined above.

The data show that after an initial, strong increase in most cases the lanthionine ratio rises continuously for all treatments. The steady increase of Lan can be explained by the limited amount of lysine. While Cys, which is necessary to form Lan, is continuously generated from CyS-SCy by alkali, lysine is used up during the treatment.

Treatments with No-Lye convert a higher percentage of degraded CyS-SCy into Lan than NaOH. This can be explained by the different pH values of the creams. The higher concentration of hydroxyl ions in the No-Lye cream effects a faster degradation of CyS-SCy. Since more dehydroalanine and Cys are formed, the probability increases that these residues interact and form Lan. This circumstance causes a higher concentration of Lan.

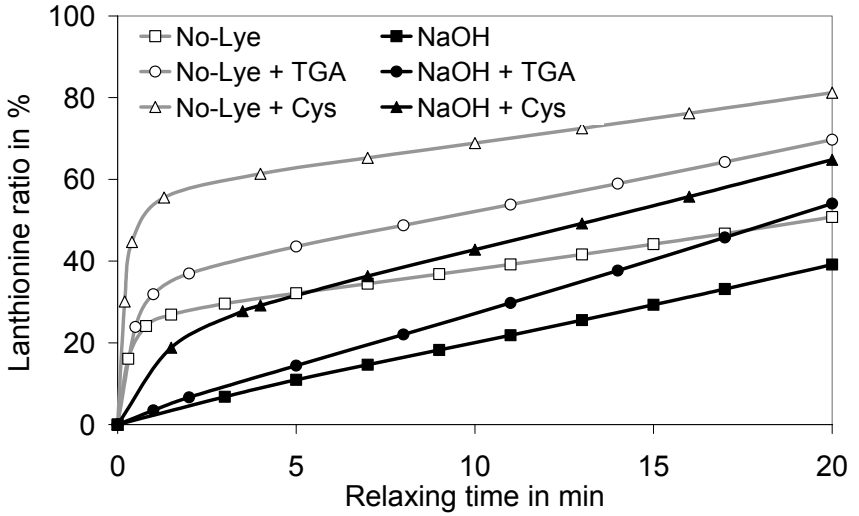


Fig. 49: Percentage of degraded cystine (by alkali) which is transformed into lanthionine (lanthionine ratio) versus relaxing time. The curves are calculated for different times by using eq. 3.8 and 3.9, and the values of Tab. 8 and 9.

Relaxer creams + Cys generate the highest percentage of degraded CyS-SCy to be transferred into Lan, followed by TGA containing creams. The smallest amount of Lan is formed by the pure relaxer creams. The thioles generate additional Cys by reduction (Scheme 7). Thus, the probability increases that a dehydroalanine residue and a Cys residue can interact to form Lan. Therefore, thiole containing treatments lead to a higher percentage of Lan. Moreover, the additionally added Cys is able to react directly with dehydroalanine into Lan. Such a Lan is obviously not a new crosslink between two protein fibers but rather a Lan side chain. AAA is not able to differ between Lan side groups and Lan bridges.

In 1994 *Wong et al*. /58/ claimed that neither the formation of Lan nor the reduction of CyS-SCy necessarily corresponds to the efficacy of hair straightening. They divided the degree of hair straightening roughly into three fractions: complete, partial and no straightening. Because the straightening effect has been determined with high precision in this work, the approach of *Wong et al*. could be investigated and evaluated. For this the change of Lan content was considered for its relationship with the straightening effect (Fig. 50). As a guide for the eye the data of the relationship between Lan content and straightening effect is re-presented by an empirical fitted curve.

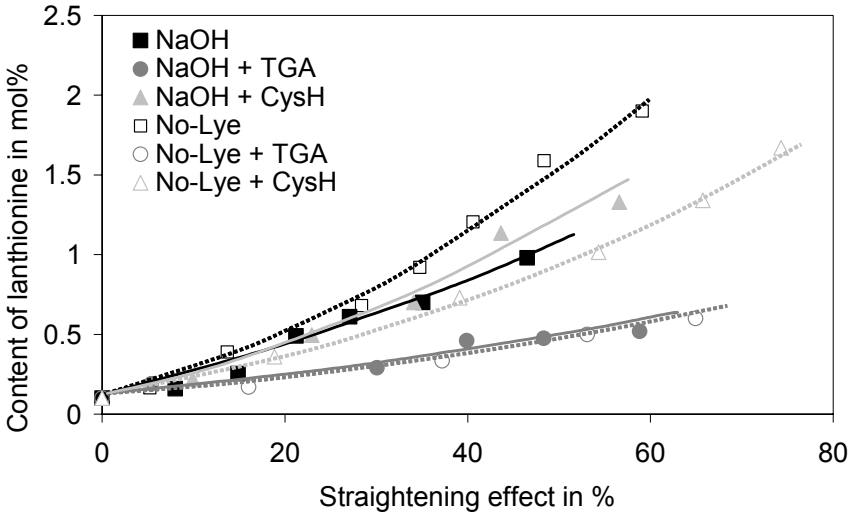


Fig. 50: Lanthionine content versus straightening effect.
The values of Lan are measured data, and the values of straightening effect are recalculated using eq. 3.6 and parameters of Tab. 6.

If the straightening effect was inherently dependent on the Lan content, the curves for all treatments would fall on top of each other. This is not observed. It is thus concluded that straightening conditions lead, as expected, to an increasing formation of Lan. This effect parallels that of hair straightening, but it is not the primary mechanism for permanent hair straightening. The above mentioned statement of *Wang et al*. /58/ is thus corroborated.

The straightening effect is mainly based on the cleavage of disulfide bridges. Since the CyS-SCy content according to the AAA reflects only the amount of CyS-SCy which is degraded by alkali, and not additionally the amount of CyS-SCy which is reduced by the thioles, the influence of the thioles cannot be determined. Thus, a comparison of the straightening effect with the CyS-SCy content is not meaningful.

3.4.2.2. Thermal properties

Denaturation enthalpy and temperature of modified relaxed Caucasian hair are measured by using high-pressure differential scanning calorimetry (HP-DSC). While the enthalpy relates to the native α-helical content, the temperature gives evidence of the thermal stability of the matrix, which is the non-helical fraction of the fiber.

Fig. 51 shows the relationship between denaturation enthalpy and time of relaxing. As a guide for the eye the data of the relationship between denaturation enthalpy and relaxing time are represented by an empirical fitted curve. The denaturation enthalpy decreases with the duration of treatment. The ranking for NaOH and No-Lye is equal in both series (Fig. 51). The strongest decline of enthalpy is observed for the TGA containing treatments. Treatments with pure and with Cys containing relaxer creams cause nearly the same reduction of denaturation enthalpy. After 20 min NaOH and NaOH + Cys samples have lost around 50 % of their helix content, NaOH + TGA samples already around 65 %. In general, the enthalpies for the No-Lye series - due to their higher pH - are lower than for the NaOH series. Applications of the pure and of the Cys containing No-Lye cream lead to only around 1/3 of the initial helix content after 20 min processing time; the TGA treated samples yield only 12 %.

The analysis of the relationship between denaturation enthalpy and CyS-SCy content, at equal relaxing times t, shows to what extent the denaturation enthalpy depends on the amount of CyS-SCy, which is cleaved by alkali. Fig. 52 represents this relationship. The content of degraded CyS-SCy, Δ[CyS-SCy], is defined as the initial amount of CyS-SCy, $[CyS\text{-}SCy]_0$, minus the determined amount of CyS-SCy at time t, $[CyS\text{-}SCy]_t$, as shown in Equ. 3.11.

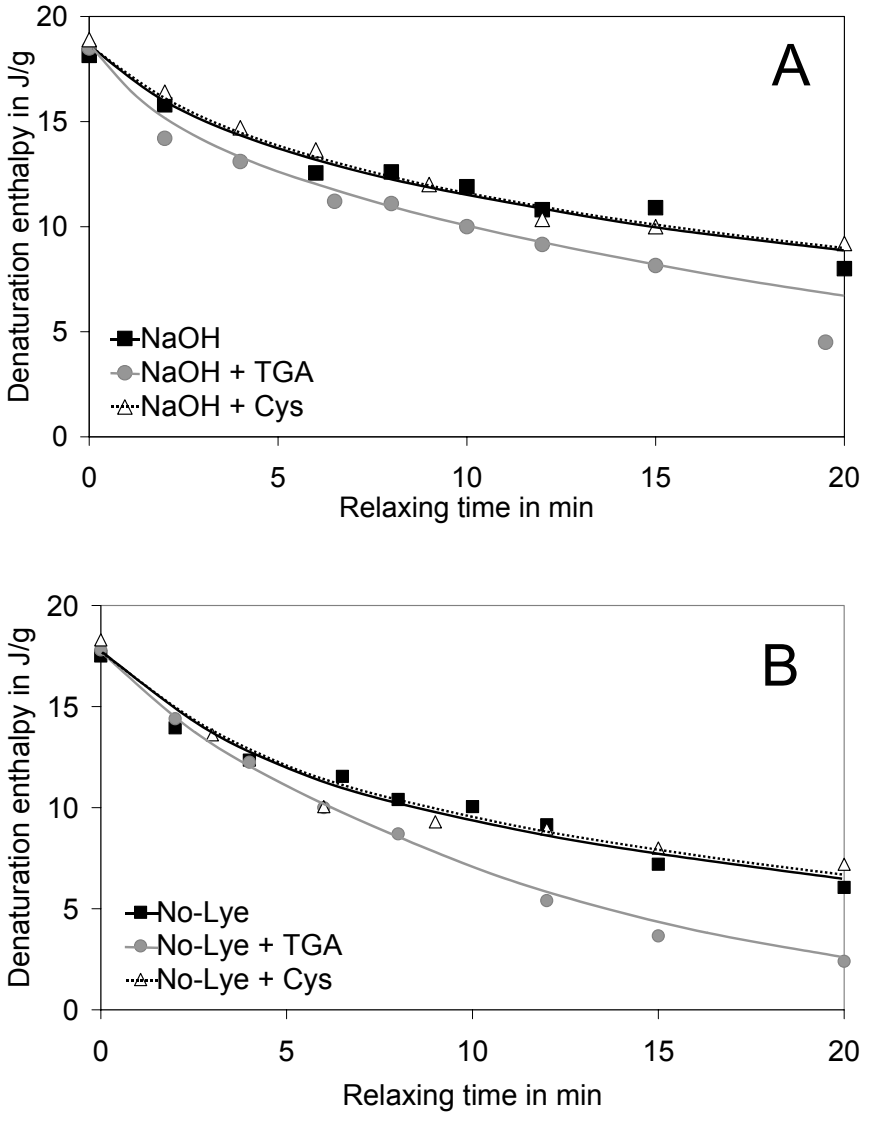


Fig. 51: Denaturation enthalpy versus relaxing time for different treatments.
A: NaOH treatments
B: No-Lye treatments

$$\Delta[\text{CyS-SCy}] = [\text{CyS-SCy}]_0 - [\text{CyS-SCy}]_t \qquad (3.11)$$

The amount of degraded CyS-SCy is calculated by using the fitted data of CyS-SCy and the initial CyS-SCy content with $[\text{CyS-SCy}]_0$ = 9.0 mol%. The data of CyS-SCy content are calculated using eq. 3.8 and 3.11, the values for k are taken from Tab. 8. The data of denaturation enthalpy are measured data. As a guide for the eye the data of the relationship between denaturation enthalpy and relaxing time are represented by an empirical fitted curves (grey, broken lines).

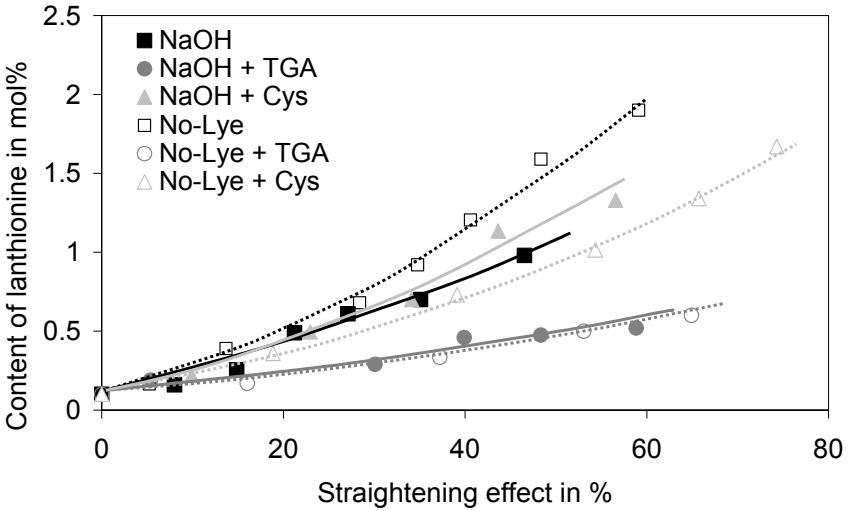


Fig. 52: Relationship between denaturation enthalpy, ΔH_D, and the amount of alkaline degraded cystine, Δ[CyS-SCy], of Caucasian hair.
The data of CyS-SCy content are calculated using eq. 3.8 and 3.11, the values for k are taken from Tab. 8. The data of denaturation enthalpy are measured data.

Generally, the more CyS-SCy is degraded by alkali the smaller the residual enthalpy. Since the denaturation enthalpy reflects the amount of helix content in hair, degradation of CyS-SCy leads to denaturation of the protein chains and thus to a decrease of the helix content.

The curves for both pure relaxer creams treated samples lie on top of each other. The same behavior can be observed for the curves of samples which were treated with Cys or TGA containing creams. The TGA containing creams cause the strongest reduction of enthalpy, the pure relaxer creams the lowest. When 2 mol% of CyS-SCy are degraded by alkali, the samples treated with pure relaxer creams have already lost 50 % of their initial native helix content; for the Cys and TGA containing creams the value reaches 60 % and 80 %, respectively. Any alteration of the protein structure leads to a change of denaturation enthalpy. The greater reduction of enthalpy by the thiole containing creams is attributed to the additional cleavage of disulfide bridges. The overlapping curves for NaOH and No-Lye treated samples show that the mechanism of the alkaline degradation of CyS-SCy is not dependent on pH for the investigated pH range.

To describe empirically the relationship between alkaline degraded CyS-SCy, Δ[CyS-SCy], and denaturation enthalpy, ΔH_D, the data are fitted by using the following simple exponential algorithm, with ΔH_D^0 as initial amount of denaturation enthalpy and h as fitting parameter:

$$\Delta H_D = \Delta H_D^0 \, e^{-\frac{\Delta[CyS-SCy]}{h}} \qquad (3.12)$$

The calculated values for the fitting parameter, h, are given in Tab. 10.

Tab. 10: Fitting parameter, h (± standard error) of eq. 3.12 (relationship between denaturation enthalpy and alkaline degraded cystine) for the different creams.

Treatment	h in mol%
NaOH No-Lye	3.2 ± 0.4
NaOH + TGA No-Lye + TGA	1.3 ± 0.2
NaOH + Cys No-Lye + Cys	2.3 ± 0.3

In 2000 *Ogawa et al*. /59/ reported on the relationship between denaturation enthalpy and degree of supercontraction, L_C, of Asian hair fibers. The extent of supercontraction was determined by measuring the length of the straight hair in a microcapillary before treatment. The extent of supercontraction, L_C, was calculated as the ratio of the length change, $(L_0 - L)$ over the initial dry length, L_0:

$$L_C = \frac{L_0 - L}{L_0} 100\% \qquad (3.13)$$

They obtained an approximately linear relationship between supercontraction and denaturation enthalpy and claimed: “Supercontraction up to a level of 10 % is due to the breakdown of the α-crystallites. Higher supercontraction is traced back to alterations resulting from the changes in the matrix components, in the cortex, as well as in non-keratin components of the cell membrane complex.”

Ogawa et al. /59/ evaluated the effectiveness of straightening as a function of supercontraction. Their results, as well as those of *Wang et al*. /58/, showed that permanent hair straightening is achieved by supercontraction above 5 %. In a practical application, a range of about 5 to 8 % supercontraction of hair has been recommended for successful straightening, while at higher contraction levels the smoothness of the fiber surface tended to be lost /60/. *Ogawa et al.* /60/ assume that supercontraction of less than 10 % is caused by randomization of the α-helix because at this level no more helical content could be found.

Ogawa et al. /59/ divided the degree of hair straightening into three fractions like *Wang et al*. /58/ has done as described above. Since in this thesis the straightening effect has been exactly determined, it is possible to investigate the relationship between the degree of hair straightening and helical content as related to changes of the denaturation enthalpy.

Fig. 53 illustrates the relationship graphically, whereby denaturation enthalpy and helical content are plotted on the abscissa. Helical content expresses the denaturation enthalpy in percent as shown in eq. 3.14, with ΔH_D^0 as initial amount of denaturation enthalpy, and ΔH_D^t as amount of denaturation enthalpy at time t:

$$Helical \quad content = \frac{\Delta H_D^t}{\Delta H_D^0} 100\% \tag{3.14}$$

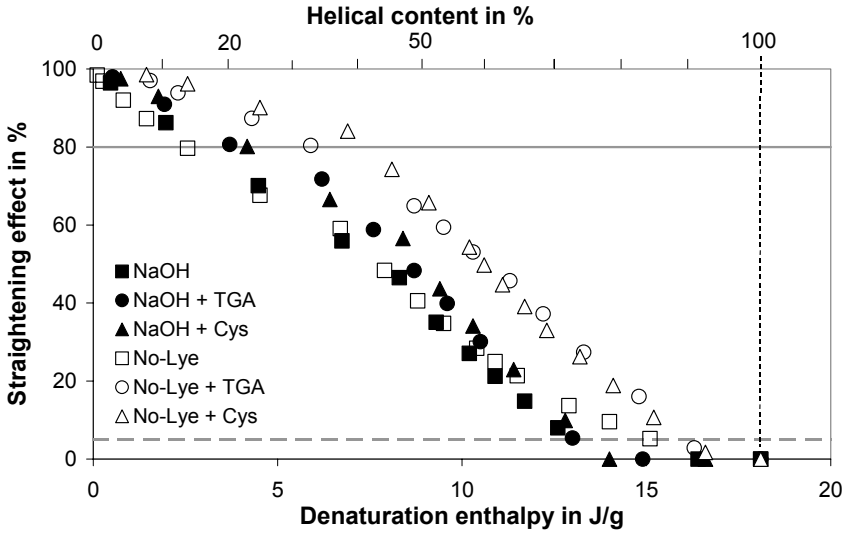


Fig. 53: Dependence of the straightening effect on the denaturation enthalpy.

The results show that the lower the enthalpy (and the lower the helical content), the higher the degree of straightening. To achieve even a small straightening effect, the helical content would have to be diminished substantially. A loss of helical content after relaxing of around 10-30 % leads only to a straightening effect of around 5 % (broken, horizontal grey line). To reach the maximum degree of hair straightening of around 80 % (horizontal grey line), 60 - 90 % of the α-crystallites must be destroyed. It strongly depends on the composition of relaxing cream to what extent the helical content must be destroyed. Pure relaxer creams reduce the amount of helical content to a higher degree than thiole containing creams to reach equal straightening effect. Thus, fewer α-crystallites must be destroyed to obtain identical hair straightening using additional thioles during relaxing. The difference between thiole containing and pure relaxer creams is not so highly developed within the NaOH -series.

For straightening effects between 5 and 80 % the curves demonstrate a nearly linear relationship between loss of enthalpy and degree of straightening. *Ogawa et al.* /60/ found an approximately linear relationship between supercontraction and denaturation enthalpy. Thus, it can be assumed that the straightening effect will have a linear dependency on the degree of supercontraction of the hair fiber.

Fig. 54 illustrates the relationship between denaturation temperature with relaxing time for different treatments. As a guide for the eye, empirical curves are plotted to represent the course of the data.

The result shows that the denaturation temperature decreases within the first 5 min for all treatments. Beyond this time the denaturation temperature remains largely unchanged. These results suggest that mainly the non-helical domains of the outer area of the fiber (especially the cuticle) are affected by alkali. However, the strong decrease of denaturation enthalpy and the only slight decrease of the denaturation temperature show that alkali has a greater effect on the helical part than on the non-helical domains of the hair fiber.

In 1987 *Wortmann* and *Souren* /61/ reported similar results for the reduction of disulfide bridges on the basis of mechanical properties. They found that reduction mainly affects the properties of the α-helical filaments in the hair fiber, while the properties of the matrix are largely unchanged.

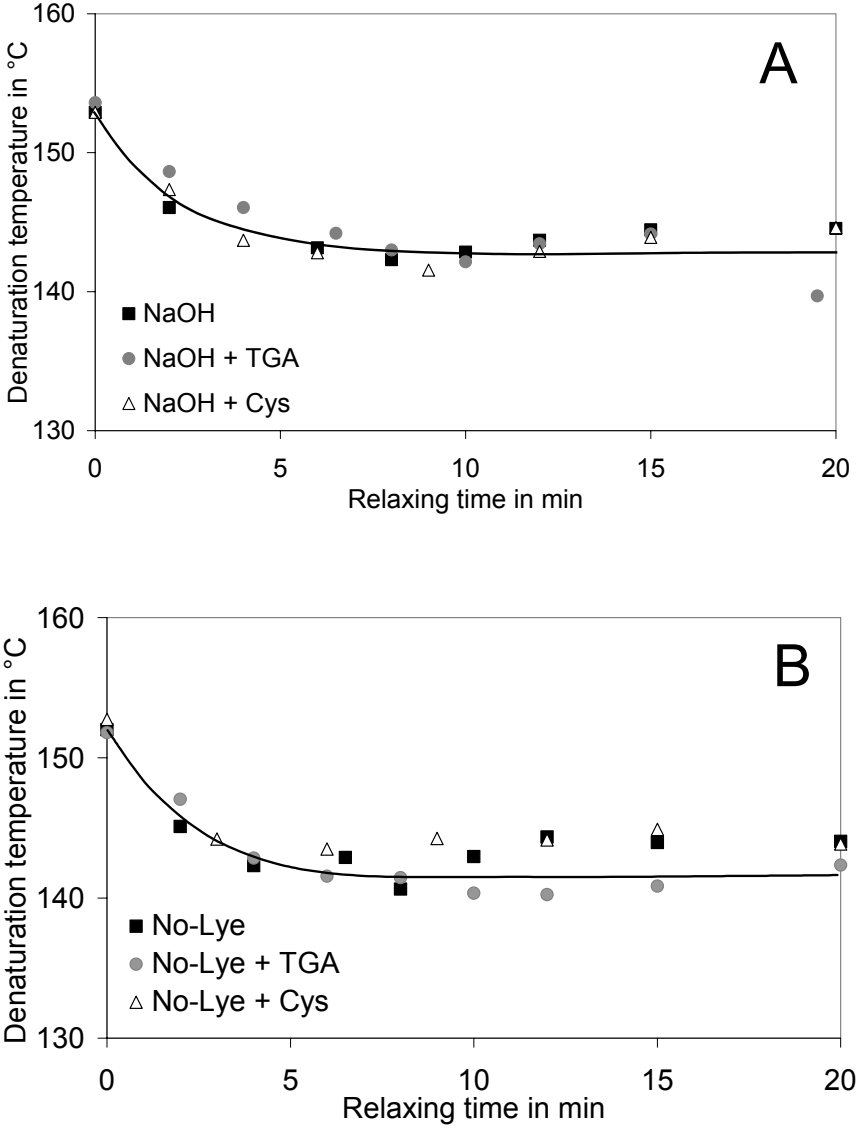


Fig. 54: Denaturation temperature versus relaxing time for different treatments.
A: NaOH treatments
B: No-Lye treatments

3.5. Permanent waving of relaxed hair

The ability to curl the hair after a relaxing treatment would be a new way to obtain desirable hair styling. In this chapter the wave behavior of relaxed hair is examined and the thus treated hair is characterized.

3.5.1. Performing single hair waving

The determination of the permanent wave set is performed through the ring test described by *Wortmann* and *Souren* /61/. Single hair fibers are wound around a steel rod and fixed. After the treatment the hairs are cut along the rod. The resulting hair rings, usually about 2 - 4 mm, are wetted and the distance of the ring ends, s, is determined (Fig. 55).

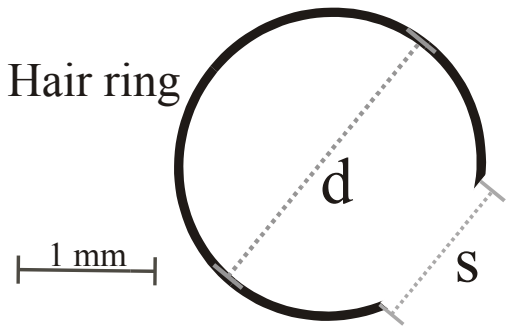


Fig. 55: Hair ring after wetting.
S = Distance between fiber ends
d = Diameter of the hair ring

The spontaneous bending relaxation, caused by wetting the hair, is complete after 10 min and the remaining set of the hair is traced back to the influence of covalent bonds. The ratio of the cylindrical rod diameter (d_R) over the average hair ring diameter ($\bar{d}$) yields the degree of set of permanent wave, S_P (perm set, eq. 3.15).

$$S_P = \frac{d_R}{\bar{d}} 100\% \quad (3.15)$$

When the set is smaller than 50 %, the diameter cannot be measured anymore directly. It is then determined by using the measurable distance between the fiber ends, s, and by the length of the hair ring, l_R. The diameter, d, and the distance between the fiber ends, s, are linked by eq. 3.16.

$$s = d \sin \frac{l_R}{d} \quad (3.16)$$

There is no precise mathematical solution for the recalculation of d. By plotting values of s versus d, the resulting curve makes it possible to assign the experimentally determined distance of ring ends, s, to the diameter, d.

One can assume that relaxed hair would show a lower perm set than untreated hair. Perm sets less then 40 % possess high standard errors because of the difficulty to determine the real distance of the ring ends. To avoid small values of perm set - even with longer relaxing treatment times - the starting point ("untreated" hair) should show a perm set around 95 %. The intensity of permanent wave depends on the pH and the concentration of the TGA solution. *Sauer* /62/ has shown that a reduction solution of pH 9.0 and a TGA concentration more than 5 % w/w produces high perm sets when the reducing species is present in a large excess.

The permanent waving process is performed in two steps: #1 reduction with a TGA solution, and #2 oxidation with an unbuffered 2.3 % w/w hydrogen peroxide solution. The concentration of the most suitable TGA solution has to be determined. The perm set should be around 95 % for this treatment. A lower initial value could lead to inappropriately small results of the relaxed and subsequently permed hair samples. Perm sets around 100 % or higher provoke partial supercontraction of hair, an additional damage which is undesirable.

Three different TGA concentrations were tested at pH 9.0. The results are summarized in Tab. 11. It was shown that a TGA concentration of 8 % w/w at pH 9.0 yields a perm set of 95.3 %; the optimum starting point of previously untreated hair for further investigations.

Tab. 11: Perm set, S_P^0, of previously untreated Caucasian hair at different TGA concentrations.
Perm parameters: Reduction with TGA solution (pH 9.0) for 10 min at 30 °C, rinsing for 15 min at RT, oxidation with H_2O_2 solution (3 %) for 10 min at 30 °C, rinsing for 10 min at RT.

Concentration of TGA in % w/w	Perm set S_P^0 in % ± standard error
6.0	88.4 ± 1.9
7.0	93.5 ± 0.6
8.0	95.3 ± 0.7

3.5.2. Quality of hair after treatment

Besides the waveability the relaxed and permed Caucasian hair samples were also investigated by AAA and DSC.

3.5.2.1. Waveability of hair

Fig. 56 shows the perm set of the hair samples which had previously been treated with the different relaxer creams. For a better differentiation, samples which were subsequently permed are annotated with “PW” (permanent wave).

As expected, perm set decreases with relaxing time. The ranking of perm set within the treatment series is as follows. The samples without thioles show the lowest perm set, followed by the samples containing Cys. The highest perm set is achieved after relaxing with the TGA-containing samples. The ranking can be explained by the number of remaining CyS-SCy bridges and Cys residues after the relaxing treatment. Permanent waving is mainly a reaction of these groups; firstly reduction of the disulfide bridges into Cys, followed by oxidation of the Cys back into CyS-SCy (Scheme 1). The remaining CyS-SCy and the Cys residues are given by the amount of CyS-SCy which can be detected after the relaxing treatment. This has been shown in the previous chapter. Thus, the ranking of perm set corresponds to the amount of CyS-SCy after the relaxing treatment (Fig. 46). The stronger the reduction of CyS-SCy during relaxing treatment, the better is the waveability of hair.

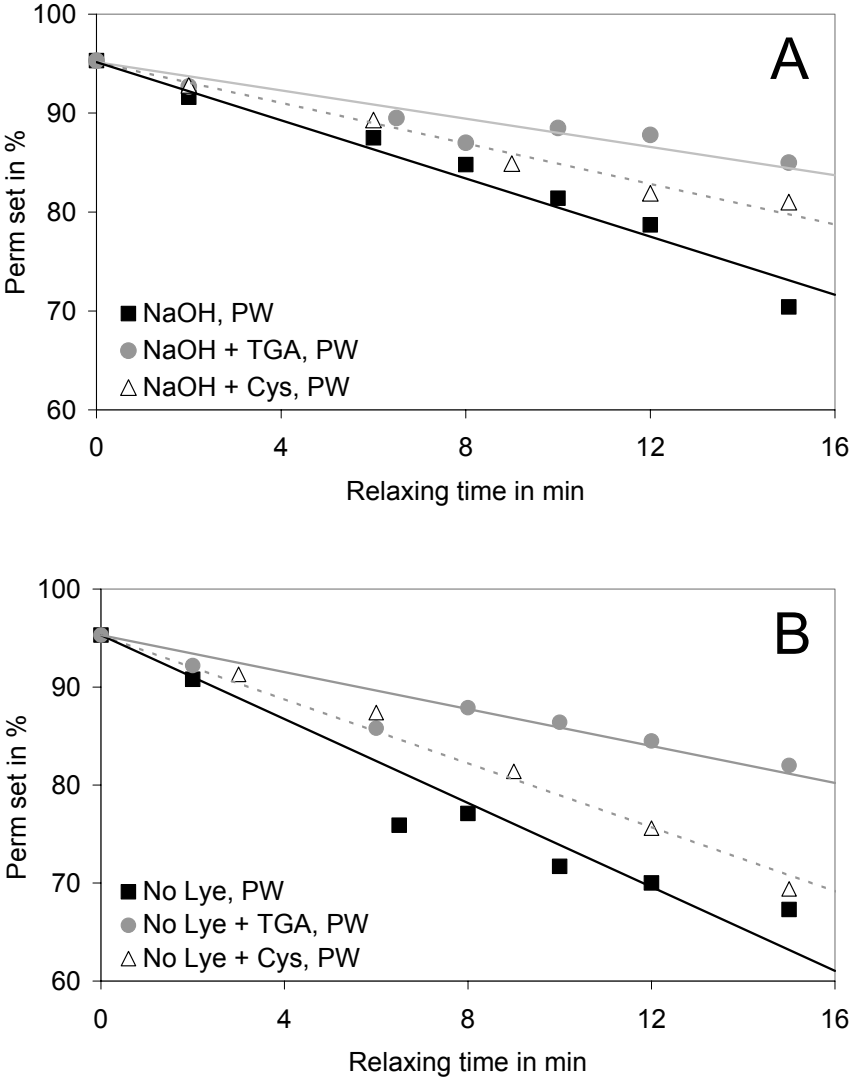


Fig. 56: Waveability of previously relaxed Caucasian hair.
A: NaOH treatments
B: No-Lye treatments

There seems to be generally a linear relationship between perm set and relaxing time, described by eq. 3.17, where $S_P(t)$ is the perm set at relaxing time t and k_S the effective rate constant.

$$S_P(t) = S_P(t_0) - k_S t \tag{3.17}$$

The slope, k_S, is given in Tab. 12 for the different treatments.

Tab. 12: Calculated rate constants for the relation between perm set and relaxing time for differently relaxed and subsequently permanent waved (PW) hair

Treatment	Rate constant, k_S ± standard deviation in min^{-1}
NaOH, PW	1.5 ± 0.2
NaOH + TGA, PW	0.7 ± 0.4
NaOH + Cys, PW	1.0 ± 0.2
No-Lye, PW	2.1 ± 0.6
No-Lye + TGA, PW	0.9 ± 0.5
No-Lye + Cys, PW	1.6 ± 0.2

The relationship between perm set and straightening effect shows to what extent hair can be sufficiently waved when it is relaxed before. The fit equations of the straightening effect (3.5) and the perm set (3.18) are compared via the relaxing time as eqs. 3.18 and 3.19 show, with $S_P(t)$ being the perm set at relaxing time t, $S_P(t_0)$ the perm set for the untreated hair, k_S the effective rate constant of perm set, Δt the time lag of straightening, τ the characteristic straightening time, $E_{St}(t)$ the straightening effect at time t, and E_{St}^{max} the maximum straightening effect (100%):

$$-\frac{S_P(t)-S_P(t_0)}{k_S}=\Delta t-\tau\ln\left(1-\frac{E_{St}(t)}{E_{St}^{\max}}\right) \tag{3.18}$$

Transformation leads to the dependence of the perm set on the straightening effect:

$$S_P(t)=S_P(t_0)-k_S\left(\Delta t-\tau\ln\left(1-\frac{E_{St}(t)}{E_{St}^{\max}}\right)\right) \tag{3.19}$$

A graphical plot of this dependency is shown in Fig. 57. The data of Tab. 6 and 12 are used for calculations of the straightening effect and the perm set at time t with $S_P(t_0)$ = 95.3 %.

Generally, the more the hair is straightened, the less it can be waved. The creams of the No-Lye-series preserve hair waveability to a higher degree than the creams of the NaOH-series for equal straightening effects. Waveability is mainly attributed to the amount of intact disulfide bridges /3/. The longer the hair is relaxed, the lower is the amount of CyS-SCy and thus the poorer the hair can be waved. Furthermore, hair which is relaxed by creams of the NaOH series has a higher amount of intact disulfide bridges than hair which is relaxed by creams of the No-Lye series (c.f. chapter 3.4.2.1.).

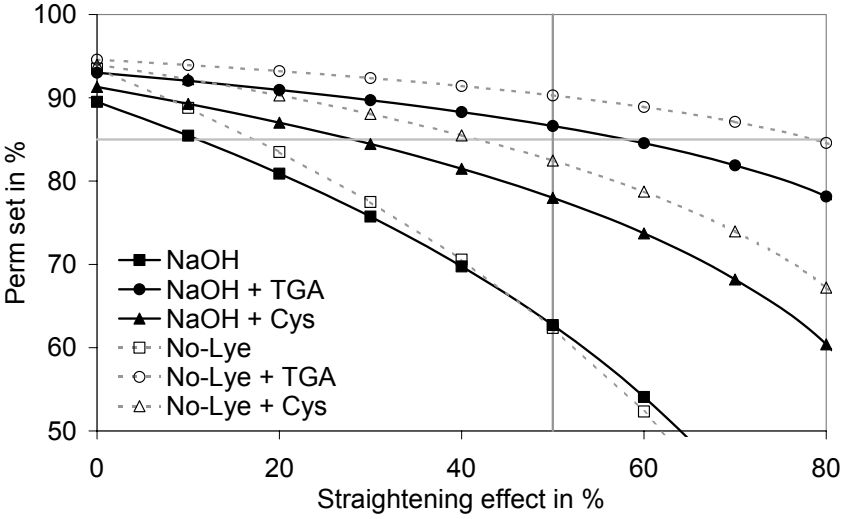


Fig. 57: Perm set versus straightening time.
The data are calculated by using eq. 3.18, with $S_P(t_0)$ = 95.3 % and the parameter values of Tabs. 6 and 12.

Generally, a perm set of around 85 % is practically an acceptable waving value. In order to obtain a perm set of around 85 % (grey horizontal line in Fig. 57), the hair must not be straightened by more than 10 -20 % with pure relaxer creams, 30 – 40 % with Cys containing creams, and 60 - 80 % with TGA containing creams. Let one assume that for Afro hair a minimum straightening effect of 50 % (grey vertical line in Fig. 57) is needed in order to be manageable as to put one's hair on curlers, only treatments with TGA containing creams reach subsequently suitable waving efficacy of around 85 %.

As an example, Tab. 13 shows the calculated values of possible perm set when the hair is 50 % straightened. Furthermore, the relaxing times which are necessary to reach a straightening of 50 % are displayed. The data are calculated by using eq. 3.18 and 3.5 with $S_P(t_0)$ = 95.3 % as well as the data of Tabs. 6 and 12.

Tab. 13: Calculated values for perm set and relaxing time for different treatments, when hair is 50 % straightened.
Eq. 3.5 and 3.18 with $S_P(t_0)$ = 95.3 % and the data of Tabs. 6 and 12 were used for the calculation.

Treatment	Perm set in %	Relaxing time in min
NaOH, PW	62.7	21.7
NaOH + TGA, PW	86.6	12.4
NaOH + Cys, PW	78.0	17.3
No-Lye, PW	62.4	15.7
No-Lye + TGA, PW	90.3	5.6
No-Lye + Cys, PW	82.5	8.0

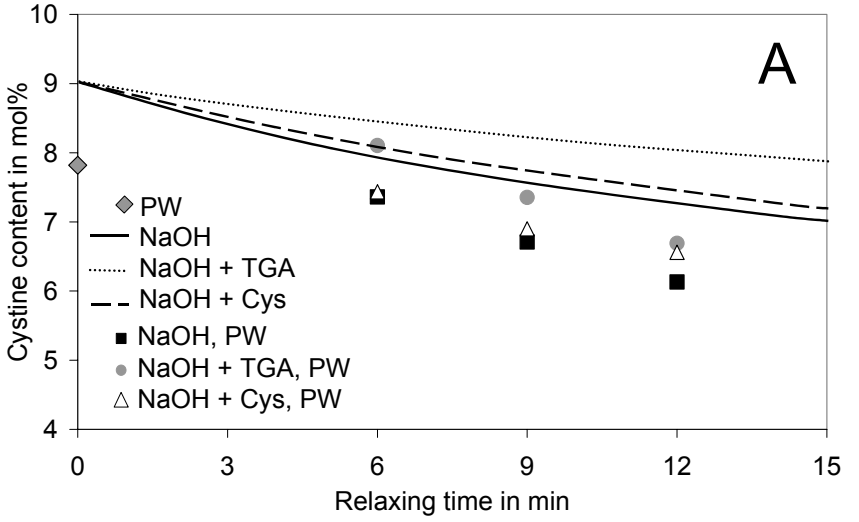
Tab. 13 shows that treatments of hair with TGA containing creams produce high perm set values at short relaxing times. Even Cys containing creams lead to better results than the pure relaxer creams.

3.5.2.2. Amino acid composition

20 different AAs were detected and determined after acid hydrolysis of the previously relaxed and permed hair samples. Tab. 14 exemplifies the AA content of permed hair which was either previously untreated hair or relaxed for 12 min with the different relaxer creams. The AAs CyS-SCy and Lan are bold typed to highlight significant changes.

The relationships between CyS-SCy and Lan content of permed hair with the previous relaxing time is shown in Fig. 58 (CyS-SCy content) and 59 (Lan content). In order to compare the AA contents of relaxed hair before and after perming, the results for both the relaxed (cf. Fig. 46/CyS-SCy and 47/Lan) and the relaxed plus permed hair are shown in the same figure. The fitted AA values for the relaxed hair (eq. 3.8 and 3.9, and data of Tabs. 8 and 9) are drawn as curves; the experimental AA contents for the relaxed plus permed hair are given as dots. Additionally, the value of only permed (relaxing time = 0) hair is shown.

The results demonstrate that a permanent wave leads to a further decrease of the CyS-SCy (Fig. 58) and increase of the Lan content (Fig. 59) for previously relaxed hair. In contrast to this, previously untreated hair shows no increase of Lan after the perm treatment.



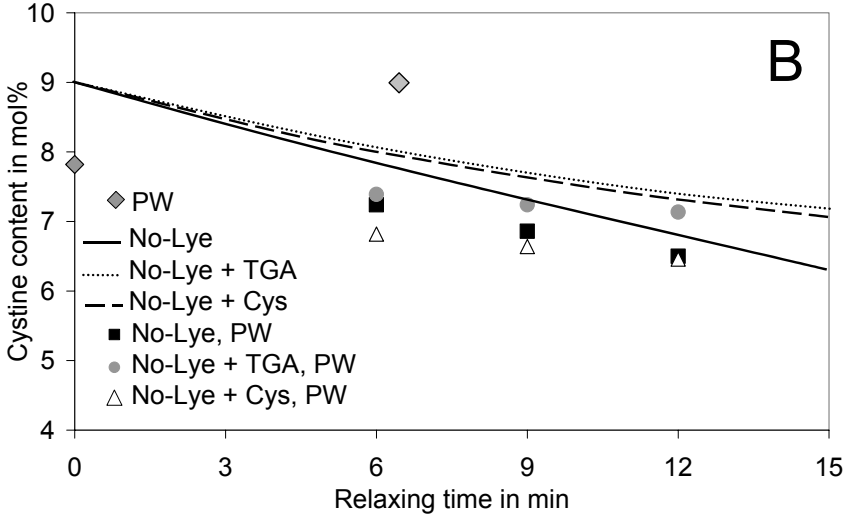


Fig. 58: Change of CyS-SCy content after a perm treatment versus previous relaxing time. The curves represent the fitted values of the only relaxed hair; the dots are the determined CyS-SCy contents of the relaxed and permed hair; = CyS-SCy content of only permed hair.
A: NaOH treatments
B: No-Lye treatments

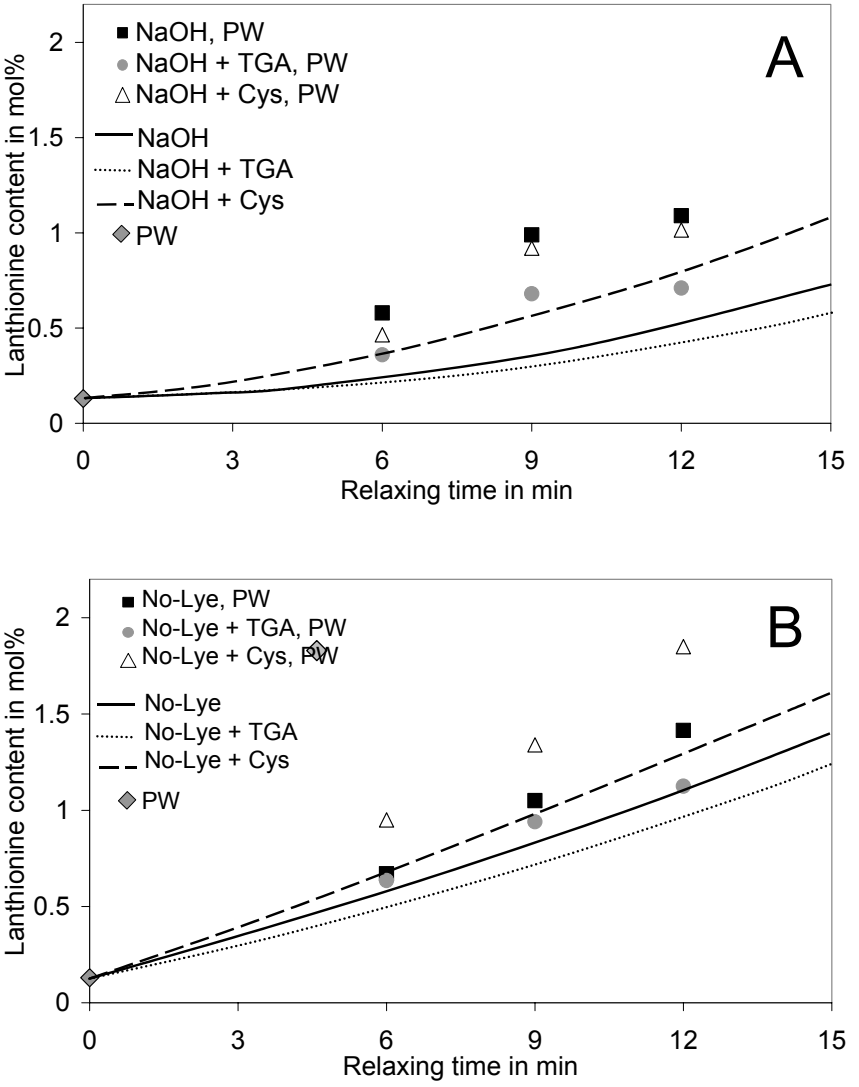


Fig. 59: Change of Lan content after a perm treatment versus previous relaxing time. The curves represent the fitted values of the only relaxed hair; the dots are the determined Lan contents of the relaxed and permed hair; = Lan content of only permed hair.
A: NaOH treatments
B: No-Lye treatments

Tab. 14: Amino acid content in mol% of permed Caucasian hair which was either previously untreated or relaxed by different treatments.

Treatment / AA	PW	NaOH PW	NaOH + TGA PW	NaOH + Cys PW	No-Lye PW	No-Lye + TGA PW	No-Lye + Cys PW
Cysteic acid	2.1	1.9	1.9	2.0	1.8	1.7	1.8
Aspartic acid + asparagine	6.2	6.3	6.4	6.3	6.2	6.1	6.3
Threonine	7.7	7.8	7.5	7.8	7.7	8.1	7.4
Serine	10.6	10.4	10.4	10.5	10.7	11.3	10.5
Glutamic acid + glutamine	14.0	13.7	12.8	13.0	13.7	13.5	13.9
Proline	8.9	8.8	8.7	9.2	8.4	7.7	8.1
Glycine	6.8	6.9	6.9	6.9	7.2	7.0	6.9
Alanine	4.8	5.1	5.2	5.2	5.1	5.2	4.7
Valine	6.5	6.5	6.6	6.6	6.5	6.5	6.3
Cystine	**7.8**	**6.2**	**6.9**	**6.7**	**6.5**	**7.1**	**6.5**
Methionine	0.5	0.4	0.4	0.3	0.2	0.1	0.3
Isoleucine	3.2	3.3	3.6	3.3	3.1	3.0	3.4
Leucine	6.6	7.1	7.5	7.4	7.2	7.1	7.4
Tyrosine	2.1	2.1	2.0	1.4	1.8	1.8	2.2
Phenylalanine	1.9	1.6	1.7	1.8	1.8	1.8	1.8
Ornithine	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Lysine	2.6	2.7	2.8	2.7	2.8	2.9	2.8
Histidine	0.9	0.9	0.9	0.9	0.8	0.8	0.9
Arginine	6.5	6.7	6.8	6.8	6.8	6.8	6.7
Lanthionine	**0.1**	**1.3**	**0.8**	**1.0**	**1.4**	**1.2**	**1.7**

Lan originates from the reaction between dehydroalanine and Cys. Dehydroalanine is rarely formed during perm treatment. The formation of Lan in the previously relaxed samples indicates that after the relaxing treatment part of the alkaline degraded CyS-SCy remains as dehydroalanine. The decrease of CyS-SCy and increase of Lan after waving is nearly equal for all relaxing treatments. The decrease of CyS-SCy varies between 0.7 and 1.2 mol%; the increase of Lan between 0.2 and 0.5 mol%. The increase of $CySO_3H$ is between 1.2 and 1.6 mol%.

Thus, alkaline degraded CyS-SCy which is not transferred into Lan, gives rise to cysteic acid formation. The damage imposed by the subsequent perm treatment corresponds to the damage associated with a normal permanent wave. The former relaxing treatment does not intensify the damage related to the permanent wave.

3.5.2.3. Thermal properties

Fig. 60 shows the changes of denaturation enthalpy after permanent wave of hair previously relaxed for different length of time. The enthalpy values as well as the curves for the just relaxed hair are given to enable a direct comparison.

The enthalpy decreases after further perm treatment. Permanent waving thus leads to an increased degradation of helical domains in hair. The decrease of enthalpy of hair is stronger for hair which has been previously treated with a TGA containing relaxer cream, than for hair which has previously treated with Cys containing relaxer cream or with pure relaxer creams. This indicates that additional TGA during the relaxing process enables a further denaturation of helix domains in the hair fiber during the waving treatment. The decrease of enthalpy after a subsequent wave is approximately the same for both kinds of creams.

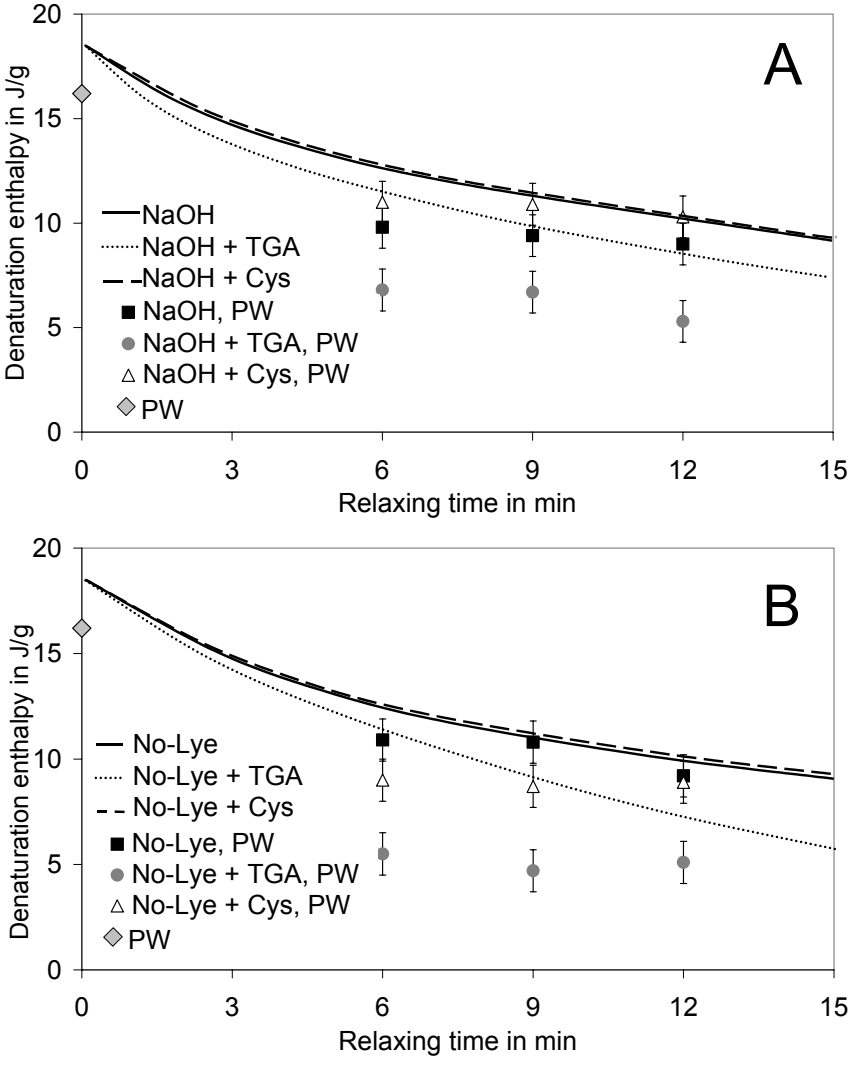


Fig. 60: Relationship between denaturation enthalpy of relaxed and subsequently permed hair and relaxing time.
The curves represent the fitted values of enthalpy for the only relaxed hair; the dots are values for the relaxed and permed hair; ◇ = enthalpy of only permed hair.
Error bars represent the standard deviation.
A: NaOH treatments
B: No-Lye treatments

A different behavior is observed for the denaturation temperature. The relationship between denaturation temperature and relaxing time is shown in Fig. 61. The denaturation temperature of permed hair is lower than that of untreated hair. Therefore, the matrix around the helical domains is altered. In contrast to this, a higher denaturation temperature is observed for previously relaxed hair after perm treatment. Whereas the denaturation temperature of the NaOH series increases by around 2 °C independent of relaxing time or of cream composition, the denaturation temperature of the No-Lye-series treatments increases with relaxing time. The increase of denaturation temperature can be caused by a strengthening of the matrix by additional covalent bonds, e.g. disulfide bridges or isodipeptide bonds.

Fig. 62 shows the dependence of perm set on the helical content of hair (before waving). The values of the fitted curves of Fig. 60 (denaturation enthalpy versus time) and of Fig. 56 (perm set versus relaxing time) are used for Fig. 62. The curves of perm set are calculated using eq. 3.18, the values for the effective rate constant, k_S, are taken from Tab. 12. The data of the relationship between denaturation enthalpy and perm set are represented by an empirical fitted curve.

The less helical domains are detected the worse is the perming performance of the hair. But perm set is not only dependent on denaturation enthalpy. It is of great importance how the helical content has been reduced. The NaOH-series and No-Lye-series differ in pH. Therefore, the overlapping curves express that the reactions during relaxing are not dependent on pH but on the ingredients of the creams.

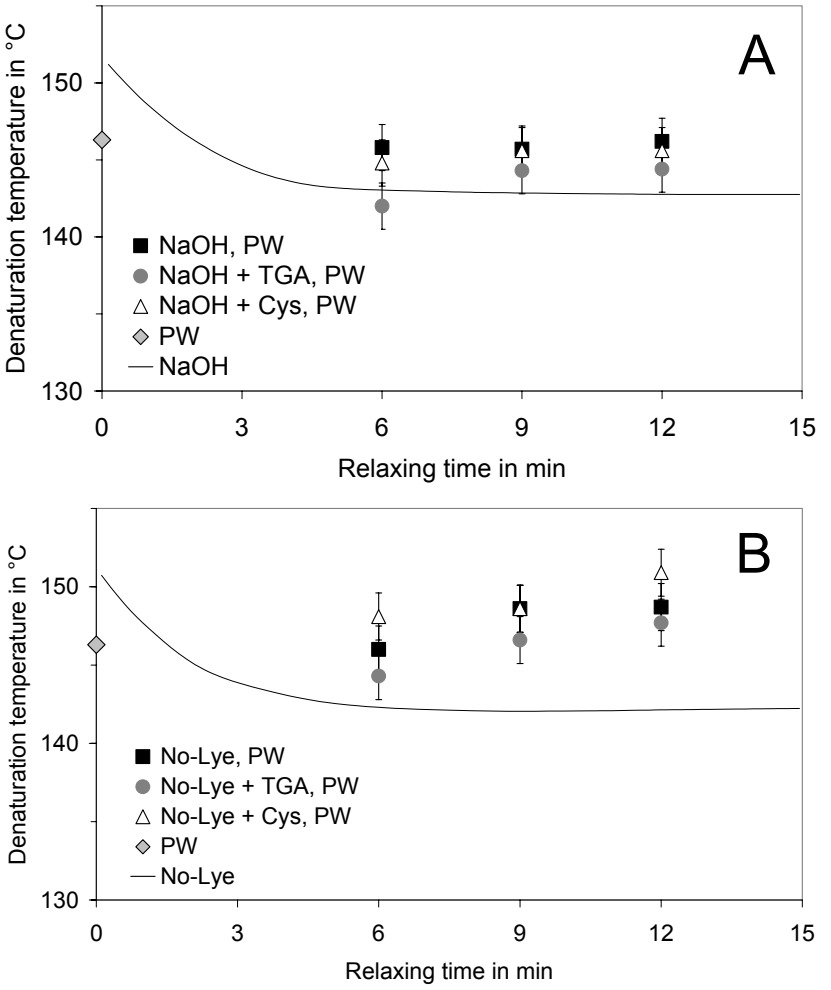


Fig. 61: Relationship between denaturation temperature of relaxed and subsequently permed hair with relaxing time.
The curve represents the fitted values of denaturation temperature for the only relaxed hair (which are nearly the same for all treatments); the dots are the determined denaturation temperatures of the relaxed and permed hair and of only permed hair, respectively. Error bars represent the standard deviation.
A: NaOH treatments
B: No-Lye treatments

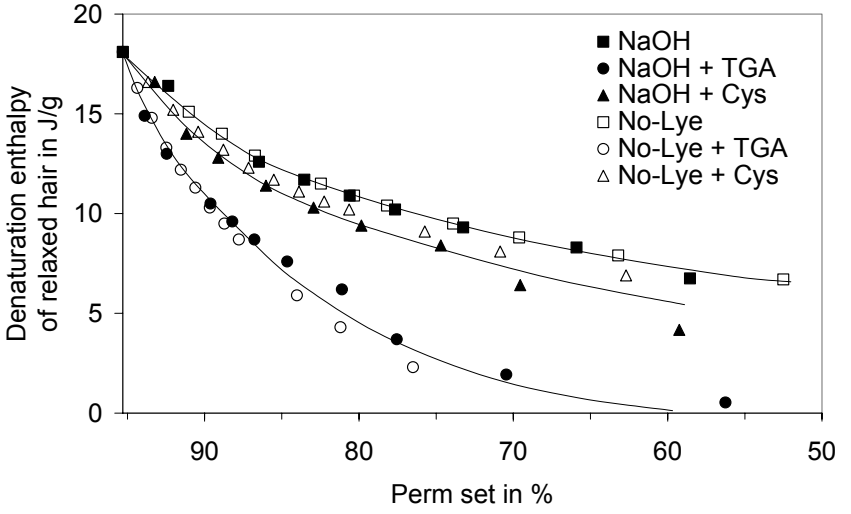


Fig. 62: Dependence of perm set on denaturation enthalpy.
The data of perm set are calculated using eq. 3.16, the parameters are taken from Tab. 12. The data of denaturation enthalpy are measured data.

Hair which is relaxed with TGA containing creams reaches higher perm set with equal helix content compared to hair which is relaxed with pure relaxer creams or Cys containing creams. TGA reduces a higher amount of disulfide bridges than Cys during relaxing. This reduction leads to change of the molecular structure of the helical domains. The domains change into amorphous structure and thus, less denaturation enthalpy is detected with DSC. Since the protein fiber itself is not completely destroyed, it can be re-structured by a permanent wave. Cys is able to reduce disulfide bonds, too, but re-oxidation of the bridges takes place more often during relaxing when additional Cys is available. Hence, the amount of helical domains does not alter considerably for Cys containing creams compared to pure relaxing creams.

3.6. Swelling and diffusion experiments

Tests for waveability of relaxed hair showed that the longer the hair was relaxed, the lower the waveability. Since the waveability of relaxed hair is dependent on the amount of not degraded CyS-SCy of hair, it is of interest to determine which CyS-SCy bridges of the fiber are destroyed by alkali. There are two possibilities for the alkaline degradation of the fiber. Either a rapid diffusion of alkali takes place and the reactions of alkali with the hair proteins occur more or less over the whole fiber, or the diffusion of the reagent is time dependent. This would lead to a diffusion front of the reagent and thus to a reaction front. To resolve this issue, *Sauer* /62/ used the method of *Herrmann* /63/ to monitor the penetration of TGA into fibers pre-dyed with iodine. The method is based on the assumption that the cleavage of disulfide bridges and the disproportion of iodine occur simultaneously. The swelling of the hair fiber and the removal of color, which is caused by the disproportion of iodine, were observed under a light microscope and recorded on video. On the assumption that the cleavage of disulfide bridges is the prerequisite for fiber swelling, for the alkali treatment the analysis of the swelling yields information on the kinetics of the β-elimination. Conclusions can be drawn about diffusion and reaction from the nature and rate of color removal.

3.6.1. Experimental aspects

Since the color of iodine dyed Afro hair is very similar to the natural color of Afro hair it is not possible to distinguish between an iodine dyed and a naturally colored area of Afro hair. Thus, blond, untreated Caucasian hair was used for the experiments.

The hair was dyed with aqueous iodine and pre-swollen with water. Excess water is then removed and replaced by the alkaline solution. The fiber

environment was kept constant by a continuous supply of fresh solution. Swelling and color changes were recorded on video and subsequently analyzed by image analysis. Alkaline solutions with pH 12.4, 12.8 and 13.4, as well as solutions which contained 1 % w/w Cys or TGA at pH 12.8 were investigated. The pH was adjusted with sodium hydroxide.

3.6.2. Analysis of swelling

In the previous chapter it was shown that degradation of CyS-SCy during relaxing follows pseudo-first order kinetics:

$$\frac{d\alpha}{dt} = k(1-\alpha) \tag{3.20}$$

Where α is the degree of conversion ($0 \leq \alpha \leq 1$). The end point of the reaction is reached when all disulfide bridges are broken. k is the rate constant. Integration results in eq. 3.21:

$$-\ln(1-\alpha) = kt \tag{3.21}$$

In a plot of $-ln(1-\alpha)$ against time the slope of the straight line provides the rate constant, k.

Reese and *Eyring* /64/, *Kubu* and *Montgomery* /65/, *Katz* and *Wakelin* /66/, and *Wickett* /67/ showed that the stress developed in hair fibers, subjected to a defined strain, is directly proportional to the concentration of intact disulfide bridges. Since swelling (and thus the increase in volume) is caused by breaking disulfide bridges, a direct relationship is assumed between the number of broken disulfide bridges, $\Delta[SS]$, and the increase of hair volume, ΔV:

$$\Delta[SS] \propto \Delta V \tag{3.22}$$

On this basis the increase of volume during the reaction is used as a measure of the extent of reaction. The conversion, α, is thus defined as follows:

$$\alpha = \frac{V_t - V_0}{V_\infty - V_0} \tag{3.23}$$

Where V_t is the volume of hair at time t, and V_0 and V_∞ are the initial and final hair volume, respectively. Instead of the volume, the diameter (d) of the hair is measured. It is assumed that the volume of hair increases homogenously during the reaction. Volume changes related to changes of fiber length are neglected. Thus, volume and diameter of hair are linked by eq. 3.24:

$$V = \frac{\pi}{4} d^2 \tag{3.24}$$

Since the reaction time is limited for practical reasons, a direct determination of the maximum diameter is not possible (hair dissolves at longer reaction times of alkaline treatments). To determine the final diameter (d_{max}) of hair, the diameter, d_t, were fitted by the exponential function 3.25, which describes first-order kinetics. Thus, parameter b is the rate constant of this reaction.

$$d_t = d_{\max}(1 - e^{-bt}) + d_0 \tag{3.25}$$

The conversion, α, is calculated by eq. 3.26, with d_0 being the diameter of the untreated, pre-swollen hair:

$$\alpha = \frac{d_t^2 - d_0^2}{d_{\max}^2 - d_0^2} \tag{3.26}$$

Fig. 63 shows the typical process of swelling of a hair fiber at pH 13.4. Always the same region of the fiber is shown. The alkali reacts with the elemental iodine (black areas) to form the colorless products iodate and iodide (scheme 6).

$$3\ I_2 + 6\ OH^- \qquad IO_3^- + 5I^- + 3\ H_2O$$

Scheme 6: Disproportionation of iodine into iodate and iodide.

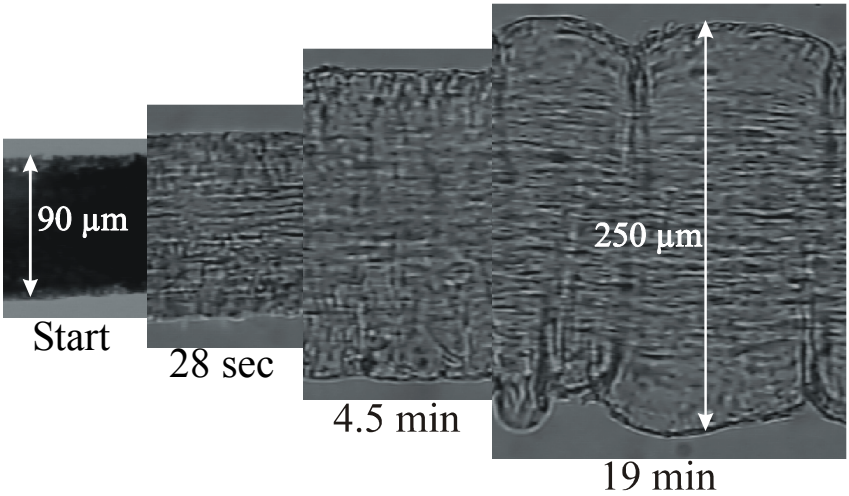


Fig. 63: Process of swelling of a blond, Caucasian hair fiber at pH 13.4. The pictures always show the same segment of the hair fiber for different treatment times.

Fig. 64 shows the results of the swelling experiments for hair which was treated with alkaline solutions at different pHs, and Fig. 65 shows the results of the swelling experiments for hair which was treated with solutions at pH 12.8 containing additional Cys or TGA. In order to compare the results of hair fibers with different initial diameters, the swelling, $S_{\%}$, is given in percent which is calculated by eq. 3.27:

$$S_{\%} = \left(\frac{d_t}{d_o} - 1 \right) 100\% \qquad (3.27)$$

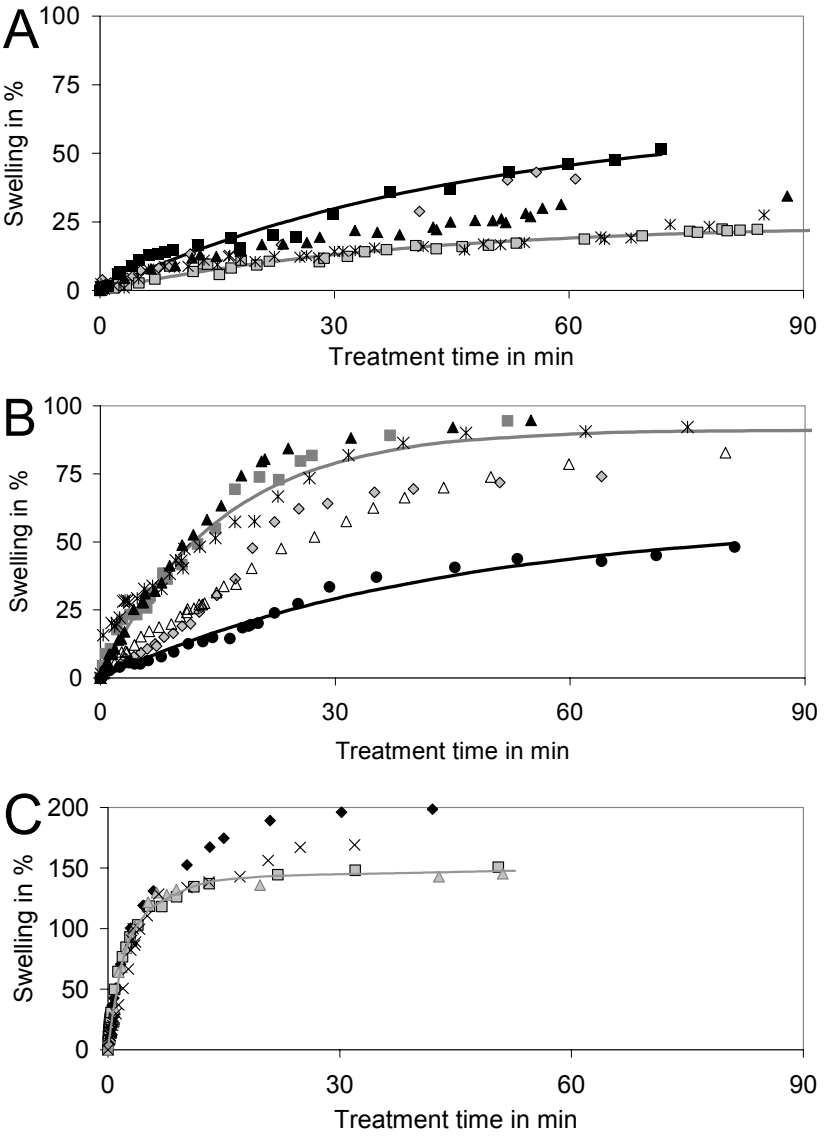


Fig. 64: Swelling of blond, Caucasian hair fibers when treated at different pHs. Each symbol shows a single experiment. Lines represent examples for fitted data by using eq. 3.25.
A: pH 12.4
B: pH 12.8
C: pH 13.3

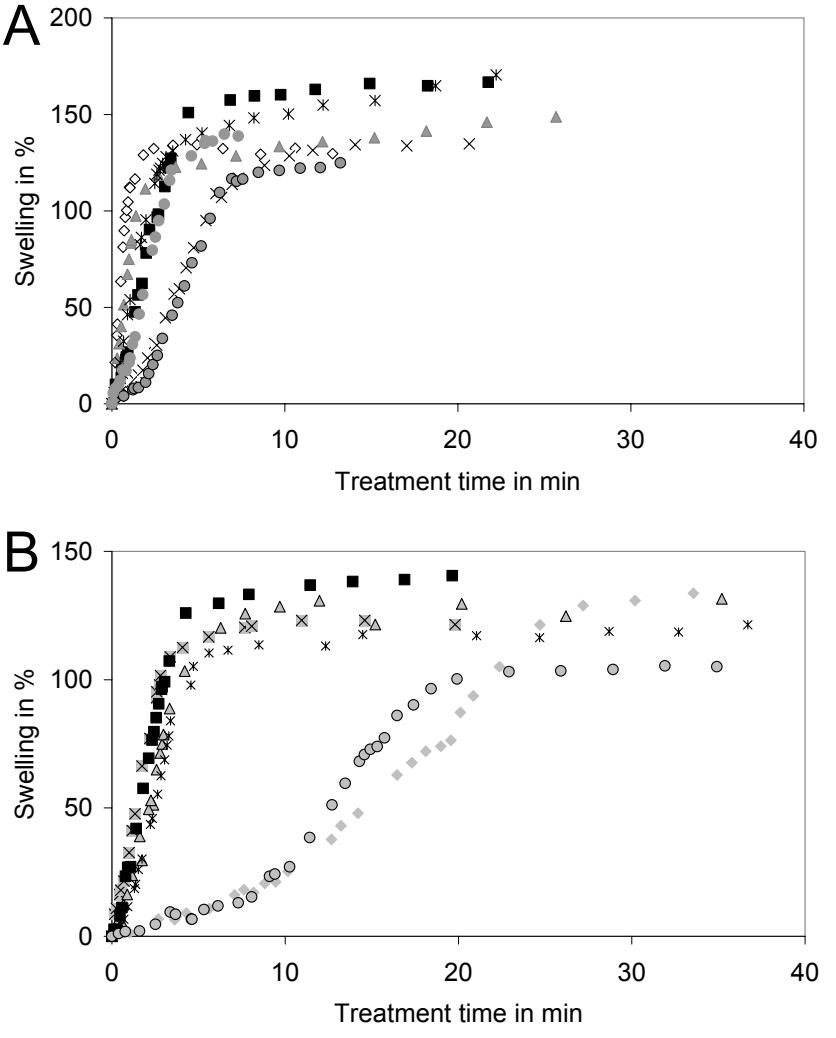


Fig. 65: Swelling of blond, Caucasian hair fibers during different treatments at pH 12.8. Each symbol shows a single experiment.
A: pH 12.8 + TGA
B: pH 12.8 + Cys

The graphs show that the swelling of hair is dependent on the treatment solution. The higher the pH, the more rapid and extensive is the swelling. Solutions which contain thioles also cause a faster and higher level of swelling of the hair fiber than solutions without thiole. Tab. 15 shows the maximum swelling of hair fiber, $S_{\%}^{max}$ for different diffusion conditions.

Tab. 15: Mean maximum swelling of hair fiber, $S_{\%}^{max}$ (± standard deviation) under different diffusion conditions (n = 5).

Diffusion conditions	Maximum swelling, $S_{\%}^{max}$ in %
pH 12.4	34 ± 11
pH 12.8	80 ± 19
pH 13.3	161 ± 29
pH 12.8 + TGA	147 ± 17
pH 12.8 + Cys	126 ± 13

As Figs. 64-65 and Tab. 15 show, alkaline solutions with a pH of 12.4 lead to a mean maximum swelling ob about 35 %. For pH 13.3 the mean maximum swelling is around 160 %. Addition of thioles leads to a greater swelling. While fibers swell on average by about 80 % at pH 12.8, with the addition of 1 % w/w TGA they swell by about 150 %, and with additional Cys about 125 % at the same pH.

The cleavage of disulfide bridges by alkali leads to negatively charged Cys residues. The protein chains of hair fibers repel each other. Since the bridges between the protein chains are broken, the distance between the protein chains is increased and the solution diffuses faster and to a greater extent into the fiber.

Thus, the hair fiber swells. With the concentration of alkali the number of negative charges increases within the fiber, and hence swelling increases. Addition of thioles leads to more cleaved disulfide bridges as well as to the formation of Cys residues and thus, to more negative charges of the protein chains. Therefore, the hair swells faster and more extensively compared to hair which is treated without additional thioles at the same pH.

To compare the results on a kinetic basis, for each experiment the average swelling result, assuming first-order kinetics, was plotted as $-ln(1-\alpha)$ against time t using eq. 3.26 for calculating the degree of conversion, α. For those experiments where a linear relationship between these two quantities was observed, the rate constant, k, was determined from the slope. The plots are shown in Fig. 66.

It becomes clear that a linear relationship is observed only for the experiments at pH 12.4 and 12.8 without thioles (but with deviation at long treatment times). Thus, swelling follows pseudo-first order kinetics for these treatments. The rate constant, k, of the experiments was determined to be 0.023 min^{-1} for pH 12.4 and pH 12.8. Thiole containing solutions at pH 12.8 and the solution at pH 13.4 show no first-order relationship.

Sauer /62/ has shown that a direct relationship exists between swelling and the amount of broken disulfide bridges, when swelling follows pseudo first-order kinetics. The degradation of CyS-SCy of relaxed hair at pH 12.4 and 12.8 – determined by AAA – within this thesis has shown that it follows pseudo first-order kinetics. Thus, the existence of a direct relationship between swelling and the amount of broken disulfide bridges is confirmed for these conditions.

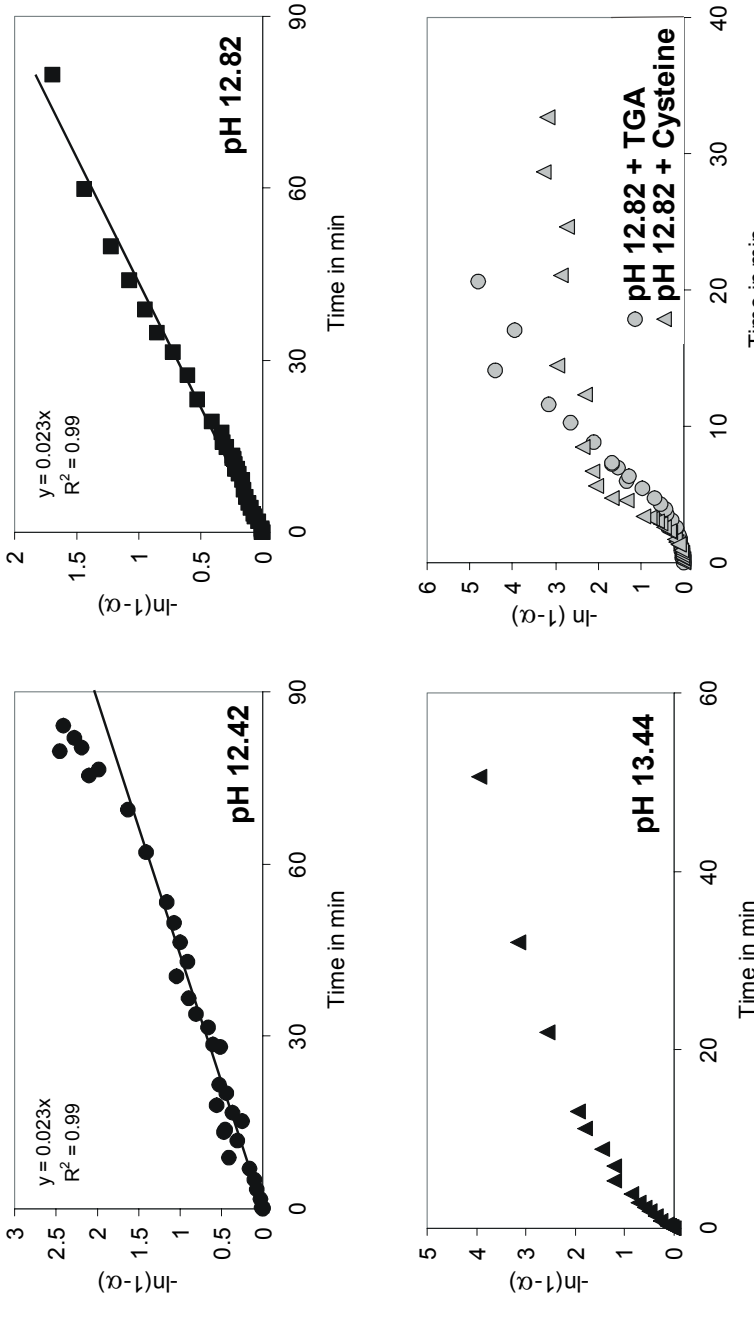


Fig. 66: Relationship between degree of conversion, α (calculated from pseudo first-order kinetics), and reaction time, t, for different treatments. The slope, k, represents the rate constant.

Swelling does not follow pseudo first-order kinetics at higher pH or with addition of thiole at pH 12.8. Therefore, one must assume that these treatments lead to further reactions beside β-elimination. These could include hydrolysis of the protein fiber at higher pH, or additional cleavage of disulfide bridges in the case of addition of thioles.

3.6.3. Analysis of diffusion

Simultaneously with the swelling, the removal of the color of the iodine resulted in the development of a sharp border line between the colorless outer area and the colored inner area of the fiber. The border line is clearly visible until it vanishes at complete fiber penetration (Figs. 67-68). Such a behavior can be often found for the swelling of vitreous polymers with or without simultaneous reaction. For example in 1947 *Hermanns* /68/ described such a process for the diffusion in gels.

Fig. 67 shows the diffusion process at different pH conditions. The diffusion processes for solutions which contain additional TGA or Cys at pH 12.8 are shown in Fig. 68. The penetration fronts were not visible from the very beginning for all experiments, but became increasingly well-defined in the course of the experiment. At the end of the diffusion process it was often not possible to separate the two areas exactly, especially at lower pH. The faster the diffusion occurred, the clearer the penetration front is.

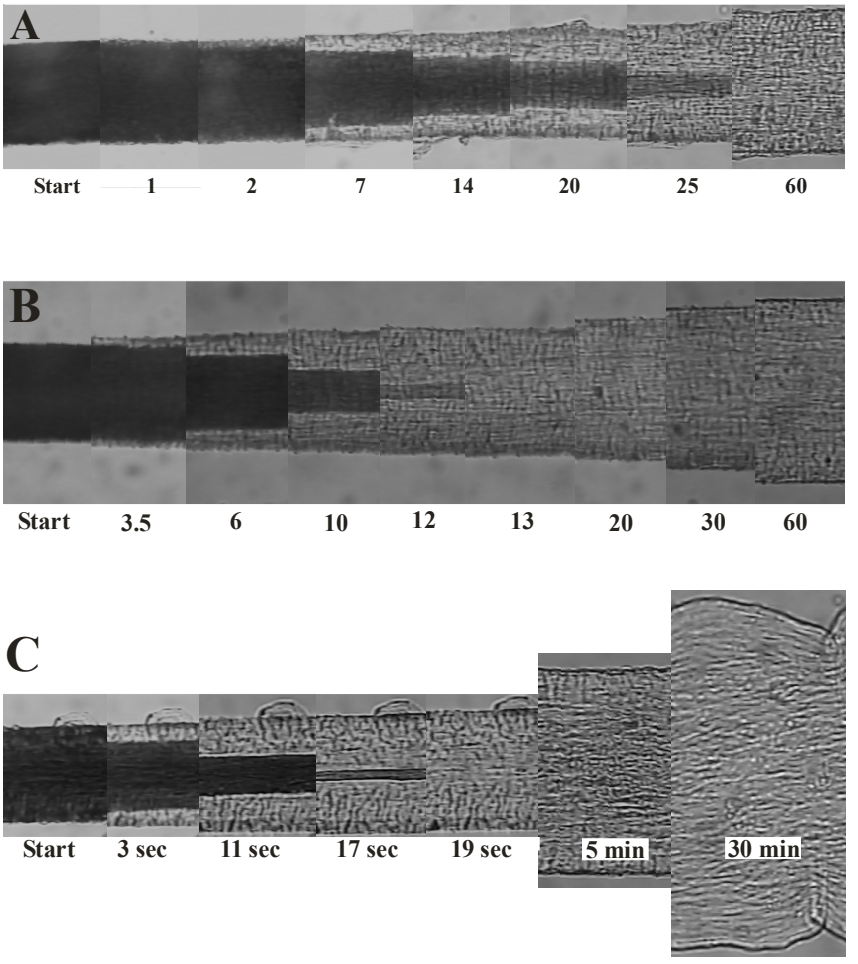


Fig. 67: Penetration of alkali into the hair fiber and swelling of the hair fiber at different pH. The same part of the fiber is always shown. Time is given in minutes, except for pH 13.4. There, the exact time is given for each picture.
A: pH 12.4
B: pH 12.8
C: pH 13.4

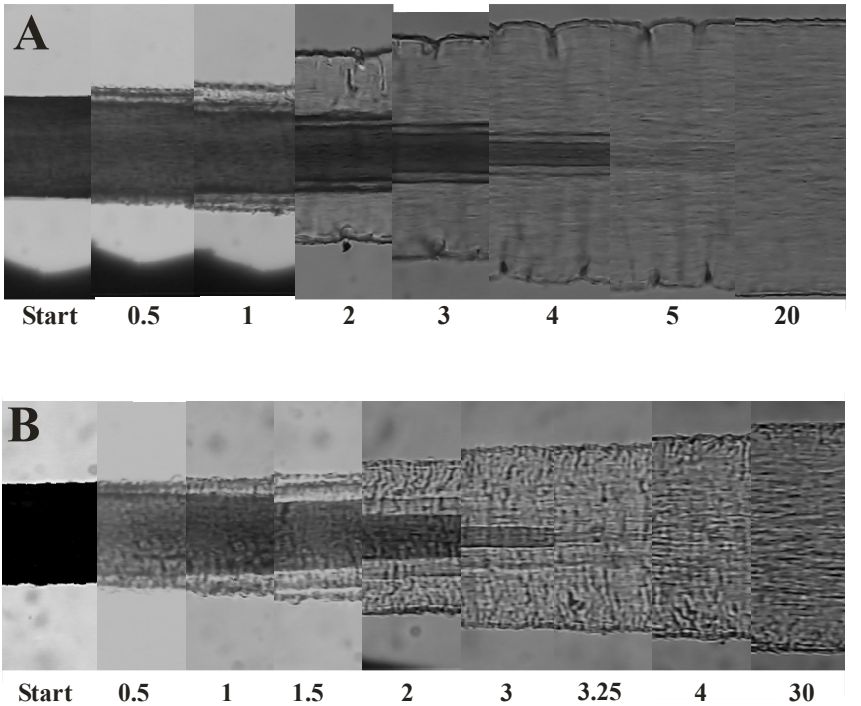


Fig. 68: Penetration of alkali and thioles into the hair fiber and swelling of the hair fiber at pH 12.8. The same part of the fiber is always shown. Time is given in minutes.
A: pH 12.8 + TGA
B: pH 12.8 + Cys

The thiole containing solutions showed two penetration fronts (Fig. 68). The distance between these fronts is nearly constant over the complete period of time. Since this behavior did not appear without thiole, one can assume that one of the penetration fronts is associated with the alkali, and the other one with the thiole. Most likely, the alkali diffuses first into the fiber because the alkaline molecules are much smaller than the thiole molecules. It is easier for the thiole molecule to penetrate into the fiber after it has been swollen by the alkali. Thus, the inner penetration fronts were used for comparisons of the rate of penetration of the alkali.

The migration of a substance is characterized by the diffusion coefficient, D. For the description of the process of diffusion of a solute into a solid cylinder, to apply *Fick's second law* is the traditional approach. It expresses the rate of change of the concentration C of substance at the point r within the cylinder with time t. This is proportional to the second derivative of the concentration gradient according to the following equation (r is the radius of the cylinder):

$$\frac{dC}{dt} = D\frac{d^2C}{dr^2} \tag{3.28}$$

Fick's equation is purely descriptive. It takes no account of molecular details and provides no basis for the prediction of the magnitude of the diffusion coefficient in a given system. However, it can be integrated to enable the evaluation of diffusion coefficients from experimental data. The complexity of the diffusion process leads to a study of diffusion on the basis of simple idealized systems.

The calculations must therefore be simplified by the inference of a number of assumptions /69, 70, 71/. Since the amount of diffusing alkali cannot be determined directly, *Sauer* /62/ used the penetration depth as a measure of the amount of diffused solute, according to eq. 3.29, where is the x_t penetration depth at time *t*:

$$x_t = \sqrt{Dt}\,\frac{4}{\sqrt{\pi}} \tag{3.29}$$

A straight line is obtained in a plot of x_t against $\sqrt{t}$. The diffusion coefficient can be calculated from the slope of the straight line. However, *Sauer* showed that the diffusion coefficient of reducing agents (TGA) at pH 8.7 is not constant, because he obtained a parabola instead of a straight line /62/. The same behavior was obtained for the results within this work. Thus, the diffusion of alkali does not show simple Fickian behavior. A diffusion coefficient cannot be determined with eq. 3.29 from the penetration depth.

Alfrey et al. /72/ described a special diffusion behavior, which they called "Case II" diffusion. It occurs in cylindrical or spherical, vitreous polymers which come into contact with a solvent. It is characterized by the formation of a sharp swelling front. This penetration front separates the vitreous nucleus from the swollen curved surface and penetrates constantly into the center of the polymer. While Fick's diffusion is described by the diffusion coefficient, Case II diffusion is characterized by the location of the penetration front which changes linearly with time. This corresponds to pseudo zero-order kinetics according to eq. 3.30 where x_t is the penetration depth at time t, and k the penetration rate constant:

$$x_t = kt \tag{3.30}$$

Fig. 69 shows the penetration curves at different pH values, and Fig 70 the curves produced for alkaline solutions with added thioles at pH 12.8. The inner penetration front was used to assess the treatments with added thiole. The plots of penetration depth versus time show that the penetration of alkali does not exhibit the same rate for all experiments at pH 12.4, 12.8 and at pH 12.8 with additional Cys. Two groups were observed under these conditions. This could be explained by differences in the aging of tips and roots. At the tip, the solvent might diffuse more rapidly into the fiber because of the damage associated with its age.

The penetration rate of alkali shows normal distributed values for both the treatments at pH 13.4 and with added TGA at pH 12.8. It may be possible that the reactions under these conditions are so fast that differences between tip and root of hair are of no consequence and thus cannot be differentiated anymore.

Treatments at pH 13.4 and at 12.8 with added TGA follow a linear course of penetration. Penetration follows the mechanism of Case II diffusion for these treatments. Another behavior occurs at pH 12.4 and 12.8 as well as for the treatment with added Cys. Within the first minutes of the experiment a linear course of penetration is observed, too, but the longer the treatment continues, the more the penetration differs from a linear course. Thus, penetration does not follow the mechanism of Case II diffusion over the whole time range of the experiment under these conditions.

To compare the differences in penetration rates, a linear regression was applied for a suitable time range. Tab. 16 gives an account of the determined penetration rates.

Tab. 16: Penetration rates for the inner diffusion front (alkali) with different treatments (± standard deviation), ($3 \leq n \leq 6$).

	Rate of (inner) penetration in 10^{-8}*ms^{-1}	
Treatment	Presumed region Tip	Root
pH 12.4	2.5 ± 0.3	0.5 ± 0.1
pH 12.8	8.4 ± 0.7	3.1 ± 0.4
pH 13.4	148.2 ± 46.1	
pH 12.8 + TGA	25.1 ± 17.1	
pH 12.8 + Cys	19.0 ± 8.1	3.4 ± 0.3

The results show that the higher the pH, the faster the penetration rate. Furthermore, the penetration of alkali was approximately two times faster when a thiole was added, though it should be noted that the penetration rate was larger with addition of TGA than with Cys. The effect of faster penetration is attributed to the greater and more rapid swelling of the fiber. The greater the extent of fiber swelling, the easier it is for the molecules to penetrate. However, the additional reduction of the disulfide bridges resulting from the presence of the thioles leads to enhanced swelling. Thus, it is a matter of mutual enhancement. Since TGA is a stronger reducing agent than Cys, more disulfide bridges are cleaved and thus the penetration of the molecules is faster.

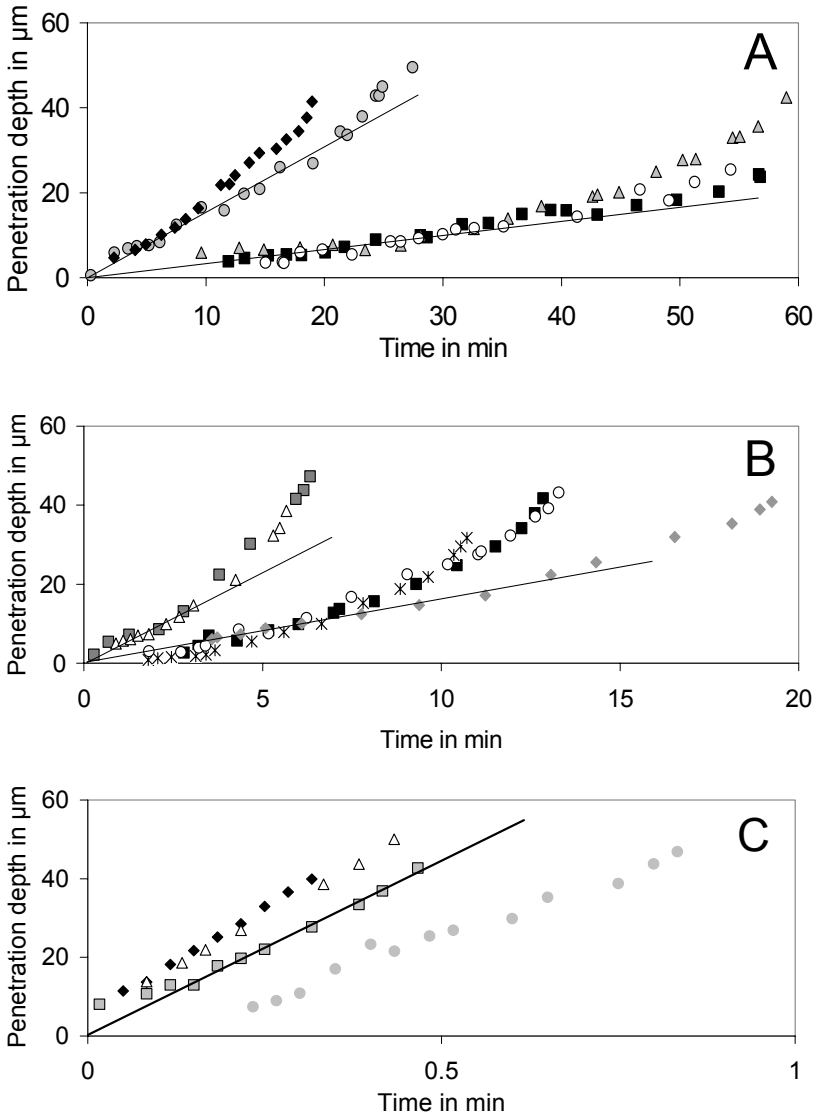


Fig. 69: Penetration depth versus time at different pH.
Each symbol corresponds to the result of one diffusion experiment.
The time scale is not equal for the different treatments.
A: pH 12.4
B: pH 12.8
C: pH 13.4

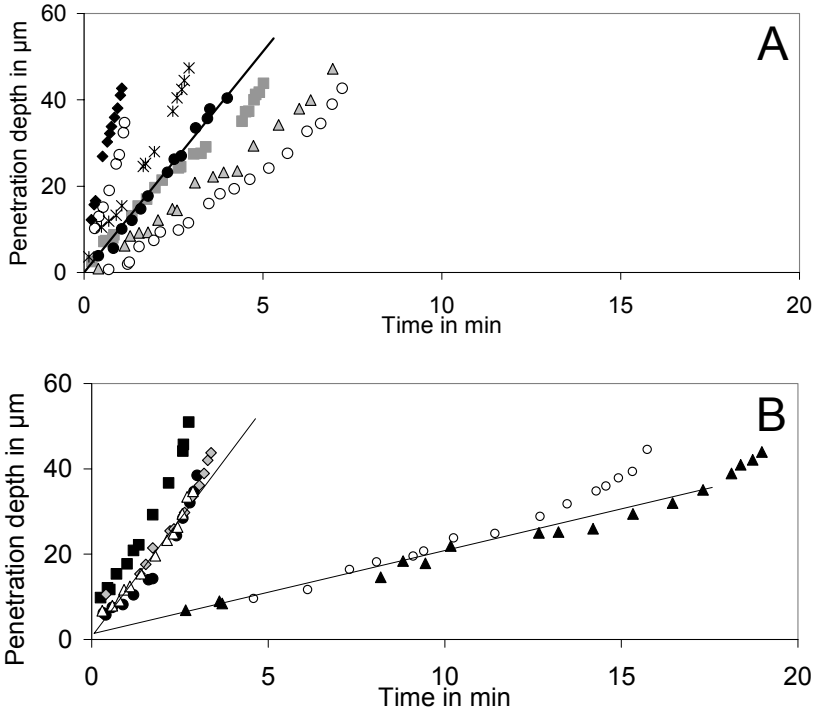


Fig. 70: Penetration depth versus time at different pH.
Each symbol corresponds to the result of one diffusion experiment.
A: pH 12.8 + TGA
B: pH 12.8 + Cys

Fig. 71 shows the penetration of the inner and outer fronts for the treatments with added thiole. Only a typical result for each kind of penetration is shown. Tab. 17 gives each of the determined penetration rates. The data show that the rate of penetration of the thiole is approximately the same as that of the alkali. However, the penetration of thioles starts with a time lag, compared to the alkali. The faster the rate of penetration, the shorter the time lag.

Tab. 17: Penetration rates of inner (alkali) and outer (thiole) penetration fronts (± standard deviation), ($3 \leq n \leq 6$).

	Rate of **inner** penetration in 10^{-8}*ms^{-1}		Rate of **outer** penetration in 10^{-8}*ms^{-1} (Time lag in min)	
Treatment	Presumed region Tip	Root	Presumed region Tip	Root
pH 12.8 + TGA	25.1 ± 17.1		27.0 ± 18.2 (0.6 ± 0.4)	
pH 12.8 + Cys	19.0 ± 8.1	3.4 ± 0.3	19.7 ± 6.9 (1.2 ± 0.8)	2.1 ± 0.2 (5.0 ± 1.1)

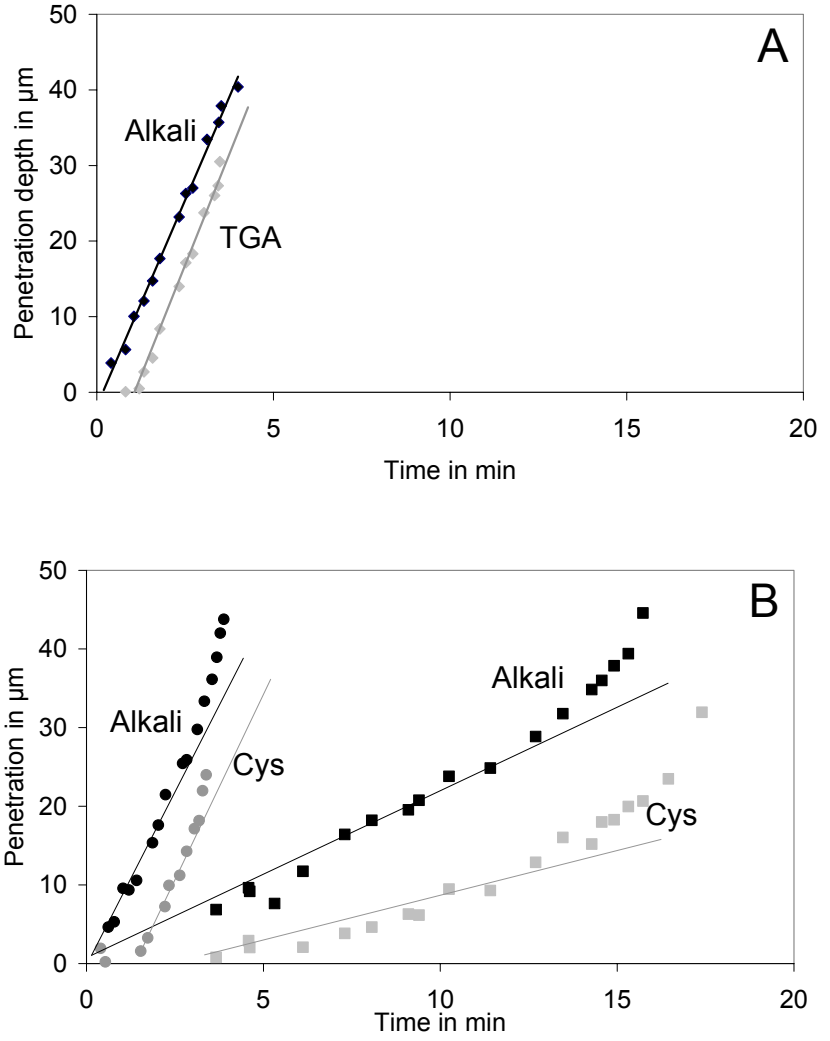


Fig. 71: Penetration depth of inner (alkali) and outer (thiole) penetration front versus time at pH 12.82 + TGA or Cys.
While the black dots represent the alkali penetration, the grey dots show the thiole penetration. Mean results are given.
A: pH 12.8 + TGA
B: pH 12.8 + Cys

In 1983 *Wickett* /73, 74/ developed a kinetic model to describes the rate of reduction of keratins by TGA at pH 10 and above. The so-called “Moving boundary”-model infers that the rate of reduction is so fast compared to the rate of diffusion that nearly complete reduction occurs as penetration of the fiber proceeds. Under these circumstances, diffusion is the rate limiting step. The penetration picture in Fig. 71 and the swelling and penetration data for the TGA containing experiment support this theory. Swelling of the hair fiber ceased, when the fiber was completely penetrated by TGA. When Cys was used as reducing agent swelling of the hair continued for a further short time. Thus, the rate of reduction of Cys does not follow moving boundary kinetics.

A comparison of swelling and penetration under different pH conditions showed that penetration and swelling did not always cease at the same time of the experiment. Whilst the swelling ceased with penetration of the alkali at pH 12.4, the swelling approximately started at the point of complete penetration of the alkali at pH 13.4. Thus, the nature of the reaction of alkali with keratin is dependent on pH.

4. Experimental part

4.1. Materials

4.1.1. Hair

The Caucasian hair sample was purchased from Kerling Internationale Haarfabrik GmbH (Backnang, Germany). The hair was about 25 cm long, untreated, medium brown and a blend of a large number of individual hair samples. The root ends of the hairs were embedded in silicon resin. Single, naturally blond hairs from a female colleague were used for the swelling experiments. This hair was about 20 cm long and untreated. The simulated Afro hair sample (so-called “Afro Hair – Natural Hair Kinked”) was purchased from DEMEO Brothers INC. (New York, USA). This black hair was about 40 cm long and the root ends were embedded in silicon resin. The diameter of the curls was about 10 mm at the roots and 5 mm at the tips. The natural Afro hair samples were either acquired privately from the USA or provided by Namasté Laboratories L.L.C. (Blue Island, IL, USA). These hair samples varied in length from 5 to 20 cm, in color from middle brown to black, and in diameter of the curls from 2 to 8 mm.

4.1.2. Chemicals

The relaxer creams was purchased from a supermarket for ethnic products in Aachen. The pH of the creams was 12.5 for the NaOH cream and 12.9 for the No-Lye cream.

NaOH relaxer cream:	sofn'free, Trend for Men (M&M Products Company, Atlanta, GA 30309, USA) Only the relaxing cream (S-Control Texturizer, pH 12.5) and the neutralization shampoo (pH 4.7) were used. The pre-conditioner sachet and the herbal oil moisturizer were not used.
No-Lye relaxer cream:	Creme of Nature, super (Revlon Professional, Inc. Dist.; New York, NY 10022, USA) For the relaxing treatments, a fresh mixture (pH 12.9) of the Relaxer Cream and the Soft Blend Activator as well as the neutralization shampoo (ph 5.0) were used. The conditioner (Corrective Salon Hair Treatment) was not used.

L-Cysteine was obtained from SIGMA Chemie GmbH, (Deisenhofen, Germany). TGA (for synthesis), iodine solution ($c(I_2)$ = 0.05 mol/l) and collodion 4 % were purchased from Merck KGaA (Darmstadt, Germany). The quality of other chemicals used in this project was pro analysi and they were purchased either from Merck or SIGMA.

4.2. General experimental technique and analyses

4.2.1. Devices

Projection microscope:

No. 66503, Carl Zeiss, Germany

Scanning electron microscopy (SEM):

SEM S 360, Cambridge Instr., acceleration voltage 15-25 kV, working distance 15 mm

Amino acid analyzer:

LC 6000 and LC 5000, Biotronic, Germany

DSC apparatus:

DSC 7, Perkin-Elmer, Überlingen, Germany

Tensile tester:

Instron 1122, Instron International LTD., Ludwigshafen, Germany

pH-meter:

PHM 83 Autocal pH.meter, Radiometer

4.2.2. Cross-section determination

The cross-sectional parameters of all hair samples were determined according to an experimental procedure developed by *Philippen* /20/. A bundle of about 100 - 150 hairs was placed in a thin shrinkage tube. During shrinkage of the tube by heating the jutting ends of the hairs were mechanical stretched. The tube was cut perpendicularly to its length and the fiber ends embedded in an ether-starch solution (collodion 4 %) by dipping the tube into the solution. Sections of 100 - 200 µm thickness were cut, though it should be noted that the more intensive the hair is pigmented the thinner it must be the cut.

The slices were fixed on a slide and investigated under a light microscope with ca. 600 x magnifications. Diameters of the long and short axes were measured, and ellipticity and equivalent diameter thus calculated.

4.2.3. Scanning electron microscopy

The dried hair fibers were fixed on a suitable holder and sputter-coated with gold. Afterwards the samples were investigated by means of the SEM.

4.2.4. Luster determination

The experiments were carried out by Fiantec GmbH (Aachen, Germany) /75/.

4.2.5. Amino acid analysis

To 8-10 mg of hair were added 8 ml of 6 N hydrochloric acid in a pressure-resistant glass tube. The tube was evaporated and sealed. It was then heated for 24 h at 110 °C. The hydrolysate was reduced *in vacuo* at 70 °C and three times washed with distilled water and finally evaporated to dryness at 70 °C. The residue was analyzed by means of cationic exchange chromato-graphy.

4.2.6. HP-DSC measurements

Samples (5-10 mg) of chopped hair were introduced into pressure-resistant capsules (steal capsules, PerkinElmer) together with 50 µl distilled water. The filled capsule and an empty one as reference were heated up to 200 °C at a rate of 10 °C/min.

4.2.7. Determination of hair straightening efficacy

Single hairs were fixed with tape in a straightened state on a glass plate with a defined length of 9 cm. They were treated with a relaxer cream for a defined period of time at 20 °C, rinsed with tap water, treated with a neutralizing shampoo for 3 min, rinsed again and air-dried. The hair was removed from the plate by lifting one of the tapes and cutting the end of the hair directly at the other tape. The fibers were soaked for at least 10 min in tap water (30-35 °C) in their unfixed state. As a result of soaking the influence of secondary cross-links (salt linkages and hydrogen bonds) was removed and the obtained straightening can be attributed to the remaining covalent bonds. Finally the effective length (l_a) of the dried hair was determined (Fig. 42).

The effect of straightening, described by the variable E_{St} in %, was calculated as the ratio of the difference between the effective length after treatment of a hair fiber (l_a) and the effective length of an untreated hair fiber (l_b) to the difference between the true length of the hair fiber (L) and the effective length of an untreated hair fiber (l_b) as shown in eq. 3.4.

4.2.8. Ring test

To determine waveability of hair, 10 hairs each with a length of 5 cm were wound around a steal rod with a diameter of 2 mm and their ends fixed with fast glue. Care was taken to ensure that the fiber loops were parallel to each other and the fiber axis perpendicular to the rod axis. Each rod was treated in a single test tube. After the treatment the hair loops were cut open with a razor blade and dropped into a Petri dish which was filled with distilled water. The loops were soaked for at least 30 min. A 400 x enlargement of the Petri dishes was made using a normal photocopier. The distance between the ends of the hair curls was determined with a ruler.

4.2.9. Diffusion and swelling measurements

To determine the degree of diffusion and swelling, blond hairs were dyed with elemental iodine. The hairs were suspended in 0.1 N iodine solutions for 4 h at 30 °C, briefly rinsed with distilled water and dried in air. About 1 cm of a dyed hair fiber was fixed on a slide, protected with a cover slide and examined under a light microscope. The fiber was pre-swollen with distilled water for at least 15 min. The water was then removed with a piece of paper tissue and replaced by the alkaline solution (22 °C). The temperature and concentration of the solution was maintained constant by minimizing the exposure to light in the microscope, and with a continuous supply of new solution during the experiment. Since the iodine reacts with the alkali according to a disproportionation into colorless IO^- and I^-, it was possible to observe the swelling and color removal and record it on video. Snapshots from this video were taken and both the fiber diameter and the front of diffusion were measured using image analysis. Thus, the swelling of the fiber and the penetration depth of the alkali were determined.

4.3. Treatment of hair

4.3.1. Preparation and preliminary treatment of the hair

The hair tresses were soaked for 5 min at 35 °C in tap water, then shampooed with a 15 % SDS-solution for 2 min and finally rinsed for 3 min under running tap water (35 °C) and subsequently air-dried.

4.3.2. Relaxing treatment with commercial relaxing products

Relaxer cream was applied on the air-dried hair samples using a cosmetic paintbrush. The hair samples were brushed from both sides to ensure that each fiber was thoroughly soaked in the cream. Durations and temperatures of the relaxing treatments are shown in Tab. 18 for the different treatments. After treatment the hair tresses were rinsed with tap water for one minute and twice washed with the neutralization shampoo. The duration of shampooing and rinsing was two minutes each. The samples were dried in air.

Tab. 18: Duration and temperature for different treatments with relaxing cream

Treatment		Processing time of cream in min	Temperature in °C
Comparison of different hair types	NaOH	10	22
	No-Lye	15	22
Hair relaxing investigations	NaOH	0 - 20	20
	No-Lye	0 - 20	20

4.3.3. Permanent waving treatments

Hair was permed two weeks after the relaxing treatment.

Treatments for the comparison of different hair types (chapter 3.2.):
The hair tresses were soaked at RT for 15 min in water. The tresses were then exposed to an 8 % w/w TGA-solution (adjusted with ammonia (30 %) to pH 8.8) for 25 min at 25 °C. Since the hair was very soft, it was rinsed by putting it in a tap-water-filled glass box for 10 min at 35 °C. The tap water was exchanged after 2 and again after 5 min. The subsequent oxidation took place upon treatment with a 3 % hydrogen peroxide solution (adjusted with phosphoric acid to pH 2.4) for 25 min at 25 °C. Afterwards, the hair was rinsed with running tap water of 35 °C for 10 min and air-dried.

Treatment for testing the waveability of relaxed hairs (chapter 3.4.):
Single hairs were wound on steel rods and soaked at RT for 5 min. Afterwards, the hairs were subjected to an 8 % w/w TGA-solution (adjusted with ammonia (30 %) to pH 9.0) for 10 min at 30 °C. Every steel rod was placed in one test tube. The rods were taken out of the TGA-solution, briefly held under running tap water, and put in a water-filled glass beaker for 15 min at RT. The hair was then subjected to 3 % hydrogen peroxide solution adjusted with phosphoric acid to pH 2.3 at 30 °C for 10 min with a, deposited in an water-filled beaker for 10 min at RT and subsequently air-dried.

All experimental solutions were used in large excess.

4.4 Statistical annotations

4.4.1 Introduction

Statistics are used to analyze and interpret data /76/. They objectively evaluate and reliably judge conclusions based on the data. In the research of human hair, the analysis of data has to consider:

- The natural variability of the material
- Differences of the raw material due to its chemical and physical prehistory
- The limited number of available data

Before data can be analyzed, they must be collected. Statistical considerations are obligatory in the design of experiments and in the conception of the hypotheses. The aim of all experiments is to find chemical or physical alterations after a treatment or to find primary differences between raw materials.

For this purpose random samples of a population are taken. Measured results establish the basis of the evaluation of the population. Three claims are linked to the evaluation:

- Unbiased statistics:
  It is desirable that if one takes an indefinitely large number of samples from a population, the long run average of the obtained statistics will equal the parameter.
- Consistent statistics:
  As the sample size increases, consistent statistics will become a better estimate of the parameter it is appraising.

- Reliability:

  The estimate should result from a variation as small as possible. Since frequently only one sample is secured from a population, it is important to arrive at a close estimate of a parameter from a single sample.

The program Statistica 5.0 is used for all statistical calculations.

4.4.2 Description of a population and sample

In samples, as well as in populations, one generally finds a preponderance of values somewhere around the middle of the range of observed values. The description of this concentration near the middle is an average to the layman, and a measure of central tendency to the statistician. The most widely used measure of central tendency is the arithmetic mean:

$$\bar{x} = \sum_{i=1}^{n} \frac{x_i}{n} \tag{4.1}$$

Where x_i represents the individual observation and n is the number of observations.

In addition to a measure of central tendency, it is generally desirable to have a measure of the dispersion/variability of data. This is an indication of the scatter of measurements around the center and is normally given by the standard deviation (eq. 4.3). The square of the standard deviation is the variance (eq. 4.2):

$$s^2 = \frac{\sum_i (x_i - \bar{x})^2}{n-1} \tag{4.2}$$

$$s = \sqrt{s^2} \tag{4.3}$$

The standard error of the mean is defined as:

$$s_{\bar{x}} = \sqrt{\frac{s^2}{n}} \tag{4.4}$$

Confidence limits: The statistical procedure for addressing a question first involves the concise statement of the hypothesis to be tested; statistically this is referred to as null hypothesis (abbreviated H_0).

It is necessary to consider how small a probability can lead one to reasonably conclude that H_0 is false. The probability conventionally used in scientific statistics is 0.05 (i.e. 5%) referred to as α, the level of significance of the statistical test.

The confidence interval for a sample mean is defined as:

$$q_{95\%} = \bar{x} \pm t_\alpha \cdot s_{\bar{x}} \tag{4.5}$$

Where t_α is tabulated for the most common α values and numbers of observations. For n equal to infinity t_α becomes 1.96 ($\alpha = 0.05$).

Hair can be analyzed in two ways. If the sample consists of single hairs the experiment must contain at least 40 hairs because of the natural variability of the hair. The results are presented in so-called *Box and Whisker plots* illustrating the arithmetic mean (Mean), standard error (1.00*Std. Err.), and the limiting value

for the 95 % confidence limit (1.96*Std. Err.). A typical legend of such a plot is shown in Fig. 79.

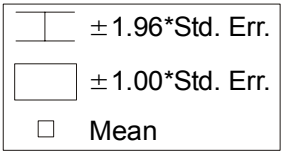


Fig. 79: Legend of a typical *Box and Whisker plot* frequently used in this work to present results.

When experiments were performed with larger amounts of hair, the natural variability of hair is averaged. To verify the results, at least two analyses have to be performed per experiment. The results of these tests are represented by the mean and the standard variation.

4.4.3 t-Test for independent samples

The terms *dependent* and *independent variable* apply mostly to experimental work where some variables are manipulated, and in this sense they are "independent" from the initial reaction patterns, features, intentions, etc. of the subjects. Some other variables are expected to be "dependent" on the manipulation or experimental conditions. That is to say, they depend on "what the subject will do" in response. *Independent variables* are those that are manipulated whereas *dependent variables* are only measured or registered. Somewhat contrary to the nature of this distinction, these terms are also used in studies where we do not literally manipulate *independent variables*, but only assign subjects to "experimental groups" based on some preexisting properties of the subjects. For example, if in an experiment, males are compared with females regarding their white cell count (WCC), Gender could be called the *independent variable* and WCC the *dependent variable*.

The t-test is the most commonly used method to evaluate the differences between the means of two groups. For example, the t-test can be used to test for a difference in test scores between a group of patients who were given a drug and a control group who received a placebo.

To reject the null hypothesis, H_0, that the samples do not differ, the calculated *t* value must be bigger than the critical value, which is a tabulated value for the most common α values and numbers of observations.

$$H_0 \; is \; false \qquad if \qquad t_{calculated} > t_{critical \; value} \tag{4.6}$$

The t value is calculated by the following equation:

$$t_{calculated} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2 + s_2^2}{n_1 + n_2}}} \tag{4.7}$$

4.4.3 Simple linear regression

The relationship between two variables may be a function of dependence of one on the other. The magnitude of one of the variables, *y*, is assumed to be determined by – i.e., is a function of – the magnitude of the second variable, *x*, whereas the reverse is not true. In the following, the dependent variable, *y,* represents the property of treated hair; the independent variable, *x,* is mostly the time of treatment.

Such a dependent relationship is termed a regression; the term simple regression refers to the fact that only two variables are being considered. In the case of simple regression, a direct straight line is described as “linear” relationship between the two variables (eq. 4.8).

$$y = a + bx \tag{4.8}$$

a represents the intercept of the line and *b* is the slope of the straight line. They are population parameters and, therefore, constants.

The proportion (or percentage) of the total variation in *y* that is explained or accounted for by the fitted regression, is termed the coefficient of determination, R^2 (eq. 4.9). It may be thought of as a measure of the strength of the straight-line relationship:

$$R^2 = \frac{\left(n(\sum x_i y_i) - (\sum x_i \sum y_i)\right)^2}{\left(n\sum x_i^2 - (\sum x_i)^2\right) \cdot \left(n\sum y_i^2 - (\sum y_i)^2\right)} \tag{4.9}$$

The maximum value of R^2 is 1. Generally, $R^2 > 0.95$ features a strong linear dependency of the variable *y* on the variable *x*.

4.4.4. General linear model

The general linear model (GLM) is a generalization of the linear regression model, such that effects can be tested (1) for categorical predictor variables (e.g. gender), as well as for effects for continuous predictor variables (e.g. age) and (2) in designs with multiple dependent variables (e.g. ellipticity or diameter) as well as in designs with a single dependent variable. GLM uses the methods of smallest squares to estimate effects and to test hypotheses.

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Lebenslauf

Jutta Maria Quadflieg geb. Bußmann

Geburtsdatum:	10.12.1971
Geburtsort:	Dortmund
Eltern:	Franz-Josef Anton Bußmann
	Barbara Bußmann geb. Hildebrand
Familienstand:	verheiratet

1976 – 1980	Kerschensteiner Grundschule, Dortmund
1980 – 1991	Mallinckrodt-Gymnasium, Dortmund
1991 – 1998	Studium der Chemie an der RWTH Aachen
	Diplomarbeit am Deutschen Wollforschungsinstitut an der RWTH Aachen e.V.
	Thema: *Enyzmbehandlung von Haaren*
1998 – 2002	Promotion am Deutschen Wollforschungsinstitut an der RWTH Aachen e.V.
05.09.2003	Tag der mündlichen Prüfung
seit 01/2003:	Wissenschaftliche Mitarbeiterin bei Institute Dr. Schrader, Holzminden